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L-Carnitine Effect on Quantity and Quality of African Black Neck Ostrich Sperm

[1]Sh. Golzar Adabi, [2]A. Haji Babaei, [3]Gh. Moghaddam, [3]A. Taghizadeh, [4]T. Farahvash,
[5]H. Lotfollahian and [6]F. Moslemi Pour
[1]Jahad Keshavarzi Organization, East Azarbijan, Tabriz, Iran
[2]Iran Ostrich Research International Company, Iran
[3]Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[4]Department of Animal Science, Faculty of Agriculture, Islamic Azad University, Shabestar Branch
[5]Department of Poultry Science, Animal Science Research Institute, Iran
[6]Department of Animal Science, Faculty of Agriculture, University of Goragan, Iran

Abstract: To investigative the effect of dietary L-carnitine on ostrich sperm quality, a research was conducted in a completely randomized design consisting of 3 treatments and 4 replicates. The total numbers of birds used in this trail was 12 African black neck male ostrich. The experimental rations were prepared using the different levels (0, 250 and 500 ppm). The metabolizable energy, protein and the nutrient of these rations were similar. In order to study semen volume, sperm motility, live sperm percent, sperm count and abnormal sperm percent, after one month adaptation, semen collected at the beginning of every three month. The results of statistical analysis of data obtained indicated that there were significant effect of L-carnitine on semen volume ($p<0.01$), sperm motility, live sperm percent, sperm count ($p<0.05$) but L-carnitine had no significant effect on abnormal sperm percent.

Key words: L-carnitine, ostrich, sperm, quantity and quality, black neck ostrich

INTRODUCTION

L-carinitine is a natural, vitamin like substance that acts in the cells as a receptor molecule for activated fatty acids. A shortage of this substance results primarily in impaired energy metabolism and membranes function. 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. The highest synthesizing capacity is found in the liver (Harmeyer, 2002). Vitamin C or Ascorbic Acid (AA) is a cofactor at the two hydroxylation steps in carnitine biosynthetic pathway (Neuman *et al*., 2000).

Good quality semen is the most important factor to implement breeding programs (Stradaioli *et al*., 2004). The effects of L-carnitine on reproductive parameters have been assessed in human and boars. Infertile men have significantly lower seminal carnitine concentrations than fertile men. When utilized as an epididymal marker and correlated with sperm concentration, L-carnitine levels are elevated in fertile vs. infertile men (Neuman *et al*., 2002). Free radicals or Reactive Oxygen Species (ROS) are deleterious to cell membranes. The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for B-oxidation (Coulter, 1995) thus dietary L-carnitine supplementation could improve fatty acid and energy utilization and therefore gain and feed efficient to meet endogenous requirements (Gropp *et al*., 1994). L-carnitine promotes the mitochondrial B- oxidation of long-chain fatty acids by facilitating their transfer across the inner mitochondrial membrane. It also facilitates the removal from mitochondria of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism (Rabie *et al*., 1998).

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) energy. This transport of fatty acids into the mitochondria for catabolism reduces the amount of lipid available for peroxidation (Kalaiselvi and Panneerselvam, 1998). Carnitine is taken from the blood stream and then released in epididymal lumen by active transporters which are regulated by androgens and

Corresponding Author: Shahram Golzar Adabi, Jahad Keshavarzi Organization, East Azarbijan, Tabriz, Iran

depletion of epididymal carnitine caused a reduction in fertilizing capacity of hamsters spermatozoa. In humans, rams and stallions, seminal carnitines are correlated with spermatozoa count and progressive motility (Stradaioli *et al*., 2004). However, little L-carnitine has been reported to be found in cereal grains and their by- products on the other hand these feed ingredients usually constitute the major portion of poultry diets (Rabie *et al*., 1998). Leibetseder (1995) has reported that the L-carnitine content in the feed of broiler breeders influenced hatchability and showed that during the three week supplementation period the hatching rate increased from 83-87% in the group receiving 50 mg L-carnitine and from 82.4-85.3% in the group with 100 mg L-carnitine. The L-carnitine concentrations in randomly sampled eggs were increased as a result of L-carnitine supplementation and yolk weight increase in response to dietary L-carnitine (Golzar *et al*., 2006). In contrast, some researchers failed to observe any favorable responses to added dietary carnitine (Cartwright, 1986; Barker and Sell, 1994). While no or little L-carnitine has been found in eggs (Leibetseder, 1995) a high concentration of L-carnitine was found in chick embryo at the first stages of development (Chiodi *et al*., 1994). Stradaioli (2004) recently produced evidence that the oral administration of L-carnitine to stallions with questionable seminal characteristics may improve spermatozoa kinetics and morphological characteristics, whereas, it seem to be ineffective in normospermic animals. Although, detailed descriptions of spermatogenesis and sperm morphology in ostriches (Soley, 1992; Malecki *et al*., 1997; Hemberger *et al*., 2001) are available, little research has been completed concerning the effect of nutritive ingredient on ostrich male fertility. This study aims investigate effect of L-carnitine on quantity and quality of ostrich sperm.

MATERIALS AND METHODS

This trail was conducted in Iran Ostrich Research International Company. A total of 12 healthy male 5.5 years old ostrich (African black neck) were used in the present study. They were examined Randomly (CRD) within 3 treatment of 4 replicates for 3 months and each experimental group included 1 male. Three levels of L-carnitine 0, 250 and 500 ppm were used in a complete random design of treatments. Birds were housed in a standard fenced yard, equipped with one feeder and water device. Composition and nutrient content of experimental rations in percent of original matter are presented in Table 1 (Aganga *et al*., 2003).

The males were kept separated from female and adaptated to semen collection. After one month adaptation, semen collected at the beginning of every three month (May, June and July). The semen collection procedure was carried out using a methods described by (Hemberger *et al*., 2001; Rozenboim *et al*., 2003). Semen volume was determined by using a scaled glass. Sperm viability expressed as percentage of dead sperm by using the eosin-nigrozin (Chalha *et al*., 1998). Natural sperm also were determined after blending. Sperm motility determined with observation new sample in the microscope. Sperm count was determined with a hemocytometer (Etches, 2001). A drop diluted semen was dropped in a Neubauer hemocytometer chamber and the sperm numbers were counted microscopically after 15 min (Brillard and McDaniel, 1985).

Statistical analysis: A completely randomized design arrangement of treatments, three levels of dietary L-carnitine (0, 250 and 500 mg Kg^{-1}) were used. The data was analyzed using the SAS program (SAS institute, 1986). After ANOVA, significantly different means for each variable were separated using Duncan's multiple-range test (Duncan, 1995).

RESULTS AND DISCUSSION

Data for sperm motility, as well as semen volume, live sperm percent, number of abnormal sperm and number of sperm are presented in Table 2-6, respectively. No mortalities occurred during the study. Significant differences were observed in sperm motility (Table 2) ($p<0.05$). Semen volume were higher in both L_2 and L_3

Table 1: Composition and nutrient content of experimental rations(%)

Ingredient (%)	L_1	L_2	L_3
Corn	22.63	22.63	22.63
Bran	18	18	18
Palnt fat powder	1.33	1.33	1.33
Sunflower meal	12.32	12.32	12.32
Alfa alfa	23.14	23.14	23.14
Soy bean meal	13.6	13.6	13.6
Salt	0.43	0.43	0.43
Di calcium phosphate	1.37	1.37	1.37
Calcium carbonate	5.93	5.93	5.93
Min. + vit premix	1	1	1
L-carnitine (ppm)	0	250	500
DL-methionin	0.25	0.25	0.25
Calculated analyses	****	****	****
ME (Kcal/Kg)	2600	2600	2600
Crude protein %	16	16	16
Methionine+cyste %	0.5	0.5	0.5
Lysine%	0.7	0.7	0.7
Ca%	3	3	3
P% (non phytate)	0.5	0.5	0.5
Na%	0.22	0.22	0.22
Crude fiber%	12.72	12.72	12.72

L_1: Control, L_2: With 250 ppm L-carnitine, L_3: With 500 ppm L-carnitine

Table 2: The sperm motility in ostrich

Treatment	May	June	July	Total period
L_1	55^b	65^b	60^c	60^b
L_2	65^a	75^a	70^a	70^a
L_3	65^a	75^a	65^b	68.3^a
SEM	2.03	2.01	1.96	1.78

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

Table 3: The Semen volume in ostrich

Treatment	May	June	July	Total period
L_1	0.46^{ab}	0.48^b	0.46^b	0.46^b
L_2	0.5^a	0.6^a	0.56^a	0.55^a
L_3	0.52^a	0.58^a	0.55^a	0.55^a
SEM	0.01	0.02	0.013	0.016

a,b Mean values in the same column with different superscript letters were significantly different ($p<0.01$)

Table 4: The live sperm in ostrich

Treatment	May	June	July	Total period
L_1	65.2^c	64.6^b	64.4^b	64.73^c
L_2	75.0^a	72.2^a	74.0^a	73.73^a
L_3	73.6^b	72.4^a	71.8^a	72.6^b
SEM	0.56	0.54	0.5	0.51

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

Table 5: The number of abnormal sperm in ostrich

Treatment	May	June	July	Total period
L_1	23.5	23.54	23.2	23.41
L_2	23.19	22.77	23.29	23.08
L_3	22.86	23.34	23.75	23.31
SEM	0.6	0.64	0.62	0.63

Table 6: The number of sperm in ostrich

Treatment	May	June	July	Total period
L_1	65.83^b	65.95^b	66.82^b	66.2^b
L_2	73.15^a	72.68^a	72.51^a	72.78^a
L_3	70.65^a	69.07^{ab}	70.46^a	70.06^a
SEM	2.06	2.03	1.87	2.07

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

group (Table 3) ($p<0.01$) in compare with control group (L_1). It should be pointed out that supplementing the diets of ostriches with 250 mg L-carnitine for 3 month had no effect on abnormal sperm number (Table 5). However, L-carnitine-treated ostriches tended to have increased live sperm percentage compared to the control groups (Table 4) ($p<0.05$). Ostriches fed carnitine (250 and 500 mg Kg^{-1}) had significantly more sperm number than control-fed birds (Table 6) ($p<0.05$).

Free radicals or Reactive Oxygen Species (ROS) are harmful to cell membranes. Contact of cell membranes to ROS induces lipid peroxidation causing membrane breakdown and loss of function. Lipid peroxidation results when intracellular production of ROS overcomes the antioxidant defense mechanisms utilized by cells including sperm and an immediate accumulation of lipid peroxides occurs in the plasma membrane. Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids than mammalian sperm cells and are therefore more susceptible to lipid peroxidation during *in vitro* handling and storage of sperm, which is the primary cause of fertility dysfunction. Carnitine has antioxidant properties, which may protect sperm membranes from toxic oxygen metabolites (Neuman *et al*., 2002; Gurbuz *et al*., 2004; Yalcin *et al*., 2004). If L-carnitine increased sperm viability, then perhaps fewer dead sperm would be reabsorbed, inherently increasing total output of spermatozoa. L-carnitine has also been implicated in buffering the cell against high concentrations of mitochondrial acetyl-CoA by converting it into acyl carnitine. Excess acetyl-CoA inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial energy metabolism. This function of L-carnitine may further improve the survival of spermatozoa and increase the total number of sperm that are ejaculated (Konzik *et al*., 2004). Neuman (2002) suggested that a possible explanation for the increase in sperm concentration of carnitine-fed birds was that carnitine facilitated the preservation of the sperm lipid membranes, thereby extending sperm longevity.

It is well known that one of the major effects of epididymal transit is the stabilization of sperm head and tail structures, in particular nuclear protamine, mitochondrial capsule and the coarse outer fibres of flagella thought to be related to the formation of intra- and inter- molecular disulfides. This process is essential for acquiring motility, ultrastructural stability and fertilizing ability; thus, an improvement in epididymal microenvironment is likely to lead to an increase in sperm quality. Most of this stabilization process is due to oxidation of protein thiols (-SH) to form disulfides(S-S) and an increase in the (-SH) and (-SH+S-S) ratio has been reported in asthenozoospermic patients, suggesting that this over oxidation reflects an abnormal maturation process of the epididymis. In this context, it is intriguing to note, that carnitine also acts as a secondary antioxidant that repairs damage occurring after oxidative noxae and its administration to aging rats improves to glutathione and over all thiols status, perhaps by exerting a sparing activity on thiol and methionine (Stradaioli *et al*., 2004). Therefore, a direct effect of carnitine on the functionality of sertoli cells is also plausible as observed by (Palmero *et al*., 1990) who reported an increase in both lipid oxidation and glucose utilization by *in vitro* cultured sertoli cells in response to carnitine and concluded that the improvement in semen quality reported after *in vivo* treatments could be related to its interactions with sertoli cell functions. Carnitine's protective role is further sustained by reducing toxicity and accelerating repair processes following physical and chemical damages on the testicular parenchyma.

The highly significant correlations among carnitine and spermatozoa concentrations could only be due to an increase in the intracellular pool of carnitine, although too slight to induce an increase of seminal levels (Stradaioli *et al*., 2004). In agreement of the acetyl carnitine with a reduction of the acetyl carnitine/L-carnitine ratio and seminal plasma carnitines levels observed in several forms of human infertility on seminal deficit (Golan *et al*., 1984; Lewin *et al*., 1981).

L-carnitine administration improves pyruvate utilization, an elective energetic substratum for sperm motility. The correct Acetyl coA/coA ratio is fundamental in order to maintain the proper functionality of the Kreb's cycle for a sufficient production of Adenosine Three Phosphate (ATP) and the high levels of Acetyl coA inhibit pyruvic dehydrogenase enzyme activity; consequently, the metabolic flow of pyruvate into the Kreb's cycle is showed down. Through Carnitine Acyl Transferase (CAT), carnitine is transformed into acetylcarnitine (buffering effect), which reduces the AcetylcoA/coA ratio and improves the metabolic flux to Kreb's cycle, with an increased production of ATP, preserving a high motility of the spermatozoa (Stradaioli *et al*., 2004).

CONCLUSION

In conclusion, oral L-carnitine administration to the ostrich could be benefit to increase semen volume, sperm quality and quantity consequently increase males fertility. A direct effect of carnitine administration on spermatozoa traits also seems plausible, but a longer treatment period should be tested in order to reach definitive conclusions.

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