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Effect of Different Levels of L-Carnitine on the Productive Performance, Egg Quality, Blood Parameters and Egg Yolk Cholesterol in Laying Hens

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Abstract

This experiment was conducted to investigate the effects of different levels of L-carnitine on productive performance, egg quality and blood parameters in laying hens. Forty-eight Hy-Line W-36 egg Layers were weighed at 90 weeks of age and randomly allocated into 16 cages (three hens per cage). Four dietary treatments were prepared by supplementing L-carnitine (0, 50, 100 and 150 mg/kg of diet) to corn-soybean meal diet and offered *ad libitum* to hens. After two weeks of acclimatization, the eggs were weighed daily and feed intake as well as egg quality traits were measured biweekly. At the end of the experiment, two hens from each cage were selected to determine blood parameters and two eggs from each replicate were collected for cholesterol analysis. Results showed that L-carnitine supplementation at 100 and 150 mg/kg significantly increased egg production and egg mass, but decreased yolk cholesterol content. Laying hens receiving diet containing 50 mg/kg L-carnitine had significantly higher Hough unit, but lower progesterone than the hens fed control diet ($P < 0.05$). The results of this study showed that supplementing hens' diet with L-carnitine had beneficial effects on productive performance and decreased yolk cholesterol concentration; so it can be used as an effective supplement in the diet of laying hens.

Introduction

L-Carnitine is a substance which is made of Lysine and Methionine that is essential for the transport of long-chain fatty acids through the mitochondrial membrane in order to increase fat metabolism (Borum, 1983; Buyse *et al.*, 2001; Xu *et al.*, 2003). L-carnitine supplementation of diets helps the body turn fat into energy with augmenting β-oxidation (Kachura *et al.*, 1995). Triacylglycerol production is reduced with supplemental L-carnitine diet, therefore, storage of long chain fatty acids decrease (Xu *et al.*, 2003). Secondary functions of L-carnitine is delimiter and scavenger of potentially toxic groups from cells, moderating ratio of free CoA and acetyl-CoA between the mitochondria and cytoplasm, especially in biological procedure including set out gluconeogenesis, encouraging fatty acid and the metabolism of ketones, branched-chain amino acids, triglycerides (TG) and cholesterol (Novotny, 1998).

The continuous addition of L-carnitine to experimental layers diets resulted in decrease of concentrations of total protein, cholesterol, calcium, and phosphorus (Thiemel and Jelinek,

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2004). Rabie *et al*. (1997) concluded that dietary L-carnitine has an advantageous effect on Haugh unit and could reclaim the ingredients of the dietary part of the egg during the late laying period. Some parameters such as body weight, feed intake, egg production and yolk cholesterol concentrations were not affected by dietary of L-carnitine and nicotinic acid in the early laying period. Hossininezhad *et al*. (2011) reported that feed conversion ratio (FCR) was significantly decreased by dietary L-carnitine supplementation. Parizadian *et al*. (2011) reported that supplementation of L-carnitine (125 and 250 mg/kg) significantly reduced egg yolk cholesterol and triglyceride in quails.

Animal organism is able to synthesis carnitine. Moreover, most portions of poultry diets are formed of vegetable sources and as regard to animal-based meals are the major exogenous source of carnitine, therefore, poultry has also contributed to carnitine deficiency. Since L-carnitine plays a pivotal role in transporting long-chain fatty acids across the inner mitochondria membrane to produce energy through β-oxidation (Durán *et al*., 2002), supplementation of L-carnitine may accelerate yolk lipid metabolism (Peebles *et al*., 2007) and thus expedite follicular development. It may also increase metabolic rate in the magnum and shell gland and therefore affects albumen deposition and shell calcification, leading to greater egg weight and shell thickness.

The objective of the present study was to investigate the effect of different levels of L-carnitine on the productive performances, egg quality, blood parameters and egg yolk cholesterol concentration of laying hens in late-phase production**.**

Materials and methods

All experimental procedures used in this experiment were approved by the Animal Care Committee of the Sari Agriculture and Natural Resources University (SANRU) of Iran. A total of 48, Hy-Line W-36 layers were randomly assigned to four treatments with four replicates and three layers per each replicate. A control corn-soybean meal based diet formulated according to Hy-line W-36 Commercial Management Guide (Hy-Line International 2009-2011) and supplemented with 0, 50, 100 and 150 mg/kg L-carnitine (Lohman, Germany) for preparing dietary treatments (Table 1). All birds were housed in caged layers and received feed and water *ad libitum*. House temperature was kept at 21°C and the light program consisted of 16 hrs light daily throughout the experiment. At 90 weeks of age, hens were selected on the basis of their egg production and body weight (BW) and assigned to dietary treatments two weeks prior to the trial, to ensure that the egg production and body weight in each group were similar. The experiment started at 92 weeks of age and lasted for six weeks.

Table 1. Ingredients and chemical composition of basal diet[1]

Ingredients (%)	
Corn	61.69
Soybean meal	22.04
Soybean oil	3.00
Calcium Carbonate	10.87
Dicalcium phosphate	1.39
Salt	0.41
Vitamin premix[2]	0.25
Mineral premix[3]	0.25
DL- Methionine	0.10
Calculated nutrients composition	
ME (Kcal/kg)	2815
Crud protein (g/kg)	150.00
Calcium (g/kg)	45.00
Nonphytate P (g/kg)	4.00
Methionine + Cystine (g/kg)	6.43
Lysine (g/kg)	8.49

[1]For preparing dietary treatments the basal diet supplemented with 0, 50, 100 and 150 mg/kg L-carnitine.
[2]Vitamin premix provided per Kg of diet: vitamin A (retinyl acetate), 8800 IU; vitmain D_3 (cholecalciferol), 2200 IU; vitamin E (DL-α-tocopheryl acetate), 11 IU; vitamin K (menadione sodium bisulfite), 2.2 mg; vitamin B_2 (riboflavin), 4.4 mg; pantothenic acid (D-calcium pantothenate), 8.8 mg; nicotinic acid, 44 mg; vitamin B_6 (pyridoxine hydrochloride), 2.2 mg; biotin, 0.11 mg; vitamin B_1 (thiamine hydrochloride), 2.5 mg; ethoxyquin, 125 mg.
[3]Mineral premix provided per kg of diet: manganese ($MnSO_4$: H_2O), 185 mg; zinc (ZnO), 62 mg; iron ($FeSO_4$: $7H_2O$), 149 mg; cupper ($CuSO_4$: $5H_2O$), 19.6 mg; iodine (KI), 1.4 mg; selenium (Na_2SeO_3), 0.22 mg.

Eggs weight was recorded daily and feed intake biweekly. Egg quality and specific gravity were determined on eggs produced during a 4-d period at the end of each two weeks. The eggs from the first and second days were used to measure egg quality and others for specific gravity after weighing all eggs (Nasiri Moghaddam *et al*., 2012). The specific gravity was determined by using the formula according

to the Archimedes method (Hempe *et al*., 1988). To measure egg quality, eggs were broken on a flat surface and yolks were separated from the albumen. The yolk rolled on a paper towel to remove adhering albumen and weighed. The shells were carefully washed with warm water and dried at 55°C for 48 hrs in the oven and then weighed. The shell thickness was measured at three locations on the eggs (air cell, equator, and sharp end) using a micrometer caliper with an accuracy of ± 1 µm (series 500, Mitutoyo, Tokyo, Japan) and the average was calculated (Tahmasbi *et al*., 2012). The surface area (SA) of the egg was calculated as indicated by Ousterhout (1980). The shell weight per unit of surface area (SWUSA) was calculated by dividing the dried shell weight to the surface area of each egg. Yolk color measured using a yolk color chart and Haugh unit calculated based on albumen height and egg weight using the formula: $HU = 100 \log10 (H - 1.7 W^{0.37} + 7.56)$, (Carter, 1975). Where: HU= Haugh unit, H= height of the albumen (mm) and W= egg weight (g).

At the end of experiment (at 7:00 am), blood samples were taken from two layers of each cage to measure aspartate aminotransferase (AST), Alanine transaminase (ALT), glucose, triglyceride, high-density lipoprotein cholesterol (HDL_C), very low density lipoprotein cholesterol ($VLDL_C$), 17-Beta estradiol and progesterone concentrations. Blood was taken from the left brachial vein and collected in plastic tubes contained EDTA. Samples were maintained on ice until plasma was separated by centrifugation (1500 × g for 20 minutes) within 1 hr of collection. Plasma was harvested and stored at -20°C until further analysis. Plasma concentrations of progesterone and estradiol were determined using ELISA kit (Diaplus, North York, Ontario, Canada) and ELISA plate reader (Stat Fax 210). Plasma concentrations of glucose (kit no. 5412, Pars Azmoon Co, Tehran, Iran), and triglycerides (kit no. 6752, Pars Azmoon Co, Tehran, Iran) were determined using Autoanalyzer (BS120, Mindray, Shenzhen, China). HDL_C measured with a spectrophotometer (Apel-PD-303S) according to Pars Azmoon kit procedure and $VLDL_C$ calculated by dividing triglyceride values to five ($VLDL_C = TG / 5$).

Eight random samples of eggs from each treatment were obtained for egg yolk cholesterol analysis at the end of the experiment. Hard boiled and separated egg yolks were used for the determination of cholesterol. After homogenization, 0.1 g of sample was extracted with isopropanol, followed by centrifugation at 1300 × g at 4°C for 10 min. Cholesterol content was determined by a UV spectrophotometer (Apel, PD-303S, Japan), and absorbance was read at 500 nm (Boehringer Mannheim, 1989).

Statistical analysis

Data were analyzed according to a completely randomized design using the GLM procedure of SAS (2003) software. Differences among treatment means were measured by Duncan's multiple range test and considered significant at $P < 0.05$.

Results and Discussion

The effects of L-carnitine supplementation on the productive performance of laying hens are shown in Table 2. Egg production was higher in hens fed L-carnitine supplemented diets compared to those hens fed control diet (from 62.23 to 66.99 *vs.* 56.25%). However, the egg production difference was only significant when hens received more than 50 mg/kg L-carnitine. Consistent with our results, Richter *et al*. (1998) reported a numerical increase in egg production by L-carnitine supplementation. Neuman *et al*. (2002) showed that L-carnitine supplementation increases β-oxidation of fatty acids to adenosine triphosphate, therefore, energy yielding increases. Thus, supplemental L-carnitine could enhance fatty acid and energy utilization (Mast *et al*., 2000). In contrast, Corduk and Sarica (2008) reported that L-carnitine supplementation in diet did not affect egg production because L-carnitine has been supplemented to low energy diets.

The hens received L-carnitine had higher egg mass than the control group, but it was significantly higher in groups with 100 and 150 mg/kg L-carnitine ($P < 0.05$). Since egg mass is related to egg production and egg weight, any changes in these two parameters will affect on egg mass. No significant effect was found by supplementing of L-carnitine on egg weight, feed intake, FCR and shape index among treatments. Consistent with our results, Yalçin *et al*. (2006) indicated that L-carnitine supplementation at 100 mg/kg had no significant effect on feed intake, egg weight and

FCR, whereas Daşkiran *et al*. (2009) reported that L-carnitine supplementation to the basal diet had a significant effect on shape index.

Table 2. Effect of different levels of L-carnitine supplementation on productive performance of laying hens

Parameters	L-carnitine levels (mg/kg)				*P*-value
	0 (Control)	50	100	150	
Hen-day egg production (%)[1]	56.25[b]	62.23[ab]	64.06[a]	66.99[a]	0.01
Egg weight (g)	62.70	62.38	62.00	61.18	0.92
Egg mass (g/hen/d)[2]	35.21[b]	38.11[ab]	39.66[a]	40.98[a]	0.04
Feed intake (g/day)	107.10	110.40	111.90	110.50	0.91
FCR (g feed intake/g egg mass)	3.04	2.84	2.83	2.69	0.27
Shape index[3]	73.30	73.14	74.38	74.16	0.73

[a,b]Means within a row without common superscript differ significantly ($P < 0.05$).
[1]Hen-day egg production = (100 × number of eggs laid) / (number of hens × days).
[2]Egg mass = (egg production × egg weight) / 100
[3]Shape index = [Egg width (mm) / Egg height (mm)] × 100

Although supplementation of L-carnitine had no significant effect on egg albumen, egg yolk, yolk color and yolk index, but Haugh unit was significantly higher in hens received diet contained 50 mg/kg L-carnitine compared to hens fed control diet (Table 3). Rabie *et al*. (1997) reported that albumen quality was improved with L-carnitine supplementation, probably due to the higher metabolic rate in magnum and/or increase in activity of the shell gland. It is obvious to improve Haugh unit when hen's diets supplemented with L-carnitine because albumen is made from ovomucin and especially β-ovomucin, secreted by the magnum. Gelatinous traits mainly depend on β-ovomucin that is responsible for the thick albumen gel and here upon secretion of β-ovomucin extremely promoting with L-carnitine supplementation to diet that increase metabolizable energy generation in the magnum (USDA, 2000). It was reported that supplementation of L-carnitine at dose of 50 ppm to drinking water significantly increased albumen weight and height (Celik *et al*., 2004). Also, these researchers pointed that yolk index and yolk color score did not significantly affect with L-carnitine supplementation, whereas yolk weight was significantly decreased. L-Carnitine has presumably altered hepatic biosynthesis of yolk precursors with a shift of their transport from the liver to the ovary. Celik *et al*. (2004) showed a lower egg yolk percentage in hens fed diets containing L-carnitine.

Table 3. Effect of different levels of L-carnitine supplementations on internal egg quality traits in laying hens

Parameters	L-carnitine levels (mg/kg)				*P*-value
	0 (Control)	50	100	150	
Egg albumen (%)	65.98	62.95	61.84	63.45	0.36
Egg yolk (%)	26.03	28.82	29.27	28.22	0.43
Yolk color	5.91	6.22	7.66	5.72	0.31
Yolk index	0.41	0.39	0.39	0.38	0.72
Haugh unit	86.78 [b]	92.12 [a]	91.20[ab]	86.97 [b]	0.04

[a,b]Means within a row without a common superscript differ significantly ($P < 0.05$).

Dietary carnitine supplementation had no significant effect on egg shell quality parameters including shell weight, shell thickness, surface area, SWUSA and specific gravity (Table 4) which agrees with results of Rabie *et al*. (1997) and Celik *et al*. (2004). Shell breaking strength may be reduced with supplementation of L-carnitine to diet because surcharge of L-carnitine increases calcium excretion owing to the β-oxidation of long chain fatty acid in metabolism (Corduk and Sarica, 2008). In contrast to our results, egg specific gravity was affected with supplementation of dietary L-carnitine and humate in Sariözkan *et al*. (2013) study.

Table 4. Effect of different levels of L-carnitine supplementations on egg shell quality in laying hens

Parameters	L-carnitine levels (mg/kg)				*P*-value
	0 (Control)	50	100	150	
Shell weight (%)	7.97	8.22	8.40	8.31	0.58
Egg shell thickness (mm)	0.43	0.44	0.44	0.44	0.97
Surface area (cm^2)	75.04	75.19	74.55	73.79	0.92
SWUSA[1] (mg/cm^2)	68.22	70.50	71.50	70.70	0.74
Specific gravity (g/cm^3)	1.06	1.06	1.06	1.07	0.72

[1]Shell weight per unit of surface area.
No significant difference was observed between treatments in each parameter ($P > 0.05$).

Table 5. Effect of different levels of L-carnitine supplementations on blood parameters in laying hens

Parameters	L-carnitine levels (mg/kg)				*P*-value
	0 (Control)	50	100	150	
Estradiol (pg/mL)	3.67	2.26	1.70	2.57	0.30
Progesterone (ng/mL)	232.60[a]	163.70[ab]	114.90[b]	175.30[ab]	0.07
Glucose (mg/dL)	212.70	211.00	213.25	211.50	0.98
AST (IU/L)	175.50	170.70	203.00	191.00	0.38
ALT (IU/L)	6.50	7.75	10.00	8.25	0.83
Triglyceride (mg/dL)	1400.0	1510.5	1950.0	1440.7	0.49
HDL_C (mg/dL)	77.25	69.75	70.50	73.50	0.80
$VLDL_C$ (mg/dL)	28.00	30.30	39.00	28.95	0.49
Yolk cholesterol (mg/g)	14.76[a]	10.36[ab]	8.80[b]	7.88[b]	0.06

[a,b]Means within a row without a common superscript differ significantly ($P < 0.05$).

Effect of L-carnitine on blood parameters and yolk cholesterol has been presented in Table 5. No significant difference was found between dietary treatments for plasma estradiol (E2) concentration. Progesterone concentration was lower in hens fed L-carnitine diets compared to the control diet, but it was significant only at the level of 100 mg/kg supplementation. Yolk cholesterol has tended to decrease by L-carnitine supplementation. Agarwal and Said (2004) reported that L-carnitine enhances the oxidation of fatty acids which stimulates estrogen and progesterone biosynthesis by increasing the regeneration of the reducing equivalents necessary for the cholesterol side-chain cleavage reaction. Walzem (1996) stated that yolk lipids are synthesized in the liver under the influence of estrogen. Since L-carnitine can reduce body fat, progesterone may be reduced by reducing fat. There was no significant difference between dietary treatments for plasma glucose, AST, ALT, TG, cholesterol, HDL, LDL and VLDL concentrations. Similar results have been reported by Arsalan *et al*. (2003) who reported that L-carnitine did not affect serum cholesterol, total lipid, triglyceride and glucose. Carnitine palmitoyl transferase is one of the main enzymes relevant to the fatty acid β-oxidation and augments of L-carnitine to the diet increases this enzyme activity in broiler chickens (Lien and Horng, 2001). However, these researchers did not observe any significant activities of other fatty acid β-oxidation enzymes by L-carnitine supplementation to broiler diets. Conversely, Bell *et al*. (1987) carried out an experiment with different levels of L-carnitine on rabbits and concluded that dietary L-carnitine decrease serum cholesterol and triglyceride concentrations. Corduk *et al*. (2007) showed that L-carnitine supplementation did not influence glucose, blood urea nitrogen, cholesterol, triglyceride and creatinine concentration in broiler chickens. The administration of L-carnitine to experimental layers increased plasma glucose (Thiemel and Jelinek, 2004). They found that concentration of AST decreased during the experimental period in breeder layers. In this respect, L-carnitine can be considered as a protecting agent, particularly in the liver parenchyma. Arsalan *et al*. (2003) showed that L-carnitine supplementation did not affect serum cholesterol, total lipid, triglyceride and glucose levels. Nevertheless, cholesterol, total lipid, triglyceride and glucose levels decreased when L-carnitine supplemented to diet. These researchers pronounced that non-significant decreases in lipid fractions (cholesterol, total lipid, and triglyceride) were probably the result of β-oxidation of long chain fatty acids with the support of additional L-carnitine. In contravention of our experiment, many studies carried out on different animal

species showed that L-carnitine administration reduced serum cholesterol, total lipid and triglyceride levels (Bell *et al*., 1987; Mondola *et al*., 1992). Lien and Horng (2001) noticed that the activity of carnitine palmitoyltransferase, which is the fatty acid β-oxidation enzyme, was significantly increased by supplementation L-carnitine in broilers diet.

Conclusion

Dietary L-carnitine supplementation did not have any effect on egg quality, most likely because of the egg quality mostly related to several factors such as calcium, phosphorus, vitamin D_3, egg size, etc. Higher concentrations of L-carnitine at the level of 100 and 150 mg/kg diet resulted in an increase of egg production and egg mass. Administrations of L-carnitine to diet have tended to decrease egg yolk cholesterol, but Haugh unit increases at the level of 50 mg/kg diet.

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