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

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Abstract: To investigate the effect of dietary L-carnitine on ostrich sperm quantity and quality, a study was conducted in a completely randomized design consisting of three treatments and four replicates (total 12 male ostriches). The experimental rations were prepared using different levels of carnitine ($L_1 = 0$, $L_2 = 250$ and $L_3 = 500$ mg kg^{-1}). Ejaculates were collected by massage method once a month for three months. The ejaculate volume, total sperm numbers per ejaculate, concentration of spermatozoa, sperm motility, live sperm percentage and abnormal sperm percentage were measured. Semen volume in both groups (250 and 500 mg kg^{-1}) was higher than control group, ($p < 0.01$), L-carnitine treated ostriches (250 and 500 mg kg^{-1}) have increased sperm count, sperm motility, live sperm ($p < 0.05$) but L-carnitine had no significant effect on abnormal sperm. According to this study it is recommended to use 250 mg kg^{-1} L-carnitine in order to increase the ejaculate quality in ostriches.

Key words: L-carnitine, spermatozoid, quality, quantity, ejaculate, ostrich

INTRODUCTION

L-carnitine 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 membrane function. The vitamins B₆, B₁₂, 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 (Cartwright, 1986; Rabie *et al.*, 1998; Golzar Adabi *et al.*, 2006). 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 effect 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.*, 2000). 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 β -oxidation thus dietary L-carnitine supplementation could improve fatty acid and energy utilization and therefore gain and feed efficient to meet endogenous requirements (Coulter, 1995). L-carnitine promotes the mitochondrial β -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 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 to 87% in the group receiving 50 mg L-carnitine and from 82.4 to 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. In contrast, some researchers failed to observe any favourable 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 *et al.* (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 description of spermatogenesis and sperm morphology in ostriches (Malecki *et al.*, 1997a; Hemberger *et al.*, 2001) is available, little research has been completed concerning the effect of nutritive ingredient on ostrich male fertility. This study aims to investigate the effect of L-carnitine on quantity and quality of ostrich sperm.

MATERIALS AND METHODS

A total of 12 healthy male 5.5 years old ostrich (African black neck) were used in the present study. The study was conducted from may to July 2007. The birds were randomly divided into three experimental groups of 4 with 4 replicate of 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 shown in Table 1 (Aganga *et al.*, 2003).

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

Ingredient*	L ₁	L ₂	L ₃
Corn	22.63	22.63	22.63
Bran	18.00	18.00	18.00
Plant 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	13.60	13.60	13.60
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	1.00	1.00	1.00
L-carnitine (ppm)	0.00	250.00	500.00
DL-methionine	0.25	0.25	0.25

L₁: Control, L₂: With 250 ppm L-carnitine, L₃: With 500 ppm L-carnitine, *Calculated analyses: ME (kcal/kg) 2600, Crude protein 16%, Methionine+cyste 0.5%, Lysine 0.7 %, Ca 3%, P (non phytate) 0.5%, Na 0.22%, Crude fiber 12.72%

The males were kept separated from female and adapted to semen collection. After one month adaptation, semen collected at the beginning of every three month (from May to July 2007). The semen collection procedure was carried out using a methods described by Hemberger *et al.* (2001). Briefly, for the purpose of semen collection, the ostrich was placed in a plucking crush and a soft bag was pulled over its head to calm it. An assistant then inserted his hand into the cloaca in order to remove the phallus. It was held in an extruded position using a soft and clean pad for a firmer grip. For ejaculating, birds required soft massage of the papillae seminales, which were located by inserting two fingers into the bird's proctodeum. Semen samples were collected in sterile glass tubes for subsequent further evaluation. A small portion of each sample was used to prepare smears for microscopic assessment. The volume of the ejaculate was measured in a 2 mL glass cylinder. The vitality (2% Eosin-B-stain) and abnormal cells were evaluated using a phase contrast microscope at magnifications of 400 x (Chalah and Brillard, 1998). Sperm cells with abnormal shapes were counted and calculated, in four microscopic fields, as a percentage of total number of sperm cells. The degree of motility was evaluated using light field illumination microscopy of ejaculate samples on prewarmed glass slides at a magnification of 200 x. Spermatozoa were counted with a Neubauer hemocytometer after dilution 1:400 with IMV poultry semen diluter. Four hemocytometer counts for each sample were made (Etches, 1996; Malecki *et al.*, 1997b).

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 analysed using the SAS program (SAS, 1986). After ANOVA, significantly different means for each variable were separated using Duncan's multiple-range test (Duncann, 1995).

RESULTS AND DISCUSSION

In accordance with the experimental design, all the main seminal characteristics differed between the groups. Significant differences were observed in sperm motility (Table 2, p<0.05). Supplemental dietary carnitine had significant effect on ejaculate volume (mL) (p<0.01) the highest ejaculate volume were observed in both L₂ and L₃ groups (Table 3) in compare with control group (L₁).

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 percent (Table 4). However, L-carnitine- treated ostriches

Table 2: The effect of feeding different levels of dietary carnitine on sperm motility (%) in ostrich

Treatments	Month			
	May	June	July	Total period
L ₁	55.00 ^b	65.00 ^b	60.00 ^c	60.00 ^b
L ₂	65.00 ^a	75.00 ^a	70.00 ^a	70.00 ^a
L ₃	65.00 ^a	75.00 ^a	65.00 ^b	68.30 ^a
SEM	2.03	2.01	1.96	1.78

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

Table 3: The effect of feeding different levels of dietary carnitine on Semen volume (mL) in ostrich

Treatments	Month			
	May	June	July	Total period
L ₁	0.46 ^{ab}	0.48 ^b	0.46 ^b	0.46 ^b
L ₂	0.50 ^a	0.60 ^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, Letter(s) were significantly different (p<0.01)

Table 4: The effect of feeding different levels of dietary carnitine on abnormal sperm (%) in ostrich

Treatments	Month			Total period
	May	June	July	
L ₁	23.50	23.54	23.20	23.41
L ₂	23.19	22.77	23.29	23.08
L ₃	22.86	23.34	23.75	23.31
SEM	0.60	0.64	0.62	0.63

Table 5: The effect of feeding different levels of dietary carnitine on live sperm (%) in ostrich

Treatments	Month			Total period
	May	June	July	
L ₁	65.20 ^c	64.60 ^b	64.4 ^b	64.73 ^c
L ₂	75.00 ^a	72.20 ^a	74.0 ^a	73.73 ^a
L ₃	73.60 ^b	72.40 ^a	71.8 ^a	72.60 ^b
SEM	0.56	0.54	0.5	0.51

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

Table 6: The effect of feeding different levels of dietary carnitine on number of sperm count (*10⁹) in ostrich

Treatments	Month			Total period
	May	June	July	
L ₁	1.99 ^b	2.02 ^b	2.03 ^b	2.01 ^b
L ₂	2.14 ^a	2.19 ^a	2.19 ^a	2.16 ^a
L ₃	2.20 ^a	2.15 ^{ab}	2.19 ^a	2.18 ^a
SEM	2.06	2.03	1.87	0.30

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

tended to have increased live sperm percentage compared to the control groups. In particular, L₂ group had a significant increase in the live sperm percentage in compare with L₁ and L₃ in total period of experiment (Table 5, p<0.05). Ostriches fed carnitine (250 and 500 mg kg⁻¹) had significantly sperm count 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 per oxidation causing membrane breakdown and loss of function. Lipid per oxidation 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 per oxidation 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.*, 2000; Xu *et al.*, 2003).

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 *et al.* (2000) 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, ultra structural 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 (Stradaoli *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 (Stradaoli *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 Krebs'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 Krebs'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 Krebs's cycle, with an increased production of ATP, preserving a high motility of the spermatozoa (Stradaoli *et al.*, 2004).

To the knowledge of the authors, this study represents the first scientific report on the effects of L-carnitine supplementation on ostrich semen characteristics. 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. According to this study it is recommended to use 250 mg kg⁻¹ L-carnitine in order to increase the ejaculate quality in ostriches.

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