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EFFECT OF SUPPLEMENTING L-CARNITINE ON SEMEN QUALITY TRAITS IN IRAQI DRAKES

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ABSTRACT

This study was conducted to determine the effect of supplementing Iraqi drakes with L-carnitine on semen quality traits. A total of 48 male Iraqi ducks, which were 30 weeks old were used in this study. Drakes were randomly allocated to four treatment groups with 12 drakes per treatment group, replicated three times, with 4 drakes in each replicate. Treatment groups were: Treatment 1 (T_1): Birds fed a diet free of L-carnitine (control group), Treatment 2 (T_2): Birds fed a diet containing 50 mg L-carnitine/kg of diet, Treatment 3 (T_3): Birds fed a diet containing 100 mg L-carnitine/kg of diet, and Treatment 4 (T_4): Birds fed a diet containing 150 mg L-carnitine/kg of diet. Drakes were only fed experimental diets during the experiment which lasted three months. The semen quality traits investigated were ejaculate volume, mass and individual motility of spermatozoa, spermatocrit, spermatozoa concentration, percentages of dead and abnormal spermatozoa and acrosomal abnormalities. Supplementing the diet of drakes with L-carnitine at the levels of 50, 100 and 150 mg / kg of 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 were decreased. However, T4 (150 mg L-carnitine/kg of diet) recorded the best results with relation to all semen quality traits included in this study. Dietary supplementation with L-carnitine improved semen quality of local drakes; therefore L-carnitine can be used as an efficient feed additive to improve reproductive performance of male ducks.

Key words: Carnitine, reproductive performance, drakes.

INTRODUCTION

L-Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is a highly polar natural compound found in microorganisms, plants, and animals (Arslan et al., 2003). It has been shown to have beneficial effects on the heart and skeletal muscles and conditions, such as disorders of the central nervous system, certain types of male sterility and some disorders in new borns (Benvenga et. al., 2004). Mammals and birds are able to synthesize carnitine from the amino acids L-methionine and L-lysine, but approximately two thirds of the human daily requirements come from an omnivorous diet (Griffin *et al.*, 1990). Carnitine is most likely found in all animal species, in numerous organisms and plants (Elgazzar *et al.*, 2012; Kleber, 1997). It has been established that an adequate supply of L-carnitine is necessary for the maintenance of good health. L-carnitine transports long-chain fatty acids across the mitochondrial membrane to be metabolized and aids in the release of stored body fat, triglycerides into the bloodstream for energy. Triglycerides are the major source for the production of energy in the heart and skeletal muscles. Access to L-carnitine is believed to increase energy levels for long-term aerobic activity. L-carnitine is also responsible for muscle contraction, regulation of protein balance and maintenance of a healthy heart. Researches also suggest that an adequate supply of L-carnitine could be instrumental in the treatment of diabetes, chronic fatigue syndrome, kidney and liver disease (Karadeniz *et al.*, 2008). It also reduces fat deposition by reducing the availability of lipids for peroxidation (Neuman *et al.*, 2002). It transports fatty acids into the mitochondria for β- oxidation to generate adenosine triphosphate (ATP) energy (Neuman *et al.*, 2002).

The effects of carnitine supplementation have been studied in trained pigeons, turkeys, broilers, and layers. Leibetseder (1995) determined the effects of supplementary dietary L-carnitine on carcass composition, performance of chickens and hatchability. He found that performance and abdominal fat were not influenced by carnitine and that hatchability of the birds fed with carnitine was higher and the carnitine levels of these eggs were higher than in the control group. Al- Hayani (2012) showed that including 300 mg/kg of L-carnitine in the diets of guinea fowl improved productive traits, such as, egg production rate, cumulative egg number, egg weight, feed conversion ratio and egg mass. It also improved egg quality traits, like yolk weight, yolk diameter, yolk height, albumen height and Haugh unit as compared with control group. Supplementation of L-carnitine may also improve energy production from fatty acids to facilitate the hatching process in chicken embryos (Zhai *et al.*, 2008a). Dietary supplementation of L-carnitine increased sperm concentration, count and motility,

semen volume and decreased lipid peroxidation of spermatozoa in breeding roosters (Neuman *et al.*, 2002; Golzar Adabi *et al.*, 2006; Zhai *et al.*, 2007). L-carnitine also plays a critical role in the maturation and motility of spermatozoa within the male reproductive tract (Ng *et al.*, 2004).

There is paucity of information on the effect of dietary L-carnitine on the reproductive performance of the male duck. Therefore, the aim of this study was to investigate the effects of dietary supplementation with different levels of L-carnitine on semen quality of drakes.

MATERIALS AND METHODS

The experiment was on 48 male Iraqi ducks that were 30 weeks olds and weighed between 1.75-2.0 kg. The males were kept in individual cages with size 0.6/0.8/0.6 m and fed *ad libitum*, except that the drakes were restricted from feed 12 h prior to semen collection. These drakes were separated into four groups with one control group and three treatment groups. Each treatment was replicated three times, four birds per replicate. The experiment was divided into two stages. During the first stage, which was the first two weeks, the birds were allowed to adapt to new diet and condition. All experimental groups were fed a mash diet. The diets were formulated to be isocaloric and isonitrogenous and their composition were determined according to the NRC (1994) and the composition of the basal diet is presented in Table 1. At the second stage four experimental diets were formulated to provide a similar nutrient profile with the exception of using four graded levels of L-carnitine (0, 50, 100 and 150 mg per kg of diet, respectively). Therefore, the arrangement of treatments was: Treatment 1 (T_1): Birds fed a diet free of L-carnitine (control group), Treatment 2 (T_2): Birds fed a diet containing 50 mg L-carnitine/kg of diet, Treatment 3 (T_3): Birds fed a diet containing 100 mg L-carnitine/kg of diet, and Treatment 4 (T_4): Birds fed a diet containing 150 mg L-carnitine/kg of diet. The drakes were fed experimental diets during this stage of the experiment, which lasted three months, during which semen samples were also collected.Thesedrakes were trained for semen collection14 days before the actual collection began. The semen collection procedure was carried out by using dorsal-abdominal manual massage procedure (Al-Daraji *et al.*, 2012). Semen traits were analysed for ejaculate volume, mass and individual motility of spermatozoa, spermatocrit, spermatozoa concentration, and percentages of dead spermatozoa, abnormal spermatozoa, and acrosomal abnormalities. These traits were evaluated according the procedures reported by Al-Dareaji (2007a, b) and Al-Daraji *et al.* (2002). Data were statistically analysed using the General Linear Model

procedure of SAS (2004). Test of significance for the differences between means of each classification were done by the Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSION

As seen in Table 2, there was an increase ($p<0.01$) in the semen ejaculate volume of drakes supplemented with L-carnitine.The highest semen volume was observed in T4 (0.41 ml), while the lowest was in T_1 (0.22 ml).The effects of supplementation of L-carnitine on mass activity of spermatozoa of drakes are illustrated in Table 3. Mass activity of spermatozoa of drakes supplemented with L-carnitine was higher ($p< 0.01$) than that of the control. Treatment group four had the highest mass activity of spermatozoa during the three-month experimental period (78.82, 79.73 and 82.27 %, respectively). However, there was no difference ($P >0.05$) noted in the mass activity of spermatozoa in T_2 and T_3 during the third month. Results in Table 4 revealed that supplementation of L-carnitine increased ($P <0.01$) the individual motility of spermatozoa of drakes compared to the control. Drakes inT_4 had the highest (86.57 %) individual motility of spermatozoa, while those inT_1 had the lowest (74.3 %).There was no significant difference between T_2 and T_3 in the individual motility of spermatozoa. Table 5 presents the effects of L-carnitine supplementation on the spermatocrit of the drakes. There was an increase ($p<0.01$) in the average spermatocrit for all treatment groups supplemented with L-carnitine, compared to the control group. Treatment group four recorded the highest values (20.22, 22.25 and 22.97 %) during the three months of the experiment.

Spermatozoa concentrations for the treatment groups are given in Table 6. Sperm concentration means ranged from 2.30×10^9cell/ml to 4.77×10^9 cell/ml. The lowest sperm concentration was observed in the control group. There were high differences ($P<0.01$) in mean concentrations of spermatozoa between the drakes in T_1 and other treatment groups, while there were no differences ($P >0.05$) among T_2, T_3 and T_4 in the third month. As shown in Table 7, the percentages of dead spermatozoa decreased ($P<0.01$) due to dietary L-carnitine supplementation. No differences were found between T_2 and T3 (20.36 and 18.9×10^9 cell/ml, respectively).The effects of L-carnitine supplementation on the percentage of abnormal spermatozoa are shown in Table 8. There was a lower ($P <0.01$) percentage of abnormal spermatozoa in T_2, T_3 and T_4 (16.76, 15.74 and 13.63 %, respectively) compared to the control (18.49 %). No differences ($P >0.05$) were observed between T_2 and T_3 in the second and third month. Results contained in Table 9 show that consumption of L-carnitine

reduced (P <0.01) the percentage of acrosomal abnormalities. The highest average of percentage of acrosomes abnormalities (12.27 %) was found in the first month of the control group, while the lowest (7.54 %) was in the second month of T_4.

The significant improvement in semen quality traits is shown by increasing in means of each of the ejaculate volume (Table 2), mass and individual motility of spermatozoa (Tables 3 and 4), spermatocrit (Table 5) and spermatozoa concentration (Table 6) and decreasing means of percentages of dead and abnormal spermatozoa and acrosomal abnormalities (Tables 7, 8 and 9, respectively). This may be due to the role of carnitine in the oxidation of long-chain fatty acids, which improved spermatogenesis within the seminiferous tubules of the testis (Neuman *et al.*, 2002). It is the sequence of events that transforms spermatogonia into mature spermatozoa and improves the semen qualities. Fat is used to build the membrane of the sperm and has an important role in sperm metabolism and fertilizing ability. The composition of fat in the sperm membrane is considered as the main factor determining the effectiveness of sperms and sperm parameters (sperm count, motility and viability) (Kelso *et al.*, 1997). In addition, carnitine has a pivotal role in the movement of sperm. During the passage of sperm from the head to the tail of the epididymis, carnitine concentration increased significantly (Enomoto *et al.*, 2002; Kobayashi *et al.*, 2007). Therefore, it is believed that the acquisition of sperm movement is synchronized with the increasing concentration of carnitine in those sperms (Jeulin and Lawrence, 1996). The carnitine itself is also an energy substrate to support the movement of sperm (Al-Daraji, 2007b).

The reason for the improvement in semen quality of males that were treated with carnitine is due to the effectiveness of carnitine as a powerful antioxidant and prevents the creation of free radicals in the semen (Agarwal and Said, 2004). This can be explained, by the presence of high quantities of long-chain polyunsaturated fatty acids (PUFA) in the membrane of avian spermatozoa. The peroxides reduce the lifespan of the sperm *in vivo* during fertilization or during laboratory conservation *in vitro* (Agarwal *et al.*, 2005). Peroxides also lead to fundamental changes in the composition of sperm, especially the acrosome area and caused a sharp decline in the motility of sperm. Free radicals reduce sperm motility and vitality, and prevent the reaction of the acrosome with the membrane (Aitken and Clarkson, 1987; Aitken *et al.*, 1993). Carnitine prevents the formation of free radicals, which form peroxides that cause oxidation or destroy free radicals formed (Sarica *et al.*, 2007). The action of carnitine in these cases is similar to the actions of vitamins A and E (Neuman *et al.*, 2002). They work on

the mid piece of sperm; thus providing protection to the sperm membrane and acrosome (Aitken *et al.*, 1993). In addition, the role of carnitine is in the metabolism of long-chain fatty acids, where it reduces their accumulation in the semen or reproductive tracts, and consequently reduces the opportunities of oxidation and formation of free radicals (Vicari and Calogero, 2001). It is noteworthy that the carnitine effective in eliminating free radicals or reactive oxygen species is more likely to be formed by the free iron in the body and works on the degradation of cell membranes. Carnitine can bind with free iron because it has a chelating property; thus decreases the chances of the formation of free radicals and active oxygen species (Kalaiselvi and Panneerselvam, 1998).

The significant improvement in semen quality of the male ducks, as a result of L-carnitine supplementation may be also attributed to the roles of FSH and LH hormones. This is evidenced by an increase in the concentration of testosterone in the blood serum of these males. The FSH hormone is directly responsible for stimulating the process of spermatogenesis and increase the testis size, Sertoli cell differentiation and seminiferous tubules size (O'Shaughnessy *et al.*, 2010). On the other hand, LH hormone plays a basic role in the differentiation and maturation of Leydig cells and testosterone production in the interstitial tissue of the testis. Testosterone has an important role in spermatogenesis and the improvement of semen quality and quantity (Weinbauer and Nieschlag, 1991; Squires, 2003). The high concentration of this hormone also enhances growth and maintenance of testes (Rommerts, 1990; Jacyno *et al.*, 2007). Positive effects of carnitine on spermatogenesis have been reported in previous studies, where sperm concentration, ejaculated volume and number of live sperms increased (Palmero *et al.*, 1990; Vitali *et al.*, 1995; Matalliotakis and Koumantakis, 2000; Neuman *et al.*, 2002; Zhai *et al.*, 2008b).

Table 1 Percentage of ingredient and calculated chemical analysis of experimental basal diet

Ingredients	**(%)**
Yellow corn	39
Wheat	33.7
Soya bean meal (44 %)	13
concentration protein *	5
Limestone	6
Vegetable oil	2

Dicalcium Phosphate	1
NaCl	0.3
Total	100
Calculated Chemical composition**	
Crud Protein (%)	15.2
Energy (kcal/kg)	2927,3
Lysine (%)	0.7
Methionine (%)	0.3
Cysteine, %	0.25
Calcium (%)	2.7
Available Phosphorus (%)	0.3

*Concentration protein (BROCON – 5 SPECIAL W) each 1kg of vit .and min. premix (imported from China) contains: 3.25 % Crud protein; 3.5 % Crud fat; 1 % Crud fiber; 6 % Calcium; 3 % Available phosphorus; 2.2 % sodium; 3.5 % methionine; 3.90 % methionine + cysteine; 3.25 % Lysine; 2100 kcal/kg metabolizable energy;200000 IUVit A; 40000 IU Vit.D_3; 500mg Vit.E; 30 mg Vit.K_3; 15 mg Vit B_1, B_2; 150 mg Vit B_3; 20 mg Vit B_6; 300 mg Vit.B_{12}; 10 mg Folic acid; 50 mg Biotin; 800 mg Zinc; 100 mg Copper; 15 mg Iodine ; 1 mg Iron; 2 mg Selenium; 1.2mg Manganese; 6 mg Cobalt; and antioxidant 90 mg.

**Calculated Chemical composition analysis adopted by NRC (1994).

Table 2 Effect of dietary L-carnitine on ejaculate volume (ml) (Mean ± SE) of rdakes

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	0.216 ± 0.012^{d}	0.230 ± 0.005^{d}	0.243 ± 0.003^{d}	0.22 ± 0.006^{d}
T2	0.276 ± 0.006^{c}	0.286 ± 0.003^{c}	0.310 ± 0.005^{c}	0.29 ± 0.004^{c}
T3	0.323 ± 0.006^{b}	0.363 ± 0.006^{b}	0.376 ± 0.009^{b}	0.35 ± 0.007^{b}
T4	0.386 ± 0.006^{a}	0.416 ± 0.003^{a}	0.446 ± 0.012^{a}	0.41 ± 0.007^{a}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different. ** ($P<0.01$).

Table 3 Effect of dietary L-carnitine on mass activity of spermatozoa (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	64.60 ± 0.57^{d}	66.65 ± 0.54^{d}	68.57 ± 0.61^{c}	66.60±0.57^{c}
T2	69.99 ± 1.31^{c}	70.81 ± 1.31^{c}	73.62 ± 1.59^{b}	71.47±0.96^{b}
T3	75.77 ± 0.69^{b}	74.85 ± 0.89^{b}	76.65 ± 0.66^{b}	75.75±0.74^{b}
T4	78.82 ± 1.15^{a}	79.73 ± 0.63^{a}	82.27 ± 0.57^{a}	80.27±0.78^{a}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** (P<0.01).

Table 4 Effect of dietary L-carnitine on individual motility of spermatozoa (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	73.81 ± 0.94^{c}	74.27 ± 0.60^{c}	74.82 ± 0.32^{c}	74.30±0.62^{c}
T2	76.82 ± 1.14^{bc}	77.55 ± 0.63^{b}	78.39 ± 1.09^{bc}	77.58±0.95^{bc}
T3	77.98 ± 0.75^{b}	79.42 ± 0.39^{b}	80.26 ± 1.11^{b}	79.22±0.75^{b}
T4	83.93 ± 1.07^{a}	86.24 ± 0.90^{a}	89.55 ± 2.26^{a}	86.57±1.41^{a}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** (P<0.01).

Table 5 Effect of dietary L-carnitine on spermatocrit (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	15.81 ± 0.36^{c}	16.22 ± 0.40^{c}	16.80 ± 0.14^{c}	16.27 ± 0.30^{c}
T2	17.72 ± 0.57^{bc}	17.69 ± 0.54^{bc}	18.40 ± 0.36^{b}	17.93 ± 0.49^{b}
T3	18.07 ± 0.43^{b}	18.46 ± 0.54^{b}	19.08 ± 0.30^{b}	18.53 ± 0.42^{b}
T4	20.22 ± 0.96^{a}	22.25 ± 0.61^{a}	22.97 ± 0.34^{a}	21.81 ± 0.63^{a}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** ($P<0.01$).

Table 6 Effect of dietary L-carnitine on spermatozoa concentration ($\times 10^9$/ml) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	2.30 ± 0.15^{c}	2.46 ± 0.47^{d}	2.33 ± 0.67^{c}	0.77 ± 0.22^{c}
T2	3.10 ± 0.37^{b}	3.63 ± 1.67^{c}	3.67 ± 1.67^{b}	1.22 ± 0.55^{b}
T3	3.60 ± 0.20^{ab}	4.10 ± 1.00^{b}	3.90 ± 1.00^{b}	1.30 ± 0.33^{b}
T4	4.30 ± 1.00^{a}	4.67 ± 1.67^{a}	4.77 ± 0.33^{a}	1.09 ± 0.11^{a}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** ($P<0.01$).

Table 7 Effect of dietary L-carnitine on Percentages of dead spermatozoa (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	23.47 ± 0.58^{a}	22.95 ± 1.00^{a}	22.08 ± 0.92^{a}	22.83±0.83^{a}
T2	20.50 ± 0.98^{b}	21.50 ± 0.82^{ab}	19.09 ± 0.64^{b}	20.36±0.81^{b}
T3	19.21 ± 0.72^{b}	19.82 ± 1.11^{b}	17.67 ± 0.01^{b}	18.9±0.61^{b}
T4	16.25 ± 0.33^{c}	16.65 ± 0.61^{c}	15.29 ± 0.38^{c}	16.06±0.44^{c}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** (P<0.01).

Table 8 Effect of dietary L-carnitine on abnormal spermatozoa (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	19.24 ± 0.69^{a}	18.08 ± 0.76^{a}	18.15 ± 0.76^{a}	18.49±0.73^{a}
T2	17.59 ± 0.58^{b}	16.31 ± 0.15^{b}	16.40 ± 0.57^{b}	16.76±0.43^{b}
T3	15.93 ± 0.26^{c}	15.71 ± 0.31^{b}	15.60 ± 0.43^{b}	15.74±0.33^{b}
T4	14.30 ± 0.15^{d}	13.29 ± 0.53^{c}	13.32 ± 0.46^{c}	13.63±0.38^{c}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** (P<0.01).

Table 9 Effect of dietary L-carnitine on acrosomal abnormalities (%) (Mean ± SE) of drakes

Treatment	Periods			Overall mean
	First month	Second month	Third month	
T1	12.27 ± 0.72^{a}	11.88 ± 0.34^{a}	10.81 ± 0.23^{a}	11.65 ± 0.43^{a}
T2	10.72 ± 0.84^{ab}	10.04 ± 0.22^{b}	09.49 ± 0.42^{b}	10.08 ± 0.49^{b}
T3	9.28 ± 0.66^{bc}	8.66 ± 0.11^{c}	8.77 ± 0.43^{bc}	8.81 ± 0.40^{bc}
T4	7.77 ± 0.13^{c}	7.54 ± 0.06^{d}	7.73 ± 0.21^{c}	7.68 ± 0.13^{c}
Level of significance	**	**	**	**

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.

Means having different superscript at the same column are significantly different.

** ($P<0.01$).

CONCLUSION

In conclusion, feeding diets containing different levels of L-carnitine resulted in significant improvement of semen quality and quantity traits in this study of Iraqi drakes. Therefore, L-carnitine can be used as an efficient feed additive to improve the reproductive performance of male ducks.

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