



Improvement in Reproductive Performance, Milk Production, and Blood Metabolites in Friesian Cows treated with Various L-Carnitine Dosages

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THIS study aimed to investigate the effect of L-Carnitine on reproductive performance, milk yield, and some blood metabolites of Friesian cows. Thirty Friesian cows were categorized into three comparable groups, each consisting of ten cows. The first group (G1) did not receive any supplementation, while the second (G2) and third (G3) groups were provided with L-carnitine supplements of 2g and 4g per cow per day, respectively, during the period from October 2020 to June 2021. The results indicate that in G3 cows, the postpartum first service interval (PFSI) and the service period length (SPL) were significantly shorter compared to the control group. The number of services per conception (NS/C) in cows was notably influenced by the dietary L-carnitine. Cows in G3 significantly the shortest days open (DO), while, G2 and control diets were nearly similar. Conception rates (CR) at successive postpartum days was higher significantly 100% and 90% of cows in L-carnitine diet than 60% of cows in control group. Average daily milk yields and monthly milk yield of cows were significantly highest in G3 than in G2 and G1. The concentration of proteins, including albumin, globulin, and lipoproteins in the blood plasma was notably higher in G3 when compared to G2 and G1. Urea-N levels were significantly lower in both G3 and G2 than in G1. Concentration of beta-hydroxybutyric acid (BHBA) was significantly reduced in both G3 and G2 compared to G1. Non-esterified fatty acids (NEFA) and T3 concentrations were significantly increased in G3 than in G2 and G1.

Keywords: Friesian cows, L-carnitine, Reproductive performance, Milk yield, Milk composition.

Introduction

The primary factor influencing dairy cattle's productivity is their regularity in reproduction. Breeders of dairy herds can accomplish their primary objective of producing healthy calves annually by increasing the reproductive efficiency of cows. The primary factors influencing dairy cow reproduction are climate, management, and nutrition. The herd's yearly income can be raised via caving interval reduction [1]. In dairy cows, the transition period is crucial because of the sharp rise in energy needed for milk production that occurs when feed intake is

insufficient to address the negative energy balance [2].

L-carnitine is extremely necessary and is endogenously generated from methionine and lysine in the kidneys and liver [3]. L-carnitine is required for energy synthesis in cells via mitochondrial oxidation. Besides, carnitine plays an important role in a variety of metabolic processes and oxidation of long-chain fatty acids such as management of ketosis, immune system support, antioxidant system enhancement, and reproductive improves [4]. L-carnitine regulated metabolic processes in high-yielding milking cows.

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These carnitines have the ability to improvement reproductive processes whether taken alone or in conjunction with other nutrients and antioxidants. A mechanism for L-carnitine-induced female fertility enhancement, which includes (a) boosting energy produce in oocytes and effectively quenching free radicals to defend reproductive cells from oxidative damage, and (b) enforcing their advantageous effects via the hypothalamic pituitary gonadal (HPG) axis to improve blood hormone concentration [5, 6]. Because of its significant functions in female reproduction, Carnitine can be utilized as fertility enhancers and as reproductive biomedicines to treat female infertility [7].

Noseir *et al.* [8] found that buffalo cows supplementing with L-carnitine resulted in shorter time intervals between first ovulation and first estrus ($P < 0.011$) than non-supplemented controls (41.50 vs. 62.20 days, respectively) (58.00 vs. 75.83 days, respectively). L-carnitine supplementation also considerably ($P < 0.01$) decreased the time interval between first service and conception (days open). The control group had 95 and 119.67 days, while the L-carnitine group had 74.35 and 81.60 days. In buffalo treated with L-carnitine (1.4 services), NS/C decreased ($P < 0.01$) in buffalo treated with L-carnitine (1.4 services) than in the control (2.0 services). The aim studies the effect of L-carnitine on reproductive performance, milk yield, milk compassion, some blood parameters and metabolites of Friesian cows.

Material and Methods

The Sakha Animal Production Research Station, which is linked with the Ministry of Agriculture's Agricultural Research Centre, the Animal Production Research Institute, and the Faculty of Agriculture's Animal Production Department at Egypt's Kafrelsheikh University, conducted the current study. The Animal Care and Ethics Committee of Kafrelsheikh University in Egypt granted permission for this study to be carried out during the months of October 2020 and June 2021 (licence number: KFS1345/10).

Management and Animals

A total of 30 healthy Friesian cows ranging in age from 28 to 58 months and weighing 607 ± 22.5 kg were used in this study. The cows were all chosen 20 days before calving, at the late pre-calving stage. The experimental cows were divided into three groups ($n=10$), at the start of the trial. Cows were divided into groups based on their BW, parity, and previous season's milk output. Cows were fed a 35 present concentrate feed combination as a baseline ration (CFM), 25% corn silage (CS) and 40% rice straw (RS) pre-partum and 55% CFM, 30% CS and 15%

RS post-partum without oil supplement in 1st group (G1) and served as control, 2nd and 3rd groups supplemented with L-carnitine supplementation 2 and 4g/cow/day, respectively from 20 days before-calving until 120 days after-calving.

System of feeding:

The experimental cows were fed a diet that included CFM, RS, and CS, as recommended by the National Research Council (NRC) [9] for dairy cows based on their live body weight and milk output. Chemical analysis of typical monthly samples of foodstuffs on a DM basis was performed using the A.O.A.C. [10].

Experimental procedures:

Detection of estrus and insemination:

To detect the onset of the first estrus, an infertile bull was presented to each group's cows for 20 minutes two times daily at 6 and 15 h beginning on day 20 postpartum. When cows demonstrated perfect sensitivity to the teaser and waited quietly to be mounted, it was determined that they were in estrus. Artificial insemination was used on cows that were found to be in heat.

From calving till conception, postpartum first estrus (PPEI) and first service (PPSI) intervals were observed. The NS/C, DO and CR % were calculated. Rectal palpation on day 60 post-insemination was used to diagnose pregnancy.

Blood sampling:

Every cow in every group had blood samples collected every two weeks from the jugular vein using heparin-containing tubes throughout the trial. The obtained blood was centrifuged at 15 g for 10 min to extract the blood plasma, which was then stored frozen at -20 °C pending chemical testing. Using commercial kits from Diagnostic System Laboratories, Inc. USA, the concentration of total proteins, albumin, lipoprotein, urea-N, glucose, and insulin in blood plasma was measured.

B-hydroxybutyric acid concentration and NEFA in plasma were analyzed according to Xu and Wang, [11]. Triiodothyronine (T3) plasma concentration was measured by using T3- 125I immunoassay procedures described by Chopra *et al.* [12].

Yield of milk and composition:

Friesian cows were milked by machine twice day at 5:40 and 17:30. Yield milk was individually recorded for the 120 days of after calving. Milk samples were obtained on a monthly basis to determine milk composition using the Milko-Scan system (Model 133B). The 4% fat corrected milk

(4% FCM) for each cow was calculated from milk yield according to the following formula:

$$4\% \text{ FCM} = \text{Actual milk yield (kg)} \times 0.4 + 15 \times \text{fat yield (kg)} \text{ Geans equation, [13].}$$

Statistical analysis

The statistically analyzed of obtained data using SAS [14]. Duncan's Multiple Range Test was used to assess significant changes between treatment groups [15]. Statistical analyzed (One way-Anova) model:

$$X_{ij} = \mu + B_i + f_{ij}$$

Where:

X_{ij} = Observed traits

μ = Means

B_i = Experimental group

f_{ij} = Error

Conception rate obtained from this study were statistically analyzed by using Chi-Square test [15].

Results and Discussion

Reproductive performance

In this study the results showed that resumption of the postpartum particular estrous activity was significantly shorter in cow's supplemented levels of L-carnitine, which may indicate beneficial effect of L-carnitine treatment on incidence of the postpartum first estrus interval (PFOI) in cows. Results revealed insignificantly effect of dietary treatment on PFOI, being shorter for cows fed L-carnitine than by about 4.2 and 7.17 days in G2 and G3, respectively, than those control cows. However, postpartum first service interval (PFSI) was significantly ($P < 0.05$) earlier in L-carnitine groups than in control group. This means earlier PFSI of cows fed L-carnitine by about 9.7 and 23.6 days in G2 and G3, respectively than those control diets (Table 1).

TABLE 1. Reproductive performance of cows fed different dietary treatments

Items	Experimental groups		
	G1	G2	G3
Postpartum first oestrus interval (PFOI, day)	41.50±5.00	37.30±8.37	34.33±3.56
Postpartum first service interval (PFSI, day)	69.50±4.0 ^a	59.80±3.5 ^{ab}	45.90±4.0 ^b
Services period (SP, day)	28.00±2.5 ^a	22.50±3.2 ^a	11.57±3.2 ^b
NS/C (services)	2.50±0.42 ^a	1.70±0.37 ^{ab}	1.30±0.33 ^b
Days open (DO, day)	99.00±9.64 ^a	81.33±4.70 ^a	56.66±6.76 ^b
Conception rate (CR, %)	60 ^b	90 ^a	100 ^a

Means in the same row with various superscripts are substantially different ($P < 0.05$).

Data showed that the service period length (SPL) in cows was affected significantly ($P < 0.01$) by dietary treatments. As a result of conceiving most cows in L-carnitine group after the 1st service, SP length was 11.57 days in G3, being significantly lower ($P < 0.05$) than in G2 and the control groups. The corresponding SP was 22.5 and 28.5 days in G2 and control groups, respectively (Table 3). These results indicated a good impact of feeding diets supplemented with L-carnitine on the service period length.

Results revealed that NS/C of cows was affected significantly ($P < 0.01$) by dietary treatment (Table 1), whereas G3 (4g L-carnitine) recorded the lowest percentage of NS/C about 1.3 services for cows. The corresponding values were 1.7 and 2.5 services for cows fed 2g L-carnitine and control diets, respectively. The low average of NS/C for cows obtained in treatment groups in this study agreed with that reported by Hegazy et al. [16], Noseir et al. [8], who found that NS/C significantly ($P < 0.01$) lower in buffalo treated L-carnitine than in the control group.

In this study the introducing male to females in all experimental groups to heat detection may be the reason in reducing number of services required per

conception. These results indicated a good impact of feeding cows on diets supplemented with L-carnitine diets supplemented on number of service per conception. On the other hand, cows G3 showed a little improvement in NS/C as compared to the control cows. Cows fed 4g L-carnitine significantly ($P < 0.05$) showed the shortest DO (56.66 days in G3), while DO for cows fed 2g L-carnitine (G2) and control diets were nearly similar, being 81.33 and 99 days, respectively (Table 1).

The results for DO are in agreement with by Hegazy, et al. [16] and Noseir et al. [8] found that DO significantly ($P < 0.01$) reduced in buffalo cows treatment with L-carnitine than in control group. The tendency of shorter length of DO in L-carnitine than in control group may reflect beneficial effect of feeding cows on diets supplemented with L-carnitine.

Conception rates (CR) at successive postpartum days of cows was significantly ($P < 0.05$) higher in cows in L-carnitine treatments (G3 and G2), than control cows (Table 2). The present results for CR are in agreement with by Pirestani *et al.* [3] found that days open was shorter significantly in carnitine treatment compare to control group. Using L-carnitine for 40 days resulted in decrease open days, a shorter calving interval, and a drop in milk factors

like ketone bodies and blood parameters like cholesterol and triglycerides, which led to a decrease in ketosis and fatty liver disease [18].

With decreased ketosis, first estrus after calving is induced, and open days is reduced, resulting in a reduction in period from calving to first service [19]. Pirestani *et al.* [3] revealed that choline and L-carnitine have a positive effect by reducing PFOI, PFSI, DO and NS/C. In dairy cows, L-carnitine (4 g/cow) supplementation improved reproductive performance either in first service, CR or in the overall conception per pregnancy rate [20]. In cows were supplementations of carnitine (2g/cow) reduce NS/C [3, 17].

Milk yield and compassion:

The improvement in the milk yield resulted from L-carnitine supplementation in G2 and G3 might be due to the improved blood protein status as shown in Table 3 or improved intake of net energy lactation or both.

Average of monthly milk yield as actual milk yield (AMMY) or 4% fat corrected milk yield (FCMY) of cows showed an improving effect ($P<0.05$) on L-carnitine levels (G2 and G3) supplementation of monthly average of AMMY and FCMY as compared to the control (Table 2). It is clear that supplemental diets with L-carnitine (G2 and G3) lead to marked increase in monthly AMMY and FCMY by 13.09; 20.26% and 18.56 and 27.03%, respectively, as compared with control group (Table 2).

The same trend was also reported by Abu El-Hamd and Ewada [6]; Carlson *et al.* [21] and Meyer

Average daily milk yield as actual milk yield (AMY) or 4% fat corrected milk yield (FCMY) of cows were significantly ($P<0.05$) increased in G3 than control (G1) cows. Overall mean of daily milk yield as AMY or FCMY of cows significantly ($P<0.05$) increased by about 20.62 and 13.09% in AMY and 27.03 and 18.56% in FCMY for cows fed diets supplemented with L-carnitine (G3 and G2) than those fed the control diets. (Table 2).

In addition, persistency of milk yield was observed in AMY of L-carnitine groups, being more consistent in L-carnitine than control. Generally, AMY and FCMY were higher in L-carnitine groups at most lactation months as compared to the control group, being almost the highest in L-carnitine group.

et al. [22] who found that milk yield was higher in L-carnitine group at most lactation months as compared to the control group in Friesian cows.

L-carnitine (G2 and G3) improved fat and protein percentages as compared to control (G1), but total solids, lactose and solids percentages not fat were not significant. This may indicate that L-carnitine has a positive reflection on the yield of fat and protein (Table 2). On the other hand, L-carnitine reduced ($P<0.05$) somatic cell count in milk of G2 and G3 as compared to G1. These results are similar to those obtained by Piepenbrink and Overton, [23], Janovick Guretzky *et al.*, [24], Carlson *et al.*, [21] and Meyer *et al.*, [22], who reported that L-carnitine was increase milk protein and milk fat percentage compared to control group.

TABLE 2. Milk yield and compassion of Friesian cows during 6 months post-partum.

Months	Dietary group			SEM
	G1	G2	G3	
Milk yield				
Actual milk yield (kg/day)	13.14 ^b	14.86 ^{ab}	15.85 ^a	0.75
Fat corrected milk yield (kg)	11.80 ^b	13.99 ^{ab}	14.99 ^a	0.72
Monthly milk yield (kg)	394.30 ^b	445.80 ^a	475.50 ^a	12.2
Monthly Fat corrected milk yield (kg)	354.10 ^b	419.70 ^a	449.80 ^a	21.7
Milk compassion (%):				
Fat	3.32 ^b	3.61 ^a	3.64 ^a	0.08
Protein	2.45 ^b	2.71 ^{ab}	2.76 ^a	0.05
Lactose	4.03	4.07	4.11	0.07
Total solids	11.25	11.39	11.48	0.26
Solids not fat	7.23	7.39	7.74	0.22
Somatic cell count	322.64 ^a	274.15 ^b	262.53 ^b	15.1

Means in the same row with various superscripts are substantially different ($P<0.05$).

Biochemical parameters

Data presented in Table (3) showed that total proteins, albumin, globulin and lipoprotein concentration in blood plasma of cows were significantly ($P<0.01$) highest in G3 than in G2 and control (G1). But, urea-N of concentrations was significantly lower ($P<0.01$) in G3 and G2 than in G1 by 27.57 and 14.64%, respectively. while, plasma glucose and insulin concentrations were nearly similar in both groups. The present values of plasma

total protein are within the normal range obtained by several investigators [25] in cows.

Data in Table (3) shown that BHBA was significantly ($P<0.05$) decreased in cows treated with L-carnitine G3 and G2 groups than in control group. However, the NEFA was significantly ($P<0.05$) increased in G3 group than in G2 and control groups (0.70 vs 0.63 and 0.62, respectively). However, there were no significant changes between G2 and control.

TABLE 3. Concentration of some biochemical parameters in plasma affected by L-carnitine treatment of post-partum

Items	Experimental groups		
	G1	G2	G3
Protein (g/dl)	6.40±0.15 ^b	6.63±0.13 ^b	8.08±0.14 ^a
Albumin (g/dl)	3.42±0.12 ^b	3.52±0.16 ^b	4.24±0.18 ^a
Globulin (g/dl)	2.98±0.13 ^b	3.11±0.15 ^b	3.84±0.17 ^a
Lipoprotein (mg/dL))	0.66±0.011 ^b	0.69±0.003 ^b	0.79±0.019 ^a
Urea-N (mg/dl)	38.66±0.60 ^a	33.00±0.93 ^b	28.00±1.47 ^c
Glucose (mg/dl)	62.88±0.96	62.44±1.07	63.44±1.13
Insulin (mg/dl)	22.22±1.03	21.00±1.08	23.66±0.95
Beta hydroxy butyrate acid (BHBA, mmol/l)	0.83±0.009 ^a	0.77±0.004 ^b	0.624±0.006 ^c
Non-esterified fatty acids (NEFA, mmol/l)	0.62±0.009 ^b	0.63±0.005 ^b	0.70±0.015 ^a
Triiodothyronine, T3 (ng/ml)	1.14± 0.009 ^b	1.15± 0.005 ^b	1.45± 0.03 ^a

Means in the same row with various superscripts are substantially different ($P<0.05$).

Our results are in agreement with Scholz *et al.* [17] who found a significant decreased on BHB in the carnitine cows than the control. A lack of L-carnitine has been suggested as a limiting factor in metabolism of fatty acid [2]. L-carnitine supply in dairy cattle a decreased TG accumulation in liver [21, 18] and increased levels of BHB in blood [21, 26], this might indicate changes in lipid and energy metabolism.

L-carnitine-supplemented cows may have a higher capacity for oxidising NEFA to BHB, which are precursors to the synthesis of milk fat [21]. In L-carnitine-supplemented cows, Meyer *et al.* [22] report

On the other hand, Pirestani and Aghakhani [26] found that L-carnitine supplementation resulted in an increase in BHBA, lipoprotein lipase activity, which may higher BHBA mobilization by L-carnitine treatment [28].

The content of free carnitine in the liver of lactating cows at 7 days post calving is much higher than at 3 weeks pre-calving, stimulation of hepatic genes involved in carnitine synthesis and absorption is most likely the cause [29]. As a result, these findings are really compelling suggest that ruminant tissue carnitine level is influenced by metabolic changes caused by physiological changes or ketosis. However, in the blood of clinically healthy cattle, compound L-carnitine has no influence on ketosis-related biochemical indicators [22, 30, 31]. As a

that serum NEFA concentrations were unaltered. Meyer *et al.* [22] found that the increased in ketogenesis was not related to lower TG accumulation in the liver of parturient cows given dietary L-carnitine. However, Pirestani and Aghakhani [26] detected an increased on BHB concentration of cows treated L-carnitine. Olgaray *et al.* [27] found that net lipid mobilization is generally low during mid-lactation with no effects on fatty acids or BHB. However carnitine supplemented hadn't any effect on of fatty acid concentrations during the postpartum period, when fatty acid mobilization is higher [18, 20].

result, greater liver function supports better glucose, urea, and other metabolic pathways metabolism.

Concentrations of T3 in blood plasma of cows was affected significantly ($P<0.05$) by L-carnitine treatment, being the highest in G3 than in G2 and control group Table (3). Mechanistically, T3 can modify growth hormone (GH) receptor binding in the liver and consequently increase insulin like growth factor (IGF-I) synthesis is stimulated by GH [20]. The T3 also acts as a regulator of the pituitary's GH synthesis [32]. Thyroid hormones may potentially play a role in the resumption of ovarian activity [33], who found that cows with greater plasma T3 levels had an earlier start to their ovarian cycle. Also, Spicer *et al.* [34] found evidence for a role of T3 in controlling steroidogenesis in bovine follicles, which

is consistent with our findings. The T3 and T4 may have a minor positive effect on FSH-induced progesterone production by cow granulosa cells and a substantial positive effect on LH-induced androstenedione production by bovine theca cells, resulting in a net rise in oestrogen production by the follicle, on the other hand, Abu El-Hamd [20] reported a little or no direct effect on aromatase activity with T3.

Conclusion

This study demonstrated that the treated during Pre- or Postpartum with levels of L-carnitine 4g/head/day (G3) has a beneficial effect on reproduction traits of Friesian cows. As well as improvement in milk yield, milk composition and blood metabolites parameters.

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الكفاءة التناسلية وإنتاج اللبن وصفات الدم في الأبقار الفريزيان المعاملة بمستويات مختلفة من الكارنيتين

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تهدف هذه الدراسة إلى معرفة تأثير L-Carnitine على الأداء التناسلي وإنتاج اللبن ، مكوناته ، وبعض نواتج الدم في أبقار الفريزيان. واستخدم في الدراسة ثلاثين بقرة فريزيان وقسمت إلى ثلاث مجموعات متشابهة (١٠ في كل مجموعة) المجموعة الأولى (G1) مجموعة مقارنة بدون أي إضافات، بينما تم إضافة ٢ و ٤ جم كارنيتين/ بقرة / يوم في المجموعة الثانية (G2) والثالثة (G3) على التوالي خلال الفترة من أكتوبر ٢٠٢٠ إلى يونيو ٢٠٢١.

وأظهرت النتائج قصر الفترة من الولادة وحتى أول تلقيح (PFSI) وطول فترة التلقيح (SPL) بشكل ملحوظ في أبقار المجموعة الثالثة مقارنة بالمجموعة الضابطة. انخفضت عدد التلقيحات اللازمة للحمل (NS / C) في المجموعات المعاملة. كما قصرت الأيام الضائعة (DO) معنويًا في المجموعة الثالثة ، بينما كانت المجموعة الثانية والكنترول متشابهة تقريبًا. كانت معدلات الحمل (CR) أعلى معنويًا ١٠٠٪ و ٩٠٪ من الأبقار في نظام L-carnitine الغذائي مقارنة ب ٦٠٪ من الأبقار في المجموعة الضابطة. كان متوسط إنتاج اللبن اليومي (AMY) وإنتاج اللبن الشهري (AMMY) للأبقار أعلى بكثير في المجموعة الثالثة منه في المجموعة الثانية و المجموعة الأولى. كان تركيز البروتينات والألبومين والجلوبيولين في بلازما دم الأبقار أعلى بشكل ملحوظ في المجموعة الثالثة عنها في المجموعة الثانية و المجموعة الأولى. وكانت تركيزات البورينا- N أقل بشكل ملحوظ في المجموعة الثالثة و المجموعة الثانية عنها في المجموعة الأولى. وانخفض تركيز BHBA بشكل ملحوظ في المجموعة الثالثة و المجموعة الثانية مقارنة بالمجموعة الأولى. وكان هناك زيادة معنوية في تركيز كل من NEFA و T3 بشكل ملحوظ في المجموعة الثالثة عنها في المجموعة الثانية و المجموعة الأولى.

الكلمات الدالة: أبقار الفريزيان والكرانتين والكفاءة التناسلية و إنتاج اللبن وتركيب اللبن.