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Performance and Semen Traits of Friesian Bulls Administrated with Free L-Carnitine as Metabolic Regulator

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ABSTRACT

This study aimed to evaluate the effect of daily oral administration of free L-carnitine (LC) for 3 months on digestibility coefficients, blood parameters and semen traits of Friesian bulls. Total of 9 bulls (360±32.1 kg LBW and 20±1.4 months of age) were assigned into 3 groups. Bulls in G1, G2 and G3 were fed the same diet and kept under the same conditions but differed in LC level (0, 1 and 2 g h^{-1} day^{-1}, respectively). Bulls were received LC for 3 months (treatment period). At the end of treatment, semen was collected from bulls twice a week for 12 weeks and evaluated for ejaculate volume, sperm progressive motility, livability and abnormality, sperm concentration and total sperm output. Also, digestibility coefficients were performed and blood samples were collected. Concentration of Total Proteins (TP), albumin (AL), globulin (GL), urea-N, cholesterol (CHO), glucose and Total Lipids (TL), activity of AST and ALT and testosterone were determined in blood serum. Results show that bulls in G3 showed highest ($p<0.05$) digestibility coefficients. Only concentration of TP and GL increased ($p<0.05$), AL/GL ratio and CHO and TL concentrations reduced ($p<0.05$) in LC groups. All semen characteristics improved ($p<0.05$) in LC groups, being better ($p<0.05$) in G3 than in G2. 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^{-1} day^{-1} for 3 months had impact to achieve high quality semen to spread the use of artificial insemination with bulls of high fertility.

Key words: Bulls, carnitine, body weight, blood, semen quality, testosterone level

INTRODUCTION

Carnitines occur in the form of L-and D-isomers; however, only the L-carnitine is biologically active, while the D-isomer may even be noxious for the organism (Szilagyi, 1998). L-carnitine (α-hydroxy-γ-trimethylammonium butyrate) is a highly polar natural compound, vitamin-like amino-acid, synthesized within the body of most animals from lysine and methionine (Groff and Gropper, 2000; Vaz and Wanders, 2002). It is very important in the metabolism of lipids leads to carry long-chain fatty acids to the mitochondria for α-oxidation which produces energy (ATP) needed for proper functioning within the cells (Hoppel, 2003). It also plays an important role in the cellular detoxification (Arrigoni-Martelli and Caso, 2001) and as antioxidant for protection of the cell membranes against oxidative damages (Kalaiselvi and Panneerselvam, 1998).

Dietary L-carnitine supplementation is recommended in domestic animals especially in cattle (Carlson *et al*., 2006) to increase animal performance. It was reported that L-carnitine increases

apparent digestibility of lipid, energy and fatty acids (LaCount *et al.*, 1995). Also, dietary supplements of L-carnitine (20-500 mg kg^{-1} diet) raised plasma L-carnitine level in plasma, liver and milk of ruminants (LaCount *et al.*, 1995).

In mammals, L-carnitine transports from the blood plasma into epididymis (Jeulin and Lewin, 1996) and the epididymis is the origin of free L-carnitine in seminal plasma and spermatozoa (Brooks, 1979). During sperm transit through the epididymis, its motility initiates in parallel with an increase in the concentration of free L-carnitine in the epididymal lumen (Jeulin *et al.*, 1994). In sperm cells, intracellular acetyl L-carnitine may be a metabolic fuel used by the spermatozoa during their short life (Jeulin and Lewin, 1996) by transporting fatty acids into the mitochondria, where they undergo α-oxidation leading to the generation of metabolic energy needed by the sperm for progressive motility (Jeulin *et al.*, 1987). 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).

In the literature, conflicted results were reported on semen characteristics and libido of animals (Akey, 2000; Kozink *et al.*, 2004; Jacyno *et al.*, 2007). Therefore, aim of this study is to evaluate the influence of daily oral administration of free L-carnitine at two levels (1 and 2 g h^{-1} day^{-1}) for 3 months on digestibility, blood parameters and semen characteristics 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 months 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 same diet and administrated with oral dose of LC at levels of 1 and 2 g h^{-1} day^{-1} for 3 months 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. Based on the chemical analysis, the basal ration contained 46.6% DM, 88.5% OM, 13.1% CP, 19.1% CF, 2.3% EE, 54% NFE and 11.5% ash.

Experimental procedures

Digestibility trials: Digestibility trials were conducted at the end of the experimental period using Acid Insoluble Ash (AIA) as a natural marker. Each digestibility trial consisted of 15 days as a preliminary period followed by 5 days collection period. Feces samples were taken from the rectum of each bull twice daily with 12 h interval during the collection period. Samples of feedstuffs were

taken at the beginning, middle and end of collection period. The samples of feedstuffs and feces were composted and representative samples were dried in a forced air oven at 70°C for 48 h, then ground. Representative samples from CFM, RS, BH, FB and feces were taken and prepared for the chemical analysis for CP, CF, EE and ash according to the methods of AOAC (1995).

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 h, thereafter centrifuged for 15 min 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 and Amerson (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 aspartate (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 months of treatment according to Jaffe and Behrman (1974).

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 am a bull was used as teaser animal for sexual preparation. Semen was collected for 12 weeks from January to March after 3 months 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 ($\times 10^9$ mL^{-1}) 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 Amann 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:

$$\text{Total sperm output} = \text{Ejaculate volume (mL)} \times \text{sperm cell concentration}$$

Statistical analysis: Data was 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

Digestibility coefficients and feeding values: Results presented in Table 1 show that digestion of DM and all nutrients significantly ($p<0.05$) improved by treatment of bulls with 1 or 2 g LC h^{-1} day^{-1} as compared to the control bulls. The pronounced effect was on increasing digestibility coefficients of CP and CF, while the lowest effect was on NFE. These finding s indicated benefits of L-carnitine treatment at a level of 1 or 2 g LC h^{-1} day^{-1} on improving nutrient digestion of bulls. In association with increasing digestibility coefficients of all nutrients, L-carnitine treatment at a level of 1 or 2 g/animal led to significant ($p<0.05$) improving feeding value of the diets as TDN and DCP.

In harmony with the present results, LaCount *et al.* (1995) found increased apparent digestibility of lipid, energy and fatty acids in multiparous Holstein cows administrated carnitine. The maximum activity of cellulolytic microorganisms can be noticed near the neutral pH value (Mehrez *et al.*, 1977). Values of pH in ruminal fluid was higher in lambs fed L-carnitine containing diets which may indicate increased rumen parameters of animals fed diets containing L-carnitine (Chalupa, 1972; Kertz *et al.*, 1982).

In this respect, LaCount *et al.* (1995) reported that total Volatile Fatty Acids (VFA) concentrations and molar proportions of propionate tended to increase and molar proportions of acetate tended to decrease, in cows fed supplemental L-carnitine. Moreover, several authors indicated that ruminal NH_3-N concentrations were higher in lambs fed the L-carnitine containing diets (LaCount *et al.*, 1995).

Blood parameters

Haemo biochemical concentration and enzyme activity: Results presented in Table 2 show that LC treatment significantly ($p<0.05$) increased concentration of Total Proteins (TP) as a result

Table 1: Effect of oral L-carnitine (LC) administration on apparent digestibility coefficients and nutritive values

	Experimental group			
Item	G1 (Control)	G2 (1 g LC h^{-1} day^{-1})	G3 (2 g LC h^{-1} day^{-1})	SEM
Digestibility coefficients (%)				
DM	67.44^{b}	70.82^{a}	72.05^{a}	0.82
OM	67.92^{b}	71.08^{a}	72.60^{a}	0.84
CP	66.13^{b}	71.02^{a}	73.35^{a}	1.25
CF	65.64^{b}	69.66^{a}	72.52^{a}	1.17
EE	68.30^{b}	71.53^{a}	72.84^{a}	0.82
NFE	69.17^{b}	71.56^{a}	72.45^{a}	0.56
Nutritive values (%)				
TDN	62.08^{b}	64.94^{a}	66.34^{a}	0.70
DCP	8.66^{b}	9.30^{a}	9.61^{a}	0.16

^{a,b}Means within the same row with different superscripts differ significantly ($p<0.05$)

Table 2: Effect of L-carnitine on some haemo-chemicals and enzyme activity in blood serum of Friesian bulls at the end of collection period

	Experimental group		
Item	G1 (Control)	G2 (1 g LC h^{-1} day^{-1})	G3 (2 g LC h^{-1} day^{-1})
Blood metabolites			
Total proteins (g dL^{-1})	7.19±0.28^{b}	8.04±0.10^{a}	8.04±0.12^{a}
Albumin (AL) (g dL^{-1})	3.52±0.13	3.62±0.10	3.64±0.08
Globulin (GL) (g dL^{-1})	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}
Total cholesterol (g dL^{-1})	211.80±4.04^{a}	194.40±6.19^{b}	193.70±2.87^{b}
Total lipids (mg dL^{-1})	539.40±12.73^{a}	523.90±6.95^{ab}	513.90±14.86^{b}
Glucose (mg dL^{-1})	77.80±2.80	78.20±1.23	73.70±2.09
Urea-N (mg dL^{-1})	31.90±2.28	29.00±0.59	28.30±1.06
Enzyme activity			
AST (IU L^{-1})	37.23±0.83	36.79±0.63	36.91±0.53
ALT (IU L^{-1})	19.01±0.75	17.96±0.38	18.70±0.46
AST/ALT ratio	1.98±0.091	2.05±0.047	1.99±0.075

^{a,b}Means within the same row with different superscripts are significantly different at $p<0.05$

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 G2 and G3 than in G1. In accordance with these results, serum AL concentration was not affected significantly by carnitine treatment in lambs (Chapa *et al.*, 2001) or in cows (Carlson *et al.*, 2007). However, others observed an increased amount of AL in blood samples of carnitine treated ewes (Citil *et al.*, 2009). The observed increase in concentration of total proteins and globulin in LC groups was associated with increasing digestibility coefficient of CP of bulls in G2 and G3.

Concerning the 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 2). These results are in agreement with those reported by Citil *et al.* (2009), who found that oral carnitine treatment in healthy suckled ewes resulted in alterations in triglycerides, cholesterol, urea, glucose which are indicators of energy metabolism. The addition of 500 mg carnitine to ewe diet led to a reduction in serum cholesterol concentration. Similar trend was observed by Kellog and Miller (1977) in cows. Also, many previous reports suggested that LC supplementation could influence lipid metabolism (Heo *et al.*, 2000). The effect of LC on reducing lipid metabolites could be associated with stimulation of lipid metabolism through transfer of acyl groups across the mitochondrial membranes (Owen *et al.*, 1996).

The obtained results revealed insignificant effect of LC treatment on glucose and urea-N concentrations in serum of bulls (Table 2). In agreement with this result, Carlson *et al.* (2007) found that plasma glucose concentration was not altered for carnitine-supplemented cows as compared to controls, regardless of carnitine intake. The effect of carnitine on plasma glucose level is controversial; some reported increase (Chapa *et al.*, 2001), decrease (Hadadinezhad *et al.*, 2008) or unchanged (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). Regard

Table 3: Effect of L-carnitine on semen traits of Friesian bulls during the collection period

	Experimental group		
Semen trait	G1 (Control)	G2 (1 g LC h^{-1} day^{-1})	G3 (2 g LC h^{-1} day^{-1})
Ejaculate volume (mL)	1.840±0.11^{c}	2.230±0.10^{b}	2.650±0.08^{a}
Progressive motility (%)	59.100±2.41^{c}	67.600±1.39^{b}	74.800±1.15^{a}
Live sperm (%)	81.200±1.52^{b}	85.800±1.72^{a}	88.800±0.46^{a}
Abnormal sperm (%)	25.300±0.62^{a}	15.170±0.87^{b}	14.920±0.95^{b}
Sperm concentration ($\times 10^{9}$ mL^{-1})	0.790±0.04^{c}	1.154±0.07^{b}	1.333±0.09^{a}
Total sperm put ($\times 10^{9}$/ejaculate)	1.454±0.14^{c}	2.573±0.19^{b}	3.532±0.21^{a}

^{a,b,c}Means within the same row with different superscripts are significantly different at $p<0.05$

to urea-N concentration, Rincker *et al.* (2003) observed no difference in urea-N in weanling pigs fed added LC. However, others showed that addition of 500 mg carnitine to ewe diet led to a reduction in serum urea level (Citil *et al.*, 2009).

Generally, the biological significance of these changes is likely minimal because concentrations were within normal ranges (Boyd, 1984).

Data in Table 2 show that activity of aspartate (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. Similar trend was reported by Citil *et al.* (2009). Contrary, Carlson *et al.* (2007) found that LC treatment resulted in elevated concentrations of AST.

Semen traits: Results presented in Table 3 show that all physical semen traits 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 traits. These results mean that increasing LC dose from 1 to 2 g h^{-1} day^{-1} had more impact on semen quality of bulls.

In accordance with the present improvement in all semen traits studied, Jacyno *et al.* (2007) found that the addition of 500 mg LC to the boar feed had a positive effect on the quality of boar semen. They found that ejaculate volume increased by 11%, the total ejaculate sperm count increased by 11.5% and number of spermatozoa with major and minor morphological changes decreased. Meanwhile, percentages of sperm motility, sperm concentration and spermatozoa with intact acrosome did not increase considerably. Also, Wahner *et al.* (2004) found that boars receiving 230 mg of LC in their daily ration showed an increase in ejaculate volume and sperm concentration. Yet, Kozink *et al.* (2004) have proved only an increase of spermatozoa concentration in adult boars. 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-vesiculo-epididymitis and elevated seminal reactive oxygen species production (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, 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). L-carnitine 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). L-carnitine 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). L-carnitine 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).

Regarding the changes in semen traits studied during 12 weeks as a collection period (Fig. 1), it is of interest to note that ejaculate volume, percentages of sperm motility and livability, sperm cell concentration and total sperm output showed the highest values, while percentage of sperm abnormality showed the lowest values in G3 during most collection weeks as compared to that in G2 and G1, respectively.

Testosterone concentration: Results illustrated in Fig. 2 show that blood serum testosterone concentration was significantly ($p<0.05$) higher in blood serum of bulls treated with both levels of LC (G2 and G3) than in the controls (G1). 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

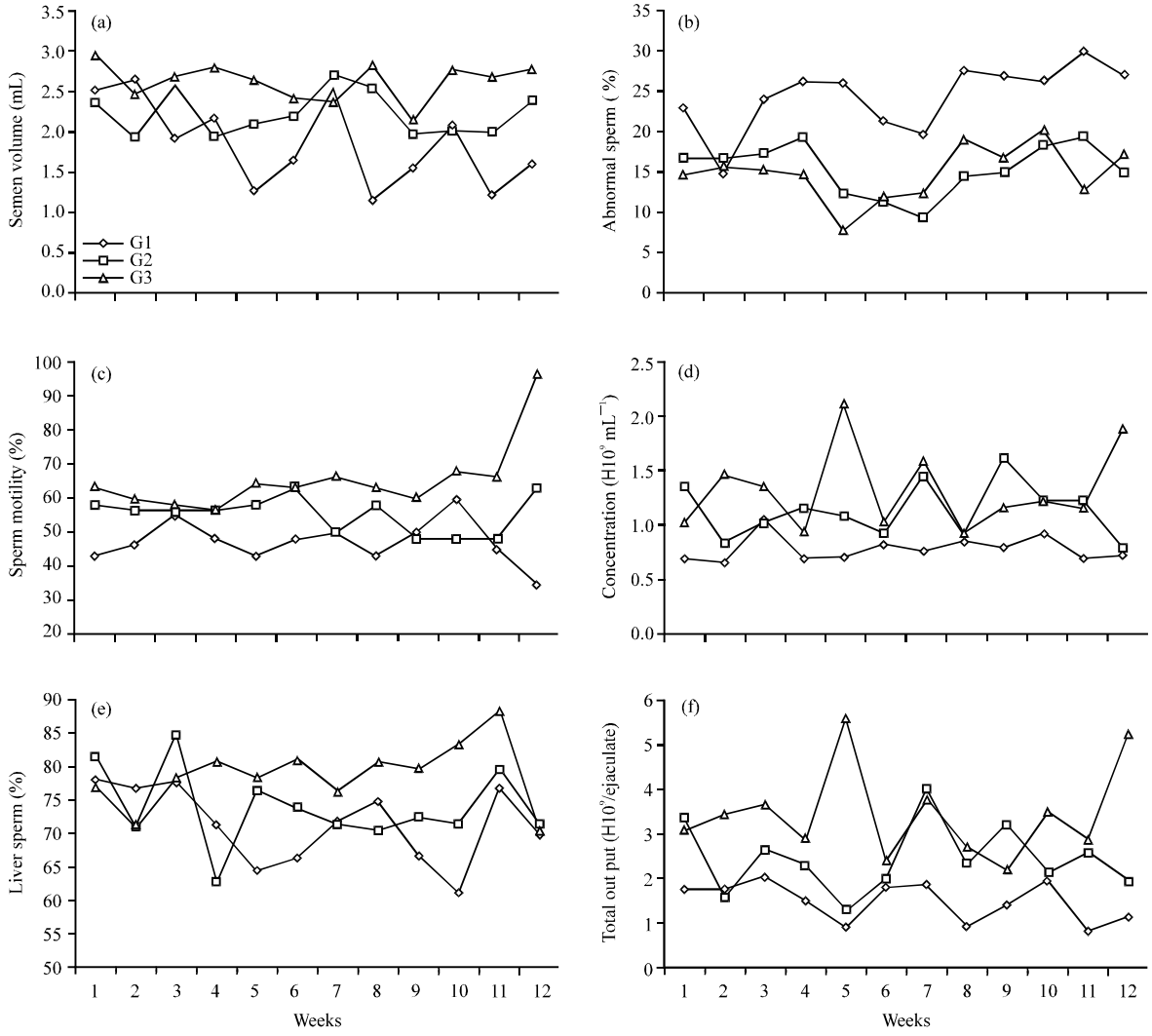

Fig. 1(a-f): Semen traits (a) Semen volume, (b) Abnormal sperm, (c) Sperm mortality, (d) Concentration, (e) Liver sperm and (f) Total output of bulls in different experimental groups during collection weeks, G1: Control, G2: 1 g LC h^{-1} day^{-1} and G3: 2 g LC h^{-1} day^{-1}

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 (Carter *et al*., 1980). 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.

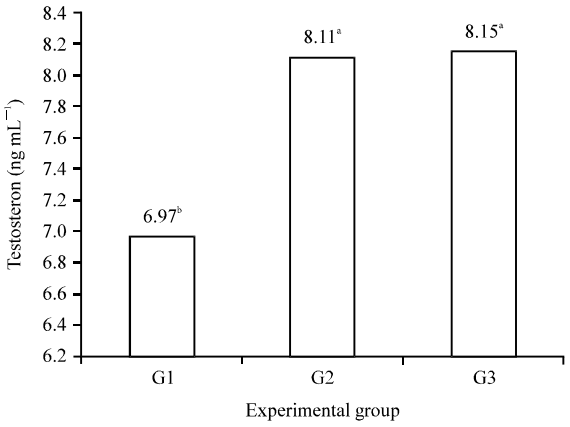

Fig. 2: Effect of L-carnitine on testosterone concentration in blood serum of Friesian bulls at the end of semen collection period, G1: Control, G2: 1 g LC h^{-1} day^{-1} and G3: 2 g LC h^{-1} day^{-1}, ^{a,b}Significant difference at $p<0.05$

CONCLUSION

In conclusion, oral dose of LC at a level of 2 g h^{-1} day^{-1} for 3 months had impact to achieve high quality semen to spread the use of artificial insemination with bulls of high fertility.

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