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Effect of L-carnitine supplementation on seminal plasma quality of Iraqi drakes

KEYWORDS: Carnitine, seminal plasma traits, drakes

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ABSTRACT *This experiment was conducted to determine the effect of different levels of L-carnitine added to drake diets on certain semen plasma characteristics. For that, 48 thirty weeks old Iraqi drakes were divided into 4 equal groups according to the L-carnitine contents added to the diet for 12 weeks (0 mg / kg of diet in control group; T1, 50, 100 and 150 mg / kg diet in the treatment groups T2, T3 and T4, respectively. Semen biochemical characteristics were determined fortnightly. Seminal plasma constituents included in this study were glucose, total protein, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The L-carnitine treatments (T2, T3 and T4) have induced significant (P < 0.01) decreases in semen plasma concentrations of glucose, total protein and cholesterol and seminal plasma activity of AST and ALT enzymes during all periods of experiment and also with relation to the overall means of these traits compared to respective control (T1) whereas seminal plasma activity of ALP was significantly (P < 0.01) increased during all periods of experiment and also with respect to the overall means of this trait in comparison with control group (T1). These results suggest that dietary L-carnitine supplementation significantly improve semen plasma constituents included in this study. Therefore, L-carnitine can be used as beneficial tool for improve semen quality of bird males.*

Introduction

Carnitine occurs in the form of L-and D-isomers; however, only the L-isomer of carnitine is biologically active, while the D-isomer may even be noxious for the organism (Szilagyi, 1998). L-carnitine is a natural, vitamin-like amino-acid, synthesised within the body from lysine and methionine (Vaz and Wanders, 2002), and is very important in the metabolism of lipids. It carries long-chain fatty acids to the mitochondria for beta-oxidation, which produces energy (ATP) needed by the cells for proper functioning (Hoppel, 2003; Ramsay et al., 2001). L-carnitine plays an important role in the processes of cellular detoxification, since it removes acyl-CoA from the mitochondria, excess of which has a toxic effect (Arrigoni Martelli and Caso, 2001). It also protects cellular membranes against oxidative damages resulting from peroxidation of polyunsaturated fatty acids that are a component of membrane phospholipids (Kalaiselvi and Panneerselvam, 1998).

L-carnitine is used as feed additive in poultry diets to increase yield and to improve feed efficiency. Thus, L-carnitine supplementation to diets reduces long chain fatty acid availability for esterification to triacylglycerols and storage in the adipose tissue (Xu et al., 2003). It also participates in biological processes for example, regulation of gluconeogenesis, stimulation of fatty acid synthesis and ketone, branched-chain amino acid, triglyceride and cholesterol metabolism (Corduk et al., 2007). Dietary plants and plant based feedstuffs generally contain very little carnitine compared with animal products (Baumgartner and Blum, 1997). The concentration of carnitine in animals varied widely across species, tissue type and nutritional status of the animal (De-Beer and Coon, 2009). There are contradictory reports in the case of the effects of L-carnitine on animals. Differences in dosage level of L-carnitine, levels of metabolisable energy, fat and cereals in the diet and physiological status of the animals may be responsible for the discrepancies between published studies (Buyse et al., 2001). Spermatozoa are very susceptible to peroxidation damage because of the high concentration of long chain polyunsaturated fatty acids within the phospholipids (Sarica et al., 2007). Many studies have established that spermatozoa and seminal leukocytes have the capability to generate high levels of Reactive Oxygen Species (ROS) with can reduce the viability and fertility of spermatozoa (Cerolini et al., 2005). Carnitine has antioxidant properties which may protect sperm membranes from toxic oxygen metabolites. It also functions to reduce the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for B-oxidation to generate adenosine triphosphate (ATP). This transport of fatty acids into the mitochondria for catabolism reduces the amount of lipid available for peroxidation (Kalaiselvi and Panneerselvam, 1998). Although, cereal grains and their by-products have a low L-carnitine content, they usually represent the major component of poultry diets. Consequently, L-carnitine supplementation in diet or in drinking water would be useful for facilitating fatty acid oxidation and reducing the storage of long-chain fatty acids in spermatozoa membrane (Rezaei et al., 2007). Feeding L-carnitine (500 mg kg^{-1} of diet) increase sperm concentration and decreased lipid peroxidation of spermatozoa in roosters (Neuman et al., 2002). Adabi et al. (2008) reported that dietary L-carnitine supplementation improved ostrich semen volume, sperm motility, live sperm percent and sperm count. Zhai et al. (2007) indicated that using of L-carnitine (125 mg kg^{-1} of diet) increase sperm concentration in comparison with control group in roosters. Boars receiving 500 mg or 230 mg of L-carnitine in their daily ration demonstrated increased ejaculate volume and sperm concentration (Währner et al., 2004). Inside a sperm cell, L-carnitine transports fatty acids to the mitochondria, where they undergo beta-oxidation leading to the generation of metabolic energy needed by the sperm cells for their progressive movement (Jacyno et al., 2007).

Scarce literature data and ambiguous results reported by other authors encouraged us to undertake this study in order to establish the effect of L-carnitine added to the daily feed ration on the seminal plasma traits of drakes.

Materials and Methods

The main aim of the present paper was to assess the effect of continuously administered feeding mixture supplemented with L-carnitine on metabolic profile of blood in Iraqi drakes.

A total of 48 Iraqi drakes, 30 weeks old, with an average weight of 1.57 – 2.00 kg were used in this study. They were randomly divided into 4 equal groups (each group contained 12 drakes) according to the dietary L-carnitine (L-Carnitine

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

Xtreme 60 ct, Dymatize Nutrition Company, USA) supplementation for 12 weeks (0 mg / kg diet; control group (T_1), 50 mg / kg diet (T_2), 100 mg / kg diet (T_3) and 150 mg / kg diet (T_4). Each group was divided into three replicate groups of 4 drakes each. A photoperiod of 24 h was maintained. Food and water were provided *ad libitum* and the diets were presented in mash form. They 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. The experiment was started with drakes aged 32 weeks. Drakes were restricted from feed 12 h prior to semen collection.

An ejaculate was collected from each of 84 Iraqi drakes according to conventional manual massage method reported by Al-Daraji (2007 a; b). Semen was caught with a glass funnel (70 mm in diameter) attached to a centrifuge tube (10-mL capacity). During the 2-wk training period, all drakes were successfully trained in all experimental groups and started to produce semen. Semen was collected from individual males two times per month for 3 consecutive months (six times for each male). Each ejaculate was pipetted into a 1.5-mL microcentrifuge tube. Tubes were centrifuged at 15,600 x g for 5 in in an Eppendorf Model 5414 microcentrifuge (Brinkmann Instruments, Inc., Westbury, NY). Following centrifugation, seminal plasma supernatants were transferred to 1.5-mL microcentrifuge tubes. Seminal plasma cholesterol was enzymatically measured using cholesterol esterase and cholesterol oxidase according to the enzymatic method described by Allain (1974). Seminal plasma glucose determination was based on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in production of hydrogen peroxide. Plasma glucose was determined according to Trinder (1969) using commercial kits of Plasmatic Laboratory Products LTD. Seminal plasma protein was determined by using colorimetric method described by Wotton and Freeman (1982). The peptide bonds of proteins react with Cu+2 in alkaline solution to form a colored

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).

complex whose absorbance, proportional to the concentration of total protein in the specimen or sample, is measured at 550 nm. The Biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution. Colorimetric method was used to determine alkaline phosphatase (ALP) activity according to Kind and King (1954). Seminal plasma asparte aminotransferase (AST) and alanine transaminase (ALT) activities were determined by using the colorimetric method according to Reitman and Frankel (1957). Commercial kits (Randox Laboratories Ltd., United Kingdom) were used for this purpose. ata were subjected to statistical analysis using a completely randomized design, four levels of L-carnitine 0, 50, 100, 150 ppm were used. The data was analyzed using the SAS program (SAS Institute, 2004). The means of variables were compared using Duncan's multiple- range test (Duncan, 1955).

Results and Discussion

Supplemental dietary carnitine had significant effect on seminal plasma glucose ($P < 0.01$) during all periods of experiments and regarding the overall mean of this trait. The highest and the lowest were obtained, respectively in levels of 250 and 0 mg kg^{-1} L-carnitine (Table 2). Al-Daraji (2001) concluded that the highly significant negative correlation between numbers of spermatozoa and glucose concentration in seminal plasma suggests the utilization of glucose by spermatozoa. Al- Daraji (2002) indicated that spermatozoa utilize the glucose in their metabolism. Al-Daraji (2007a) reported that there was significant positive correlation between glycolysis rate and motility and sperm density. Agarwal (2004) reported that L-carnitine play a key role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and the spermatogenic process. This beneficial effect is mediated by the transport of long chain fatty acids across the inner membrane of the mitochondria for utilization in metabolism through β- oxidation (Matalliotakis et al., 2000). Epididymal sperm use fatty acid oxidation as the main source of energy metabolism, carnitine is crucial to transport fatty acids into mitochondria matrix within spermatozoa for energy production (Enomoto et. al., 2002).

The content of protein in seminal plasma of Iraqi drakes was decreased ($P < 0.01$) by supplementing with 50, 100 or 150 mg L-carnitine/kg diet during all periods of experiment and also as regards the overall means of this

Table 2. Effect of dietary L-carnitine on seminal plasma glucose (mg/dl) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	53.00 ± 2.08^{a}	50.67 ± 2.18^{a}	58.00 ± 1.53^{a}	53.89±1.^{9}3a
T2	46.00 ± 1.00^{b}	42.67 ± 1.67^{b}	43.33 ± 1.76^{b}	44.00±1.4^{7}b
T3	35.67 ± 0.33^{c}	38.00 ± 1.15^{b}	37.33 ± 0.67^{c}	37.00±0.^{7}1c
T4	32.00 ± 1.00^{c}	31.33 ± 0.88^{c}	29.67 ± 1.33^{d}	31.00±1.^{0}7d

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 ($P<0.01$).

trait (Table 3). The significant decrease in seminal plasma protein due to the addition of carnitine to diets of drakes is an important indicator for predicting fertility and hatchability rates and semen quality, as Moustafa and

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

Table 3. Effect of dietary L-carnitine on seminal plasma **protein** (g/dl) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	2.10 ± 0.21[a]	2.00 ± 0.10[a]	1.91 ± 0.04[a]	2.00±0.11a
T2	1.47 ± 0.27[b]	0.95 ± 0.02[b]	1.70 ± 0.02[b]	1.37±0.10b
T3	0.94 ± 0.02[b]c	0.80 ± 0.03[b]	0.82 ± 0.04[c]	0.85±0.03c
T4	0.59 ± 0.09[c]	0.54 ± 0.04[c]	0.61 ± 0.03[d]	0.58±0.05d

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).

Meszaros (1980) found an inverse relationship between seminal plasma protein and spermatozoa motility and fertility and hatchability rates, and on this basis the males with low seminal plasma protein could be selected to improve semen quality and fertility rates and hatchability rates (Thurston et al, 1982; 1992). Čeřovský et al. (2007) found significantly negative relationship between the morphologically abnormal spermatozoa content and the concentration of seminal plasma protein (r = –0.60, P < 0.01). Al- Biar (2010) recorded significant positive correlation between seminal plasma protein and percentages of dead and abnormal spermatozoa and acrosomal abnormalities. Carnitine is a water-soluble antioxidant mostly derived from the diet that may play a role in sperm energy metabolism and provide the primary fuel for sperm motility. Spermatozoa exhibit increased L-carnitine and L-acetyl carnitine content during epididymal passage and acquisition of motility (Jeulin and Lewin, 1996). Carnitines enhance the cellular energetics in mitochondria by facilitating the entry and utilisation of free fatty acids within the mitochondria and also restore the phospholipid composition of mitochondrial membranes by decreasing fatty acid oxidation (Lenzi et al., 2003). In addition, carnitines protect sperm DNA and cell membranes from ROS-induced damage and apoptosis (Lenzi et al., 2004). However, Cavallini et al. (2004) demonstrated improved morphology after 3 and 6 months of treatment with carnitine.

The results of this investigation showed that L-carnitine supplementation induced a marked decrease of seminal plasma cholesterol during all periods of experiment and with relation to the overall means of this trait as compared to those of control group (Table 4), and this decrease was directly proportional to the dose of L-carnitine. Examinations indicate that there is a connection between spermatozoa cholesterol content and certain qualitative traits in male semen (Cerolini et al., 1997).The high concentration of cholesterol in the seminal plasma is usually associated with the increase of the percentages of dead and abnormal spermatozoa and acrosomal abnormality (Al - Daraji et al., 2002 a, b). Also the high concentrations of cholesterol in the seminal plasma had an inverse relationship with fertilizing ability, as cholesterol play inhibitory effect on fusion of membranes during acrosome reaction as a result of its entering into the fatty layers consisting of the cell membrane (Vicari and Calogero, 2001). In the boars was found a positive relationship between seminal plasma cholesterol content and spermatozoa motility, whereas in poultry a negative correlation (r=−0.279) was reported between spermatozoa motility and sperm cholesterol content

Table 4. Effect of dietary L-carnitine on seminal plasma cholesterol (µg/dl) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	1.50 ± 0.21[a]	1.17 ± 0.14[a]	0.92 ± 0.03a	1.19±0.12a
T2	1.00 ± 0.01[b]	0.85 ± 0.06[b]	0.71 ± 0.02[b]	0.85±0.03b
T3	0.84 ± 0.02[c]	0.67 ± 0.01[c]	0.59 ± 0.03[c]	0.70±0.02c
T4	0.52 ± 0.04[d]	0.34 ± 0.04[d]	0.31 ± 0.03[d]	0.39±0.03d

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).

(Cerolini et al., 1997). The presence of secretions of cholesterol in the lumen of the seminiferous tubules lumen and closely adherent to proteinaceous materials considered as a clear indication of cracking the seminiferous cells and release of cholesterol from these cells (Flesch et al, 2001). There appears to be no correlation between the amount of cholesterol in blood serum and that in sperm or seminal plasma, suggesting that sperm cholesterol content is regulated locally within the male reproductive tract (Grizard et al., 1995). The carnitine content of seminal fluid is directly related to sperm count and motility. Several studies indicate that carnitine supplementation may improve sperm quality. The reported benefits may relate to increased mitochondrial fatty-acid oxidation (providing more energy for sperm) and reduced cell death in the testes (Ng et al., 2004; Vicari et al., 2001). The improved semen quality in L-carnitine supplemented animals is related to the bioenergy coming from the beta-oxidation of activated long-chain fatty acids in the inner mitochondrial matrix. Mayes (2000) detailed that L-carnitine plays a major role as a cofactor in the transportation of free fatty acids from cytosol to the mitochondria.

The effect of trail diets on seminal plasma AST and ALT are presented in Table 5 and 6. The content of AST and ALT in seminal plasma decreased significantly (P<0.01) in response to L-carnitine supplementation during all periods of experiment and also respecting the overall means of this trait. The extra cellular activity of transaminases is due to their leakage into seminal

Table 5. Effect of dietary L-carnitine on seminal plasma AST activity (IU /L) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	33.00 ± 2.08[a]	30.67 ± 2.18[a]	34.67 ± 3.17[a]	32.78±2.47a
T2	25.00 ± 1.73[b]	23.33 ± 3.17[b]	21.00 ± 2.51[b]	23.11±2.47b
T3	25.33 ± 0.67[b]	22.67 ± 3.52[b]	24.00 ± 1.00[b]	24.00±1.73[b]
T4	23.33 ± 0.33[b]	20.00 ± 2.08[b]	19.67 ± 1.33[b]	21.00±1.24b

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).

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

Table 6. Effect of dietary L-carnitine on seminal plasma ALT activity (IU /L) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	23.00 ± 2.08[a]	24.00 ± 4.50[a]	28.00 ± 1.52[a]	25.00±2.07a
T2	15.00 ± 1.73[b]	16.67 ± 0.33[b]	14.33 ± 0.88[b]	15.33±0.98b
T3	15.33 ± 0.67[b]	16.00 ± 1.15[b]	14.00 ± 1.00[b]	15.11±0.94b
T4	13.33 ± 0.33[b]	13.33 ± 1.45[b]	13.00 ± 2.00[b]	13.22±1.26b

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).

plasma caused by damage inflicted upon spermatozoa (Kapila, 1992), hence seminal plasma transaminases are evaluated as an index of measurement of injury to spermatozoa incurred during different conditions (Singh et al., 1996). The enzyme release from spermatozoa has generally been viewed as cellular injury (Ingale et al., 2000), whereby membrane become inactive with altered permeability or destroyed resulting into loss of material therein (Sidhu et al., 1996). Sperm damage through oxidative stress results in increased membrane permeability to enzymes and other substances, and therefore, reduced metabolic activity of sperm (Storey, 1997). Changes in the activity of enzymes such as AST or ALT in semen plasma are associated with defects of sperm membranes (Colebrander et al., 1992). Cavallini et al. (2004) reported tha carnitine protect sperm DNA and cell membranes from ROS-induced damage and apoptosis. Xuan et al. (2003) documented that maturation, respiration, motility and fertility are dependent on the progressive increase in epididymal and spermatozoa carnitine, critical for mitochondrial fatty acid oxidation, as sperm pass from caput of the epididymis. Newsletter (2000) showed that L- carnitine increased the number of viable sperm cells. During spermatogenesis, sperm cells lose a large amount of their associated cytoplasm along with antioxidant substances contained in it, and subsequently become sensitive to oxidative stress (OS). However, these cells are suitably protected against OS process because of immersion in sperm fluid containing many antioxidants such as ascorbic acid, urate, Turin, sulfhydryl (thiol) groups, catalases, superoxide dismutase and L-carnitine (Peyvandi et al., 2009). Shahrzad et al. (2013 concluded that the use of L-carnitine as a substance with antioxidant properties can at least affect some sperm parameters such as reduction of DNA and cell membrane damage through preventing more production of free radicals and enhancing antioxidant defense of cell.

Table 7 shows the effect of dietary L-carnitine supplementation on seminal plasma activity of alkaline phosphatase. The supplement of 50, 100 or 150 mg/kg L-carnitine increased ($P \leq 0.01$) the seminal plasma activity of alkaline phoshatase during all periods of experiment and with respect to the overall means of this trait compared to control group. Alkaline phosphatase is a main secretion of the epididymis, the organ that plays a crucial role in the sperm cells' maturation, but the activity of this enzyme was also determined in the cytoplasmic droplets of the sperm cells, which suggests its association with glycogen metabolism in epididymal epithelium, thus supplying the maturing spermatozoa with energy (Arangasamy et. al., 2005). Alkaline phosphatase also participates in producing free semen fructosis, which, after fructolysis, provides the energy indispensable for the sperm cells' motility (Borkowski and Strzeżek, 1994). Variable levels of alkaline phosphatase activity have been reported in the semen plasma of men, dogs, toms, bulls, rabbits, rams, goats, buffalo, cocks, turkeys, boars and camels where it is believed to be involved in sperm glycolytic reactions and fructose

Table 7. Effect of dietary L-carnitine on seminal plasma alkaline phosphatase activity (IU /L) (Mean ± SE) of drakes.

Treatments	Periods			Overall mean
	First month	Second month	Third month	
T1	9.67 ± 2.33[c]	10.67 ± 2.18[b]	9.67 ± 0.33c	10.00±1.61c
T2	12.67 ± 0.67[b]	13.33 ± 0.33[b]	12.67 ± 0.88[b]	12.89±0.62b
T3	15.33 ± 0.67[b]	16.00 ± 11.5[b]	15.33 ± 0.33[a]	15.55±4.16b
T4	18.67 ± 0.67[a]	22.00 ± 2.64[a]	16.67 ± 2.96[a]	19.11±2.09a

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).

formation (Turner and McDonnell, 2003). Alibawi et al. (2012) concluded that the level of ALP in the seminal plasma and sperms correlated with the concentration of the sperms. Stasiak et al. (201) found that the seminal plasma acid and alkaline phosphatase activity correlated positively with the sperm concentration ($r = 0.5676$; $r = 0.6302$; $P < 0.01$) and negatively with the ejaculate volume ($r = –0.3456$; $r = –0.2783$, $P < 0.01$). The sperm quality and function improves with the intake of complementary L-carnitine. A positive correlation has been reported between free L-carnitine and sperm count ($r= 0.617$; $P < 0.01$), sperm motility ($r= 0.614$; $P < 0.01$), and the number of motile spermatozoa / ml ($r= 0.646$; $P < 0.01$) (Agarwal, 2004). L-carnitine, which is an essential cofactor for fatty acid metabolism, is present in epididymal plasma and spermatozoa at a concentration of 1-63 mM, while the blood plasma concentration is about 50 mM. L-carnitine provides valuable support for the male reproductive system and plays an important role in sperm energy metabolism and support semen quality (El-Nattat et al., 2011).

Conclusion

In conclusion the administration of L-carnitine has ameliorated the semen plasma characters of Iraqi drakes, therefore L-carnitine can be used as an efficient feed additive for improve reproductive performance of drakes.

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

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