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EFFECT OF ORAL L-CARNITINE TREATMENT ON BODY WEIGHT, BLOOD PARAMETERS AND REPRODUCTIVE PERFORMANCE OF FRIESIAN BULLS

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ABSTRACT

To establish the effect of daily oral treatment with different levels of L-Carnitine (LC) for 3 mo on body weight, blood parameters and reproductive performance of Friesian bulls, total of 9 bulls (360±32.1 kg LBW and 20±1.4 mo of age) were assigned into 3 groups. All bulls were fed the same diet and kept under the same condition, but differed in LC treatment. Bulls were received oral dose of LC for 3 mo at levels of 0, 1 and 2 g/h/d in G1, G2 and G3, respectively. At the end of treatment, semen was collected from bulls twice a week for 12 wk and evaluated for ejaculate volume, sperm motility, sperm livability, sperm cell concentration and total sperm output. At the end of semen collection, blood samples were collected from all animals for determination of total protein (TP), albumin (AL), globulin (GL), urea-N**,** cholesterol (CHO), glucose and total lipids (TL) concentration and activity of AST and ALT in blood serum. Also, testosterone concentration was determined in blood serum after 1 and 3 mo of treatment. Results show that bulls in G3 showed the heaviest weights and gain as compared to G1, but did not differ significantly from those in G2. In blood serum of bulls, concentration of TP and GL increased ($P<0.05$), while AL/GL ratio and concentration of CHO and TL reduced ($P<0.05$) in G2 and G3 as compared to G1. Concentration of AL, glucose and urea-N or activity of AST and ALT were not affected by LC treatment. All semen characteristics of bulls improved ($P<0.05$)in G2 and G3 as compared to G1, being better ($P<0.05$) in G3 than in G2 for most characteristics. Serum testosterone concentration was higher ($P<0.05$) in G2 and G3 than in G1. In conclusion, oral dose of LC at a level of 2 g/h/d for 3 mo had impact to achieve high quality semen to spread the use of artificial insemination with bulls of high fertility.

Keywords: Bulls, carnitine, body weight, blood, semen quality, testosterone level.

INTRODUCTION

Carnitine can be synthesized in the body from protein-bound lysine and methionine by most animals. Carnitine occurs in the form of L-and D-isomers; however, only the L-carnitine (LC) is biologically active, while the D-isomer may even be noxious for the organism (Szilagyi, 1998). Carnitine can be synthesized in the body from protein-bound lysine and methionine by most animals. LC (*β*-hydroxy-*γ*-trimethylammonium butyrate) is a highly polar natural compound, vitamin-like amino-acid, synthesized within the body from lysine and methionine (Vaz and wanders, 2002) and it found in microorganisms, plants, and animals (Bremer, 1983). It is very important in the metabolism of lipids and carries long-chain fatty acids to the mitochondria for beta-oxidation, which produces energy (ATP) needed by the cells for

proper functioning (Hoppel, 2003). LC plays an important role in the processes of cellular detoxification (Arrigoni-Martelli and Caso, 2001) and protects cellular membranes against oxidative damages (Kalaiselvi and Panneerselvam, 1998).

In mammal semen, the origin of free LC in seminal plasma and spermatozoa is mainly epididymis (Brooks, 1979), and is transport from the blood plasma into epididymis (Jeulin and Lewin, 1996). Therefore, LC is found with very high concentrations in the epididymis, seminal plasma and spermatozoa (Golan *et al.*, 1982) and it passes from the epididymal lumen through the sperm plasma membrane by passive diffusion during the transit time of 1–10 days (Jeulin *et al.,* 1994). Inside sperm cells, LC transports medium and long-chain fatty acids into the mitochondria where they undergo beta-oxidation leading to the generation of metabolic energy needed for the sperm cells for their progressive movement (Jeulin *et al.,* 1987).

Spermatozoa which enter the epididymis are immotile and their free LC content is very low or undetectable. During their transit through the epididymis, they become capable of initiating flagellar motion and accumulate a very high concentration of free LC. The initiation of sperm motility occurs in parallel with an increase in the concentration of free LC in the epididymal lumen (Jeulin *et al.,* 1994).

It was reported that dietary supplements of LC (20 to 500 mg/kg feed) raised plasma LC levels in domestic animals, in plasma, liver and milk In ruminants (La Count *et al.*, 1995). In boars, feeding the high level of LC improved volume and quality of fresh semen (Akey, 2000; Jacyno *et al*., 2007), but did not show any beneficial effects on libido, sperm production or maintenance of sperm motility during liquid storage (Kozink *et al.,* 2004).

Despite some studies indicate that supplemental LC in the diet is not required, its use is recommended in domestic animals especially in cattle (La Count *et al*., 1995; Carlson *et al*., 2006) to increase performance and to support medical treatment There are scant information in literature regarding the effect of LC on male reproductive processes, especially on spermatogenesis and consequently on semen quality of bulls.

The objective of this study is to establish the effect of daily oral treatment with two levels of LC (1 and 2 g/h/d) for 3 mo on growth and blood parameters reproductive performance of Friesian bulls.

MATERIALS AND METHODS

The current study was carried out at Sakha Animal Experimental Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture in cooperation with the Department of Animal Production, Faculty of Agriculture, Mansoura University, during the period from October 2011 to March 2012.

Animals:

A total of 9 Friesian bulls with an average live body weight (LBW) of 360.4±32.1 kg and 20±1.4 mo of age was assigned randomly into 3 groups

nearly similar in LBW and age (3 animals in each). Bulls in the 1st group (G1) were fed the control diet, while those in the 2nd (G2) and 3rd (G3) groups were fed the control diet and treated with oral dose of LC at levels of 1 and 2 g/h/d for 3 mo as treatment period, respectively. All the experimental bulls were housed under sheds and kept under the same environmental and managerial conditions.

Feeding system:

Animals were fed individually on a basal diet composed of concentrate feed mixture (CFM), fresh berseem (FB) and rice straw (RS). The CFM composted of 35% undecorticated cottonseed cake, 5% linseed cake, 25% ground yellow corn, 20% wheat bran, 10% rice bran, 3% molasses, 1% limestone and 1% common salt. Chemical analysis of different feedstuffs in the basal diet is shown in Table (1).

Table (1): Chemical composition of different feedstuffs in the basal diet fed to bulls in all groups.

Feedstuff	DM %	Chemical analysis (%, on DM basis) %					
		OM	CP	CF	EE	NFE	ASH
Concentrate feed mixture	88.62	91.64	16.72	11.25	2.42	61.25	8.36
Fresh berseem	16.06	87.87	15.31	22.31	3.10	47.15	12.13
Rice straw	89.63	82.21	3.36	33.98	1.27	43.60	17.79
Basal ration	46.62	88.53	13.10	19.14	2.27	54.02	11.47

Growth parameters:

Throughout the treatment period, bulls were monthly weighed, and then average daily gain (ADG) was monthly calculated.

Semen collection:

At the end of LC treatment period, semen was collected from bulls in each group twice a week using the conventional artificial vaginal method. The collected ejaculates from each bull per collection day were taken immediately to the laboratory and kept in water bath at 37°C for performing individual physical characteristics in raw semen.

Semen was collected before feeding at 8 a.m. A bull was used as teaser animal for sexual preparation. Semen was collected for 12 wk from January to March after 3 mo of LC treatment.

Semen evaluation:

Ejaculate volume of raw semen was measured and percentages of individual motility, livability and abnormality of spermatozoa were measured. Also, sperm cell concentration (x 10^6/ml) in each ejaculate was determined and then total sperm output per ejaculate was calculated.

The percentage of sperm motility was assessed using research microscope supplied with a hot stage adjusted to 37°C. Semen was extended with sodium citrate (2.9%) at a rate of 1:1 according to Amman and Hammerstedt (1980). Smear from fresh semen was made on a glass slide and stained by eosin (1.67%) and nigrosin (10%) mixture stain (Hackett and

Macpherson, 1965) for count of live (unstained ones) and dead spermatozoa (stained ones), then percentage of live spermatozoa was calculated. During the examination of live /dead sperm percentage, the morphological abnormalities of spermatozoa according to the classification adopted by Blom (1983) were also determined

Number of spermatozoa in each ml of ejaculate was counting using haemocytometer (Neubauer), and then total sperm outputs were calculated as the following:

Total sperm output = Ejaculate volume (ml) x sperm cell concentration.

Blood sampling:

Blood samples were collected from all animals at the end of the collection period. Blood samples were collected from the jugular vein before morning feeding into dry clean glass tubes. The collected blood was left to clot for 4 hours, thereafter centrifuged for 15 minutes at 15 *g* to obtain blood serum. Serum samples were kept in deep freezer till chemical analysis for concentration of some biochemical and activity of aminotransferases in blood serum. Biochemical blood parameters in serum were determined calorimetrically using commercial kits (diagnostic system laboratories, INC, USA) and spectrophotometer. Total protein (TP) and albumin (AL) concentrations were determined as methods described by Tietz (1994) and Tietz (1990), respectively. Concentration of urea-N (Patton and Crouch, 1977) cholesterol (CHO, Watson, 1960), glucose (Trinder, 1969) and total lipids (Zollner and Kirch, 1962) was also determined in blood serum.. While, concentration of globulin was calculated by subtracting the albumin form the total protein concentration. Activities of asprtate (AST) and alanine (ALT) aminotransferases in blood serum were determined (Reitman and Frankal, 1957). However, testosterone concentration in blood serum was determined after 1 and 3 mo of treatment according to Jaffe and Behrman (1974).

Statistical analysis:

Data were statistically analyzed by the methods of least square analysis of variance using the General Linear Model procedures of SAS (2004). Duncan multiple range test was used to test the differences among means (Duncan, 1955) at $P<0.05$. The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Growth parameters:

Live body weight:

Data presented in Table 2 show that LBW of bulls was affected significantly ($P<0.05$) by LC treatment during semen collection period. At all experimental months, bulls treated with 2 g LC (G3) had significantly ($P<0.05$) higher weights than the control bulls. Also, bulls fed 1 g LC (G2)

showed higher LBW than the control ones, but the differences were not significant. The differences in LBW between bulls in G2 and G3 were not significant.

Table (2): Effect of L-Carnitine on monthly live body weight (kg) of Friesian bulls during treatment period.

Treatment month	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)	SEM
0	360.001	360.03	360.00	3.83
1	380.00	381.70	383.3	2.22
2	403.00^{b}	407.03^{ab}	411.30^{a}	2.88
3	425.30^{b}	435.36^{ab}	443.30^{a}	3.51

a and b: Means within the same row with different superscripts are significantly different at P<0.05.

It is of interest to note that LBW of bulls in all groups showed pronounced increase with advancing treatment month, because these bulls were at younger ages (20 mo), therefore it still to grow and gained.

Body weight gain:

Results shown in Table 3 revealed significant effect of LC treatment on total weight gain (TWG) and average daily gain (ADG) throughout the experimental intervals. Bulls in G3 showed significantly (P<0.05) the highest TWG and ADG during the intervals from 1-2 or 2-3 mo of treatment and during the entire length of treatment period.

Table (3): Effect of L-Carnitine on weight gain of Friesian bulls at monthly intervals of treatment period.

Treatment month	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)	SEM
Total weight gain (kg):				
0-1	20.00	21.67	23.33	1.44
1-2	23.00^{b}	25.33^{ab}	28.00^{a}	1.22
2-3	25.33^{b}	28.33^{ab}	32.00^{a}	1.82
0-3	68.33^{b}	75.33^{ab}	83.33^{a}	3.40
Average daily gain (g/h/d):				
0-1	667	722	778	32.4
1-2	767^{b}	844^{ab}	933^{a}	28.3
2-3	844^{b}	944^{ab}	1.07^{a}	34.8
0-3	759^{b}	837^{ab}	926^{a}	51.6

a and b: Means within the same row with different superscripts are significantly different at P<0.05.

In accordance with improvement of growth parameters in bulls, carnitine addition to complex nursery diets has been shown to improve growth performance of early-weaned pigs (Heo *et al*., 2000). Adding up to 1,000 ppm of carnitine to nursery diets containing soybean oil improved feed efficiency 3 to 5 wk post weaning (Owen *et al.,* 1996). Similar results were obtained by Li *et al*. (1999), who reported that LC supplementation could benefit growth performance of weaning pigs. Therefore, weanling animals

supplemented with LC grew faster and improved weight gain (Newton and Haydon, 1989). In growing animals, Owen *et al.* (2001) found that feeding carnitine can result in greater lean muscle deposition and reduced back fat thickness. Supplemental LC has improved growth in grazing beef calves fed liquid supplements containing urea (White *et al*., 2001) and possible effects of oral LC supplementation to growing and finishing steers have also been examined (Greenwood *et al*., 2001).

Other studies reported that LC supplementation and various protein sources decrease dry matter intake and gain: feed ratio in Holstein calves fed broiler litter (White *et al*., 1998). On the other hand, addition of LC had no effect on average daily gain and feed conversion ratio (Greenwood *et al*., 2001) or on growth performance of weaning and growing-finishing pigs (Owen *et al*., 2001) or of neonatal and young pigs (Hoffman *et al*., 1993).

These conflicting results appear to be characteristic of studies conducted on vitamin requirement estimates. It is likely that factors such As age, health, environment, lean growth potential, and diet influence responses to added carnitine. The variations in the abovementioned studies can be ascribed to several aspects such as diet (Owen *et al*., 2001), age (Chen *et al*. 2008).

Blood parameters:

Blood biochemicals:

Results presented in Table (4) show that LC treatment significantly (P<0.05) increased concentration of total protein (TP) as a result of significant (P<0.05) increase in globulin (GL) and insignificant increase in albumin (AL) concentrations. Such trend led to significant reduction in AL/GL ratio in LC treated bulls than in control bulls. Similar to the present results in bulls, it was found that serum AL concentration was not affected significantly by carnitine treatment in lambs (Chapa *et al*., 2001) or in cows (Carlson *et al*., 2007). This is in contrast to the study of Citil *et al*. (2009), who observed an increased amount of AL in blood samples of carnitine treated ewes.

As the main effect of LC on lipid metabolism, the present results indicated significant (P<0.05) reduction in cholesterol and total lipids concentrations in blood serum of bulls (Table 4).

Table (4): Effect of L-Carnitine on some blood biochemicals in blood serum of Friesian bulls at the end of experimental period.

Item	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)
Total proteins (g/dl)	7.19±0.28^{b}	8.04±0.10^{a}	8.04±0.12^{a}
Albumin (g/dl)	3.52±0.13	3.62±0.10	3.64±0.08
Globulin (g/dl)	3.67±0.19^{b}	4.42±0.07^{a}	4.40±0.07^{a}
AL/GL ratio	0.98±0.55^{a}	0.82±0.32^{b}	0.83±0.29^{b}
Cholesterol (g/dl)	211.8±4.00^{a}	194.4±6.19^{b}	193.7±2.87^{b}
Total lipids (mg/dl)	539.4±12.73^{a}	523.9±6.95^{ab}	513.9±14.86^{b}
Glucose (mg/dl)	77.8±2.80	78.2±1.23	73.7±2.09
Urea-N (mg/dl)	31.9±2.28	29.0±0.59	28.3±1.06

a and b : Means within the same row with different superscripts are significantly different at P<0.05.

Similarly, Citil *et al*. (2009) reported that oral carnitine treatment in healthy suckled ewes resulted in alterations in triglycerides, cholesterol, urea, glucose, which are indicators of energy metabolism. Addition of 500 mg carnitine to ewes' diet led to a reduction in serum cholesterol level. Similar results were reported by Kellog and Miller (1977). Furthermore, many previous reports suggested that LC supplementation could influence lipid metabolism (Heo *et al*., 2000). Therefore, it can be concluded that LC decreased tissue lipid content (Chen *et al.*, 2008).

This effect of LC could be associated with stimulation of lipid metabolism through transfer of acyl groups across the mitochondrial membranes (Owen *et al*., 1996).

In addition, the obtained results revealed insignificant effect of LC treatment on glucose concentration in serum of bulls. In accordance with this result, Carlson *et al*. (2007) found that plasma glucose concentration was not altered for carnitine-supplemented cows than for the control, regardless of carnitine intake. In general, the effect of carnitine on plasma glucose level is controversial; some reported increase (Chapa *et al*., 1998, 2001), decrease (Hadadinezhad *et al*., 2008) or unchanged (La Count *et al*., 1995; Carlson *et al*., 2007). The likely mechanism was related to a direct effect of LC resulting in increased pyruvate dehydrogenase enzyme activity and an indirect effect on increased receptor sensitivity to insulin and post-insulin receptor defects (Hadadinezhad *et al*., 2008). The illustrated results in Table (4) also showed that LC had no effect on urea-N concentration in serum of bulls. In the same line, Rincker *et al*. (2003) observed no difference in urea-N in weanling pigs fed added LC. However, others reported that addition of 500 mg carnitine to ewes' diet led to a reduction in serum urea level (Citil *et al*., 2009).

The positive effect of oral LC treatment was observed 3 weeks after the initial carnitine treatment (Citil *et al*., 2009). However, Chapa *et al*. (2001) suggested that a 2-week adaptation period is required when feeding ruminants with oral carnitine. This could be explained by adaptation of ruminal microflora to the applied carnitine. The biological significance of these changes is likely minimal because concentrations were within normal ranges (Boyd, 1984).

Activity of amino-transferases (AST and ALT):

Data in Table (5) show that activity of asprtate (AST) and alanine (ALT) aminotransferases and AST/ALT ratio in blood serum of bulls were not affected significantly by LC treatment. Only, LC treatment slightly decreased activity of AST and ALT and slightly increased AST/ALT ratio.

Table (5): Effect of L-Carnitine on activity of aminotransferases (AST and ALT) in blood serum of Friesian bulls at the end of experimental period.

Enzyme activity	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)
AST (IU/l)	37.233±0.83	36.792±0.63	36.908±0.53
ALT (IU/l)	19.011±0.75	17.961±0.38	18.700±0.46
AST/ALT ratio	1.98±0.09	2.05±0.04	1.99±0.07

All group differences are not significant (P≥0.05).

Similar insignificant reduction in liver enzymes (AST, ALT) was reported by Citil *et al*. (2009). Contrary, Carlson *et al*. (2007) found that LC treatment resulted in elevated concentrations of AST.

Reproductive performance:

Physical semen characteristics:

Results presented in Table (6) show that all physical semen characteristics of bulls significantly (P<0.05) improved by both LC treatments (G2 and G3) as compared to the control group (G1), being significantly (P<0.05) better in G3 than in G2 for most characteristics. This result means that increasing LC dose from 1 to 2 g/h/d had more impact on semen characteristics.

In accordance with the present improvement in all physical semen characteristics of bulls, Jacyno *et al.* (2007) found that the addition of 500 mg LC to the boars' feed had a positive effect on the quality of boar semen. The total ejaculates volume increased by 11%; the total ejaculate sperm count increased by 11.5% (P < 0.05). Number of spermatozoa with major and minor morphological changes decreased. Sperm concentration and motility, as well as normal acrosome sperm percentage, did not increase considerably.

The positive effect of LC on boar semen quality was observable as early as after one week of its application. Wahrner *et al.* (2004) found that boars receiving 230 mg of LC in their daily ration showed an increase in ejaculate volume and sperm concentration. Kozink *et al*. (2004) have proved only an increase of spermatozoa concentration in adult boars.

Table (6): Effect of L-Carnitine on overall mean of physical semen characteristics of Friesian bulls during the collection period.

Characteristics	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)
Ejaculate volume (ml)	1.84 ± 0.11^{c}	2.23 ± 0.10^{b}	2.65 ± 0.08^{a}
Sperm motility (%)	59.1 ± 2.41^{c}	67.6 ± 1.39^{b}	74.8 ± 1.15^{a}
Sperm livability (%)	81.2 ± 1.52^{b}	85.8 ± 1.72^{a}	88.8 ± 0.46^{a}
Normal sperm (%)	74.8 ± 0.62^{b}	84.8 ± 0.87^{a}	85.1 ± 0.95^{a}
Abnormal sperm (%)	25.3 ± 0.62^{a}	15.17 ± 0.87^{b}	14.92 ± 0.95^{b}
Sperm concentration ($x10^9$/ml)	0.790 ± 0.04^{c}	1.154 ± 0.07^{b}	1.333 ± 0.09^{a}
Total sperm put ($x10^9$/ejaculate)	1.454 ± 0.14^{c}	2.573 ± 0.19^{b}	3.532 ± 0.21^{a}

a, b and c: Means within the same row with different superscripts are significantly different at P<0.05.

Content of LC in seminal fluid is correlated positively to sperm concentration and motility (Lenzi *et al*., 2003). Further, previous clinical studies have reported an increase in sperm motility and sometimes sperm count in patients treated with oral carnitine. In addition, improvements in motility have been reported in patients with a bacterial prostate-visiculo-epididymitis and elevated seminal reactive oxygen species production, but only in those with normal seminal white blood cell concentrations (Vicari and Calogero, 2001).

Feeding the high level of LC to boars increased semen volume; number of viable sperm cells produced and resulted in extra 2 doses of semen produced per boar per week for artificial insemination (Akey, 2000). Also, LC increases sperm concentration and motility in men with idiopathic asthenozoospermia (Matalliotakis *et al*., 2000).

Conversely, boars that were randomly selected for LC treatment and received a feed mixture supplemented with 500 mg per day for 16 weeks did not show any beneficial effects on boar libido, semen quality, sperm production or maintenance of sperm motility during liquid storage (Kozink *et al.,* 2004). Also, Sigman *et al.* (2006) reported that, it would be difficult to recommend oral carnitine supplementation for improving sperm motility in infertile men with low sperm motility.

In addition, LC content in seminal fluid is correlated positively to sperm cell concentration (Lenzi *et al*., 2003). Previous studies have shown that seminal fluid free carnitine content is directly related to sperm count, further suggesting that carnitine may be used in the treatment of male infertility (Matalliotakis *et al*., 2000).

Oxidative stress in the male germ line leads to the induction of damage in the spermatozoa and loss of integrity in the nucleus and mitochondria (Aitken *et al.,* 2003). Saturated and monounsaturated fatty acids are reduced with a concomitant increase in the proportion of polyunsaturated fatty acids (PUFA), leading to a potential increase in the fluidity of the sperm membrane and perhaps increasing susceptibility to lipid peroxidation (Ladha, 1998). Because LC is involved in fatty acid transport for energy metabolism, it reduces lipid availability for peroxidation. Its antioxidant properties likely preserve other antioxidants (e.g., ascorbic acid), including antioxidant enzymes, against potential peroxidative damage (Kalaiselvi and Panneerselvam, 1998).

According to the present results and those reported in the literature, the observed improved effects of LC treatment on physical semen characteristics of males, dietary carnitine has antioxidant properties that may preserve sperm membranes, thereby extending the lifespan of sperm (Newman *et al.*, 2002). LC possesses antioxidant properties which increase the sperm concentration by preventing lipid peroxidation (Zhai *et al*., 2007) and reduce reactive oxygen species (ROS) to increase sperm forward motility and viability in infertile patients (Vicari and Calogero, 2001). Also, LC plays a critical role in the maturation and motility of spermatozoa within the male reproductive tract (Ng *et al*., 2004). LC accumulates in spermatozoa as they progress to the caudal region of the epididymis (Jeulin *et al*., 1994), whereas spermatozoa simultaneously gain motility and fertilizing capabilities (Kirby

and Froman, 2000). LC plays a key role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects in sperm motility (Matalliotakis *et al*., 2000). A secondary role of LC, as an antioxidant, which can counteract and eliminate various kinds of oxidation factors in the body and protect the cell normal status and physiologic function (Dokmeci, 2005).

Testosterone concentration:

Results in Table (7) show that blood serum testosterone concentration was significantly (P<0.05) higher in bulls treated with both levels of LC than in the controls after one month and 3 months of treatment and as an average of these ages.

In young bull calves, the testis produces androgens such as androstenedione and 5α-reduced androgens as well as testosterone. In the adult, testosterone is the major product (Rawlings and Cook 1986). The mammalian epithelium secretes LC into the epididymal fluid, and it is subsequently transported into spermatozoa, where it accumulates as free LC and acetylated LC (Jeulin and Lewin, 1996). It has been demonstrated that a major function of carnitine in spermatozoa is to store “acetyl units” for aerobic oxidation and energy production when needed (Van Dop *et al*., 1977). Therefore, blood plasma testosterone concentration was significantly negatively correlated (r=-0.91, P<0.05) with blood plasma carnitine concentrations among dairy bulls of varying fertility levels. However, blood plasma testosterone was positively correlated with spermatozoa total carnitine (r=0.32) spermatozoa acyl carnitines (Lee Carter *et al.,* 1980).

Table (7): Effect of L-Carnitine on testosterone concentration in blood serum of Friesian bulls during treatment period.

Item	G1 (Control)	G1 (1 g LC/h/d)	G3 (2 g LC/h/d)
One mo of treatment	7.02±0.167^{b}	7.99±0.190^{a}	8.03±0.194^{a}
Three mo of treatment	6.92±0.463^{b}	8.23±0.157^{a}	8.28±0.161^{a}
Mean	6.97±0.221^{b}	8.11±0.123^{a}	8.15±0.126^{a}

a and b: Means within the same row with different superscripts are significantly different at P<0.05.

Conversely, Kozink *et al.* (2004) found that boars that were randomly selected for LC treatment and received a feed mixture supplemented with 500 mg per day LC for 16 weeks did not show any beneficial effects on boar libido, semen quality, sperm production or maintenance of sperm motility during liquid storage**.**

CONCLUSION

In conclusion, oral dose of LC at a level of 2 g/h/d for 3 mo had impact to achieve high quality semen to spread the use of artificial insemination with bulls of high fertility.

REFERENCES

Aitken, R.; M. Baker and D. Sawyer (2003). Oxidative stress in the male germ line and its role in the etiology of male in fertility and genetic disease. Reproductive Bio Medicine Online, 7: 65–70.

Akey S.N. (2000). Akey's field trial experience with l-carnitine supplementation of sow diets.Lewisburg, OH:Akey Inc., Germany,1–2.

Amman, R. P. and R. H. Hammerstedt (1980). Validation of a system for computerized measurements of spermatozoa velocity and percentage of motile sperm .Biol. Reprod., 23: 647-656.

Arrigoni-Martelli, E. and V. Caso (2001). Carnitine protects mitochondria and removes toxic acyls from xenobiotics. Drug Exp. Clin. Res., 27: 27-49.

Blom, E. (1983). Sperm morphology with reference to bull infertility. proceedings of the 1st All-India Symposium on Animal Reproduction, (AISAR'83), Ludhiana, India pp: 61-81.

Boyd, J.W. (1984). The interpretation of serum biochemistry test results in domestic animals. Vet. Clin. Pathol., 13:7–14.

Bremer, J. (1983). Carnitine metabolism and functions. Physiol. Rev., 63:1420−1480.

Brooks, D.E. (1979). Carnitine, acetyl carnitine and the activity of carnitine acyl transferases in seminal plasma and spermatozoa of men, rams and rats. Journal of Re-production and Fertility, 56: 667–673.

Carlson, D.B.; J.W. McFadden; A. D'Angelo; J.C. Woodworth and J.K. Drackley (2007). Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. J. Dairy Sci., 90:3422–3441.

Carlson, D.B.; N.B. Litherland; H.M. Dann; J.C. Woodworth and J.K. Drackley (2006). Metabolic effects of abomasal l-carnitine infusion and feed restriction in lactating Holstein cows. J. Dairy Sci. 89:4819–4834.

Chapa, A.M.; J.M. Fernandez; T.W. Whtte; L.D. Bunting; L.R. Gentry; J.C. Lovejoy and K.Q. Owen (2001). Influence of dietary carnitine in growing sheep fed diets containing non-protein nitrogen. Small Rumin.Res.40:13-28.

Chapa, A.M.; J.M. Fernandez; T.W. White; L.D. Bunting; L.R. Gentry; T.L. Ward and S.A. Blum (1998). Influence of intravenous L-carnitine administration in sheep preceding an oral urea drench. J. Anim. Sci. 76:2930-2937

Chen, C.P.; S.H. Chan; T.C. Chao; C. Ting and B.T. Ko (2008). Low-bandgap poly (thiophene–phenylene–thiophene) derivatives with broaden absorption spectra for use in high-performance bulk-heterojunction polymer solar cells.

Citil, M.; K. Mahmut; M.E. Hidayet; Y. Rahsan; A. Emine and A. Onur (2009). Effect of orally administered l-carnitine on selected biochemical indicators of lactating Tuj-ewes. Small Ruminants Research, 81:174–177.

Dokmeci, D. (2005). Oxidative stress, male infertility and the role of carnitines. Folia. Med., 47(1):26–30.

Duncan, D. B. (1955). Multiple range and multiple F-tests. Biometrics, 11:1-42.

Golan, R.; B.P. Setchell; P.V. Burrow and L.M. Lewin (1982). A comparative study of carnitine and acylcarnitine concentration in semen and male reproductive tract fluids. Comparative Biochemistry and Physiology B. 72:457–460.

Greenwood, R.H.; E.C. Titgemeyer; G.L. Stokka; J. S. Drouillard and C.A. Loest (2001). Effects of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers.J. Anim. Sci., 79:254-260.

Hackett, A.J. and J.W. Macpherson (1965). Some Staing Procedures for Spermatozoa. A. Rreview. Can Vet J. Mar., 6(3):55–62.

Hadadinezhad, S.; N. Ghazaleh and Z. Razavi (2008). Effects of l-carnitine on glycemic control and C-peptide levels in patients with type 2 diabetes mellitus. Turk. Jem., 12:1–3.

Heo, K.; J. Odle; I.K. Han; W. Cho; S. Seo; E. van Heugton and D.H. Pilington (2000). Dietary l-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J. Nutr., 130:1809–1814.

Hoffman, L. A.; Ivers, D. J.; Ellersieck, M. R.; and T. L. Veum. (1993). The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. J. Anim. Sci., 71:132–138.

Hoppel, C. (2003). The role of carnitine in normal and altered fatty acid metabolism. Am. J. Kidney. Dis. 41(Suppl.4): S4-12.

Jacyno, E.; A. Kołodziej; M. Kamyczek; M. Kawęcka; K. Dziadek and A. Pietruszka (2007). Effect of L-Carnitine Supplementation on Boar Semen Quality. Acta Vet. Brno., 76:595-600.

Jaffe, B.M. and N.R. Behrman (1974). Methods of normone radio immuno assay Academic pross.

Jeulin, C. and L.M. Lewin (1996). Role of free l-carnitine and acetyl-L-carnitine in post gonadal maturation of mammalian spermatozoa. Journal of Reproduction Update, 2:87–102.

Jeulin, C.; J.L. Dacheux and J.C. Soufir (1994). Uptake and release of free l-carnitine by boar epididymal spermatozoa in vitro and subsequent acetylation rate. Journal of Reproduction and Fertility, 100:263–271

Jeulin, C.; J.C. Soufir; J. Marson; M. Paquignon and J.L. Dacheux (1987). The distribution of carnitine and acetylcarnitine in the epididymis and epididymal spermatozoa of the boar. Journal of Reproduction and Fertility, 79:523–529.

Kalaiselvi, C.J. and C. Panneerselvam (1998). Effect of L-carnitine on the status of lipid peroxidation and antioxidants in ageing rats. J Nutr Biochem 9:575-581.

Kellog, D.W. and D.D. Miller (1977). Effects of thyroxin, carnitine and pan to the nic acid plus cystein on milk production and certain blood metabolites of cows receiving a low energy ration, New Mexico State Univ. Las Cruces. Agric. Exp. Sta. Bull. 654:1–23.

Kirby, J.D. and D.P. Froman (2000). Avian Male Reproduction, In Sturkie's Avian Physiology, 5 th edition, G.C. Whittow, e. d. Academic Press, N. Y.

Kozink, D.M.; M.J. Estienne; A.F. Harper and J.W. Knight (2004). Effects of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology, 61:1247-1258

La Count, D.W.; J.K. Drackley and D.J. Weigel, (1995). Responses of dairy cows during early lactation to ruminal or abomasal dministration of L-carnitine. J. Dairy Sci., 78:1824-1836.

Ladha, S. (1998). Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. Journal of Membrane Biology, 165:1-10,

Lee Carter, A.; M. Susan; F. Hutson; W. Stratman and R.V. Jr. Haning (1980). Relationship of Carnitine and Acylcarnitines in Ejaculated Sperm to Blood Plasma Testosterone of Dairy Bulls1 Biology of Reproduction, 23:820-825.

Lenzi, A.; F. Lombardo; P. Sgro; P. Salacone; L. Caponecchia; F. Dondero and L. Gandini (2003). Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertility and Sterility, 79 (2): 292–300.

Li, D.; Q. Qiao; E.W. Johnson; J. Jiang; F. Wang; R. Blum and G. Allee (1999). Effect of L-carnitine and source of dietary fat on growth performance and serum biochemical parameters of piglets weaned at 35 days of age. Asian-Aus. J. Anim. Sci., 12:1263–1272.

Matalliotakis, I.; Y. Youmantaki; A. Evageliou; G. Matalliotakis; A. Goumenou and E. Koumantakis (2000). L-Carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. International Journal of Fertility, 45:236–240.

Newman, D.J.; G.M. Cragg; S. Holbeck and E.A. Sausville (2002). Natural products as leads to cell cycle pathway targets in cancer chemotherapy. Current Cancer Drug Targets, 2:279–308.

Newton, G.L. and K.D. Haydon (1989). Carnitine supplementation for finishing pigs. J. Anim. Sci. 67 (Suppl. 1):267(Abstr.)

Ng, C.M.; M.R. Blackman; C. Wang and R.S. Swerdloff (2004). The role of carnitine in the male reproductive system. Ann. NY Acad. Sci., 1033:177-188.

Owen, K.Q.; J.L. Nelssen; R.D. Goodband; T.L. Weeden and S.A. Blum (1996). Effect of l-carnitine and soybean oil on growth and performance and body composition of early-weaned pigs. J. Anim. Sci., 74:1612–1619.

Owen, K.Q.; J.L. Nelssen; R.D. Goodband; M.D. Tokach and K.G. Friesen (2001). Effect of dietary l-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J. Anim. Sci., 79:1509–1515.

Patton, C.J. and S.R. Crouch (1977). Spectoro-photmetric and kinetics investigations of Berthelot reaction for the determination of Ammonia. Analyt Chem., 49: 464-469.

Rincker, M.J.; S.D. Carter; D.E. Real; J.L. Nelssen; M. D. Tokach; R.D. Goodband; S.S. Dritz; B.W. Senne; R.W. Fent; L.A. Pettey and K.Q. Owen (2003). Effects of increasing dietary l-carnitine on growth performance of weanling pigs. J. Anim. Sci., 81:2259-2269.

Rawlings, N.C. and S.J. Cook (1986). Plasma concentration of testosterone, androstenedione, dihydrotestosterone, 5α-androsterone in bull calves: Response to hCG. Canadian J. Anim. Sci., 66:975-982.

Reitman, A. and Frankal, S. (1957). Determination of GOT and GPT in blood serum. J. Clin. Path., 28: 56.

SAS. (2004). SAS/STAT User's Guide: Volumes 1-7. SAS Institute Inc., Cary, NC.

Sigman, M.; S. Glass; J. Campagnone and J.L. Pryor (2006). Carnitine for the treatment of idiopathic asthenospermia: A randomized, double-blind, placebo-controlled trial. Fertil. Steril., 85:1409-1414.

Szilagyi, M. (1998). L-carnitine as essential methylated compound in animal metabolism. Acta Biol. Hung., 49:209-218.

Tietz, N.W. (1990). Clinical guide to laboratory tests. 2 nd ed. Philadelphia:WB Saunders.

Tietz, N.W. (1994). Fundamentals of Clinical Chemistry. 2nd ed. N.W. Tietz, editor, p 692.

Trinder, P. (1969). Determination of blood serum glucose, Ann. Cl. Biochem., 6: 24-31.

Van Dop, C.; S.M. Hutton and H.A. Lardy (1977). Pyruvate metabolism in bovine epididymal spermatozoa. J. Biol. Chern., 252:1303-1308.

Vaz, F.M. and R.J.A. Wanders (2002). Carnitine biosynthesis in mammals. Biochem. J., 36:417–429.

Vicari, E. and A.E. Calogero (2001). Effects of treatment with carnitines in infertile patients with prostatovesiculoepididymitis. Human Reproduction, 16 (11):2338–2342.

Wahrner, M.; M. Geyer; G. Hallfarth and U. Huhn (2004). Der Einfluss von Zulagen einer Vitaminemulsion mit L-Carnitin auf die Spermaeigenschaften von Besamungsebern. Zuchtungskunde 76: 196-207.

Watson, D. (1960). Method of determination of blood serum cholesterol and urea. Clin. Chem. Acta, 5 : 637.

White, T.W.; J.M. Fernandez; G.T. Gentry; L.R. Gentry; P.T. DeRouen and M.A. Froetschel (2001). Influence of urea alone or combined with fish solubles, fish meal,or feather meal in liquid supplement with and without L-carnitine on performance and ruminal and metabolic parameiers of weanling calves. Anim. Sci., 7:145-153.

White, T.W.; J.M. Fernandez; G.K. Armes and P.T. DeRouen (1998). Influence of L-caranitine on growth rate and blood metabolites of weaning calves. J. Anim. Sci., 76:(Suppl.1), 325 (Abstr.).

Zhai, W.; S.L. Neuman; M.A. Latour and P.Y. Hester (2007). The effect of dietary L-carnitine on semen traits of white leg horns. Poult. Sci., 86:2228-2235.

Zollner, N. and K. Kirsch (1962). Uber die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natiirlichen Lipoiden (allen bekannten Plasma lipoiden) gemeinsamen Sulpho-phospho-vanillin Reaktion. Z. Ges. Exp. Med., 135, 545-561.

تأثير المعامله عن طريق الفم بالكارنيتين علي وزن الجسم، وخصائص الدم والأداء التناسلي لطلائق الفرزيان.

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تمت الدراسة لتقيم مستويات مختلفة من ل- كارنيتين (LC) يوميا عن طريق الفم لمدة ٣شهوروتأثيرها على وزن الجسم وبعض خصائص الدم والأداء التناسلي لثيران الفريزيان.

استخدم فى هذه التجربه ٩ طلائق فريزيان (كان الوزن الحي عند بداية التجربة ٣٨٠±٣٢,١ كجم والعمر ٢٠±١,٤شهر)، تم تقسيمها إلى ٣ مجاميع متماثلة في الوزن والعمر . تم تغذية جميع الثيران ووضعها تحت نظام غذائي وظروف واحدة ، ولكن اختلفت في المعامله ب LC وقد عوملت الثيران بجرعة LC عن طريق الفم يوميا لمدة ٣ شهور عند مستوى صفر، ١ و ٢ جم/راس/يوم في المجموعة الأولي والثانية والثالثة ، على التوالي . في نهاية فترة المعاملات تم جمع السائل المنوي من الثيران مرتين في الأسبوع لمدة ١٢ أسبوع وتقييم حجم القذفه، والنسبه المؤيه لحركة الحيوانات المنوية ، الحيوانات المنوية الحية والشاذه ، وتركيز الخلايا المنوية و اجمالى عدد الحيوانات المنوية/القذفه. تم جمع عينات الدم من جميع الحيوانات في نهاية فترة جمع السائل المنوي ، لتحديد تركيز البروتينات الكليه، الألبيومين، الجلوبيولين، اليوريا ،الكولسترول، الجلوكوزو الدهون الكلية ونشاط انزيمات AST و ALT في سيرم الدم . أيضا ، تم تحديد تركيز هرمون التستوستيرون في سيرم الدم بعد ١ و ٣ شهور من المعاملات.

أظهرت النتائج الآتى:

١- أظهرت المجموعة الثالثة أعلى الأوزان وأعلي معل نمو كلى ويومى معنويا بالمقارنة مع المجموعة الأولي ، ولكن لم تكن الفروق معنويه مع المجموعه الثانيه.

٢- زاد تركيز البروتينات الكليه والجلوبيولين عند مستوي ($P < 0.05$) وانخفض كل من نسبة الألبيومين الى الجلوبيولين، تركيز الكوليسترول والدهون الكليه معنويا فى المجموعات الثانيه والثالثه عن المجموعه الأولى عند مستوى ($P < 0.05$) ولم يتأثر تركيز الألبيومين، الجلوكوز و اليوريا أو نشاط AST وALT عن طريق المعاملة ب LC.

٣- تحسنت جميع خصائص السائل المنوي عند مستوي ($P < 0.05$) في المجموعة الثانية والثالثة بالمقارنة مع المجموعة الأولي زكانت المجموعة الثالثة أفضل عند مستوي ($P < 0.05$) من المجموعة الثانية في معظم الخصائص.

٤- زاد تركيز هرمون التستوستيرون في سيرم الدم عند مستوي معنويه ($P < 0.05$) في المجموعة الثانية والثالثة عن المجموعة الأولي.

ونستخلص من هذه الدراسة أن معامله طلائق القريزيان بجرعة من LC عند مستوي ٢ جم/راس/ يوم عن طريق الفم لمدة ٣شهور لها تأثيرايجابى على تحسيين جودة السائل المنوي لنشر استخدام التلقيح الاصطناعي مع الثيران ذات الخصوبة العالية.

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