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Research article

Effects of dietary L-carnitine on puberty indices in the young breeder rooster

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ABSTRACT

The aim of current study was to investigate the effect of dietary L-Carnitine (LC) in immature roosters on reproductive hormones, lipid profile and testicular histology at the time of maturity. Eighteen 12-wk-old breeder roosters (Ross 308) of similar weights were randomly allocated into 3 dietary treatments (LC-0: basic diet, LC-250: basic diet + 250 mg LC/kg of diet, LC-500: basic diet + 500 mg of LC/kg of diet) in 6 replicates. The feeding program and photoperiod regimen were performed based on ROSS 308 management handbook. Dietary LC supplementation markedly improved testicle weight and testicle index ($p < 0.05$). Comb height was also affected by LC supplementation ($p < 0.05$). The testicle weight and index, comb height, and shank lengths improved linearly with increasing levels of dietary LC ($p < 0.05$). The LC-250 and LC-500 diets significantly improved the number of sertoli cells (NSC), height epithelium seminiferous tubules (HEST), seminiferous tubules diameter (STD), spermiogenesis index (SI) and tubular differentiation index (TDI) of rooster's testis tissue ($p < 0.05$). The number of seminiferous tubules (NST) was affected by of the amount of LC ($p < 0.05$). The roosters on the LC-250 mg/kg diet had longer HEST compared to roosters that received the LC-500 mg/kg diet ($p < 0.05$). Testicular histology parameters increased in a linear and quadratic manner in response to increasing levels of LC ($p < 0.05$). Dietary LC significantly increased ($p < 0.05$) plasma concentrations of testosterone, GnRH, LH, FSH and High-Density Lipoprotein (HDL), but reduced the plasma concentration of Low-Density Lipoprotein (LDL). However, no significant differences were observed between LC-250 and LC-500 groups in these parameters. Plasma testosterone, GnRH, LH, LDL and HDL were affected in a linear and quadratic manner in response to increasing levels of LC ($p < 0.05$). Similarly, FSH increased linearly with increasing dietary LC ($p < 0.05$). Thus, adding up to 250g of LC per kg of the rooster chicken can improve reproductive hormones, blood lipids and testicular histology parameters at the time of maturity.

1. Introduction

Production of fertile hatching eggs is considered one of the key goals of broiler breeders. From an economic perspective, the fertility of the roosters in a breeder flock is of greater importance than that of the hens, because the male is responsible for fertilizing the eggs from a number of females. In general, in commercial flocks, some males have high fertility whereas others have low fertility (sub-fertile), the latter leading to a reduction in overall flock fertility.

Birds with low body weight often have underdeveloped testes, which can result in subfertility [1,2]. During testis development in chicken (from 2 to 15 weeks of age), although there is no significant increase in testicular weight in the early stage [3], it is the most important period for testicular development [4]. A mature testis has seminiferous tubules with a multilayered epithelium representing the different stages of spermatogenesis [5].

Sexual maturity is associated with the highest testis weight and consequently with the highest plasma concentration of reproductive hormones [6,7]. The main effects of luteinizing hormone (LH) and follicle stimulating hormone (FSH) are on the Leydig cells and Sertoli cells, respectively. FSH stimulates testicular growth and development by increasing seminiferous tubule diameter and stimulating Sertoli cells proliferation and differentiation [8]. The Leydig cells contain the steroidogenic enzymes needed for the generation of testosterone hormone (TH) [9].

In birds, TH is necessary for spermatogenesis, keeping the excurrent ducts and secondary sexual characteristics, reproductive behavior, and modifying the template of gonadotropin-releasing hormone (GnRH)

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secretion [9]. Several studies have been performed on the effect of factors that affect sexual maturity of male chicken such as the photoperiod [10, 11, 12], dietary protein levels [13], treatment with 6-N-Propyl-2-Thiouracil [14], and chronic treatment with tamoxifen [15]. However, data on the effects of multi-functional compounds on puberty regulation in broiler breeder roosters are scarce.

L-carnitine (LC) is an important quaternary amine (3-hydroxy-4-N,N,N-trimethyl amino butyrate) that is stored in skeletal muscles, heart, brain, and testes. LC plays an essential role in cellular energetic metabolism, acting as a shuttle for activated fatty acids into the mitochondria, where β-oxidation takes place [16]. High concentrations of LC have been observed in the male reproductive tract, especially in the epididymis, where its concentration is about 2000 times higher than that in plasma [17], suggesting its vital role in energy metabolism and in the maturation of sperm [18]. More recently, it has been shown that LC increases the activities of reproductive hormones in aged roosters by improving the hypothalamic-pituitary-testicular axis [19].

Aromatase is an enzyme that converts TH to estrogen. It has been shown that the decomposition of long-chain fatty acids reduces the activity of aromatase in the adipose tissue [20], and thereby LC. Thus, the decomposition of long-chain fatty acids can affect TH concentration by reducing aromatase activity. The diet of birds is typically vegetarian and is therefore poor in term of LC concentration; on the other hand, Met and Lys (LC precursors) are the first and second limiting amino acids, respectively, in birds [21]. To the best of our knowledge, the question of whether supplementing diet with LC affects during puberty "when the reproductive organs are developing" has not been addressed. Therefore, the current study was designed to determine whether dietary LC supplementation influences puberty indices such as sex hormones, lipid profile and testicular histology parameters in young broiler breeder roosters.

2. Materials and methods

Animal ethics

All procedures used in the experiments were confirmed by the Committee of Animal Welfare of the Department of Animal Science, University of Tehran. All husbandry practices and euthanasia were carried out under full consideration of animal welfare. All chemicals were purchased from sigma unless those mentioned.

2.1. Birds housing and treatments

Eighteen 12-wk-old breeder roosters (Ross 308) with the same weight were obtained from a commercial broiler breeder farm and randomly allocated into three dietary treatments including 3 dietary LC inclusion rates (LC-0: basic diet, LC-250: basic diet + 250 mg LC/kg of diet, LC-500: basic diet + 500 mg of LC/kg of diet) in 6 replicates. Basal diets (mash form) were formulated to meet the nutrient requirements of roosters recommended by ROSS 308 management manual book (Table 1). Each pen was equipped with a bell drinker and a tube feeder. The roosters were raised under environmentally controlled conditions following a standard temperature regimen (25 ± 2 °C) and an 8L: 16D lighting program. The roosters were photo-stimulated at 21 weeks of age by increasing the photoperiod from 8L:16D to 14L:10D.

2.2. Body weight and testes index

At 24 W of age, four roosters from each treatment were randomly selected, weighed and blood samples were taken from their brachial vein. Then the birds were killed by cervical dislocation. From each euthanized bird, testes were quickly dissected out and their weights were measured to determine the testis index [testis weight (g)/body weight (kg)] [22]. A graduated tube containing water was used for testes volume evaluation.

2.3. Testicular histology

Before histological examination, the left testicles of four birds per treatment were fixed in 10% neutral buffered formalin for three days. Afterward, the samples were imbedded in paraffin, sectioned to a thickness of 5 μm, affixed to a microscope slide and stained with hematoxylin and eosin [2]. Then, the samples were used for evaluation of Number of Seminiferous Tubules (NST), Number of Sertoli Cells (NSC), Height of Epithelium Seminiferous Tubules (HEST), Seminiferous Tubules Diameter (STD), Spermatogenesis Index (SI), and Tubular Differentiation Index (TDI) [23].

The number of active mutilated tubules (adult) in a circle with a radius of 500 μm was considered as NST. Tubular differentiation index was calculated as the percentage of seminiferous tubules containing more than three layers of germ cells derived from type A of spermatogonia. To find the spermiogenesis indices, the ratios of the number of seminiferous tubules with spermatozoids to the empty tubules, were computed. Sertoli cells were counted based on the accumulation of spermatids at triangular points in seminiferous tubules (Figure 1).

2.4. Measurement of blood parameters

At 24 W of age, blood samples from four birds per treatment were collected and transferred into K2EDTA tube. Blood plasma was collected by centrifugation at 1500×g for 10 min and stored at -20 °C until assay. The plasma concentrations of the GnRH, LH, FSH and TH were measured using an ELISA kit (Nanjing, Jiangsu, China) according to the

Table 1. Ingredients and nutrient composition of basal diet.

Ingredients	g/kg
Corn	690
Soybean meal	85
Wheat bran	191.9
Di-calcium phosphate	14.4
Sodium chloride	3.25
Calcium carbonate	8.45
D-L-Methionine	1.14
L-Lys Hcl	0.86
Mineral premix[1]	2.5
Vitamin premix[2]	2.5
Calculated Composition	
Metabolism energy (kcal/kg)	2754
Crude protein (%)	12
Calcium (%)	0.7
Available phosphorus (%)	0.35
Sodium (%)	0.15
L-Lysine (%)	0.45
D-L-Methionine (%)	0.29
Methionine + cysteine (%)	0.49
Actual Composition(measured)	
Crude protein (%)	11.74
Calcium (%)	0.72
Available phosphorus (%)	0.34
Sodium (%)	0.16
L-Lysine (%)	0.43
D-L-Methionine (%)	0.31
Methionine + cysteine (%)	0.54

[1] Supplied per kilogram of diet: Fe, 60 mg; Mn, 6 mg; Zn, 100 mg; 12 mg; cu, 10 mg; and Se, 0.2 mg.

[2] Supplied per kilogram of diet: vitamin A, 12000 IU; vitamin E, 100 IU; vitamin K3, 5 mg, B1, 3 mg; riboflavin, 12 mg; niacin, 15 mg; vitamin B12, 0.04 mg; vitamin D, 3,000 IU; pantothenic acid, 55 mg; pyridoxine, 4 mg; biotin, 0.25 mg and Choline chloride, 1 gr.

manufacturer's instruction. As the same way, the concentrations of triglyceride (TG), Low-density lipoprotein (LDL) and High-density lipoprotein (HDL), Very Low-density lipoprotein (VLDL) and total cholesterol (CHOL) were analyzed with commercial enzyme kits (Pars Azmoon Co., Tehran-Iran).

2.5. Statistical analysis

The data were assessed for normality using the Shapiro-Wilk test. All data were analyzed by the GLM procedure of SAS software [24] and statistical differences among the treatments was conducted using Tukey Test, when the p value was less than 0.05.

3. Results

Data related to the body weight, testicle weight, testicle index, testicular volume, comb height and shank lengths are presented in Table 2. Dietary LC supplementation markedly improved testicle weight and testicle index ($p < 0.05$). In line with testicle weight and testicle index, comb height also increased with dietary LC. However, there were no significant differences in these parameters between the LC-250 and LC-500 groups. Linear and quadratic analysis in testis index, testis weight and comb height were significant ($p < 0.05$). However, no differences were found in body weight and testicular volume between linear and quadratic analysis. As shown in Table 2, the best results in relation to testicular characteristics (weight and index) and comb height were observed in birds fed with 250 mg of LC.

Nevertheless, on increasing the dose of LC, the shank lengths continued to increase linearly. Orthogonal contrasts between LC groups (LC-250 and LC-500) *vs* control indicated the effect of LC on body weight, testicle weight, testicle index, comb height and shank lengths ($p < 0.05$).

The effects of dietary LC supplementation on the testicular histology of roosters are shown in Table 3. LC significantly improved NSC, HEST, STD, SI, and TDI in the testis tissue of the roosters ($p < 0.05$). Birds fed with LC-250 mg/kg had higher NST than control birds (LC-0) ($p < 0.05$). The roosters fed with LC-250 mg/kg diet had longer HEST compared to roosters that received the LC-500 mg/kg diet ($p < 0.05$). Also, roosters that were fed the LC-500 mg/kg diet ($p < 0.05$) had larger STD. There were no significant differences between the LC-250 and LC-500 treatments in terms of NST, NSC, SI, TDI. Although the quadratic analyses results of STD, SI and TDI were significant ($p < 0.05$), these parameters improved linearly, so that, the best average was observed in birds fed 500 mg/kg LC. Response curves in HEST, NST, NSC were altered with increasing dosage of LC. This means that more responses have seen in birds fed with LC-250 mg/kg diet. Orthogonal contrasts between C-250 and LC-500 groups vs control demonstrated the effect of LC on all of the testis histology parameters ($p < 0.05$).

The effects of dietary LC supplementation on plasma concentration of testosterone, GnRH, LH and FSH are shown in Table 4. Dietary LC significantly increased plasma concentrations of testosterone, GnRH, LH,

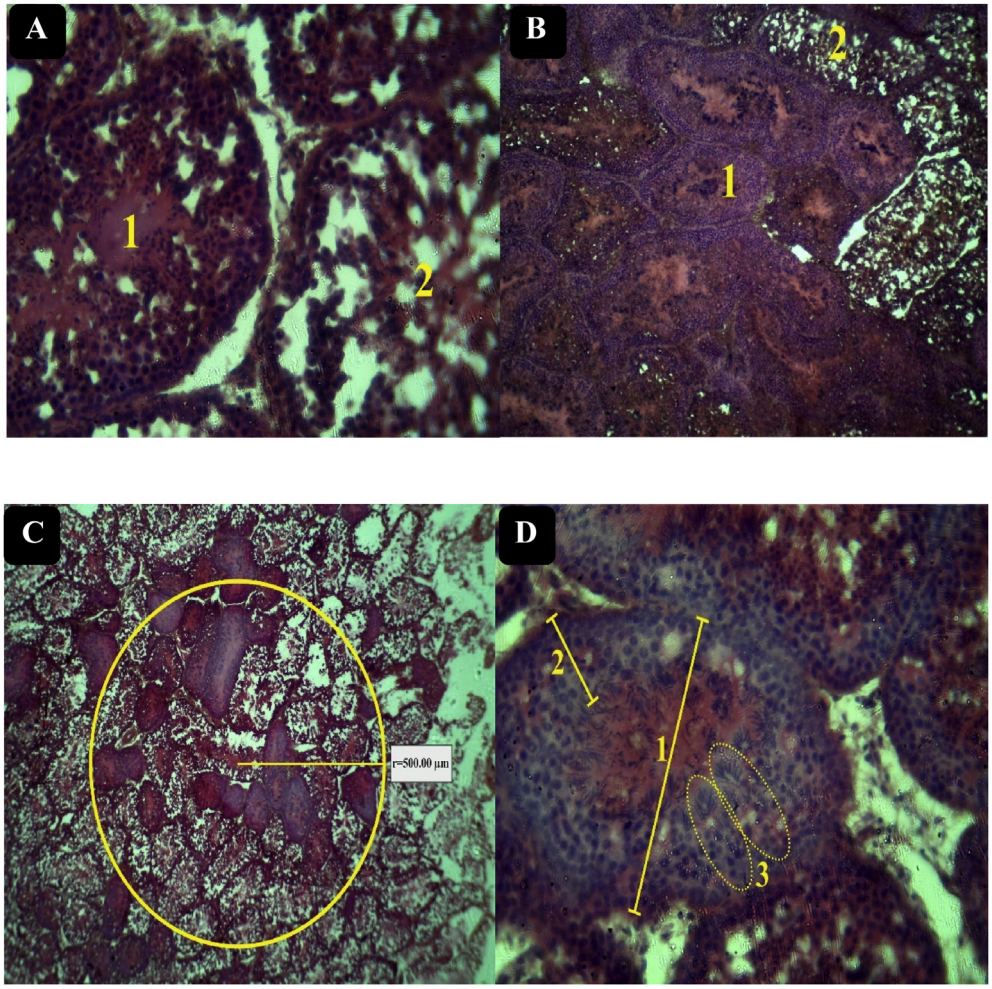

Figure 1. Photomicrographs of cross-sections of testes from rooster were stained with hematoxylin and eosin (HE). A. Tubular Differentiation Index (1 = TDI^{+}, 2 = TDI^{-}). B. Spermiogenesis Index (1 = SI^{+}, 2 = SI^{-}). C. Number of active seminiferous tubules (adult) in a circle with a radius of 500 µm. D [1]Seminiferous tubules diameter, [2] Height of epithelium seminiferous tubules, [3] Sertoli cells.

and FSH ($p < 0.05$). However, no significant differences were observed between LC-250 and LC-500 groups on these parameters. The results of linear and quadratic analyses of testosterone were significant ($p < 0.05$), which shows that testosterone response to the various doses of LC increases linearly and then decreases. This study indicated that the LC-250 mg/kg of diet resulted in highest levels of reproductive hormones.

The plasma concentrations of HDL and LDL were significantly ($p < 0.05$) affected by dietary supplementation with LC (Table 5). Plasma LDL decreased and plasma HDL increased significantly in birds fed diets supplemented with LC ($p < 0.05$). However, no significant differences were observed between LC-250 and LC-500 groups on these parameters. Supplementing the diets with LC had no significant effects on the plasma concentration TG, CHOL and VLDL. LDL concentration decreased as LC was added to the diet. Thus, the highest HDL and the lowest LDL concentrations were observed in response to the LC-250 mg/kg diet. However, no difference was found in CHO, TG, and VLDL in both linear and quadratic analyses. Orthogonal contrasts between LC-250 and LC-500 groups VS control showed the effects of LC on the plasma concentration of TRG, HDL and LDL ($p < 0.05$).

4. Discussion

This research aimed to investigate the effect of dietary LC supplementation in growing broiler breeder roosters, on the reproductive hormones, lipid profile and testicular histology parameters at the time of maturity. In the present study, testis weight and testis index were found to be respectively ~25 % and ~21% higher in the groups fed with LC supplemented diet than in the control group. In contrast to the results of this study, it was earlier reported that the LC has no effects on weight and index of testis in mature White Leghorn roosters [25]. A trend to increase ($P = 0.07$) in body weight (~3.5 %) was observed on increasing LC. The same results have been reported for pigeons [26], native Turkish geese [27], pheasant [28], broiler chickens and laying hens [29,30], in all of which, growth performance improved when fed with LC. The results obtained from the current study show that LC has a positive effect on the growth performance of roosters at the time of maturity. Birds receiving LC-250 mg/kg of LC in their diet showed the highest increase in body weight, testis weight and testis index. It has been found that LC to be present in high concentrations in the male genital system, including Sertoli cells, epididymis, and spermatozoa [31]. Spermatozoa use several substrates as energy sources during epididymal transit, but fatty-acid oxidation involving the carnitine dependent system seems to be a major energy supplying process [32]. The improvements in body weight, testis weight and testis index of roosters observed in this study in response to dietary LC may be attributed, at least partly, to improved utilization of dietary nitrogen, achieved through more efficient fat oxidation by LC [33]. It has been reported that testicular growth and development are delayed in underweight chicken [34]. The development of the testis is important for estimating the growth rate in birds because bird's testes are located within the body cavity [35].

In most birds, comb height and shank lengths have been used as external indicators of sexual maturity [36]. It has been shown that secondary characteristics like comb height are reliable indicators of fertility in roosters [37]. In this study, birds fed with LC (LC-250 or LC-500) had higher comb heights (~16 %) and shank lengths (~6 %) than birds of the control group. Regarding the fact that growth of combs is stimulated by TH [38], it seems that increasing concentration of testosterone in this study would be a reason for taller combs observed when fed with LC.

The growth and development of Gonads depend on the level of reproductive hormones in the blood; there is a positive correlation between the concentration of testosterone and the size of the testicles coefficient [39]. Our findings showed that the highest reproductive hormones were in response to LC-250 or LC-500 mg/kg of LC in diet. This in agreement with earlier studies that have shown that LC increased blood FSH, LH, and TH levels in male rats [40].

There are various mechanisms explaining the effect of LC on improving reproductive hormones. Increasing the production of free radicals and reducing the antioxidants enzymes leads to oxidative stress, which subsequently decreases LH, FSH, and TH levels [41]. LC can act as an antioxidant by inhibiting free radicals and increasing expression of antioxidant enzymes such as GSH and CAT, leading to reduced oxidative stress [42]. It has been reported that pulsatile GnRH secretion from perfused hypothalamic cells and GT1-1 neuronal cells significantly increased after culture in medium containing 100 μM acetyl-l-carnitine (ALC). This action of ALC can be attributed to an increase in the spike amplitude of GnRH release [43]. It has been reported that among the neural centers, the highest LC concentration is seen in the hypothalamus [44]; this suggests a possible role of LC in brain as a regulator of neurotransmitters. LC acts indirectly by affecting the HPG axis to regulate reproductive hormone secretion [45]. The highest LC concentration in neuronal cells is in the hypothalamus where it decreases neuronal cell death [46].

Data related to the histology showed that LC highly affects testicular tissue development. The results of this study showed that the birds on the LC-250 or LC-500 diets had more complete testicles than control groups (see Figure 2).

Different metabolic processes happen in the testis before the onset of maturity; these include proliferation of functional cells and increase in testicular volume; lack of development can be a sign of infertility in adulthood [47]. LC acts as a cytoprotective agent with antioxidant, anti-apoptotic, and anti-inflammatory properties [48] and it protects germ cells from apoptosis involving the Sertoli cell metabolism [49]. It seems that the improvement of testicular histology with dietary LC was not unexpected in the present study.

More than 90% of the variation on testicular development in roosters has been reported by altering in FSH, and 35% of the variation in testicular development has been brought about by altering in LH concentrations [4]. FSH stimulates testicular growth and development by increasing seminiferous tubule diameter and stimulating Sertoli cells proliferation and differentiation [8]. It has been suggested that increased

Table 2. Effect of l-carnitine on body weight and sexual maturity indices.

Item	Treatment*			SEM	P-value			
	LC-0 (Cont)	LC-250	LC-500		Treat.	Linear	Quadratic	LC vs. Cont
Body weight(g)	3515	3628	3613	68.28	0.08	0.07	0.15	0.03
Testicle weight(g)	19.75^{b}	24.75^{a}	23.5^{a}	1.33	0.001	0.003	0.004	0.0005
Testicle index (g/kg)	5.62^{b}	6.82^{a}	6.50^{a}	0.42	0.008	0.01	0.01	0.003
Testicular volume (cm3)	15.50	17.75	18.00	1.75	0.14	0.07	0.36	0.06
Comb height (cm)	5.67^{b}	6.62^{a}	6.32^{ab}	0.38	0.02	0.04	0.02	0.008
Shank lengths (cm)	8.67	9.12	9.20	0.29	0.07	0.03	0.43	0.02

^{ab}Means within the same row with different superscripts are significantly different ($p < 0.05$).
SEM: standard error of mean.
* Treatment: LC-0 (0 mg L-Carnitine per kg of diet) (Control), LC-250 (250 mg L-Carnitine per kg of diet), LC-500 (500 mg L-Carnitine per kg of diet).

Table 3. Effect of l-carnitine on histology of rooster's testis.

Item[#]	Treatment[*]			SEM	P-value			
	LC-0(Cont)	LC-250	LC-500		Treat.	Linear	Quadratic	LC vs. Cont
NST (n)	9^{b}	15^{a}	12^{ab}	2.00	0.002	0.03	0.002	0.002
NSC (n)	15^{b}	23^{a}	20^{a}	2.29	0.003	0.01	0.005	0.001
HEST (μm)	22.7^{c}	67.7^{a}	56.8^{b}	3.09	<.0001	<.0001	<.0001	<.0001
STD (μm)	85.3^{c}	163.8^{b}	198.4^{a}	9.49	<.0001	<.0001	0.004	<.0001
SI (%)	52^{b}	84.4^{a}	85.1^{a}	1.52	<.0001	<.0001	<.0001	<.0001
TDI (%)	73.9^{b}	80.9^{a}	81.7^{a}	1.74	0.0008	0.0001	0.01	<.0001

[abc]Means within the same row with different superscripts are significantly different ($p < 0.05$).
SEM: standard error of mean.
[*] Treatment: LC-0 (0 mg L-Carnitine per kg of diet) (Control), LC-250 (250 mg L-Carnitine per kg of diet), LC-500 (500 mg L-Carnitine per kg of diet).
[#] NST: Number of Seminiferous Tubules, NSC: Number of Sertoli Cells, HEST: Height of Epithelium Seminiferous Tubules, STD: Seminiferous Tubules Diameter, SI: Spermiogenesis Index, TDI: Tubular Differentiation Index.

Table 4. Effect of l-carnitine on sex hormones profile of plasma.

Item	Treatment[*]			SEM	P-value			
	LC-0(Cont)	LC-250	LC-500		Treat.	Linear	Quadratic	Cont vs. LC
Testosterone (nmol/l)	5.34^{b}	8.75^{a}	7.72^{ab}	1.23	0.01	0.02	0.01	0.004
GnRH (ng/l)	132.75^{b}	155.25^{a}	153.25^{a}	7.39	0.002	0.003	0.02	0.001
LH (mIU/ml)	2.52^{b}	3.49^{a}	3.30^{a}	0.30	0.001	0.005	0.01	0.001
FSH (IU/l)	3.74^{b}	5.71^{a}	5.33^{ab}	0.95	0.04	0.04	0.07	0.015

[ab]Means within the same row with different superscripts are significantly different ($p < 0.05$).
SEM: standard error of mean.
[*] Treatment: LC-0 (0 mg L-Carnitine per kg of diet) (Control), LC-250 (250 mg L-Carnitine per kg of diet), LC-500 (500 mg L-Carnitine per kg of diet).

Table 5. The effect of l-carnitine on triglycerides, cholesterol and lipoproteins.

Item[#](mg/dl)	Treatment[*]			SEM	P-value			
	LC-0(Cont)	LC-250	LC-500		Treat.	Linear	Quadratic	Cont *vs.* LC
CHOL	123.50	119.75	121.50	2.32	0.12	0.26	0.08	0.07
TRG	42.00	37.50	38.50	2.58	0.08	0.07	0.12	0.03
HDL	90.50^{b}	101.25^{a}	100.00^{a}	3.01	0.001	0.001	0.01	0.0004
LDL	21.50^{a}	10.25^{b}	12.25^{b}	3.42	0.002	0.004	0.01	0.0009
VLDL	11.50	7.43	9.25	2.50	0.11	0.23	0.07	0.06

[ab]Means within the same row with different superscripts are significantly different ($p < 0.05$).
SEM: standard error of mean.
[*] Treatment: LC-0 (0 mg L-Carnitine per kg of diet) (Control), LC-250 (250 mg L-Carnitine per kg of diet), LC-500 (500 mg L-Carnitine per kg of diet).
[#] CHOL: Cholesterol, TRG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very Low -density lipoprotein.

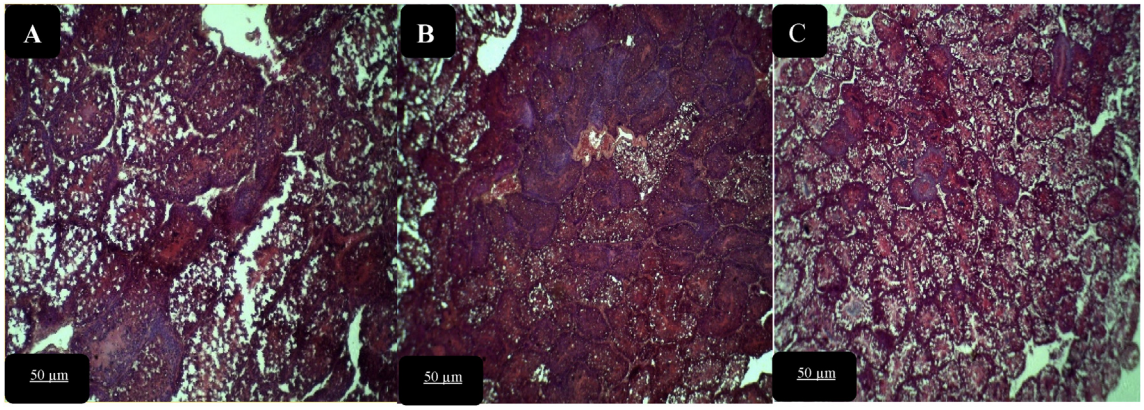

Figure 2. Effects of l-carnitine on testis histology of young broiler breeder roosters. A. Control; B. 250 mg l-carnitine, and C. 500 mg l-carnitine. Histological parameters of the testis were affected by the treatments ($p < 0.01$). So that, the least development of testicular tissue observed in control group birds, Magnification, × 400; Bar = 50 μm.

serum TH concentration resulted from increased Leydig cell numbers and also the amount of smooth endoplasmic reticulum within the Leydig cells [50]. In the present experiment, birds on LC-250 and LC-500 mg/kg diets had more complete testicles than control groups.

In line with the hormonal results, lipoprotein data showed that birds fed with LC (LC-250 or LC-500) had the highest plasma HDL and the lowest plasma LDL. It is known that LC is involved in lipid metabolism [50], indeed, LC increases the blood HDL and decreases the blood levels of total CHO and LDL. In the present study, the increase in Leydig cell numbers may be relative as a result of the increment of the HDL and reduction of the LDL. HDL is a major substrate source in human [51], and in Goat [52], for the steroidogenic process. Sertoli cells play a pivotal role in delivering the necessary nutrients such as lipid substrates to the sperm throughout its differentiation. In addition, LDL and VLDL particles cannot pass through the blood-testis barrier, thus their access to the germ cells is excluded [53].

5. Conclusions

The present study showed that the addition of 250 mg of LC/kg body weight of immature rooster chicken can lead to improved sexual maturity indices in young roosters. Further studies are required to confirm these observations.

Declarations

Author contribution statement

Vahid Mohammadi: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Seyed Davood Sharifi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mohsen Sharafi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdollah Mohammadi-Sangcheshmeh: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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