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EFFECT OF ORAL L-CARINITINE ADMINISTRATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCES OF CROSSBREED CATTLE

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SUMMARY

The objective of this study was to evaluate the effects of oral L-carnitine (LC) administration on milk yield and composition, estrous activity and reproduction performance of crossbreed cows. A total of 30 crossbreed cows at early post-partum, weighing 432.7±23.5 kg LBW, aging 3.5-4 years and in 1-3 parities were divided into two similar groups according to LBW and age. Cows in the 1st group were fed a basal diet and was considered as a control group (G1). While, cows in the 2nd group (G2) were fed the same diet and treated orally with 3 g LC/cow/day for 120 days postpartum. Cows were machine milked and milk yield was calculated as actual (AMY) or 4% fat corrected milk (FCMY) yield. Milk composition and somatic cell count (SCC) were determined. Estrus was detect and cows in heat were artificially inseminated. Then, number and length of estrous cycles from calving up to conception was recorded. Postpartum 1st estrus (PPFEI) and 1st service (PPFSI) intervals, number of services per conception (NSC), service period length (SPL), days open (DO) and conception rate (CR%) were calculated. Pregnancy was diagnosed by rectal palpation on day 60 post-insemination. Results show that daily milk yield as AMY or FCMY of cows increased ($P<0.05$) by about 19.3% in G2 than in G1. Percentage of fat and protein in milk increased ($P<0.05$), while SCC decreased ($P<0.05$) by about 16.9% in milk of G2 as compared to G1. Average number of estrus cases and estrous cycles/cow was lower ($P<0.05$) in G2 than in G1. Average P4 concentration and P4 peak during the estrous cycle was higher ($P<0.05$) in G2 than in G1. Average P4 concentration during PPFEI was lower ($P<0.05$) in G2 than in G1. Average of PPFEI, PPFSI, SPL, DO and NSC were lower ($P<0.05$) in G2 than in G1. CR was higher ($P<0.05$) in G2 (93.33%) than in G1 (66.67%). In conclusion, the present study indicated beneficial effects of L-carnitine treatment (3 g/cow) for 120 days during early lactation period on milk production and reproductive performance of crossbreed cows during the first four months of lactation.

Keywords*: Cattle, L-carnitine, milk production, fertility.*

INTRODUCTION

During peak milk production, cows expend more energy than what is consumed through their diets, reaching a negative energy balance. To maintain adequate milk production, high-producing dairy cows consume a high energy diet. Concentrate feeds are necessary as an energy source added to the diet and substitute for forage content of the diet (Andrew *et al*., 1991).

Carnitine is synthesized endogenously from lysine (carbon backbone) and methionine (methyl group donor); however, these amino acids are not directly converted to carnitine but are required for synthesis of the endogenous carnitine precursor tri-methyl lysine (Vaz and Wanders, 2002). Carinitine naturally occurring quaternary amine compound, is important to transport long chain fatty acids across the inner mitochondrial membrane (Devivo and Tein, 1990). Carnitine is an essential substrate for carnitine palmitoyl transferase-I, a mitochondrial enzyme that condenses carnitine with fatty acyl-CoA to form acylcarnitine. The carnitine-acylcarnitine system is required for mitochondrial oxidation because the inner mitochondrial membrane is impermeable to long-chain fatty acids (McGarry and Brown, 1997). Additional functions of carnitine include modulating the ratio of acetyl-CoA: CoA, modulating the toxic effects of poorly metabolized acyl groups, shuttling activated medium- and short-chain organic acids from peroxisomes to mitochondria, and altering branched-chain amino acid metabolism (Owen *et al*., 2001). The rate of protein turnover and the trimethyllysine content in proteins are the main factors determining the rate of endogenous carnitine synthesis (Vaz and Wanders, 2002).

Insufficient endogenous carnitine synthesis might contribute to fatty liver development, despite increased hepatic carnitine concentration around calving (Grum *et al*., 1996).There are two forms of carnitines, L-and D-isomers, but only L-carnitine (LC) is biologically active, while the D-isomer may even be noxious for the organism (Szilagyi, 1998). LC is effective in increasing level of LC in blood plasma, liver, and milk when cows were administered directly with LC into either the rumen or abomasums, but LC had little effect on milk yield and composition (LaCount *et al*., 1995). Effect of LC on reproduction was studied by several authors. In this respect, LC improved fertility of buffalo cows (Freemaut *et al.,* 1993; Harmeyers, 1993) and in rams (Noseir and El-Amrawi, 2001). Improvement in energy balance of buffalo cows treated with LC form its nadir towards a positive state may provide an important signal for resumption of ovarian activity in terms of shortening the interval to 1st ovulation, estrus and service, and recducing number of services per conception (Noseir *et al*., 2003). Also, Musser *et al*. (1999) and Eder *et al*. (2001) observed beneficial effects of LC on sow reproductive performance. Dietary LC fed during gestation and lactation increased the number of pigs born live at birth (Musser *et al*., 1999) and weaning (Eder *et al*., 2001).

Aim of this study was to evaluate influence of oral L-carnitine administration during early lactation on milk yield and composition, estrus activity and reproductive performances of cross-breed Friesian cows.

MATERIALS AND METHODS

A total of 30 crossbreed cows (Friesian x Balady) at early post-partum weighing 432.7±23.5 kg LBW, aging 3.5-4 years and in 1-3 parities were used in this study. Cows were free of any diseases with healthy appearance and housed in group under semi-open sheds. Animals were divided into two groups according to live body weight (LBW) and age. In the 1st group, cows were fed commercial diet and served as a control group (G1), while in the 2nd group; cows were fed the same diet and orally treated with 3 g L-carnitine/cows/day for 120 days starting from calving (G2).

Cows were fed on equal amounts of diet containing concentrate feed mixture (CFM), rice straw and fresh berseem according to the NRC (2001). The daily allowances were adjusted every 15 days according to LBW, milk yield and fat milk percentage. Animals were fed twice daily at 8 a.m. and 2 p.m., while fresh water was available all daytime. Ingredients of CFM are presented in Table (1).

Table (1): Ingredients of CFM fed to cows in the experimental groups.

Feed stuff	%
yellow corn	37.5
soybean meal	20.0
corn gluten	15.0
wheat bran	22.5
Molasses	03.0
Premix*	00.5
Common salt	01.5
Total	100

** One kg of premix contained 3.3 x 10^6 IU Vit. A. 3.3 g Vit. E; 3.3 x 10^6 IU Vit. D_3; 0.33 g Vit. K; 0.33 g Vit B_1; 1.33 Vit. B_2; 6.67 g Vit B_5; 0.50 g Vit B_6; 3.3 g Vit. B_{12}; 3.3 g Pantothenic acid; 0.33 g folic acid; 16.67 mg Biotin; 166.67 g Cholin; 1 g Copper; 10 g Iron; 13.3 g Mn; 15 g Zn; 0.1 g Iodin; 0.03 g Se and carrier $CACO_3$ to 1 kg).*

During 120 day-lactation as an experimental period, milk yield was individually recorded twice daily at 6 a.m. and 5 p.m. Representative monthly milk samples were taken from evening and morning milk for determination of fat, protein, lactose and total solids percentages. Milk composition was performed using milko-scan (Milko Scano, 150, Italy). Fat corrected milk (4% FCM) for each cow was calculated from milk yield according to the following formula (Abou-Raya, 1967): 4% FCM (kg) = Actual milk yield (kg) x 0.4 +15 x fat yield (kg).

Beginning on day 10 postpartum, an infertile bull was introduced to cows of each group for 20 minutes three times daily at 6, 12 and 15 h to recognize the onset of the 1st estrus. Estrus was identified when cows showed complete receptivity to the teaser and stood quietly to be mounted. Cows observed to be in heat were artificially inseminated. Returned cows were re-inseminated and then, number and length of estrous cycles from calving up to conception were recorded. Postpartum 1st estrus (PPFEI) and 1st service (PPFSI) intervals, number of services per conception (NSC), service period length (SPL), days open (DO) and conception rate (CR%) were calculated. Conception rate was calculated as the proportion of conceived cows relative to inseminated cows multipliable by 100. Pregnancy was diagnose by rectal palpation which taken place on day 60 post-insemination.

Blood sample were taken at 3-day intervals from 10 day post-partum up to conception. Blood plasma were prepared for progesterone determination in plasma

Direct radioimmunoassay technique (RIA) was performed for determination of plasma progesterone (P4) concentration using antibody-coated tubes kit (Diagnosis systems, laboratories Texas, USA) according to the procedure outlined by the manufacture. According to the manufacture's information, the radioimmunoassay of progesterone is a competition assay. Sample and standards are incubated with 125I-labeled progesterone, as tracer, in antibody-coated tubes. After incubation the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are calculated by interpolation from the curve. The standard curve of P4 concentration ranged from 0 to 20 ng/ml. The intra-and inter assay coefficient of variation were 5.4 and 9.1%, respectively.

The obtained data were statistically analyzed according to Snedecor and Cochran (1980). For milk yield and compassion as well as all reproductive parameters, the statistical model was: $Y_{ij} = U + A_i + e_{ij}$. Where: Y_{ij} = observed values, U = overall mean, A_i = experimental group, and e_{ij} = random error

RESULTS AND DISCUSSION

Milk yield:

Results presented in Table (1) revealed that cows treated with L-carnitine(LC) showed significantly (P<0.05) higher daily or monthly milk yield as actual milk yield (AMY) and 4% fat corrected milk yield (FCMY) than the control cows. Average daily milk yield as AMY and FCMY of LC cows significantly (P<0.05) increased by about 19.13 and 25.75% as compared to control cows, respectively.

Results illustrated in Fig. (1) showed that monthly AMY peak was similar in both groups, being in the 3rd month of lactation. At this peak, LC cows showed the highest increase as compared to control cows. In less extend, monthly AMY was higher in LC cows than in controls at most lactation months.

Table (1): Average of actual milk yield, 4% fat corrected milk yield and milk compassion of cows during 6 months post-partum.

Item	Experimental group		±SEM
	Control	LC	
Actual milk yield (kg/day)	13.54^b	16.13^a	0.81
4% fat corrected milk yield (kg /day)	11.96^b	15.04^a	0.76
Monthly milk yield (kg)	406.2^b	483.9^a	13.4
Monthly 4% fat corrected milk yield (kg)	358.8^b	451.2^a	22.6

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

In accordance with the present results, Roos *et al*. (1992) and Citil *et al*. (2003) indicated improvement of lactation performance in ruminants supplemented with LC. Baumgartner and Jacobs (1999) found that an adequate supplementation of LC in animal nutrition enables to overcome an energy bottleneck with minimal performance losses. Also, improvement in milk production due to LC treatment was reported in sows (Freemaut *et al.*, 1993), during the first 120 days of lactation in buffalo cows (Noseir *et al*., 2003). LC has a number of functions, such as increasing milk rate, supporting immune system, and by this way protecting body against infections (Harmeyer, 2001). Also, LC affects metabolic processes in high yielding lactating cows and ewes in an advanced stage of pregnancy (Harmeyer and Schlumbohm, 1997). Moreover, Freemaut *et al.* (1993) suggested that the positive effects of LC on milk production were probably resulted from better energy utilization. In this line, Harmeyer (2001) reported that LC has a number of functions, such as transforming fatty acids into energy, preventing ketosis, carrying ATP from mitochondria to cytosole. Noseir *et al*. (2003) found that LC supplementation led to improvement in body condition score, dry matter and energy intakes during early lactation.

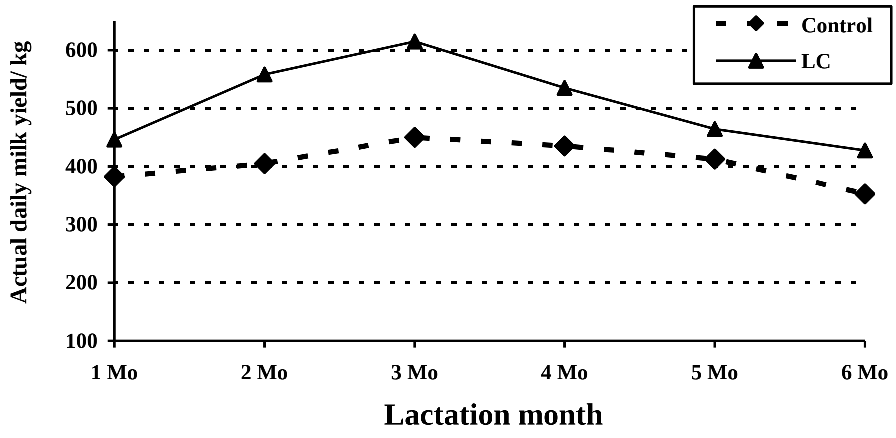

Fig. (1): Monthly milk yield of cows during 6 lactation months lamonths in different groups.

Milk composition:

Results shown in Table (2) revealed that percentage of both fat and protein contents in milk of cows was significantly ($P<0.05$) higher and somatic cell count was significantly ($P<0.05$) lower in LC than in the control group. However, both groups showed nearly similar percentages of lactose, total solids and solid not fat. In accordance with the present results, Carlson *et al*. (2007) found that lactose and total solids percentages in milk were not affected by carnitine treatment. Carlson *et al*. (2006) found that abomasal carnitine infusion (20 g/d) during short-term feed restriction did not affect milk fat percentage. In another study, Carlson *et al*. (2007) found that protein percentage in milk was not affected by carnitine treatment. Darragh and Mouigha (1998) indicated that the milk composition, i.e., the concentrations of protein, fat and lactose in the milk of the sows, also is consistent. In general, milk composition was not affected by post-ruminal infusion of carnitine to cows fed *ad. libitum* (La Count *et al*., 1995 and 1996).

Table (2): Milk compassion of cows in the experimental groups during 6 months post-partum.

Milk compassion (%):	Experimental group		±SEM
	Control	LC	
Fat	3.21^{b}	3.54^{a}	0.09
Protein	2.33^{b}	2.56^{a}	0.06
Lactose	3.99	4.07	0.07
Total solids	11.10	11.35	0.25
Solid not fat	7.07	7.50	0.21
Somatic cell count ($x10^3$)	338.69^{a}	281.33^{b}	24.2

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

Somatic cell count is one of the milk classification criteria, among others factors in the aspect of milk suitability for processing. It is also an indicator of the healthy condition of the mammary gland. Somatic cells are mostly composed of leucocytes which are natural protectors of organs in all body fluids and blood circulation. Somatic cell count is one of the milk classification criteria, among others from the aspect of milk suitability for processing. It is also an indicator of the healthy condition of the mammary gland (Jozwik *et al.*, 2004). Based on these finding, LC had beneficial effects on somatic cell count of cows.

***Ovarian activity**:*

Results in Table (3) showed that LC **treatment** significantly ($P<0.05$) decreased average number of estrus cases per cow as compared to the control group, but did not affect estrous cycle length. Also, **LC** treatment significantly ($P<0.05$) decreased average P4 level during postpartum 1^{st} estrus interval, while significantly ($P<0.05$) increased average P4 level and P4 peak during each estrous cycle as compared to controls. However, interval to P4 peak during the estrous cycles was not affected significantly by LC treatment.

Table (3): Postpartum estrous activity of cows in the experimental groups.

Item	Experimental group		±SEM
	Control	LC	
Number of estrus cases /cow	4.5[a]	3.00[b]	0.24
Average estrous cycle length (day)	20.94	20.65	3.61
Average P4 level up to 1st estrus interval (ng/ml)	0.496[a]	0.312[b]	0.06
Average P4 level during estrous cycle (ng/ml)	2.821[b]	3.739[a]	0.19
P4 peak during estrous cycle (ng/ml)	6.090[b]	8.701[a]	0.56
Interval to P4 peak during estrous cycle (day)	11.56	11.05	1.53

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

Reproductive performance:

Data presented in Table (4) showed significant ($P<0.05$) effect of LC treatment on reproductive performance of cows in terms of significant ($P<0.05$) reduction in average of postpartum 1st estrus (PPFEI) and 1st service (PPFSI) intervals, service period length (SPL) and days open (DO) of LC cows as compared to controls. Also, LC treatment significantly ($P<0.05$) decreased, number of services per conception (NSC) and increased conception rate (CR) of cows as compared to controls. It is of interest to note that 83.3% of cows received LC were conceived from the 1st service, leading to marked reduction in NSC to 1.20 services/cow.

The present results indicated beneficial effects of LC on most reproductive measurements of cows. Average of PPFEI and PPFSI was earlier by about 14.35 and 47.52 days in LC than in control group. In agreement with the present results, Noseir *et al*. (2003) reported that postpartum 1st ovulation interval significantly ($P<0.01$) decreased in buffalo cows receiving LC as compared to controls (41.50 and 62.20 vs. 58. and 75.83 d). Also, PPFSI and DO significantly ($P<0.01$) reduced by LC supplementation, being 95.0 and 119.67 d in the control group compared with 74.35 and 81.60 d in LC group, respectively. Number of services per conception also decreased ($P<0.01$) to 1.4 services/cow as compared to 2.0 services/cow in controls. Also, Hegazy *et al*. (2002) found that DO and NSC significantly ($P<0.01$) decreased in buffalo cows receiving LC compared to non-supplemented group. Moreover, Carlson *et al*. (2007) and Pirestani *et al*. (2011) indicated that LC have beneficial effect on reducing PPFEI, PPFSI, DO and NSC in Holstein dairy cows.

Table (4): Reproductive measurements and conception rate of cows in the experimental groups.

Item	Experimental group		±SEM
	Control	LC	
Postpartum first estrus interval (day)	45.67[a]	31.32[b]	2.013
Postpartum first service interval (day)	115.17[a]	67.65[b]	3.31
Service period length (day)	50.38[a]	4.04[b]	4.03
Number of services per conception	2.75[a]	1.20[b]	0.3234
Days open (day)	165.55[a]	71.70[b]	13.51
Number of inseminated cows	30	30	-
Number of conceived cows	20	28	-
Conception rate (%)	66.67[b]	93.33[a]	-

[a and b] *Group means denoted with the same superscripts within the same row are not significantly different at (P<0.05).*

CONCLUSION

The present study indicated beneficial effects of L-carnitine treatment (3 g/cow) for 120 days during early lactation period on milk production and reproductive performance of crossbreed cows during the first four months of lactation.

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تأثير تجريع الكارنيتين علي الأداء الانتاجى والتناسلي لخلطان الماشية

حلمى قطب زغلول

المعهد العالى للتعاون الزراعى – بشبرا- مصر

تهدف هذه الدراسة معرفة تأثير تجريع الكارنتين على إنتاج وتركيب اللبن والأداء التناسلي للأبقار الخليطة. استخدم في هذه الدراسة 30 بقرة خليطة (الفريزيانx البلدي) في الفترة المبكرة ما بعد الولادة متوسّط أوزانها 432.7 ±23.5 كجم وتتراوح أعمارها بين 3.5– 4 سنة وفى موسم 1-3. قسّمت الأبقار إلى مجموعتين متماثلتين على حسب الوزن الحي والعمر. غذيت الأبقار في المجموعة الأولى على العليقة الأساسية بينما الأبقار في المجموعة الثانية غذيت على نفس العليقة ثم تم تجريعها 3جم/رأس/يوم كارنتين لمدة 120 يوم بعد الولادة. كانت الأبقار تحلب آليا وإنتاج اللبن يحسب كأنتاج فعلي أو كمية اللبن معدلة الدهن. وتم تقدير مكونات اللبن وعدد الخلايا الجسدية. تم كشف الشياع وتلقيح الأبقار التي في شياع صناعيا. كذلك تم تسجيل عدد وطول دورات الشياع من الولادة حتى الحمل. بعد الولادة وفي بعد الولادة تم حساب كل من الفترة من الولادة وحتى أول شياع والفترة حتى أول تلقيحه وعدد التلقيحات اللازمة للإخصاب والأيام المفتوحة وفترة التلقيح وتم تشخيص الحمل عن طريق المستقيم عند اليوم 60 بعد التلقيح وحساب معدل الحمل. ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

1- زاد إنتاج اللبن اليومي وإنتاج اللبن المعدل (4% دهن) لمجموعة الكارنتين بنسبه 19.3% مقارنة بالكنترول.
2- أرتفعت نسبة الدهن والبروتين فى اللبن معنويا (P<0.05) في مجموعة الكارنيتين مقارنا بالكنترول. بينما عدد للخلايا الجسدية نقصت معنويا (P<0.05) في اللبن بحوالي 16.9% في الأبقار المعاملة بالكارنيتين مقارنة بالكنترول.
3- كان متوسط تركيز هرمون البروجسترون اعلي معنويا (P<0.05) خلال فترة ماقبل حدوث الشياع في مجموعة الكارنيتين مقارنة بالكنترول.
4- متوسط تركيز هرمون البروجسترون خلال الفترة من الولادة وحتى أول شياع انخفض معنويا (P<0.05) في الأبقار المعاملة بالكارنيتين مقارنة بالكنترول.
5- انخفضت الفترة الفاصلة من الولادة وحتى الشياع وحتى أول تلقيحه وطول فترة التلقيح وفتر الأيام المفتوحة وعدد التلقيحات لكل حمل معنويا (P<0.05) في مجموعة الكارنيتين مقارنة بمجموعة الكنترول.
6- كان معدل الحمل أعلى بمعنوية في مجموعة الكارينتين (93.33%) عن الكنترول (66.67 %)

نستخلص من هذه الدراسة ان معاملة الأبقار الخليطة بالكارنيتين (3جم/بقرة) لمدة 120 يوم في الفترة المبكرة بعد الولادة كان له اثر مفيد على إنتاج اللبن والأداء التناسلي للأبقار الخليطة