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# Investigation the Effects of Dietary L-Carnitine Supplementation on Characteristics of Rooster Semen During Liquid Storage

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**Abstract:** The objective of present study was to investigate the effects of various levels of dietary L carnitine supplementation (0, 125, 250 and 500 mg $kg^{-1}$) on rooster semen characteristics during liquid storage. Semen were collected from 16 rooster using abdominal massage and suitable samples were mixed together and sperm characteristics including percentage of motile, viable, abnormal, semen pH, volume and concentration were assessed. This experiment was carried out on the basis of completely randomized design. Results showed that during liquid storage, the effect of L carnitine on motility and viability percentage of sperm in beltsville extender were significant ($p<0.05$). Mean comparison on the basis of duncan test showed that the highest motility and viability of spermatozoa were obtained from roosters that were fed with rations included 250 mg $kg^{-1}$ L-carnitine supplementation. Semen characteristics such as volume, pH and abnormal percentage of sperm did not differ significantly ($p>0.05$). Furthermore, semen concentration of birds fed dietary carnitine significantly differ from controls during experiment ($p<0.05$). The highest concentration of semen were obtained in level 250 mg $kg^{-1}$ L-carnitine. Therefore, use of L-carnitine supplementation (250 mg $kg^{-1}$) in broiler breeder male feeding is recommended to improve quality of rooster semen.

**Key words:** L-carnitine, sperm, peroxidation, motility, b-oxidation, Iran

## INTRODUCTION

L-carnitine (β-hydroxy γ-trimethyl amino butyrate) is a water-soluble quarternary amine that exists naturally in micro-organisms, plants and animals and is required for the long chain fatty acid transfer from cytoplasm to mitochondrial matrix for subsequent β-oxidation and energy production (Miah *et al*., 2004).

L-carnitine can be formed in the animals body. The amino acids lysine and methionine act as precursors. The vitamins $B_6$, $B_{12}$, C, folic acid and niacin and the trace element iron are also necessary as catalysis of the endogenous synthesis of L-carnitine (Nouboukpo *et al*., 2010).

The highest synthesizing capacity is found in the liver. A shortage of this substance results primarily in impaired energy metabolism and membrane function (Harmeyer, 2002).

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

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polyunsaturated fatty acids within the phospholipids (Sarica *et al*., 2007; Kodama *et al*., 1996). 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). Golzar Adabi *et al*. (2008) reported that dietary L-carnitine supplementation improved ostrich semen volume, sperm motility, live sperm percent and sperm count.

In humans, L-carnitine supplementation (3 g $day^{-1}$) increase sperm concentration and sperm motility of idiopathic astheno-zoospermia patients (Costa *et al*., 1994). Zhai *et al*. (2007) reported that using of L-carnitine (125 mg $kg^{-1}$ of diet) increase sperm concentration in comparision with control group in roosters.

The objective of the current study were to determine the effects of dietary L-carnitine supplementation on broiler breeder males semen characteristics during liquid storage.

## MATERIALS AND METHODS

This experiment was conducted from Jun 15, 2010-Aug 10, 2010 at Ghaemshahr University, Iran. A total of 16 roosters were selected and randomly assigned to 4 groups of similar mean weight each of which included 4 replicates of 4 rooster. The roosters were kept on wire cages. A continous lighting program was provided during the experiment.

The experimental diets were formulated to meet minimum nutrient requirements of roosters as established by the National Research Council. They were fed a diets supplemented with L-carnitine at 0, 125, 250 and 500 mg $kg^{-1}$ from 32-36 weeks of age (Table 1). The experimental diets in mash form and drinking water were provided *ad libitum*. Beginning at 30 weeks of age, all roosters were trained to the semen collection process using the abdominal massage method (Donoghue and Wishart, 2000).

Table 1: Ingredient composition (dry matter (%)) and calculated analysis of the basal diet

| Ingredients (%) | Diet |
|---|---|
| Corn | 70.96 |
| Soybean meal (44% CP) | 13.00 |
| Wheet bran | 9.00 |
| Vegetable oil | 3.40 |
| Dicalcium phosphate | 1.58 |
| Limestone | 1.34 |
| Salt | 0.30 |
| Vitami premix | 0.25 |
| Mineral premix | 0.25 |
| **Calculated composition** | |
| Metabolizable energy (kcal $kg^{-1}$) | 2950.00 |
| Crude protein (%) | 12.77 |
| Calcium (%) | 0.90 |
| Available phosphorus (%) | 0.45 |
| Sodium (%) | 0.15 |
| Lysine (%) | 0.71 |
| Methionine (%) | 0.39 |
| Methionine + cystine (%) | 0.53 |

Each kg of vitamin premix contained: Vitamin A, 3,500,000 IU; Vitamin $D_3$, 1,000,000 IU; Vitamin E, 9000 IU; Vitamin $K_3$ 1000 mg; Vitamin $B_1$, 900 mg; Vitamin $B_2$, 3,300 mg; Vitamin $B_3$, 5,000 mg; Vitamin $B_5$, 15,000 mg; Vitamin $B_6$, 150 mg; Vitamin $B_9$, 500 mg; Vitamin $B_{12}$, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg and each kg of mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg

Semen was collected from 16 rooster once weekly for 4 weeks then raw semen was diluted 1:1 with Beltsville Poultry Semen Extender (BPSE). Afetr dilution, semen samples were transferred to 64 well culture plates and sperm characteristics including percentage of motile, viable, abnormal, semen pH, volume and concentration were assessed.

Semen volume was determined by using a scales glass and pH measured with using of P 731 pH meter. Sperm concentration was determined using a hemocytometer procedure (Bakst and Cecil, 1997). Total sperm cells produced/rooster were calculated as sperm cells $mL^{-1}$ x mL of semen volume. The percentage of motile spermatozoa was determined by compound microscope at 10x magnification after placing a cover slip over 2-3 mm drop of semen on a warmed microscope slide (Biswas *et al*., 2009).

For sperm viability, expressed as percentage of dead sperm, number of viable spermatozoa in total sperm was determined using the eosin staining method as described

by Ozkoca (1984). The staining solution was prepared by adding 2 g of eosin stain and 3 g of sodium citrate into distilled water.

The solution was filtered with a paper filter before being used. The staining was performed with 1 drop of fresh semen into 2 drops of staining solution on a microscope slide. Using another slide, a smear was made and allowed to dry.

Unstained (intact) and red-colored (with damaged membranes) spermatozoa were counted as a counterstain. Dead spermatozoa retained more stain and appeared dark whereas the viable ones appeared clear. The percentage of abnormal spermatozoa was evaluated in the same by examining the morphology of a total count of 100 spermatozoa.

**Statistical analysis:** The data obtained from the experiment were analyzed by using SAS (1999) statistical programs with the ANOVA.

Significant differences among treatment means were separated using Duncan's multiple range test with a 5% probability (Duncan, 1955).

## RESULTS AND DISCUSSION

The supplementation of dietary L-carnitine to a basal diet increase sperm motility during liquid storage ($p<0.05$). The using of L-carnitine at level of 250 mg $kg^{-1}$ significantly improve sperm motility at storage times of 4, 8 and 24 h in comparison with control group however, the supplementation of dietary L-carnitine to a basal diet did not influence sperm motility at storage time of 12 h (Table 2).

Supplemental dietary carnitine had significant effect on sperm viability at storage times of 4, 8 and 12 h ($p<0.05$). The highest and the lowest sperm viability at storage times of 4, 8 and 12 h were obtained, respectively in levels of 250 and 0 mg $kg^{-1}$ L-carnitine (Table 3). It should be pointed out that supplementing the diets of roosters with 125, 250 and 500 mg L-carnitine had no effect on abnormal sperm percentage at storage times of 4, 8, 12 and 24 h in comparison with control group (Table 4).

Semen characteristics (Table 5) in terms of volume and pH did not differ statistically between the different dietary treatment groups ($p>0.05$).

Semen concentration of birds fed dietary carnitine significantly differ from control during experiment ($p<0.05$).

Mean comparison on the basis of duncan test showed that the highest concentration of semen were obtained in level 250 mg $kg^{-1}$ L-carnitine (Table 5).

Lipids are a basic component of semen, contributing to the membrane structure of spermatozoa, the metabolism of the sperm cells and their ability to fertilize the female gamete.

There is considerable evidence to indicate that the lipid composition of the sperm membrane is a major determinant of the cold sensitivity, motility and overall viability of spermatozoa (Kelso *et al*., 1997).

Table 2: Effect of L-carnitine on sperm motility (%) in different storage times

| Treatment carnitine (mg $kg^{-1}$) | Storage times (h): 4 | 8 | 12 | 24 |
|---|---|---|---|---|
| 0 | $73.970^b$ | $66.38^b$ | 36.55 | $20.28^b$ |
| 125 | $74.740^b$ | $66.67^b$ | 37.82 | $20.14^b$ |
| 250 | $79.220^a$ | $70.88^a$ | 38.55 | $24.31^a$ |
| 500 | $75.410^b$ | $67.91^{ab}$ | 37.19 | $22.16^{ab}$ |
| SEM | 0.890 | 1.17 | 0.79 | 0.87 |
| p-value | 0.006 | 0.04 | 0.39 | 0.03 |

$^{a, b}$Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Table 3: Effect of L-carnitine on sperm viability (%) in different storage times

| Treatment carnitine (mg $kg^{-1}$) | Storage times (h): 4 | 8 | 12 | 24 |
|---|---|---|---|---|
| 0 | $76.720^b$ | $69.050^b$ | $39.390^b$ | 22.88 |
| 125 | $78.180^b$ | $70.100^b$ | $40.240^b$ | 23.45 |
| 250 | $82.880^a$ | $75.200^a$ | $43.680^a$ | 23.33 |
| 500 | $78.130^b$ | $71.000^b$ | $40.210^b$ | 23.43 |
| SEM | 1.020 | 0.930 | 0.640 | 0.77 |
| p-value | 0.033 | 0.003 | 0.029 | 0.92 |

$^{a, b}$Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Table 4: Effect of L-carnitine on sperm abnormal (%) in different storage times

| Treatment carnitine (mg $kg^{-1}$) | Storage times (h): 4 | 8 | 12 | 24 |
|---|---|---|---|---|
| 0 | 10.80 | 15.43 | 23.22 | 32.17 |
| 125 | 8.88 | 14.19 | 22.89 | 31.52 |
| 250 | 8.51 | 14.42 | 22.84 | 32.01 |
| 500 | 8.37 | 14.08 | 22.15 | 31.51 |
| SEM | 0.59 | 0.61 | 0.84 | 1.18 |
| p-value | 0.07 | 0.47 | 0.84 | 0.97 |

$^{a, b}$Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Table 5: Effect of L-carnitine on semen characteristics

| Treatment carnitine (mg $kg^{-1}$) | Volume (mL) | Concentration ($n\times10^9$ $mL^{-1}$) | pH |
|---|---|---|---|
| 0 | 0.480 | $2.900^b$ | 7.050 |
| 125 | 0.490 | $3.060^{ab}$ | 7.010 |
| 250 | 0.500 | $3.130^a$ | 7.080 |
| 500 | 0.500 | $2.970^b$ | 7.000 |
| SEM | 0.013 | 0.042 | 0.063 |
| p-value | 0.750 | 0.031 | 0.830 |

$^{a, b}$Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

The presence of the high concentration of long chain Polyunsaturated Fatty Acid (PUFA) of the n-6 series in avian spermatozoa increases their susceptibility to lipid peroxidation and limits the viability of chicken and turkey spermatozoa.

Lipid peroxidation plays a key role in the aging of spermatozoa by shorting its lifetime *in vivo* as well as during the *in vitro* conservation of sperm for artificial insemination.

The peroxidation process comes with extensive structural alterations especially in the acrosomal section of the spermatozoa, fast and irreversible loss of motility, extensive metabolic changes and high rate of leakage of intracellular spermatic constituents. L-carnitine supplementation increased overall antioxidant enzyme activities as a function of the duration of treatment thus decreasing the levels of free radicals available for lipid peroxidation (Sarica *et al*., 2007).

A possible explanation for the increase in sperm concentration of carnitine-fed birds is that carnitine facilitate the preservation of the sperm lipid membranes, thereby extending sperm longevity. These results are in agreement with those reported by Neuman *et al.* (2002) and Zhai *et al*. (2007).

Neuman *et al*. (2002) observed that supplementation of dietary L-carnitine at level of 500 mg $kg^{-1}$ to a basal diet significantly increased semen concentration in roosters. In the study by Zhai *et al.* (2007), adding carnitine at level of 125 mg $kg^{-1}$ of diet increase sperm concentration in comparision with control group in roosters.

Generation of Reactive Oxygen Species (ROS) such as the superoxide anion, the hydroxyl radical and hydrogen peroxide can cause oxidative damage to liver, kidney, brain, lung and are particularly responsible for sperm dysfunction (Hsu *et al*., 1998) in humans (Aitken *et al*., 1989), cattle (Beconi *et al*., 1991), rats (Shang *et al*., 1999) and chickens and turkeys (Surai *et al*., 1998).

Intracellular production of ROS results in the immediate accumulation of lipid peroxides in the plasma membrane and cytosol of cells (Aitken *et al*., 1989). This accretion has also been correlated with the impaired ability of sperm to partake in the acrosome reaction and sperm-oocyte fusion and may be responsible for a reduction in motility (Aitken *et al*., 1989).

To reduce the incidence of peroxidation, organisms have evolved antioxidants and antioxidant enzymes that prevent this chain reaction by interfering with the process of peroxidation by scavenging for ROS (Halliwell *et al*., 1984).

Catalase, SOD and glutathione peroxidase are three types of antioxidant enzymes, the latter two identified in birds that scavenge for biological oxidants and protect cells from peroxidative damage (Forman and Thurston, 1981).

Neuman *et al*. (2002) reported that carnitine-fed birds produced significantly lower amounts of Malonaldehyde (MAL) as compared to control-fed birds.

Circulating free iron is able to catalyze ROS leading to lipid membrane degradation. Carnitine has iron-chelating properties which may allow carnitine and acetylcarnitine to partially prevent the generation of ROS by binding with free iron.

Because carnitine also functions as an antioxidant and participates in fatty acid transport for energy metabolism, it likely preserves other antioxidants such as amino acids and antioxidant enzymes against potential peroxidative damage, therby reducing the availability of lipids for peroxidation (Kalaiselvi and Panneerselvam, 1998).

## CONCLUSION

These results shown that dietary L-carnitine supplementation at 250 mg $kg^{-1}$ significantly improve rooster sperm motility, viability and semen concentration during liquid storage. The further studies are essential to confirm and extend these findings.

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