The effects of L-carnitine-loaded solid lipid nanoparticles on performance, antioxidant parameters, and expression of genes associated with cholesterol metabolism in laying hens

Masoud Eskandani,[*] Bahman Navidshad,[*] Morteza Eskandani ,[†,1] Somayeh Vandghanooni,[‡] Farzad Mirzaei Aghjehgheshlagh,[*] Ali Nobakht,[§] and Amir Ali Shahbazfar[#]

[*]*Department of Animal Science, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran;* [†]*Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran;* [‡]*Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran;* [§]*Islamic Azad University-Maragheh Branch, Maragheh, Iran; and* [#]*Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran*

ABSTRACT The purpose of this study was to investigate the production performance, antioxidant parameters, egg yolk cholesterol content, and expression of genes related to cholesterol metabolism in laying hens fed L-carnitine (**LC**) and L-carnitine-loaded solid lipid nanoparticles (**LC-SLNs**). A total of 350 Hy-Line (w-36) laying hens at 50 wk of age (1520.0 ± 0.7 g) were randomly assigned to 35 units (5 replicates and 50 hens in each treatment) with seven dietary treatments as a completely randomized design. The dietary treatments were corn-soybean meal-based diets, including 1) Control (basal diet); 2) Basal diet +50 mg/kg LC (50LC); 3) Basal diet +100 mg/kg LC (100LC); 4) Basal diet +150 mg/kg LC (150LC); 5) Basal diet +50 mg/kg LC-SLNs (50LC-SLNs); 6) Basal diet +100 mg/kg LC-SLNs (100LC-SLNs) and 7) Basal diet +150 mg/kg LC-SLNs (150LC-SLNs). Results showed that the 50LC-SLNs had the least feed conversion ratio (**FCR**) in all groups ($P < 0.05$). The dietary supplementation of 100LC-SLNs decreased ($P < 0.01$) the egg yolk cholesterol concentration from 14.71 to 11.76 mg/g yolk (25%). The 50LC-SLNs group produced the most total antioxidant capacity with a difference of 58.44% compared to the control group ($P < 0.01$). The greatest amount of total superoxide dismutase was found for 50LC-SLNs ($P < 0.05$), while the glutathione peroxidase was not affected by the experimental treatments ($P > 0.05$). Serum malondialdehyde levels were reduced by 50.52% in laying hens fed 50LC-SLNs compared to the control group ($P < 0.05$). The transcript level of 3-hydroxy-3-methylglutaryl coenzyme A reductase was significantly decreased ($P < 0.01$) in the LC and LC-SLNs groups. The expression of cholesterol 7α-hydroxylase was significantly increased ($P < 0.01$) in the plain LC (~83%) and LC-SLNs (~91%) groups. The inclusion of LC-SLNs in the diet increased ($P < 0.05$) the villus height and decreased villus width in all three parts of the small intestine. Dietary inclusion of LC was found to reduce egg yolk and serum cholesterol content by improving the production performance and antioxidant status. The LC-SLNs groups were more affected than the plain LC groups, which may be attributed to the increased bioavailability of LC.

Key words: cholesterol, egg, laying hens, L-carnitine, solid lipid nanoparticles

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INTRODUCTION

Obesity, cardiovascular disease, and non-alcoholic fatty liver are the 3 major public health problems associated with cholesterol (Enjoji et al., 2012; Geiker et al., 2018; Zhuang et al., 2021). Approximately 15 to 30% of people worldwide have a hyper-response to dietary cholesterol (Elkin, 2006). The egg is widely consumed, but with caution, owing to its high cholesterol content (~ 200 mg cholesterol/egg; Zhuang et al., 2020). Efforts have been made over the last four decades to reduce the cholesterol content of egg yolks. However, many of these strategies were ineffectual (reduced by 10%; Elkin, 2017). L-carnitine (**LC**) (β-hydroxy-

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[1]Corresponding author: eskandanim@tbzmed.ac.ir, morteza.eskandani@gmail.com (ME)

γ-trimethyl-aminobutyrate, $C_7H_{15}NO_{3)}$ plays an important role in lipid metabolism and acts as an essential cofactor for the β-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane as acyl-carnitine esters (Tekeli, 2019'; Kuter and Önol, 2021; Liu et al., 2021). According to reports, LC is a potent antioxidant that protects antioxidant enzymes from oxidative damage (Canbolat et al., 2017). LC treatment can also decrease serum cholesterol and triglycerides in rats and domestic fowls (Eskandari et al., 2004; Malaguarnera et al., 2009; Adabi et al., 2011; Jang et al., 2014; Fallah and Mirzaei, 2016; Rehman et al., 2017). The addition of LC to the laying hen diet reduced egg yolk cholesterol and triglycerides for unknown reasons (Adabi et al., 2006; Rehman et al., 2017; Placidi et al., 2022).

Because of the hygroscopic properties of LC, it is extremely difficult to formulate it in medicinal dosage forms with optimal stability and bioavailability (Bhosle et al., 2006). The absolute bioavailability of LC after 1 to 6 g oral dosages is 5 to 18% (Evans and Fornasini, 2003). Moreover, its hydrophilic nature limits its diffusion over the bilayer lipid layer of the intestinal epithelium, diminishing bioavailability. In this context, lipid-based carriers mainly have broad usage to enhance the bioavailability of drugs (e.g., LC) undergoing hepatic metabolism (Desai and Thakkar, 2018). Solid lipid nanoparticles (**SLNs**) have been studied as an alternative colloidal drug delivery technology for decades. SLNs increase the cell or tissue uptake of drugs, mainly due to their small size, improve the bioavailability of drugs by increasing their diffusion through biological membranes and protect drugs against enzyme activation (Genç et al., 2015).

The use of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (**HMGR**) has been demonstrated to be one of the most successful strategies for lowering egg cholesterol content (Puertas and Vázquez, 2019). HMGR regulates cholesterol production in the liver (Deng et al., 2020) and some studies showed that LC inhibits HMGR activity (Wu et al., 2015; Bagherzadeh et al., 2019). It appears that LC either inhibits HMGR in the liver of laying hens or induces excessive cholesterol excretion through bile by raising the activity of the cholesterol 7 alpha-hydroxylase (**CYP7A1**) enzyme. CYP7A1 catalyzes the first and rate-limiting step in the bile acid biosynthetic pathway, which turns cholesterol into bile acids and plays a key role in maintaining cholesterol and bile acid homeostasis (Chambers et al., 2019; Chiang and Ferrell, 2020a). We tried to find the role of LC in decreasing cholesterol in egg yolk by measuring the expression of HMGR and CYP7A1 genes and by quantifying cholesterol in the blood, eggs, and bile in layer hens. The present study aimed to evaluate the dietary supplementation of laying hens with LC and LC-SLNs on the production performance traits, antioxidant parameters, intestine histomorphology, and blood hematology. We aimed to see whether LC treatments could affect the expression of genes involved in cholesterol metabolism and the cholesterol content of egg yolks in laying hens.

MATERIALS AND METHODS

The protocol for the animal experimental procedures was approved by the Animal Care and Use Committee of Animal Science College of Mohaghehgh Ardabili University (Permit Number: AM98052003-2/12).

Chemicals and Reagents

L-Carnitine (LC; C7H15NO3 HCl) was purchased from Sigma-Aldrich (Sigma-Aldrich, ≥98% L-Carnitine, Cas. No. 6645-46-1; St. Louis, MO). L-Carnitine-loaded solid lipid nanoparticles (spray-dried LC-SLNs, with a content of ≥20% LC) were prepared and various physicochemical characterizations were evaluated (Eskandani et al., 2022). A serum cholesterol detection Assay kit was purchased from a Biosis (Athens, Greece). Yolk and bile cholesterol, total antioxidant capacity (**TAOC**), total superoxide dismutase (**TSOD**), and glutathione peroxidase (**GSH-Px**) detection kit was purchased from a Randox (Antrium, UK). To assay the levels of glucose (**GLU**), alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), triglycerides (**TG**), and high-density lipoprotein (**HDL**), detection kits bought from Pars Azmoon (Tehran, Iran). Malondialdehyde (**MDA**) was assessed by using an MDA kit that was purchased from Sigma-Aldrich (Poole, UK). For total mRNA extraction from the liver tissues, the TriReagent kit was bought from Sigma-Aldrich (St. Louis). All other materials not mentioned here were pharmaceutical grades and provided by Merck Co (Darmstadt, Germany).

Animals and Treatments

A total of 350 Hy-Line (w-36) laying hens at 50 wk of age were weighed individually (mean body weight ± SD: 1,520.0 ± 0.7 g) and randomly allocated to 35 units (each with 2 cages, and 5 birds/cage). They were then randomly assigned to 7 dietary treatments with 5 replicates and 50 hens in each treatment as a completely randomized design (five units/ treatment). Each cage had 40,549 cm^3 (41 × 23 × 43) floor space and was equipped with 2 nipple drinkers and one feeder. All diets were formulated based on Hy-Line (w-36) laying hens nutrition recommendation (Hy-Line international, 2018) (Hy-Line) according to the basis of linear programming using UFFDA software (ver .1992) (University of Georgia, GA) (Pesti et al., 1992). Diets were prepared by mixing a control diet based on corn and soybean-meal thoroughly with the designated supplements including the spray-dried LC-SLNs: 1) Control (basal diet); 2) Basal diet +50 mg/kg LC (50LC); 3) Basal diet +100 mg/kg LC (100LC); 4) Basal diet +150 mg/kg LC (150LC); 5) Basal diet +50 mg/kg LC-SLNs (50LC-SLNs); 6) Basal diet +100 mg/kg LC-SLNs (100LC-SLNs) and 7) Basal

diet +150 mg/kg LC-SLNs (150LC-SLNs) (Table 1). Cages were randomly located in a ventilated room with a temperature between 21 and 23°C and 16 h of illumination (20 lx). Diets were offered twice daily for ad libitum intake and laying hens had free access to water throughout the experimental period.

The experiment lasted 12 wk, including 2 wk acclimation period and 10 wk of experimental periods, respectively. Mortality and health status were visually inspected and documented frequently during the experiment.

Feed Mixing Procedures

On a weekly basis, the LC and LC-SLNs were carefully incorporated into the basal diet. To make sure the diets were well mixed, LC and LC-SLNs were accurately weighed and then thoroughly hand-blended and homogenized with all other minor ingredients (amino acids, sodium chloride, and vitamin-mineral premix). Before adding them to the mixer, the homogenized ingredients were then divided into 4 portions and blended in a small mixer with soybean meal using the quartering technique (Teo and Tan, 2007). The mixture was then added to the basal diet and mixed in a vertical mixer for 5 min.

Table 1. Ingredient and nutrient composition (as- fed basic) of the basal diet.

Item	
Ingredient, % of diet	
Corn	60.62
Soybean meal (42%)	20.20
Corn gluten (52%)	2.45
Wheat bran	1.00
Vegetable oil	2.85
Carbonate calcium	9.70
Dicalcium phosphate	1.95
Salt	0.32
Sodium bicarbonate	0.10
DL- Methionine	0.21
L- Lysine	0.07
L- Threonine	0.03
Mineral premix[1]	0.25
Vitamin premix[2]	0.25
Nutrient[3] (calculated values), %	
AME_n, kcal/kg	2,822
Crude protein	14.82
Calcium	4.35
Available phosphorus	0.45
Sodium	0.18
Potassium	0.55
SID[4] methionine	0.44
SID methionine + cysteine	0.65
SID lysine	0.71
SID threonine	0.50
SID arginine	0.84
Analyzed nutrients, %	
Crude protein	14.2
Crude fat	7.12

[1]Supplied per kilogram of diet: 40 mg of iron as ferrous sulfate; 90 mg of manganese as manganese oxide; 8 mg of copper as copper sulphate; 1.2 mg of iodine as calcium iodate, 0.22 mg of selenium as sodium selenite and 100 mg of zinc as zinc sulphate.

[2]Supplied per kilogram of diet: 8,000 IU of vitamin A as retinyl acetate; 3,300 IU of vitamin D3 as cholecalciferol; 20 IU of vitamin E (dl-α-tocopherol acetate); 2.5 mg of vitamin K3 (2-methyl-1, 4-naphthoquinone); 2.5 mg of thiamin; 5.5 mg of riboflavin; 30 mg of niacin; 8 mg of Ca pantothenate; 4 mg of vitamin B6 (pyridoxine); 0.75 mg of biotin; 1.0 mg of folic acid; 300 mg of choline; and 0.23 mg of vitamin B12 (cyanocobalamin).

[3]Brazilian Tables for Poultry and Swine were used for calculation (Rostagno et al., 2011).

[4]Standardized ideal digestible amino acids.

Sample Collection

For the collection of blood samples, at the age of 62 wk, after 12-h fasting (water was offered ad libitum) blood samples of 5 hens from each replicate were collected from wing veins into additive-free blood tubes or heparinized polyethylene tubes. Heparinized whole blood samples were kept at +4 ± 2°C for quantitative in vitro evaluation of antioxidant properties. Serum was obtained by centrifugation of the blood samples at 1,000 × *g* for 10 min at 20 ± 2°C. Serum samples were stored at −20 ± 2°C for further analyses. On d 70 of the experiment, one randomly chosen hen from each replicate (5 hens per treatment and a total of 35 hens) were then sacrificed by conventional neck cutting and the body cavity was excised (Leary et al., 2013). Evaluation of carcass traits was made according to previous work (Jones, 1984). For histomorphometric assays, one-centimeter tissue segments were taken from the middle parts of the duodenum (started at the gizzard and formed an elongated loop that is approximately 20 centimeters long), jejunum (midsection between Meckel's diverticulum and the duodenum), ileum (midsection between Meckel's diverticulum up to 1 cm proximal to the ileocecal junction) sections. Tissue samples were taken from the same area of each section of the tract, for each bird. Samples were stored in 10% (v/v) buffered neutral formalin for fixation, where they were tenderly shaken to expel any following intestinal substance (Gamble, 2008). Furthermore, for bile cholesterol testing, after the birds were slaughtered, the bile samples were collected from the gall bladder with direct aspiration using an insulin syringe and frozen at −20°C (Strasberg et al., 1990). The liver samples were constantly taken from the dorsal part of the left lobe and collected as well from layer hens, snap-frozen in liquid nitrogen, and stored at −70°C for total RNA extraction (Ebrahimi et al., 2014). Moreover, 15 eggs for each treatment (3 eggs from each replicate) were randomly selected for egg yolk cholesterol content determination.

Production Performance

Birds were weighed at the beginning of the experiment to determine their body weight. Feed residues were collected and weighted weekly to estimate the average daily feed intake (**ADFI**; g/hen/day). Eggs were collected twice (9:00 and 16:00) and irregular (broken, soft, and misshapen) eggs were recorded every day. Eggs from each replicate were counted and weighted daily to calculate the egg production (**EP**, g/kg), egg weight (**EW**, g/hen/day), and egg mass (**EM**, g/hen/day). Moreover, the feed conversion ratio (**FCR**) was determined per

week according to the methodology described by Ribeiro et al (Ribeiro Jr et al., 2014).

Determination of Serum, Bile, and Egg Yolk Cholesterol

The cholesterol detection assay kit was used to photometrically quantify serum cholesterol at 510 nm using a Shimadzu spectrophotometer (Kyoto, Japan). The yolk lipid was extracted at the end of the study from three eggs per replicate using Folch et al and Washburn and Nix method with minor modification (Folch et al., 1957; Washburn and Nix, 1974). In the modified extraction procedure 15 mL of chloroform-methanol (2:1) was added to 1 g egg yolk, and then the sample was shaken vigorously. Then distilled water (5 mL) was added and the sample was again shaken vigorously. The samples were centrifuged and the aqueous-methanol layer was removed and the cholesterol content of the chloroform layer was determined. Yolk cholesterol was estimated by the colorimetric Libermann-Burchard method at 560 nm (Boka et al., 2014) using the commercial kit. To determine the cholesterol concentration in bile, a simple, sensitive, and rapid method based on the combination of an enzymatic technique and spectrophotometry was used (Roda et al., 1975) by using a commercial cholesterol reagent kit.

Blood and Serum Parameters

The serum GLU, TG, HDL, ALT, and AST values were determined using a Zhuoyue 310, Kehua Bio autoanalyzer (Shanghai, China), and the corresponding kits. Low-density lipoprotein (**LDL**) was achieved by the William Friedewald formula (Friedewald et al., 1972). Furthermore, heparinized whole blood samples were utilized to determine antioxidant capacity, T-AOC, SOD, and GSH-Px using the kit procedure. Serum SOD activity determination was conducted by using the xanthine oxidase methodology previously reported (Winterbourn et al., 1975). The MDA levels in the sera were determined using the MDA detection test kit, as previously described (Maurya et al., 2021). Briefly, 20 μL of the sera samples were mixed with 4.0 mL of sulfuric acid (N/12) and 0.5 mL of phosphotungstic acid (10%). The samples were kept at room temperature (5 mins) and then centrifuged at 3,000 × g for 10 min. The sediment was again mixed with sulfuric acid (2.0 mL) and 10% phosphotungstic acid (0.3 mL) and the mixture was centrifuged at 3,000 × g for 10 min. The supernatant was discarded and the sediment was suspended in 4.0 mL of distilled water and 1.0 mL of TBA (thiobarbituric acid) reagent and heated at 95°C for 60 min. After cooling, 5.0 mL of n-butanol was added and the absorbance was measured at 553 nm by the Shimadzu spectrophotometer. The lipid peroxidation values were expressed as MDA nmol/mL of serum.

Reverse Transcription-Polymerase Chain Reaction

To explore the molecular processes that may be responsible for the observed alterations in lipid metabolism, quantitative real-time PCR was utilized to evaluate the mRNA levels of 2 genes associated with lipid metabolism in the liver of layer hens. Total mRNA was extracted from the liver by the Invitrogen kit. Reverse transcription-polymerase chain reaction (**RT-PCR**) was performed using a Roche LightCycler system (Basel, Switzerland). The integrity of the extracted RNA with respect to its 18s rRNA and 23s rRNA was confirmed. The complementary DNAs (**cDNA**) were then synthesized (Vandghanooni et al., 2011). Real-time PCR amplification reactions were performed in a 25 μL total volume containing 1 μL cDNA, 1 μL primers (100 nM primers each), 12.5 μL 2X Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), and 10.5 μL RNase/DNase-free water. Data analysis was performed by the Pfaffl method and by normalizing the cycling thresholds (**Ct**) of genes to the β-actin gene. The sequences of the primers used are shown in Table 2.

Intestinal Histomorphology Assay

Morphological indices were determined according to the previous study (Heidarieh et al., 2012). In this regard, 8 complete intestinal villi of each slice were randomly selected to measure the height and width of villus and crypt depth. The formalin-fixed samples were washed with water, dehydrated in alcohol, clarified in xylene, and embedded in paraffin. Cross segments of each intestinal portion (5 μm thick) heated (55−60°C), dewaxed with xylene, hydrated, stained with hematoxylin and eosin, fixed with neutral balsam, heated for 4 h (55−60°C), and observed by employing a BX51 Olympus light microscope (Tokyo, Japan). The histomorphological evaluation was conducted and interpreted by qualified staffs.

Table 2. Primer sequence of 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGR) and cholesterol 7 alpha-hydroxylase (CYP7A1).

Gene name[1]	Primer sequence (5′→3′)	Length	GenBank Accession Number
HMGR F	GGTACTATGT CAGGAGTGCG	128	NM_204485.2
HMGR R	CGAGAAAGC TCTAATACCAA GGAC		
CYP7A1 F	GCCTCGCAA GCTAACACC	141	NM_001001753.1
CYP7A1 R	GTTGCCATCT AAGCTGAT CTTC		
β-actin F	CTTGGGTATGG AGTCCTGTGG	142	X00182.1
β-actin R	CATCCTGTCAGC AATGCCAG		

[1]Abbreviations: CYP7A1, cholesterol 7 alpha-hydroxylase; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase.

Statistical Analyses

All analyses were performed using SAS software (Ver. 9.2, Cary, NC;). The homogeneity of variances and normality of the data was tested first. Shapiro-Wilk test was used to analyze the normality. The data were subjected to one-way ANOVA with 7 treatments and 5 replicates with 10 hens in each replicate, as a completely randomized design using the general linear models (**GLM**) procedure of SAS software for windows. Significant differences among all treatments were separated by the Duncan test for multiple comparisons, and probability values of less than 0.05 were considered significant.

RESULTS

Production Performance

Table 3 presents the effects of LC and LC-SLNs feeding treatments, on laying hens' production performance. No significant differences were observed in the body weights between the experimental groups ($P > 0.05$; Table 3). Just one bird died during the whole of the experimental period in the group fed with the diet containing LC-SLNs. FCR was significantly decreased ($P < 0.05$) in the dietary supplemented groups compared with the control group. The 50LC-SLNs group showed the least FCR and the difference (4.1%) was significant compared to the control group ($P < 0.05$). Egg weight for 150 LC-SLNs fed groups was 1.6 g (2.67%) lighter ($P < 0.05$) than the control group. Compared with the basal diet (control), dietary supplementation with LC-SLNs at a level of 100 mg/kg increased (3.9%) egg production ($P < 0.05$). Besides, the lowest amount of ADFI was observed in 50LC-SLNs treatments, while the highest amount was observed in the control group with a difference of 2.76% ($P < 0.01$). It is interesting to note that except for the egg weight index, other indices were not influenced by the source of plain LC and LC-SLNs. However, the results showed that the effects of LC were relatively enhanced ($P > 0.05$) when formulated in SLNs compared to plain LC.

Egg Yolk Cholesterol

Table 4 presents the effects of feeding maize-soybean meal-based diets with different levels of plain LC and LC-SLNs on egg yolk cholesterol. Egg yolk cholesterol content was highly affected ($P < 0.001$) by the dietary addition of plain LC and LC-SLNs. The lowest and the highest egg yolk cholesterol concentrations were observed in 100LC-SLNs and the control group, respectively. The dietary supplementation of 100 mg/kg LC-SLNs decreased ($P < 0.001$) the egg yolk cholesterol concentration from 14.71 to 11.76 mg/g yolk (25%), corresponding to 241.30 mg/yolk and 203.37 mg/yolk (18.65%), compared to the control group.

Biochemical Parameters

Table 5 summarizes the results of ALT, AST, GLU, TG, TC, BC, HDL, and LDL in different dietary groups. ALT was significantly improved by the treatments ($P < 0.05$). In this regard, the recorded value for the 50LC-SLNs group was 20.64% lower than the control group. Compared to the control group, plain LC and LC-SLNs treatments reduced ALT parameters, while the mean of the ALT in the LC-SLNs groups was lower than in the plain LC groups. The serum GLU levels increased significantly in the LC-SLNs group compared to the plain LC and control group ($P < 0.05$; Table 5). The lowest and highest serum GLU levels were observed in the control and 50LC-SLNs groups, respectively, with a difference of 21.30%. The 50LC-SLNs group had the largest reduction in serum TG, which was 23.08% lower than the control group. The greatest concentration of TG was found in the group fed 100LC-SLNs. In the 100LC-SLNs fed group, TC decreased by 16.49% compared to the control ($P < 0.01$). Conversely, a highly significant ($P < 0.01$) increase in the BC was recorded with the 50LC-SLNs group by ~27% compared with the control group. Serum HDL content was significantly influenced ($P < 0.05$) by dietary treatments (Table 5). The lowest amount of serum HDL content was observed in the control group, while the highest amount was observed in the 50 and 100 LC-SLNs groups. The HDL level of the laying hens' blood serum fed with 50LC-SLNs increased by 30.88% compared to those fed the control diet.

Table 3. Effect of experimental diets on production performances in laying hens[1].

		LC			LC-SLN				
Item	Control	50	100	150	50	100	150	*P*-value	SEM
Body weight (g)	1,531.0	1,518.5	1,523.0	1,499.0	1,510.5	1,555.0	1,502.0	0.7046	2.8896
ADFI[2] (g/d/bird)	108.96^{a}	106.04^{c}	107.99^{ab}	108.19^{ab}	106.03^{c}	108.61^{a}	107.34^{b}	0.0001	0.3311
Egg production (%)	86.66^{b}	87.70^{ab}	88.90^{a}	89.20^{a}	90.00^{a}	90.02^{a}	89.44^{a}	0.0260	0.7340
Egg weight (g)	61.50^{a}	60.77^{abc}	60.24^{bc}	61.41^{ab}	60.11^{c}	60.43^{abc}	59.90^{c}	0.0314	0.3789
Egg mass (g/d/bird)	53.13	53.27	53.52	54.78	54.11	54.39	53.57	0.0765	0.4168
FCR[3]	2.04^{a}	1.99^{ab}	2.02^{a}	1.99^{ab}	1.96^{b}	1.99^{ab}	2.00^{ab}	0.0313	0.0149

Abbreviations: Control, diet without L-Carnitine supplementation; LC, L-Carnitine; LC-SLN, L-Carnitine loaded solid lipid nanoparticle
$^{a-c}$Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Data were the mean of 5 replicates (10 birds of each replicate).
[2]Average daily feed intake.
[3]Feed conversion ratio.

Table 4. Effect of experimental diets on egg yolk cholesterol content in laying hens[1].

Item	Control	LC			LC-SLN			*P*-value	SEM
		50	100	150	50	100	150		
Yolk weight (g)	17.87	16.29	16.84	16.73	16.63	17.31	17.00	0.2150	0.4192
Yolk cholesterol (mg/g of yolk)	14.71[a]	12.86[bc]	13.90[b]	12.65[bc]	12.31[cd]	11.76[d]	12.09[cd]	0.0001	0.3683
Yolk cholesterol (mg/yolk)	241.30[a]	219.67[bc]	233.76[b]	218.46[bc]	209.49[cd]	203.37[cd]	205.84[cd]	0.0001	6.9956

Abbreviations: Control, diet without L-Carnitine supplementation; LC, L-Carnitine; LC-SLN, L-Carnitine loaded solid lipid nanoparticle.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Data were the mean of 5 replicates (3 eggs of each replicate).

Antioxidant Indices

Table 5 represents the effects of dietary treatments on the serum antioxidant status of laying hens. The highest TAOC index was observed in 50 and 100LC-SLNs, while the lowest TAOC index was observed in the control group (Table 5). The 50LC-SLNs group produced the most TAOC with a difference of 58.44% compared to the control group ($P < 0.05$). Similar results were obtained for the TSOD, where the greatest amount of TSOD was found for 50LC-SLNs ($P < 0.05$) and the lowest was for the control group with a difference of 29.28%. The lowest serum MDA content was observed in the 50LC-LSNs group, while the highest serum MDA concentration index was observed in the control group. Feeding the laying hens with 50LC-SLNs resulted in a 50.52% reduction in serum MDA concentration compared with the control group.

HMGR and CYP7A1 mRNA Expression

Table 6 summarizes the effects of different levels of LC and LC-SLNs addition on *HMGR* and *CYP7A1* mRNA gene expression. The transcript level of *HMGR* was significantly decreased in the LC and LC-SLNs group ($P < 0.01$) compared to the control group. While it greatly decreased in the LC-SLNs group ($P < 0.01$) as compared to the plain LC groups. These downregulating changes were about 93% ($P < 0.01$) for 100LC-SLNs treatment and about 68% ($P < 0.01$) for 100LC treatment, compared to the control group. In this aspect, the LC-SLNs group, particularly the 100LC-SLNs treatment, showed better effects than plain LC treatments ($P < 0.01$). The expression of cholesterol 7α-hydroxylase (*Cyp7a1*), which encodes the rate-limiting step in bile acid production from cholesterol, was significantly increased in the plain LC (~83%) and LC-SLNs (~91%) groups as compared with that in the control group ($P < 0.01$). However, the expression of this gene in the 100LC-SLNs group was significantly increased (4%) when compared to the 100LC group ($P < 0.01$).

Carcass Traits and Histomorphological Assessment

Table 7 presents the effects of LC and LC-SLNs treatments on small intestine histomorphometric parameters. Figure 1 illustrates the small intestinal samples. In this experiment, the inclusion of LC-SLNs, in particular 150LC-SLNs treatment, in the diet increased the villus height and decrease villus width in all 3 parts of the small intestine ($P < 0.01$; Figure 1). In this regard, 150LC-SLNs treatment increased the villus length to 30.71, 31.66, and 33.33% in duodenum, jejunum, and ileum segments, respectively. On the other hand,

Table 5. Effect of experimental diets on the serum biochemical and blood antioxidant indices in laying hens[1].

Item	Control	LC			LC-SLN			*P*-value	SEM
		50	100	150	50	100	150		
AST (U/L)	471.97	430.41	474.40	395.97	392.37	395.79	386.17	0.0773	27.484
ALT (U/L)	15.08[a]	13.06[bc]	14.32[ab]	12.92[bc]	12.50[c]	12.78[bc]	12.65[bc]	0.0185	0.5550
GLU (g/dL)	281.60[b]	295.20[b]	309.60[ab]	314.80[ab]	321.60[a]	290.00[b]	308.00[ab]	0.0419	12.313
TG (g/dL)	124.03[a]	101.00[b]	106.87[b]	132.14[ac]	100.20[b]	141.73[ac]	110.35[ac]	0.0401	8.316
TC (g/dL)	224.6[a]	199.8[cd]	206.2[abc]	218.0[ab]	214.6[ab]	192.8[d]	199.4[cd]	0.0007	6.3358
BC (g/dL)	62.40[b]	77.80[ab]	68.60[ab]	70.80[ab]	79.20[a]	72.60[ab]	63.00[b]	0.0065	4.1283
HDL-C (mmol/L)	13.60[b]	15.00[ab]	14.80[ab]	14.80[ab]	17.80[a]	17.20b[a]	14.00[ab]	0.0202	1.2895
LDL-C (mmol/L)	70.17	53.40	63.73	75.66	53.20	72.47	74.65	0.1530	13.723
Antioxidant indices									
T-AOC (mmol/lit)	1.54[b]	1.85[ab]	1.62[b]	1.91[ab]	2.44[a]	2.40[a]	2.01[ab]	0.0072	0.0988
T-SOD (U/g Hb)	676.03[b]	723.27[ab]	677.41[b]	857.78[ab]	873.99[a]	759.05[ab]	725.92[ab]	0.0141	25.555
GSH-Px (U/g Hb)	27.71	31.42	28.95	28.73	29.50	30.02	25.17	0.4842	1.4229
MDA (nmol/mL)	2.86[a]	2.01[ab]	1.96[b]	2.68[a]	1.90[b]	2.26[ab]	2.00[ab]	0.0037	0.3325

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BC, bile cholesterol; Control, diet without L-Carnitine supplementation; GSH-Px, glutathione peroxidase; GLU, glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LC, L-Carnitine; LC-SLN, L-Carnitine loaded solid lipid nanoparticle; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Data were the mean of 5 replicates (one birds of each replicate).

Table 6. Effect of experimental diets on HMGR and CYP7A1 mRNA expression in laying hens liver[1].

Item	Control	LC			LC-SLN			*P*-value	SEM
		50	100	150	50	100	150		
HMGR[2]	1.0050[a]	0.7427[b]	0.5995[c]	0.7604[b]	0.5705[c]	0.5210[c]	0.5637[c]	0.0001	0.0348
CYP7A[3]	1.0087[c]	1.2255[bc]	1.8468[a]	1.8362[a]	1.4681[b]	1.9241[a]	1.8687[a]	0.0001	0.0671

Abbreviations: Control, diet without L-Carnitine supplementation; LC, L-Carnitine; LC-SLN, L-Carnitine loaded solid lipid nanoparticle.
[a-c]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Data were the mean of 5 replicates (one birds of each replicate).
[2]3-Hydroxy-3-methylglutaryl coenzyme A reductase.
[3]Cholesterol 7 alpha-hydroxylase.

compared to the control group, the lowest villus width was observed in the 150LC-SLNs group with a difference of 26.31, 34, and 43.48% in the duodenum, jejunum, and ileum, respectively. The experimental treatments did not affect the crypt-depth parameter or the ratio of villus height to crypt-depth in the duodenum ($P > 0.05$). The highest ratio of villus height to crypt-depth was observed in LC-SLNs treatments and the lowest ratio was observed in the control treatment. This increase was 32.77% in the jejunum at 50LC-SLNs treatment and 28.87% in the ileum at 150LC-SLNs treatment.

The length of the duodenum and jejunum increased by 100LC-SLNs when compared to the control group, with a difference of 12.76 and 9.42%, respectively. As Table 7 summarizes, there are significant differences in heart and pancreas weight affected by experimental diets ($P < 0.05$). It was observed that 100LC-SLNs increased the weight of the heart with a difference of 9.00% compared to the control group. The lowest heart weight was related to 150LC-SLNs treatment, which was 28.62% less than the control treatment. In addition, 50LC-SLNs increased pancreatic weight by ~21% compared to the control group. While, the lowest pancreas weight was related to the 150LC treatment, which was 13.83% less than the control treatment. Dietary treatment did not affect the other parameters such as liver and gizzard weight ($P > 0.05$).

DISCUSSION

Production Performance

The investigations have shown that dietary LC supplementation improves layers' productive performance,

Table 7. Effect of experimental diets on the carcass traits and small intestine parameters in laying hens[1].

Item	Control	LC			LC-SLN			*P*-value	SEM
		50	100	150	50	100	150		
Carcass traits (%)[2]									
Heart	0.355[b]	0.385[a]	0.385[a]	0.293[d]	0.318[c]	0.387[a]	0.276[d]	0.0001	0.00104
Pancreas	0.181[b]	0.185[b]	0.212[a]	0.159[c]	0.219[a]	0.180[b]	0.173[bc]	0.0015	0.00369
Liver	1.814	2.196	2.085	2.030	1.873	2.090	1.865	0.0623	0.04820
Gizzard	1.803	2.616	1.803	1.924	1.865	1.977	2.583	0.1041	0.10633
Small intestine									
Weight (%)[2]									
Duodenum	0.562[c]	0.866[a]	0.720[ab]	0.696[bc]	0.648[bc]	0.693[bc]	0.740[abc]	0.0361	0.02781
Jejunum	0.917	1.207	1.114	1.155	1.075	0.996	0.981	0.6364	0.07633
Ileum	0.927	1.250	0.920	1.000	0.929	0.733	0.911	0.1357	0.06269
Total	2.177	3.174	2.674	2.852	2.752	2.426	2.612	0.3800	0.17807
Length (%)[3]									
Duodenum	19.56[ab]	20.98[a]	19.86[a]	17.21[b]	20.33[a]	22.06[a]	20.56[a]	0.0432	0.46903
Jejunum	36.81[c]	38.53[bc]	39.03[ab]	40.65[a]	36.64[c]	40.28[ab]	39.10[ab]	0.0094	0.36161
Ileum	41.63	40.49	41.11	42.13	41.79	39.40	40.34	0.2676	0.47834
Duodenum section									
Villus length (μm)	1,313.33[cd]	1,300[cd]	1,233.3[d]	1,366.67[bcd]	1,500[bc]	1,566[ab]	1,716.67[a]	0.0016	51.5105
Villus width (μm)	240[ab]	243.3[a]	253.33[a]	213.34[bc]	215[bc]	206[c]	190[c]	0.0018	6.8833
Crypt-depth	306.67	296.66	310.33	313.33	306.66	300	323.33	0.3700	11.1696
Villus height/crypt-depth	4.293	4.393	4.027	4.377	4.903	5.223	5.340	0.0500	0.2317
Jejunum section									
Villus length (μm)	1,000[b]	1,033.33[b]	1,200[ab]	1,150[ab]	1,316.66[a]	1,050[b]	1,083.33[a]	0.0235	60.0239
Villus width (μm)	203.33[a]	203.33[a]	193.34[a]	196.67[a]	186.67[a]	183.33[a]	151.67[b]	0.0024	5.56348
Crypt-depth	343.33	346.67	336.66	350	340	351.60	365	0.8917	12.0281
Villus height/crypt-depth	2.920[c]	2.983[c]	3.567[ab]	3.273[bc]	3.877[a]	2.980[c]	3.793[ab]	0.0037	0.1322
Ileum section									
Villus length (μm)	900[c]	906.67[c]	996.66[bc]	1,083.34[abc]	901.67[c]	1,143.30[ab]	1,200[a]	0.0097	45.6200
Villus width (μm)	220[a]	215[a]	186.67[b]	173.33[bc]	218.34[a]	170[bc]	153.33[c]	0.0002	6.51000
Crypt-depth	351.67	356.66	343.34	360	345	376.67	354.28	0.1392	6.41800
Villus height/crypt-depth	2.563[c]	2.547[c]	2.913[abc]	3.017[abc]	2.617[bc]	3.108[ab]	3.303[a]	0.0414	0.1373

Abbreviations: Control, diet without L-Carnitine supplementation; LC, L-Carnitine; LC-SLN, L-Carnitine loaded solid lipid nanoparticle.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Data were the mean of 5 replicates (one birds of each replicate).
[2]Relative weight of the trait to live body weight.
[3]Relative length of the intestine segments to total intestine length.

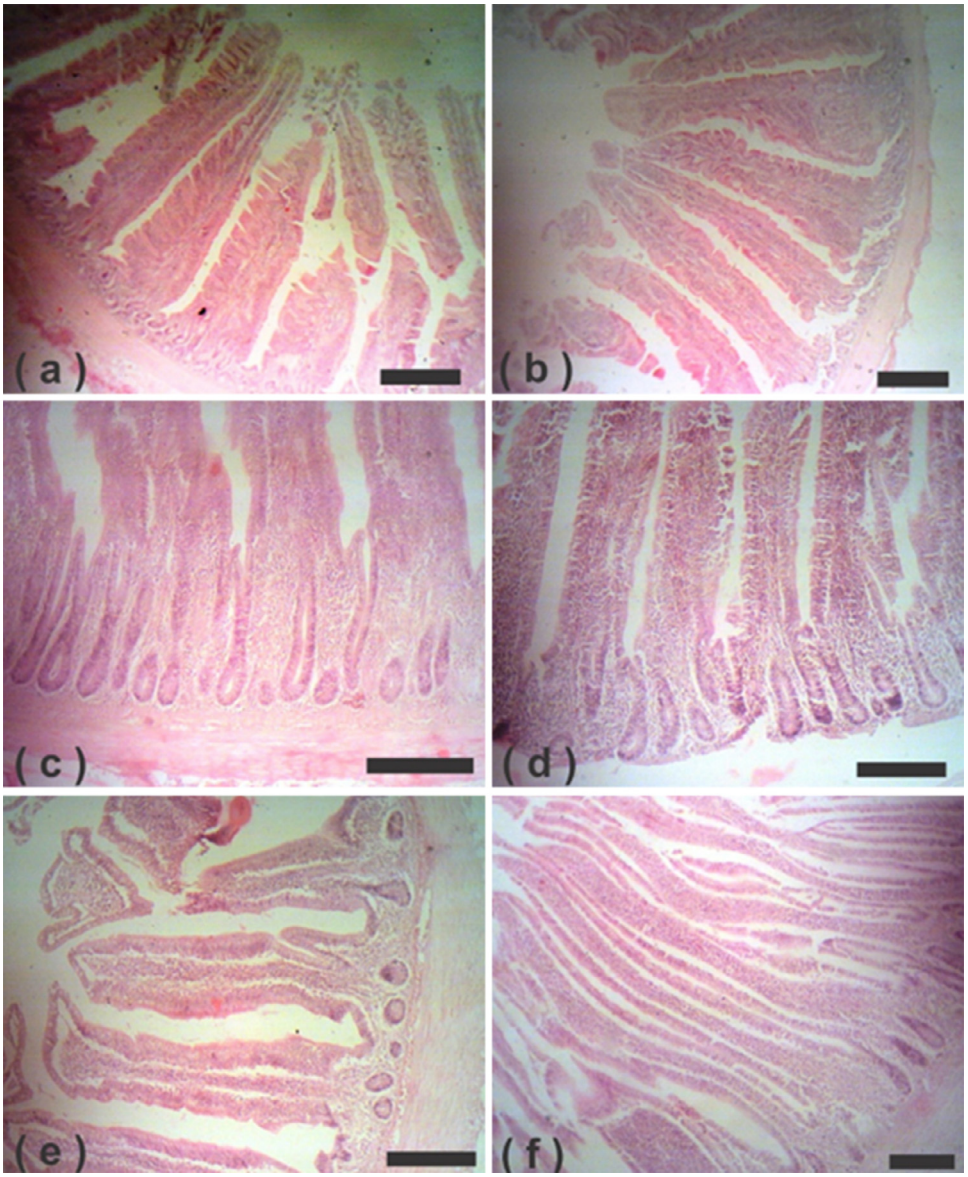

Figure 1. Photomicrographs of deoudanal sections (a; control group, b; 150LC-SLN), jeojenal sections (c; control group, d; 100LC-SLN) and ileal sections (e; control groups, f; 150LCSLN). Scale bars: 50 μm.

including egg production and FCR (Rehman et al, 2017). LC is well-known for its involvement in lipid metabolism and its potential to cause certain favorable changes in poultry products (Murali et al., 2015). In this research, dietary LC supplementation had a substantial impact on egg production, egg weight, feed intake, and feed conversion rate. The change in egg production was significant when the hens were given more than 50 mg/kg LC. Rouhanipour et al. (2022) observed that 200 mg/kg LC supplementation decreased egg weight while not influencing feed consumption or feed conversion ratio (Rouhanipour et al., 2022). According to previous work, LC supplementation boosted β-oxidation of fatty acids to adenosine triphosphate, which improved energy production (Madsen et al., 2018). LC has the potential to boost egg production by supplying energy from carbs, lipids, and proteins (Agarwal et al., 2018). The current findings contradicted those of previous studies which found that adding 100 mg/kg LC to layer hen and quail diets had no influence on feed intake, egg weight, or feed conversion rate (Erol, 2006; Daşkıran et al., 2009; Kazemi-Fard et al., 2015). In thermoneutral settings, Leibetseder (1995) found that supplementing a conventional layer's diet with 50 mg LC did not affect egg production, feed intake, or body weight during the early laying stage (Leibetseder, 1995). LC supplementation in low-energy diets did not affect egg production, according to previous findings (Corduk and Sarica, 2008). In contrast to Kazemi-Fard et al. (2015) and Nofal et al. (2006) works (Nofal et al., 2006; Kazemi-Fard et al., 2015), no significant impact of LC supplementation on egg mass was reported (Nofal et al., 2006). Contradictions in production performance findings from several studies may be attributed to factors like variances in the breed, age, and strain of birds utilized, as well as housing conditions and dietary status.

Increased fatty acid oxidation caused by LC may reduce the availability of long-chain fatty acids for esterification to triacylglycerol while also raising acetyl-CoA levels in the mitochondria (Roseiro and Santos, 2019).

The activity of pyruvate carboxylase, an acetyl-CoA-dependent enzyme that may provide carbon chains for amino acid biosynthesis might be affected in this circumstance (Dahash and Sankararaman, 2022). LC also acts as a buffer for excess acyl residues in the body's metabolism. This function of LC benefits the cell by increasing the mitochondrial acetyl-CoA/CoA ratio, for example. To maintain a high substrate flow in the citric acid cycle, sufficiently high quantities of free CoA are necessary (Giudice et al., 2022). Additional LC may improve production performance in part due to its amino acid sparing action in addition to its involvement in fatty acid metabolism. On the other hand, stimulates the biosynthesis of estrogen and progesterone by increasing the oxidation of the fatty acids, increasing the regeneration of the reducing equivalents required for the cholesterol side-chain cleavage reaction, which together act on the growth and maturation of the small ovarian follicles and the Accelerate ovulation process (Agarwal et al., 2018). Exogenous LC may theoretically reduce the demand for LC production from methionine, freeing up methionine for other biological purposes (Mirzaei et al., 2022).

Except for the egg weight index, other indices were not affected by the plain LC treatment. The results of LC-SLNs, on the other hand, were superior to those of plain LC. This could probably be explained by the poor cell membrane permeability of plain LC due to its water solubility in contrast to the hydrophobic LC-SLNs (Rasouliyan et al., 2021). Overall, the results demonstrated that the experimental treatments, particularly the 50LC-SLNs-treatments, improved the production performance index of laying hens.

Cholesterol

The addition of different quantities of LC to the diet of hens for ten weeks dramatically reduced egg yolk and serum cholesterol, according to our findings. Surprisingly, LC-SLNs therapies outperformed plain LC in blood total cholesterol and yolk cholesterol concentration. The administration of 100 mg/kg LC-SLNs decreased cholesterol in egg yolks by 25% and blood cholesterol by 16.49%, which is inconsistent with Rouhanipour et al results (Rouhanipour et al., 2022). Supplementing with LC has been shown to decrease a variety of blood metabolites, including liver enzymes, cholesterol, and triglycerides, which the results of this study confirmed previous findings (Parizadian et al., 2011; Awad et al., 2016; Fallah and Mirzaei, 2016). In contrast, Kazemi-Fard et al. (2015) and Arslan (2006) conducted an experiment with various amounts of LC on laying hens and found that there was no significant change in plasma glucose, AST, ALT, TG, cholesterol, HDL, LDL, and VLDL concentrations across dietary regimens (Arslan, 2006; Kazemi-Fard, et al., 2015). Reduced serum cholesterol in LC supplemented broiler (Rezaei et al., 2008; Zhai et al., 2008; Hassan et al., 2011; Azizi-Chekosari et al., 2021) and duck (Rizk et al., 2019) diet has been well proven. An inverse association has been found between egg yolk cholesterol and egg production rate (Elkin, 2006); this was also demonstrated in our investigation.

The association between LC's cholesterol-lowering effects and egg yolk and blood total cholesterol levels is not fully understood. The principal sites of cholesterol production in the laying bird are the liver and the ovary (Hu et al., 2020). In the production of cholesterol, HMGR is the rate-limiting enzyme (Marahatha et al., 2021). We evaluated the expression of *HMGR* and *Cyp7a1* genes in the liver to investigate the influence of LC on the molecular mechanism of lipid metabolism. The findings revealed that LC-enriched diets downregulated the expression of *HMGR* and upregulated the expression of *Cyp7a1*. This study points to a significant reduction in hepatic lipogenesis, as well as a drop in egg yolk and serum cholesterol. Our results showed that the laying hens treated with 100LC-SLNs had the lowest *HMGR* gene expression in their liver tissue. LC would help to inhibit hepatic HMG-CoA reductase activity, lowering cholesterol accumulation in the egg yolk. These findings might be explained by LC's influence on cholesterol synthesis and serum cholesterol levels.

LC helps to raise HDL cholesterol while decreasing LDL cholesterol. Because LC promotes fatty acid oxidation, it causes an increase in acetyl-CoA production and inhibits cholesterol production. In contrast, NAD+ or acetyl-CoA would be activated, allowing biliary acid production from cholesterol and amplifying its catabolism and excretion. We examine the amount of bile acid cholesterol and the expression of the *Cyp7a1* gene to support this theory. The increased conversion of cholesterol to bile acids via upregulation of *Cyp7a1* gene expression in the liver is a crucial step in decreasing blood cholesterol levels (Chiang and Ferrell, 2020). The removal of cholesterol from the body is considerably aided by bile acid production and excretion (Wang et al., 2019). LC lowers bile acids, which has a hypocholesterolemic effect. From cholesterol, hepatocytes produce cholic and deoxycholic bile acids, which are conjugated with glycine and taurine, respectively. These acids enter the small intestine, where they are absorbed and transported to the liver. Because cholesterol is used in the production of bile acids, a decrease in bile acid recycling could inhibit the absorption and acceleration of cholesterol resulting in lower serum cholesterol content (Wang et al., 2019; Cheng et al., 2020). Better results of LC-SLNs can be due to the improvement of their bioavailability. The development of optimized lipid-based nanoformulations for the delivery of hydrophilic LC is the most confident approach to improve LC bioavailability and pharmacokinetics, as well as enhance their natural action while reducing the possibility of adverse side effects (Mirchandani et al., 2021; Vandghanooni et al., 2021).

Antioxidant Indicates

The majority of pressures in poultry production at the cellular level are linked to oxidative stresses (Surai et al.,

2019). The primary goal of this study was to examine the effects of dietary LC and LC-SLNs on the oxidative stress indicators and antioxidant enzyme activity of laying hens. At the cellular level, there are three primary layers of antioxidant defense. The first stage consists of three important antioxidant enzymes: superoxide dismutase (**SOD**), glutathione peroxidase (**GPx**), and catalase, all of which are responsible for radical detoxification at the start of their production process (Surai, 2020). LC is well-known for its powerful antioxidant properties (Murali et al., 2020). The results obtained in the current study demonstrated that adding the lowest level of LC-SLNs (50 mg/kg) to the diet, significantly improved erythrocyte SOD activity and total antioxidant capacity (T-AOC); which could be attributed to the high bioavailability of LC-SLNs. Previous research found that supplementing with LC increased SOD activity at high stocking density challenged laying hens (Çetin and Güçlü, 2020) and broiler (Liu et al., 2021) which is similar to our findings. These findings suggest that LC plays an important role in antioxidant defense control. Increased SOD and GPx activity may be associated with higher blood protein levels. It was proposed that in this environment, LC increased ATP generation and improved total protein synthesis, including SOD and GPx. Although differences in GPx means were not statistically significant in this study, the findings tended to raise values. These results support previous findings by confirming the protective role of both plain LC and LC-SLNs against intracellular ROS production, as well as demonstrating the superior biological activity of LC-SLNs over plain LC, which may contribute to the enhanced cell permeability and availability of LC-SLNs over plain hydrophilic LC.

The current study found that dietary LC supplementation resulted in significant reductions in plasma MDA levels, indicating that LC-SLNs at 50 mg/kg had adequate antioxidant activity to reduce the elevated MDA level. Malondialdehyde (MDA) is one of the end products of polyunsaturated fatty acid peroxidation in cells. The increase in free radicals in cells has been linked to MDA overproduction (Maurya et al., 2021). The results of this study agree with those of Aziz et al. (2019), who reported that dietary supplementation with LC significantly reduced MDA levels in broiler serum and liver (Aziz et al., 2019).

LC acts as a cofactor during transferring of free fatty acids (**FFA**) from the cytosol to the mitochondria (Roseiro and Santos, 2019). FFA degrades to acyl-CoA via β-oxidation and enters the tricarboxylic acid (**TCA**) cycle (Ringseis et al., 2018). A significant amount of oxygen is used and ATP is produced during the electron transport chain and oxidative phosphorylation (Ahmad et al., 2022). Following the TCA cycle, oxygen is converted to H_2O, and oxygen concentration decreases, reducing ROS production (McCann et al., 2021). The drop in MDA levels seen in this research might be attributable to LC's iron-chelating action (Gülcin, 2006). Carnitine improves fatty acids transport into mitochondria for energy generation, reducing the availability of lipids for peroxidation (Zidan et al., 2018), and it keeps the membrane stable. As a result, the cell and mitochondria are protected (Modanloo and Shokrzadeh, 2019).

This study is the first investigation to show that supplementing laying hens with 50 mg/kg of LC-SLNs has a beneficial antioxidant effect on their oxidative stress state. Based on the findings of this study, we envision LC-SLNs could be a valuable dietary supplement for poultry to protect against oxidative stress caused by fatty liver syndrome. The results of this study show that the 50LCSLNs diet improves the health of laying hens; as a result, the 50LCSLNs diet is recommended over the other doses. Discrepancies in the findings of the various investigators could be attributed to differences in LC doses, animal species, and measurement methods.

Carcass Traits and Histomorphological Assessment

The LC-SLNs and LC groups in the current study had no visible histological lesions in the laying hens' intestines. Energy production in intestinal tissue may play a role in intestinal morphology. The current study found that LC improved the development of the histomorphological structure of the small intestine of laying hens, which is consistent with Mahmoud et al results (Mahmoud et al., 2020). This improvement was more effective on the duodenum and jejunum. The digestive function of the small intestine is closely related to mucosal architecture and villi structure (Ensari and Marsh, 2018). In all three parts of the intestine, LC-SLNs (50 and 150LC-SLNs) increased villus height and the proportion of villus height to crypt-depth. Feeding 50LC-SLNs to laying hens raised the villus height to crypt-depth ratio, demonstrating that the greater this ratio, the better the nutrient digestion and absorption (Jiang et al., 2020). Because this rise occurred in the jejunum, which is the primary location of digestion and nutrient absorption in birds, this factor might be one of the reasons for improved chicken performance when utilizing LC-SLNs supplements.

There are a few studies that look at the influence of different LC forms on avian intestinal shape. Some studies have found that supplementing with LC causes an increase in intestinal villus height (Xu et al., 2003). The histomorphological study demonstrated that dietary 600 mg/kg LC significantly enhanced duodenal villus height and decreased crypt depth, resulting in an elevated villus height to the crypt-depth ratio in developing Japanese quails, according to the previous report (Mahmoud et al., 2020). However, it is unclear why LC lengthens the villus. A study performed on mice found that villous epithelial cells are metabolically active and require ATP to power numerous tasks such as nutrition transport, epithelial barrier maintenance, and immunological activity (Shekhawat et al., 2013). These findings have profound implications for gut health because of the obligatory role played by carnitine in gut energy production.

It was previously observed that increasing dietary LC concentrations in chicken diets enhanced plasma IGF-I concentrations (El-Saway et al., 2022). Investigators claim that IGF-1 in various physiological forms plays a crucial role in cell proliferation and organ development (Neves et al., 2020). We envision that the improvement in villus condition is linked to the increased level of IGF-1. Also, as mentioned earlier, due to the antioxidant properties of LC (Çetin and Güçlü, 2020), it improves antioxidant capacity. We envision that LC may alleviate GPx and SOD depletion as well as lower MDA activity levels in laying hen intestinal tissues. As a result, it has the potential to be a prophylactic and preventative agent against oxidative stress. In conclusion, our findings suggest that LC may be useful in reducing intestinal damage.

CONCLUSIONS

L-carnitine (LC) plays an important role in lipid metabolism and acts as a powerful antioxidant. Also, LC can decrease serum and egg yolk cholesterol and triglycerides in laying hens. Lipid-based carriers mainly have broad usage to enhance the bioavailability of medicines. The results obtained here showed that dietary LC-SLN supplementation had a substantial impact on production performance. Besides, adding LC-SLNs to the diet considerably increased antioxidant status in laying hens and downregulated the expression of *HMGR,* and upregulated the expression of *Cyp7a1* enzyme genes. Furthermore, histomorphological analyses revealed that LC-SLNs not only had no negative effects on intestinal tissues but increased intestinal absorption capacity. The preparation of optimized SLNs for the delivery of hydrophilic LC can be considered the most hopeful approach to improve LC bioavailability and pharmacokinetics and enhance its biological activity while reducing the probability of unwanted off-target side effects. Our findings indicate that SLNs are promising nanosystems for increasing the availability of LC and improving its therapeutic effects.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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