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Effect of Dietary L-Carnitine Supplementation on Characteristics of Cobb's Semen

Research Article

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ABSTRACT

Good semen quality plays a very important role in promoting poultry breeding programs; due to its antioxidant properties and role in fat metabolism, L-carnitine seems to be effective in improving semen quality. Therefore, this study was performed to evaluate the effect of dietary L-carnitine supplementation on the motility parameters and qualitative traits in semen Cobb breed roosters. A total of twenty roosters, 24 weeks-old Cobb roosters randomly assigned to four experimental groups. The experimental design was conducted in a completely randomized design with 4 experimental treatments (T1: 0, T2: 125, T3: 250, and T4: 500 mg L-carnitine per kg diet) with 5 replications and sampling twice (semen collection) with an interval of 14 days. The collected semen was immediately evaluated for motility parameters and sperm quality traits. The results showed that there is no significant difference between the levels of 250 and 500 mg of L-carnitine in semen parameters, but there are significant differences with other groups (P≤0.05). Semen collection time did not affect sperm parameters (P≤0.05). In conclusion, the addition of L-carnitine at a rate of 250 mg/kg diet can improve motility parameters and sperm quality traits in the Cobb rooster.

KEY WORDS Cobb, L-carnitine, membrane integrity, rooster, sperm motility.

INTRODUCTION

Assisted reproductive techniques specialists are looking for non-invasive methods to increase semen quality in infertile individuals and animals (Santolaria *et al.* 2023). Part of this infertility is related to damage caused by the production of free radicals during the production and storage of gametes, which cause metabolic disorders in gametes, as well as changes in epigenetic status. These changes eventually lead to a reduction in the function of the sex cells (spermatozoa and oocytes) (Alahmar, 2019). On the other hand, the use of chemical compounds to reduce oxidative stress may cause disorders in sex cells. Therefore, reproductive researchers are considering the use of strategies such as adding compounds containing natural antioxidants to culture media, especially diets; In addition to reducing damage, such can also increase sperm antioxidant capacity and sperm energy metabolism (Walczak-Jedrzejowska *et al.* 2013; Aboul-Naga *et al.* 2020; Santolaria *et al.* 2023).

L-carnitine is a biologically active stereoisomer of 3-carboxy-2-hydroxy-N, N, N-trimethyl-1-propanaminium (l-acetyl carnitine) that exists as an extremely polar and small zwitterion (Micic *et al.* 2019). L-acetyl carnitine plays an essential role in bioenergy production, acting as a long-chain fatty acid transporter in the mitochondria, protecting cell membranes, and exerting anti-apoptotic actions (Ko *et al.* 2014). Small amounts of L-carnitine (C7H15NO3) can be biosynthesized endogenously in the liver and kidneys of all animals from the two essential amino acids lysine and methionine. Water-soluble vitamins such as B6, B12, C, and folic acid, as well as trace elements such as iron, are essential as catalysts for L-carnitine biosynthesis (Li *et al.*

2007). Studies have shown that L-carnitine can act as an antioxidant and may serve as a scavenger of free radicals that cause lipid peroxidation. Therefore, L-carnitine plays multifunctional roles in the intermediary metabolism of poultry, which improves growth performance, immune responses, antioxidant activities, and semen quality (Golzar Adabi *et al*. 2011; Al-Daraji and Tahir, 2014; Elokil *et al.* 2019) and affects the blood biochemical parameter of female Japanese quails (Abedpour *et al*. 2017).

The effect of L-carnitine on semen quality is now well recognized to have an important role in the proper functioning of mitochondrial oxidation, membrane integrity preservation, sperm energy balance, and apoptosis inhibition. Previous studies have shown the beneficial effects of L-carnitine on sperm quality traits especially motility and viability in different species, such as humans (Haseen Ahmed *et al*. 2017), boar (Yeste *et al*. 2009; Yang *et al*. 2019), rat (Abd-Elrazek and Ahmed-Farid, 2018), quail (Sarica *et al*. 2007), duck (Al-Daraji, 2014), ostrich (Adabi *et al.* 2008) and cock (Neuman *et al*. 2002; Elokil *et al*. 2019). On the other hand, L-carnitine treatment has shown improvement against the metabolic troubles caused by different factors. As a result, spermatozoa cells become more sensitive to oxidative stress and improper functioning of the reproductive axis (Almeida *et al*. 2017; Bisht *et al*. 2017). Thus, the natural anti-oxidative compounds (i.e., L-carnitine), Catalase (CAT) and glutathione peroxidase (GSH-PX) activities have key functions in protecting sperm against oxidative stress by preventing the production of free radicals (Martínez-Páramo *et al*. 2012). L-carnitine is involved in fatty acid transport for energy metabolism, it reduces lipid availability for peroxidation.

The positive relationship between carnitines and sperm quality is widely documented (Moradi *et al*. 2010; Yang *et al*. 2020). A direct relation between carnitines and sperm motility has been proven in multiple studies (Moradi *et al.* 2010). A study evidenced the positive correlation between seminal L-carnitine and sperm count levels, motility, and morphology (Haseen Ahmed *et al*. 2017). In a case-control study that compared fertile and infertile men, the fertile group had higher seminal carnitine levels (108.43 mg/L), higher sperm counts (66.66×106/mL), and higher motility (50.45%) than the infertile group that showed carnitines values of 80.6 mg/L, sperm counts of 52.56 × 106 and motility of 32.31% (Sheikh *et al*. 2007).

A study showed that feeding drakes with L-carnitine at the levels of 50-150 mg/kg diet significantly increased ejaculate volume, spermatocrit, mass and individual motility of spermatozoa, and concentration of spermatozoa, while percentages of dead and abnormal spermatozoa and acrosomal abnormalities decreased, and in this study, the best result was at 150 mg L-carnitine/kg diet (Al-Daraji, 2014). The *ad libitum* consumption of 500 ppm of dietary L-carnitine to young and aging White Leghorns for 5 weeks improved sperm concentration during the last half of supplementation and reduced sperm lipid peroxidation. Multinucleated giant cells, composed mainly of aggregates of degenerated spermatocytes and spermatids, were reduced in the testes of roosters consuming L-carnitine as compared with control-fed birds. Collectively, these results suggest that dietary L-carnitine has antioxidant properties that may preserve sperm membranes in roosters, thereby extending the life span of sperm (Neuman *et al*. 2002). According to Adabi *et al*. (2008), L-carnitine has antioxidant properties so it can protect sperm against reactive oxygen species (ROS). He reported that dietary L-carnitine supplementation improved semen volume, sperm motility, live sperm percentage, and sperm density in ostriches. This study was aimed at investigating the effect of carnitine on the semen quality of rooster Cobb semen.

MATERIALS AND METHODS

This study was performed by the international guidelines for the care and use of animals (Olfert *et al*. 1993).

Location of experiment and management of experimental poultry

The study was conducted on 20 Cobb 500 breed roosters that were 24 weeks old and weighed 3.7 ± 0.2 kg. The roosters were kept in individual cages on a research farm in Karaj Iran and all semen evaluation steps were performed in the Royal Institute's laboratory in Iran. Semen was collected from all roosters after 42 days (6 weeks) of *ad-libitum* feeding with experimental diets, twice at intervals of 14 days using the abdominal massage method. The collected semen was placed in a flask at a temperature of 35-37 ˚C and immediately transferred to the laboratory for initial testing.

Bird management and feeding

The 20 experimental roosters were randomly divided into four groups, with 5 roosters in each group, and kept individually in cages (65×60×70 cm) inside the enclosed farm with 16 hours of lighting with an intensity of 100 lux during 24 hours a day. All roosters were kept under the same management during the experiment and fed with iso-caloric and iso-nitrogenous diets and drinking water was provided *ad libitum*. During the pre-semen collection period (6 weeks) and the experiment (2 weeks), all roosters were fed the same basal diet according to the breed catalog Cobb (Table 1).

Table 1 Basic ration fed to roosters during the experiment

Ingredients	%
Yellow maize	62
Soybean meal (44 %)	13.4
Wheat bran	5
Barley	10
$CaCO_3$	7.28
DCP	1.3
Salt	0.27
$NaHCO_3$	0.15
Vitamin premix[1]	0.3
Mineral premix[2]	0.3
Calculated chemical composition	
Dry matter	88.75
Metabolizable energy (ME)	2.74 kcal/g
Crude protein (CP)	12.65
P (available)	0.38
Ca	3.07

[1] Supplied per kg of diet: vitamin A: 12000 IU; vitamin E: 100 IU; vitamin K_3: 5 mg; B_1: 3 mg; Riboflavin: 12 mg; Niacin: 15 mg; vitamin B_{12}: 0.04 mg; vitamin D: 3000 IU; Pantothenic acid: 55 mg; Pyridoxine: 4 mg; Biotin: 0.25 mg and Choline chloride: 1 g.#

[2] Supplied per kg of diet: Fe: 60 mg; Mn: 6 mg; Zn: 100 mg; I: 2 mg; Cu: 10 mg and Se: 0.2 mg.

Experimental design and data collection

The experiment was laid out in a completely randomized design with 4 experimental treatments (T1 (control): 0 mg L-carnitine, T 2: 125 mg L-carnitine per kg diet, T 3: 250 mg L-carnitine per kg diet, and T 4: 500 mg L-carnitine per kg diet) and 5 replications each group (To reduce the measurement error, each semen sample collected from each rooster was divided into four parts and evaluated, and their average was considered as replication) and sampling twice (semen collection) with an interval of 14 days (sampling time of roosters: day 0 and day14 after the start of the experiment (without adaptability time) or weeks 30 and 32 (with adaptability time)). Roosters were randomly assigned to each treatment and individually caged in the cages.

Semen collection from roosters was performed using the abdominal massage method (Kucera and Heidinger, 2018). The technique involved restraining the rooster and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. A gentle pressure caused semen to drain out of the vas deferens and eventually out through the phallic folds. Semen was collected in a 1.5 mL microtube labeled according to the number of replications, group, and sampling period, and immediately transferred to the laboratory for evaluation.

Evaluation of samples

Semen volume

The volume between 0.5 to 1 mL was considered normal semen volume. The volume of semen samples collected was measured and recorded using a calibrated tube mL (Garcia-Herreros, 2016).

Sperm concentration

A concentration of more than 2.5×10^9 sperm/mL was considered normal. Sperm concentration was determined with the hemocytometer method. Five µL of the semen sample was diluted with distilled water in a ratio of 1: 200, and a drop of it was placed on a hemocytometer slide, after dispersing, under a light microscope, the number of sperm in 25 cells middle was counted. According to the degree of dilution, sperm concentration per mL was calculated (Garcia-Herreros, 2016).

Determination of sperm motility

Motility of more than 70% was considered as normal. To evaluate sperm motility, semen samples from each experimental group were diluted with Extender- Beltsville (K_2HPO_4;12.70g/L, Sodium glutamate; 8.61g/L, fructose; 5.00g/L, CH_3COONa; 4.30g/L, Tris; 1.95g/L, Potassium citrate; 0.64g/L, KH_2PO; 0.65g/L, $MgCl_2$; 0.34), containing at least 50×10^6 sperm per mL. Then, using a variable sampler, 5 µL of semen was placed on a special slide, and sperm motility parameters such as percentage of total motile (TM), percentage of progressive motility (PM), velocity average path µm/s (VAP), velocity of straight line µm/s (VSL), curvilinear velocity µm/s (VCL), lateral amplitude µm/s (ALH) And the percentage of linearity (LIN) was measured using a computer-aided sperm analyzer (CASA) made in Russia (Test Sperm 1.2; Video Test T) (Garcia-Herreros, 2016).

Abnormal morphology (AM)

Abnormal morphology of less than 10% were considered normal. Abnormal sperm morphology was determined using the staining method as described by Hancock (1951). This was done by adding 3 drops of the sample to microtubes containing 1 mL of Hancock solution. Then a drop of this solution was placed on the slide and covered with a slide. By counting at least 400 spermatozoa under a contrast phase microscope with a magnification of 400, the percentage of abnormal sperm and sperm with abnormal acrosomes was calculated. The average of the three observations was considered as single data (Garcia-Herreros, 2016; Yang *et al*. 2019)

Membrane integrity (MI)

HOST test was used to determine the percentage of sperm membrane integrity. The HOST test is based on the osmolarity of the medium in which the sperm are located. The osmolality of the HAST medium is 100 osmol/L and the osmolality required for sperm is 425 osmol/L. Therefore, by placing live sperms in a medium with low osmolarity, they react rapidly and their tail ends are tied; but dead sperms exposed to this medium do not react after this test,

sperm with a tied tail were considered as live sperm, and sperm with a flat tail were considered as dead sperm. In summary, 10 µL of semen was added to 100 µL of HOST hypo-osmotic medium containing fructose and sodium citrate. It was then incubated at room temperature for 30 minutes. After this time, at least three drops (10 µL) of the incubated sample were examined using a light microscope at a magnification of 400. At least 400 sperms were counted in each sample and the percentage of sperm with a tied (live) tail compared to an untied (dead) was calculated (Pena *et al*. 2002; Prochowska *et al*. 2022).

Flow cytometry procedure

Flow cytometry analyses of apoptosis were performed using the FACSCalibur (Becton Dickinson, San Khosoz, CA, USA) flow cytometer equipped with standard optics. A minimum of 10000 sperms were examined for each assay at a flow rate of 100 cells/s. The sperm population was gated using 90 and forward-angle light scatter to exclude debris and aggregates. The excitation wavelength was 488 nm supplied by an argon laser at 250 mW. Green fluorescence (FL1) was measured using a 530/30 nm bandpass filter was measured using a 527/25 nm filter (FL3). The analysis of flow cytometry data was performed using FlowJo software (Treestar, Inc., San Carlos, CA) Pena *et al*. (2002).

Statistical model

For uniformity of variance, the Shapiro-Wilk test was used, and all data had a normal distribution. Not all data were rejected for the Mauchly Sphericity Test, which indicates that the variances were the same between treatments. Data from all variables using repeated measures and SAS9.4 software (SAS, 2004), MIXED method, statistical model were analyzed.

$$y_{ijk} = \mu + \tau_i + \delta_{ij} + \beta 1(tk) + \beta_{2i}(\tau * t)_{ik} + \varepsilon_{ijk}$$

y_{ijk}: observation of ijk.
μ: total average.
τ_i: group effect or treatment i.
$(\tau * t)_{ik}$: effect of interference between treatment i and period k.
t_k: effect of the period k.
ε_{ij}: random error with a mean of zero and variance σ^2, the variance between measurements within animals.
δ_{ij}: random error with mean zero and variance σ^2_δ.

The mean comparison of variables between groups was analyzed by Duncan's multivariate method at a statistical level of 5%.

RESULTS AND DISCUSSION

The results of Table 2 show that by increasing the level of L-carnitine in diets, all variables related to sperm motility parameters increase significantly between groups. Other sperm variables such as viability percentage, sperm volume, and sperm abnormality had a significant increase compared to the control group (0 mg L-carnitine) ($P \leq 0.05$). The best sperm motility parameters correspond to the group fed with a diet containing 250 mg carnitine/kg of diet. There is no significant difference between the groups fed with 250 and 500 mg carnitine/kg of diet, in sperm motility parameters except total motility ($P \leq 0.05$).

The results show that the sampling time on most sperm motility parameters except ALH and LIN parameters (first sampling time for ALH; 34.97 ± 0.205^a, second sampling time for ALH; 35.83 ± 0.205^b; first sampling for LIN 47.48 ± 0.391^a, the second sample for LIN; 50.13 ± 0.391^b) had no significant effect ($P \leq 0.05$). There was no significant effect of interference between treatments and sampling time ($P \leq 0.05$).

The results of Table 3 show that increasing the level of dietary L-carnitine significantly improves sperm quality characteristics (sperm concentration, viable sperm (%), membrane integrity (%), and abnormal morphology (%) compared to the control group and the group fed with 125 mg carnitine/kg of diet ($P<0.05$); Of course, there was no significant difference between the groups fed with 250 and 500 mg carnitine/kg of diet ($P<0.05$). The best sperm quality characteristics correspond to the group fed with a diet containing 250 mg carnitine/kg of diet. The results show that the sampling time on most sperm quality characteristics except apoptosis % (first sampling time; 7.17 ± 0.156^a, the second sampling time; 7.59 ± 0.156^b) had no significant effect ($P \leq 0.05$).

The results of Annexin V/PI analysis of sperm under different concentrations of LC are shown in Figure 1. The viability rates were significantly ($P<0.05$) higher in groups containing 250 and 500 mg CL/ kg diet compared to control. Furthermore, groups containing 250 and 500 mg CL/kg diet produced the lowest significant percentage of apoptotic-like changes compared to the control group. There was no significant difference between the levels of 250 and 500 mg carnitine/kg diet ($P \leq 0.05$) (Figure 1). There was no significant effect of interference between treatments and sampling time ($P \leq 0.05$).

Recent reports show that semen quality can significantly decrease under the influence of various factors (Moradi *et al*. 2010; Al-Daraji, 2014; Yang *et al*. 2019), for which various reasons have been discussed.

Table 2 The effect of different experimental treatments regardless of sampling time on sperm parameters

Measurement[1]	L- carnitine (mg/kg of diet)				SEM	P-value
	0	125	250	500		
TM (%)	64.95[a]	66.32[b]	83.20[c]	84.60[d]	0.487	0.015
PM (%)	58.2[a]	62.1[b]	77.1[c]	77.2[c]	2.222	0.0015
VAP (µm/s)	13.48[a]	14.20[b]	21.64[c]	21.99[c]	0.157	0.025
VSL (µm/s)	7.99[a]	10.69[b]	18.71[c]	18.61[cd]	0.534	0.0035
VCL (µm/s)	24.57[a]	25.49[a]	28.39[b]	28.46[b]	0.772	0.003
ALH (µm/s)	27.71[a]	29.68[a]	42.28[b]	43.23[b]	0.971	0.0001
LIN (%)	30.23[a]	38.03[b]	63.84[c]	64.36[c]	1.036	0.0051

TM: total motile; PM: progressive motility; VAP: velocity average path; VSL: velocity of straight line; VCL: curvilinear velocity; ALH: lateral amplitude and LIN: linearity.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effect experimental treatments regardless of sampling time on sperm quality characteristic

Measurement	L-carnitine (mg/kg of diet)				SEM	P-value
	0	125	250	500		
Sperm concentration (10^9 sperm/mL)	4.31[a]	4.12[a]	5.21[b]	5.00[b]	0.188	0.007
Live sperm (%)	68.08[a]	67.86[a]	80.99[b]	79.32[b]	0.857	0.036
Membrane integrity (%)	68.75[a]	70.14[b]	78.76[c]	78.71[c]	0.382	0.0013
Abnormal morphology (%)	11.24[a]	9.84[b]	9.16[c]	9.62[b]	0.197	0.028

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

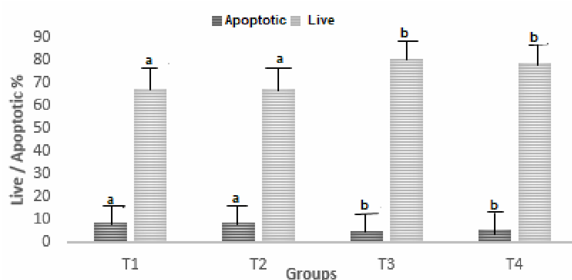

Figure 1 Viability and apoptosis status of sperm rooster in different groups
T1: 0, T2: 125, T3: 250, and T4: 500 mg L-carnitine per kg diet respectively

Environmental factors (such as toxicity of metals, chemicals, and other pollutants, radiation, heat, etc.), as well as obesity, inflammation, and exposure to ROS, are considered some of the causes of reduced spermatogenesis, sperm concentration, and sperm DNA integrity (Bisht *et al*. 2017; Alahmar, 2019; Torres-Arce *et al*. 2021). Sperm quality and quantity determining factors for male fertility can be affected by excessive ROS in the reproductive tract and semen. Therefore, adequate antioxidant levels must be continuously maintained in the body to prevent excessive ROS damage to sperm proteins, lipid membranes, and DNA integrity (Yeste *et al*. 2009). The present study shows that an antioxidant supplement containing 250 mg of L-carnitine per kg of diet can improve the quality of semen.

Many studies have investigated several supplements, including vitamins C and E, L-carnitine, coenzyme Q10, pentoxifylline, and trace elements (such as zinc and selenium), for their protective effects against ROS, either individually or in combination. Accordingly, some of these antioxidant supplements have a positive effect on the quality and quantity of sperm in sperm samples, DNA integrity of sperms, antioxidant capacity of total seminal plasma, apoptosis rate, and *in vitro* fertilization results (Aitken *et al*. 1989; Al-Daraji, 2014; Ko *et al*. 2014; Bisht *et al*. 2017; Haseen Ahmed *et al*. 2017; Elokil *et al*. 2019). However, the exact mechanism of the role of antioxidants in maintaining sperm concentration has not yet been identified, suppression of ROS-induced sperm damage has been repeatedly suggested as a possible mechanism.

The present study showed that treatment with a dietary L-carnitine supplement (containing 250 mg of L-carnitine per kg of diet for 42 days) can improve sperm concentration, sperm motility and morphology in Cobb roosters (Table 2, 3). Previously, in a study by Kozink *et al*. (2004), they showed that treatment of L-carnitine (500 g/day) in boars was not affected by treatment on semen volume, sperm concentration, total spermatozoa and sperm motility. Contrary to their findings, our study showed that antioxidant supplements containing L-carnitine (250 mg) for 42 days could improve sperm volume, concentration, motility, and morphology in Cobb breed roosters. In other studies, treatment with a dietary antioxidant supplement containing 100-500 mg of L-carnitine/kg of diet or kg of weight body for 40-60 days increased sperm concentration, motility, and morphology in roosters (Yeste *et al*. 2009; Ahangari *et al*. 2014; Al-Daraji, 2014; Haseen Ahmed *et al*. 2017; Elokil *et al*. 2019). So that Ahangari *et al*. (2014) and Al-Daraji and Tahir (2014) respectively, reported the best quality of se-

men at the level of 125 mg/kg diet for quail and 150 mg/kg of diet for duck. A study by Elokil *et al.* (2019) shows that the addition of feed with two different doses of LC (50 and 150 mg/kg body weight per day) for 12 weeks significantly increases the reproductive activity of roosters compared to the control group, and semen analysis shows that LC supplementation significantly ($P \leq 0.05$) increases sperm motility, concentration, viability, semen quality factor, sperm malondialdehyde concentration, catalase and glutathione peroxidase activity ($P<0.05$) and the biggest increase in semen quality was related to the level 150 mg LC/kg body weight per day.

However, some studies report no effect on semen parameters due to antioxidants (Kozink *et al.* 2004; Shanmugam *et al.* 2022). Comparing the consistent and conflicting results of different studies is challenging for several reasons. One of the reasons is that the negative results obtained from some similar studies are not reliable enough due to the small population, insufficient doses of antioxidants, and/or short duration of treatment (Kozink *et al.* 2004; Shanmugam *et al.* 2022). The origin of antioxidant supplementation also contributes to its effectiveness on seminal fluid parameters, so some studies report no significant improvement in sperm parameters with oral administration of antioxidants (Kozink *et al.* 2004; Shanmugam *et al.* 2022).

In the present study, increasing the level of L-carnitine had no significant effect on reducing the percentage of abnormal morphology of sperm, although, with increasing L-carnitine, the percentage of abnormal morphology was slightly reduced (Table 3). It should be noted, however, that sperm morphology is more influenced by genetic defects, and less exposed to environmental conditions. However, in most parameters, the two treatments containing 250 and 500 mg of L-carnitine were not significantly different from each other, which shows that the level of 250 mg of L-carnitine can meet the reproductive needs of roosters in this breed. The addition of L-carnitine to the diet reduced apoptosis in sperm, which could be due to increased semen antioxidant capacity and thus reduced free radicals and ROS levels in experimental groups. On the other hand, increased sperm concentration and motility can be due to increased energy available to sperm. Because L-carnitine plays an important role in the transfer of fatty acids to the mitochondria and in increasing beta-oxidation function. The addition of L-carnitine has increased the level of sperm membrane integration, which can be effective in sperm motility and penetration of sperm into the ovum and increase herd fertility.

The results of this study are in agreement with the results of (Ahangari *et al.* 2014; Al-Daraji and Tahir, 2014; Elokil *et al.* 2019). Lipid peroxidation plays a key role in the aging of spermatozoa by shortening its lifetime *in vivo* as well as during the *in vitro* conservation of sperm for artificial insemination (Sarica *et al.* 2007; Elokil *et al.* 2019). The peroxidation process induces structural alterations in the acrosomal section of the spermatozoa and consequently reduces the motility and viability of spermatozoa (Dimitrov *et al.* 2007).

Furthermore, a possible explanation for the increase in sperm concentration in carnitine-fed birds is that carnitine facilitates the preservation of the sperm lipid membranes, thereby extending sperm longevity. These results are in agreement with those reported by Neuman *et al.* (2002), Sarica *et al.* (2007) and Zhai *et al.* (2007). Neuman *et al.* (2002) observed that supplementation of dietary L-carnitine at the 500 mg/kg level to a basal diet significantly increased semen concentration in roosters.

In the study by Sarica *et al.* (2007), adding carnitine at the 250 and 500 mg CL kg^{-1} levels of the diet increased sperm viability in comparison with the control group in male Japanese quail. Elokil *et al.* (2019) showed that supplementation of the feed with two different doses of L-carnitine (50 and 150 mg/kg body weight/day) for 12 weeks showed significantly increased in the reproductive activity of cock, in comparison to the control group. Seminal analysis showed that supplementation of L-carnitine significantly increased ($P \leq 0.05$) the sperm motility, concentration, livability, semen quality factor, seminal malondialdehyde concentration, catalase, and glutathione peroxidase activities (Elokil *et al.* 2019).

CONCLUSION

The results showed that L-carnitine supplementation in the diet can improve the characteristics of Cobb rooster sperm. The results obtained at the levels of 250 and 500 mg of L-carnitine increased the concentration, motility, and integrity of the sperm membrane and decreased apoptosis in the sperm cells. Therefore, according to the results, the level of 250 mg of L-carnitine per kg of diet is recommended to improve the quality of Cobb rooster semen.

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