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Effect of l-carnitine supplementation on productive and reproductive performance of buffalo cows at antepartum period.

Effect of L-carnitine supplementation on some blood traits and reproductive performance of buffalo cows.

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Abstract

The study aims to evaluate the effect of L-carnitine supplementation on some blood parameters and reproductive performance of buffaloes at late pregnancy period. A number of twenty one buffaloes (3-4 lactations) were chosen to carry out the experimental work. The animals were divided into three groups (7 buffaloes each):- Group (G0) served as (control) don't receive any supplementation. Group (G1) supplemented with 5.0 gm L- carnitine /h/day. Group (G2) supplemented with 7.0 gm L-carnitine h/day. Both groups (G1 and G2) were supplemented with L-carnitine before parturition with two months and continued three months after parturition. During the experimental period, close observation was undertaken at the late pregnancy, one day before delivery buffaloes were kept in the delivery units until time of calving. Blood samples were collected weekly via the jugular vein from each buffaloes through the experimental period and used for assay of plasma Glucose, total protein, blood urea nitrogen, creatine, creatinine, ALT, AST,Total lipids, cholesterol, triglycerides, progesterone (P4) and estradiol 17β hormones. All animals were observed for heat detection. The number of days open (DO) was recorded for each dam as the period elapsed from parturition to next conception. Number of services per conception (NSPC) was recorded as the total number of services divided by the total number of animals conceived. Results indicated that buffaloes supplemented with different levels of L-carnitine had an improved blood parameters in postpartum period except triglycerides. Although, concentrations of total protein, creatine and ALT activity in pre-partum period were not significantly ($P>0.05$) affected by L-carnitine supplementation. L-carnitine supplementation enhanced oestrous sings especially mounting, response to finger massage, vaginal mucus discharge and intensity of oestrous activity. For reproductive performance, a significant differences were recorded among the groups in studied parameters such as NSPC, CI, mean period elapsed from calving until placenta drop, the interval required for each of pregnant uterus to return intra-pelvic, postpartum cervical closure and uterine horns symmetry and ovulation time. It can be concluded that, L-carnitine can be used for buffalo cows during late pregnancy and early lactation to enhance their reproductive performance.

Key words: L-crnitine supplementation, blood, reproductive performance, buffalo cows.

Introduction

Inadequate dietary energy in the short term or as a consequence of a prolonged depletion of body reserves during early lactation in both dairy cows and buffaloes can have deleterious effects on resumption of ovarian activity postpartum and other markers of reproductive success such as conception rate to first service, services per conception as well as calving-to-conception intervals (**De Vries *et al.*, 1999**). Carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) was first isolated from bovine muscle and only the L-isomer was found bioactive (**Zhou *et al.*, 2007**). Carnitine synthesis is a multiple-step process, which is synthesized from two essential amino acids lysine and methionine (**Steiber *et al.*, 2004**). It is a small water-soluble molecule important for normal oxidation of fatty acid by mitochondria in mammalian fat metabolism (**Vanella *et al.*, 2000**). Free L-Carnitine is synthesized in several tissues (liver, kidney, and brain) and is absorbed from dietary sources. L-carnitine has a prime role in energy provision. As a cofactor, it catalyses the transport of fatty acids through the mitochondria membrane. It also increases the bio-availability of free CoA in the cells, which is essential for the optimal progression of metabolic processes (**Di Lisa *et.al.*, 1995**). This optimizes the provision of ATP and assists its transport through the mitochondrial membrane (**Scholte *et al.*, 1996**). Moreover, L-carnitine has an antioxidant activity by acts as a free radical scavenger in aging, and also it can increase the levels of vitamin C, vitamin E (**Rani and Panneerselvam, 2001**), superoxide dismutase (**Cetinkaya *et al.*, 2006**) and catalase (**Izgut-Uysal *et al.*, 2001**) which play an important role in defence mechanism to protect cells from oxidative stress induced at the myocardial and endothelial cell level (**Cederblad *et al.*, 2008**). Also, L-carnitine has a number of functions, such as regulation of ketosis, increasing milk production, supporting immune system, and by this way protecting body against infections (**Harmeyer, 2001**), enhancement of the antioxidant system and improvement of reproductive processes which are particularly

dependent on adequate energy provision (**Baumgartner and Reuse, 1996**). If L-carnitine is inadequate, β-oxidation of long chain fatty acids impaired. β-oxidation of fatty acid to support the energy need is important during the late pregnancy period (**Newton and Burtle, 1992**). Therefore, L-carnitine added to feed has been used to supporting the energy need appearing in late pregnancy period. Researchers reported that L-carnitine regulates metabolic processes in high yielding lactating cows and also ewes in an advanced stage of pregnancy. **Liedtke *et al.* (1982)** mentioned that during growth or pregnancy, the requirement of carnitine might exceed its natural production. **Noseir and El-Amrawi (2001)** found an improvement effect of L-carnitine supplementation on fertility of normal and sub-fertile rams. **Pirestani *et al.*, (2011)** reported that choline + L-carnitine treatment group was indicated significant decrease on open days, calving to first visible oestrus, calving to first service and service per conception compare to other groups. It was concluded that choline + Lcarnitine combination has beneficial effect on improved reproduction performance than other treatment groups in Holstein dairy cattle. **Bayoumy, (2010)** reported that l carnitine supplemented to Friesian cows at 10 days postpartum improved reproductive performance (postpartum first oestrus interval- postpartum first service interval- service period length- number of service per conception- days open and conception rate %) than that control group. **Scholz *et al.* (2014)** reported that carnitine supplementation to dairy cows influenced fertility parameters. Whereas there was a trend for a lower insemination index and the conception rate was significantly improved in the carnitine supplemented group as compared to the control. Also, **Somfai *et al.* (2011)** reported that L-carnitine supplemented enhancement nuclear maturation and cleavage ability of porcine oocytes, significantly increased the rates of MII stage oocytes and cleavage of embryos and the density of active mitochondria was significantly higher and the density of lipid droplets was significantly lower.

This study aims to evaluate the effect of l-carnitine supplementation during pre and postpartum periods on some blood parameters and reproductive performance of buffalo cows.

Materials and Methods

The experimental procedures:-

This study was carried out at EL-Gemmaiza Experimental Station belonging to Animal Production Research Institute, Agricultural Research Centre, Giza, Egypt.

Twenty one Egyptian lactating buffalo cows (3-4 lactations) were used in this study. Animals were chosen in late pregnancy period at approximately 60 days pre-partum and divided randomly into three similar experimental groups, (7 animals in each group) to evaluate the effect of L-carnitine supplementation on some blood parameters and reproductive performance of buffalo cows. The control ration consisted of concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) which was given to buffaloes, (G0) without supplementation, while the other groups G1 and G2 were fed the control ration supplemented with two levels of L-carnitine : 1st is 5.0 gm per /h/d L - carnitine and 2nd is 7.0 gm per/h/d L-carnitine as a oral dose, respectively (Carniking, Lonza Ltd., Basel, Switzerland). Animals were individually fed according to **Kearl (1982).** The animals were left for 4 weeks (as a preliminary period) on the same diet before receiving any supplementations. The experimental period lasted five months (two months before the expected calving date and continued up to three months of lactation period) to investigate the effect of L-carnitine supplementation on some blood parameters and reproductive performance of buffalo cows.

Management and feeding:-

All animals were housed in semi-shaded open pens until time of delivery then they were transferred to the maternity unit. Water was offered freely in water troughs except at the milking time. After delivery all buffalo cows were allowed to nurse their calves for only one week postpartum (period of colostrum intake) thereafter, the dams were transferred to the milking unit and milked twice daily at 7a.m and 5 p.m. and they were subjected to the regular managerial practices of the breeding stock.

Table (1): The chemical composition of feed ingredients.

Items	Chemical composition as DM basis (%)						
	DM	OM	CP	CF	EE	Ash	NFE
CFM*	91.21	93.18	16.56	11.02	3.65	6.82	61.95
BH	88.91	82.92	13.85	24.91	1.14	17.08	43.02
RS	92.32	81.19	2.81	42.14	1.07	18.81	35.17

*CFM; concentrate feed mix contained in percentage ; 37% yellow corn , 30% undecortecated cotton seed , 20% wheat bran, 6.5% rice bran, 3% molasses , 2.5% limestone, 1% common salt.

Blood sampling:-

Blood samples were collected weekly via the jugular vein from each buffalo cows during pre and postpartum period. Blood plasma was carefully separated after centrifugation at 3000 r.p.m. for 20 minutes, and then stored at -20 C° until analysis. Blood plasma was used for the determination of Glucose (**Trinder, 1969**), total protein (**Armstrong and Carr 1964**), blood urea nitrogen (**Faweat and Scott, 1960**), creatine and creatinine was measured using the colorimetric method according to **Husdan (1968**), Liver function was assessed by measuring the activities of alanine amino transferase (ALT), aspartate amino transferase (AST) as described by **Reitman and Frankel (1957**),Total lipids (TL) were measured according to **Zollner and Kirsch (1962),** cholesterol (**Kostner *et al.*, 1979)** and triglycerides (**Schalm *et al.*, 1975**). Direct radioimmunoassay technique was performed for the determination of progesterone, (P4) estradiol 17β hormones in representative plasma samples. Kits of ''Diagnostic Products Corporation, (DCP) Los Angles, USA '' with ready antibody coated tubes were used according to the procedure outlined by the manufacturer. First ovulations were verified by occurrence of two consecutive observed P4 values > 1.0 ng/ml serum.

Measurement of reproductive parameters:-

Immediately after parturition, the time elapsed for complete fetal membranes drop (hour) were recorded. During postpartum period, the reproductive tract was rectally palpated once every two days till 21 postpartum and once every three days after that in order to assess the uterine involution period (day) and ovarian changes according to **El-Fadaly (1978).** As regular farming system, the experimental buffaloes were included in mating groups one month after parturition. Animals were examined for pregnancy by rectal palpation after 60 days of insemination. The interval from calving to first detected oestrus (day), service period length (day), number of services per conception (NSPC) was determined as the total number of services divided by the total number of animals conceived, days open(DO) was determined for each dam as the period elapsed from parturition to next conception, pregnancy rate (%) and calving interval (CI/day) was computed as the gestation period length plus days open were recorded. Ovulation was determined exactly for buffalo cows observed in estrus (ovulation = oestrus day + 1). Oestrous cycle length was estimated as the interval between successive ovulations. Ultrasound scanning was performed on day 45 after insemination to confirm pregnancy. Conception rate was defined as the proportion of buffalo cows that were detected in oestrus and inseminated that were pregnant on day 60 post insemination. Ovaries of buffaloes were examined by ultrasonography using ULTRSCAN. Model 900, 5MHz and "Falco,Easote/Piemedical,Maastricht, the Nether lands", 6-8 MH2 Linear array transducer (Alliance Medical Int.) in midmorning on every second day during the second estrous cycle of the experiment. Size and number of ovarian follicles > 3 mm were recorded . In this way, the size of the largest and second largest follicles could be followed during the preovulatory period. Follicles were grouped into three diameter classes for analyses: class 1 (3.0 to 5 mm), class 2 (5.0 to 10 mm), and class 3 (≥10 mm). Size, number, and position of corpus luteum (CL) and large luteinized follicles also were recorded.

Estrus evaluation and fertility traits:-

Response of different animals groups to various treatments was evaluated. Buffalo cows were observed twice daily at a 12 hr interval by experienced herdsman for at least one hour for estrous signs, especially the acceptance of buffalo cows to the bull, mounting, bellowing, restless and sniffing of external genitalia. The day at which the female stand to be mounted was considered the day of estrus. Buffalo cows come in heat were inseminated 12 h after estrus detection by a fertile bull. The females were checked for pregnancy 60 days after insemination by palpation per rectum and ultrasound sonar. Fertility measures including the number of animal responded the mean interval between initiation of treatment and onset of oestrus and conception rates were recorded.

Statistical analysis:

Data were analysed using GLM procedures of the **SAS, 9.1(2010)**. Means were separated by using Duncan's multiple range test (**Duncan, 1955**).

Yij = μ+Ti + eij

Where μ= means T= treat

E=error

Results and discussion

Effect of L-carnitine supplementation on the relevant blood parameters :

Blood plasma constituents including concentration of total protein, glucose, urea-nitrogen, creatine, creatinine, AST, ALT, T.lipids, total cholesterol and triglycerides in pre and postpartum periods are presented in Tables (2 and 3). Results show that only concentration of total protein, creatine and ALT were not significantly (P>0.05) affected by L-carnitine supplementation in pre-partum period. On the other hand, postpartum period

showed that only triglycerides was not significantly (P>0.05) affected by L-carnitine supplementation. In accordance, some studies attributed the differences between pre-partum and postpartum periods in level of blood total protein and glucose to milk protein synthesis (**Rakesh Kumar *et al.*, 2001**) or increased proteins break down required for gluconeogensis (**Abdul Gani *et al.*, 2003**). The differences in all blood parameters due to treatment and month of sampling were highly significant (P <0.01) except of blood parameters mentioned above in both periods.

Table (2) Pre-partum blood plasma parameters of buffalo cows in different experimental groups.

Items	Treatments			Sig.
	G0	G1	G2	
T. protein (g/dl)	6.53 ±0.08	6.74±0.11	6.79±0.07	NS
Glucose (mg/dl)	63.31^{c}±0.57	65.25^{b}±0.58	68.09^{a}±0.80	**
Blood urea nitrogen (mg/dl)	22.98^{b}±0.43	26.06^{a}±0.21	26.76^{a}± 0.30	**
Creatine (mg/dl)	47.83±0.47	49.28±1.11	50.36±1.45	NS
Creatinine (mg/dl)	2.61^{b}±0.04	2.91^{a}±0.07	2.86^{a} ±0.076	**
AST (IU/L)	87.63^{b} ±0.63	88.40^{b}±0.48	90.73^{a}±0.27	**
ALT (IU/L)	19.77±0.46	20.39±0.38	21.12±0.52	NS
T. lipids (mg/dl)	380.05^{c}±1.58	416.52^{b}±5.97	436.60^{a}±4.24	**
Cholesterol (mg/dl)	84.30^{b} ±1.49	88.17^{ab}±1.92	90.19^{a}±1.62	**
Triglycerides (mg/dl)	16.74^{c}±0.14	17.16^{b}±0.10	17.84^{a} ±0.16	**

Means bearing different superscripts within the same raw are significantly different (P < 0.05).

Table (3) Postpartum blood plasma parameters of buffalo cows in different experimental groups.

Items	Treatments			Sig.
	G0	G1	G2	
T. protein (g/dl)	6.69^{b}±0.07	7.47^{a}±0.11	7.56^{a}±0.10	**
Glucose(mg/dl)	64.96^{b}±0.47	68.06^{a}±0.66	69.16^{a}±1.10	**
Blood urea nitrogen (mg/dl)	23.66^{b}±0.25	27.59^{a}±0.20	27.20^{a}±0.25	**
Creatine (mg/dl)	48.38^{b}±0.92	51.50^{a}±1.02	49.72^{ab}±1.07	*
Creatinine (mg/dl)	2.47^{c}±0.046	3.03^{a}±0.04	2.82^{b}±0.04	**
AST (IU/L)	88.21^{b}±0.59	89.01^{b}±0.43	91.09^{a}±0.33	**
ALT (IU/L)	19.58^{b}±0.31	20.85^{a}±0.28	20.96^{a}±0.36	**
T. lipids (mg/dl)	391.00^{c}±2.95	413.87^{b}±3.92	436.38^{a}±3.97	**
Cholesterol (mg/dl)	82.28^{b}±1.032	86.29^{a}±1.01	89.11^{a}±1.83	**
Triglycerides (mg/dl)	17.76±0.19	18.03±0.18	18.23±0.13	NS

Means bearing different superscripts within the same raw are significantly different (P < 0.05).

Generally, several reports found that L-carnitine supplementation could influence blood metabolites, **Abdel-Khalek *et al.* (2015 and 2013) and Sherief (2014)** reported that L-carnitine significantly (P<0.05) increased concentration of total protein in treated groups. On the other hand, **Mehrez *et al.* (2015)** supplemented Rahmani lambs with oral L-carnitine and found that only concentration of total proteins in blood plasma decreased (P<0.05) in group receiving (350 mg/h/d L-carnitine). For other blood parameters, **Abdel-Khalek *et al.*, (2015 and 2013)** found that L-carnitine supplementation groups reduced (P<0.05) concentration of cholesterol and total lipids as compared to untreated group. But concentration of glucose, BUN or activity of AST and ALT were not affected by L-carnitine treatment in blood serum of bulls. **Khaled and Ismail (2006)** found that blood metabolites related to protein and energy status of ewes had no effect with dietary supplementation except serum total lipids which significantly decreased in ewes supplemented with L-carnitine and CSFA. **Heo *et al.* (2000)** found that L-carnitine supplementation could influence lipid metabolism and decreased tissue lipid content (**Chen *et al.*, 2008).** This effect of L-carnitine could be associated with stimulation of lipid metabolism through transfer of acyl groups across the mitochondrial membranes (**Owen *et al* ., 1996).** In general, 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**) regardless of carnitine intake. The likely mechanism was related to a direct effect of L-carnitine 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**). Increases in blood glucose in response to L-carnitine supplementation have been attributed to increased fatty acid oxidation and subsequent reduction in the oxidation of gluconeogenic precursors (**Greenwood *et al.*, 2001**).

Previous study reported a reduced plasma total carnitine level in dairy cattle during lactation period (**Citil *et al.*, 2009**). It was suggested that discharge of L-carnitine along with milk secretion

results in decreased levels of L-carnitine in dairy cows during lactation. Therefore supplemental L-carnitine may help to improve lactation performance in ruminants.

In buffaloes, **Noseir *et al.* (2003)** found that serum concentrations of AST during the first month of lactation was significantly reduced in the L-carnitine group compared to the control. Whereas serum concentration of cholesterol was significantly increased by supplementation. Similar results for liver enzymes (AST, ALT) were found by **Citil *et al.* (2009)** they reported that insignificant reduction in liver enzymes. Contrary, **Carlson *et al.* (2007)** found that L-carnitine treatment resulted in elevated concentrations of AST. **Mehrez *et al.* (2015)** supplemented Rahmani lambs with oral L-carnitine and found that triglycerides concentration decreased (P<0.05) in both groups receiving (350 or 700 mg/h/d L-carnitine), respectively, by about 52.6 and 50.3% as compared to untreated group. Also, L-carnitine supplementation had no effect on BUN concentration in blood plasma. In the same line, **Rincker *et al.* (2003)** observed no difference in BUN in weanling pigs fed added L-carnitine. However, others reported that L-carnitine addition (500 mg) to ewe diet led to a reduction in serum urea level (**Citil *et al.*, 2009**). In contrary to this study reduction in cholesterol and triglycerides concentrations were reported by **Mehrez *et al.* (2015)** in lambs and **Sherief (2014)** in bulls. Similarly, **Citil *et al* .(2009)** reported that oral carnitine treatment in healthy suckled ewes resulted in alterations in cholesterol and triglycerides, which are indicators of energy metabolism. Addition of 500 mg L-carnitine to ewe diet led to a reduction in serum cholesterol level. Supplementation of L-carnitine reduced the concentration of triglycerides in blood plasma (**Hausenblasz *et al.*, 1996**), because it lowered esterification rate of palmitate to triglycerides (**Drackley *et al.*, 1991**), which showed the essential role of L-carnitine for fatty acid oxidation in ruminant liver. L-carnitine administration did not induce statistically significant changes in blood serum concentrations of cholesterol and HDL. **Foroozandeh *et al.*, (2014)** reported that fat type interacted with L-carnitine administration for cholesterol and LDL concentrations (P<0.05). L-carnitine administration significantly decreased both of them in lambs fed the soybean oil diets without L-carnitine. Interestingly, cholesterol and LDL in lambs fed the diet supplemented with soybean oil and L-carnitine were 25% and 50% lower than in lambs fed the soybean oil diet without L-carnitine. **Hajilou *et al.*(2014)** found that plasma concentrations of glucose, cholestrol and aspartate aminotransferase were not affected by L-carnitine treatments.

Effect of L-carnitine supplementation on birth weight of new born calves:

As shown in Table (4), differences among groups in initial live body weight were not significant (P>0.05). Percentages of the loss in LBW of buffaloes in proportion to the pre-partum weight were (9.61, 9.76 and 10.02 %) for the studied groups G0,G1 and G2, respectively. In this respect, **Sherief (2014)** found significant (P<0.05) improvement in LBW of bulls treated with 2 g LC/h/d, but LC treatment at lower level (1 g/h/d) had no significant effect on LBW. **Marston *et al.* (1995)** reported that the loss in calving LBW of cattle was 60 kg, which is comparatively similar to that obtained in the present study. **Awara (2006)** working on buffaloes found that loss in weight was 9.30 to 10.41% depending on pre-partum feed supplementation. Differences between groups in calf birth weight were significant at (P<0.05).Table (4) show that different levels of L –carnitine supplementation were significantly (P<0.05) increased birth weight of calves compared with the control group. However, relative weights of calves to their dams were significantly (P<0.05), higher whereas, treated groups (G1 and G2) showed (7.18 and 7.80%) comparing with (7.02%) in group (G0), respectively.

Table (4) Body weight of buffalo dams and new born calf in different experimental groups.

Treatments	Dams body weight (kg)					Calf birth weight (kg)	Calf body weight /Dam
	Initial weight	Late pregnancy	postpartum	Loss	% of loss in LBW		
G0	515.64±3.52	540.30^{b}±4.65	488.40^{c}±3.85	51.90^{c}	9.61	34.30^{c} ±1.51	7.02^{b}
G1	513.85±3.14	546.10^{b}±4.72	492.80^{b}±3.53	53.30^{b}	9.76	35.40^{b} ±1.44	7.18^{a}
G2	516.52±3.69	552.70^{a}±5.22	497.30^{a}±4.10	55.40^{a}	10.02	38.80^{a} ±1.31	7.80^{a}

Means bearing different superscripts within the same raw are significantly different (P < 0.05).

From this result, it could be assumed that L-carnitine included in the diet of dams can be improved efficiency of growing calves whereas facilitating fat metabolism agents. Several studies **Ramanau, *et al.* (2008)** have shown that L-carnitine supplementation of sows during

pregnancy increases birth weights of litters. This effect is slightly greater at a dose of 50 mg/kg than at a dose of 25 mg/kg of supplemented L-carnitine. The favourable effect of L-carnitine supplementation on piglet and litter birth weights was observed in sows across the whole range of parities.

Foroozandeh *et al.* (2014) found that L-carnitine administration tended (P=0.13) to improve body weight, average daily gain and feed conversion ratio (P<0.05) of lambs. Production and metabolic responses to supplemental L-carnitine have been variable in ruminants. **Chapa *et al.* (2001)** did not find effects of supplementation L-carnitine on feed intake, gain and efficiency in lambs and steers. **Hajilou *et al.* (2014)** found that there were no differences between both of L-carnitine and rumen-protected choline diets for average daily gain and gain: feed for finishing Holstein calves. **Mehrez *et al.* (2015)** found that L-carnitine supplementation has no effect on live body weight, averages of total and daily body gains of lambs during all intervals of the experimental period. In contrast, **White *et al.* (2002)** showed an improvement in gain when L-carnitine was supplemented to grazing calves. Also, indicated that L-carnitine led to faster and more efficient gain.

Effect of L-carnitine supplementation on signs of oestrus:-

In the present study, the various signs of oestrus recorded in the buffalo cows at oestrus were mounting, bellowing, segregation and restlessness, standing female to female, frequent urination, response to finger massage, vaginal mucose discharge and intensity of oestrous activity (Table 5).

Although bellowing is considered the most reliable sing of oestrus behaviour in Egyptian buffaloes under field condition of small holders (**Aboul –Ela *et al.*, 2000**) or in large herds in absence of bull (**Barkawi *et al.*, 1992**), mounting, response to finger massage, vaginal mucus discharge and intensity of oestrous activity were prominent signs of oestrus in the groups of buffalo cows that responded treatment. The frequency of occurrence of mounting, response to finger massage, vaginal mucus discharge and intensity of oestrous activity were found highest in the buffalo cows of group (G2) (97.57,95.28, 54.28 and 71.14% respectively) followed by group (G1) (94.14, 90.57, 54.71 and 68.43% respectively).

Table (5) Frequency of the occurrence of different signs of oestrus in the different groups of buffalo cows .

Items	Treatments			Sig.
	G0	G1	G2	
Response to teaser bulls (mounting)	$83.43^{b}\pm3.82$	$94.14^{a}\pm1.37$	$97.57^{a}\pm1.57$	**
Bellowing	81.0±2.78	83.85±2.15	86.71±1.94	NS
Segregation& restlessness	$78.86^{a}\pm1.40$	$74.14^{b}\pm0.96$	$73.57^{b}\pm0.86$	**
Standing female to female	81.57±3.33	87.14±3.96	90.71±0.99	NS
Frequent urination	65.29±2.89	68.71±1.67	65.43±2.29	NS
Response to finger massage	$73.57^{c}\pm1.59$	$90.57^{b}\pm2.69$	$95.28^{a}\pm1.58$	**
Vaginal mucus discharge	$40.85^{b}\pm3.77$	$54.71^{a}\pm2.08$	$54.28^{a}\pm2.29$	**
Intensity of estrous activity	$59.28^{b}\pm2.91$	$68.43^{ab}\pm1.62$	$71.14^{a}\pm4.23$	**

Means bearing different superscripts within the same raw are significantly different (P < 0.05).

Other signs of oestrus viz., bellowing, standing female to female and frequent urination were observed non- significant in comparison with the other premier signs of oestrus. Higher frequencies of occurrence of the signs of oestrus were observed in the cows of group (G2) treated with 7.0 gm L –carnitine per/h/d. Both of mounting and response to finger massage showed higher percentage in comparison with other sings could be due to the incorporation of additional genital massage which had stimulated the hypothalamo pituitary ovarian axis which could cause difference in endocrine status. The signs of oestrus observed in the present study appeared to be physiological and comparable with the classical signs of oestrus as stated by (**Zeedan *et al.*, 2009**). The higher intensity of estrous symptoms of buffaloes in both groups (G1and G2) my reflect differences in ovarian activity and release of ovarian hormones responsible for the manifestation of symptoms of estrus. Generally L –carnitine supplementation increased intensity of estrous activity from 59.28% in G0 to (68.43 and 71.14 %) in groups (G1 and G2), respectively.

Effect of L-carnitine supplementation on some reproductive traits:

Data in Table (6) showed that volume of fetal fluids reached its maximum value in G0 group comparing with the other groups. The differences among buffaloes groups were significantly at (P<0.01). Pre-partum L-carnitine supplementation lower the period of fetal membrane expulsion compared to untreated group and the differences were not significant. Also, both treated groups were significantly lowered (P<0.05) in period elapsed for pregnant uterus to return intra-pelvic cavity after calving (28.71, 18.86 and 35.57 respectively) and the postpartum cervical closure

(36.57, 22.71 and 41.14 days), respectively, than the control group. Postpartum uterine horns symmetry occurred significantly (P<0.05) earlier in the treated groups G1 and G2 than control group by about 3.57 and 18.57days, respectively. The interval required for uterine horns to prove symmetry is associated with both periods elapsed for placental expulsion and the uterus return to its normal condition in the pelvic. The uterine involution is considered to be complete when both uterine horns return to equal or almost equal its non-graved size (**Aboul-Ela *et al.*, 1985). Abd-El-Azeez (2000)** found that the uterine involution ranged from 54 to 61 days for Egyptian buffaloes.

Table (6) Estimates of some reproductive parameters in the different experimental groups of buffalo cows.

Items	Treatments			Sig.
	G0	G1	G2	
Placenta weight kg.	$4.30^{a}\pm1.1$	$4.80^{a}\pm1.1$	$3.30^{b}\pm1.4$	**
Fetal fluid (L)	$13.60^{a}\pm1.4$	$13.10^{b}\pm1.1$	$13.30^{a}\pm1.2$	**
Placental expulsion (hr)	5.75	5.61	5.57	NS
Uterine return in pelvic	$35.57^{a}\pm1.38$	$28.71^{b}\pm0.92$	$18.86^{c}\pm1.74$	**
Normal uterine position	$39.57^{a}\pm0.65$	$33.29^{b}\pm1.61$	$20.28^{c}\pm1.19$	**
Postpartum cervical closure	$41.14^{a}\pm1.12$	$36.57^{b}\pm1.72$	$22.71^{c}\pm1.06$	**
Uterine horns symmetry	$41.28^{a}\pm0.94$	$37.71^{a}\pm2.13$	$22.71^{b}\pm0.75$	**
Number of heat onsets	$2.71^{a}\pm0.18$	$2.29^{ab}\pm0.18$	$2.0^{b}\pm0.0$	**
Postpartum first oestrus interval (day)	$54.43^{a}\pm3.32$	$48.29^{ab}\pm3.35$	$41.0^{b}\pm1.85$	**
Service period length (day)	$61.14^{a}\pm2.06$	$44.71^{b}\pm1.04$	$39.57^{c}\pm2.77$	**
Number of services per conception	$2.14^{a}\pm0.26$	$1.57^{ab}\pm0.20$	$1.29^{b}\pm0.18$	*
Calving-to-conception interval	$102.71^{a}\pm3.87$	$82.86^{b}\pm1.47$	$61.0^{c}\pm3.53$	**
Gestation period (days)	314.2	310.3	310.0	NS
Calving interval (day)	$411.52^{a}\pm4.21$	$390.14^{b}\pm3.74$	$370.32^{c}\pm3.21$	**

Means bearing different superscripts within the same raw are significantly different (P < 0.05).

Data showed that there were a significant differences (P<0.05) among the tested groups for number of heats detectable, where the lowest value was achieved with group G2 followed by G1 and then G0. L- carnitine improved the appearance of estrous postpartum, postpartum first estrus interval was higher significantly between groups, longer for (G0) than those of treated groups (P<0.05). Average of postpartum first estrus interval of buffaloes in treated group (G2) was earlier by about 7.29 and 13.43 days than those in groups (G1 and G0), respectively. Length of postpartum first estrous interval showed wide variation in Egyptian buffaloes. **El-Shafie *et al.* (1983)** reported that postpartum first oestrus interval ranged between 30 to 44 days, whereas, **Barkawi *et al.* (1998)** reported longer averages (55 to 91 days).

Data showed that service periods length in group G2 were (39.57±2.77) days, and required 1.29 service while the corresponding service periods were (44.71±1.04 and 61.14±2.06 days) and (1.57 and 2.14 services per conception), in groups G1 and G0, respectively. It seems that positive impact of L- carnitine supplementation were shown on reproduction in buffaloes.

Treated groups (G2 and G1) showed a shorter period of calving to conception interval (61.0 and 82.86 days) compared with untreated group G0 (102.71days). The obtained values of calving to conception interval may also suggest a beneficial effect of L-carnitine with different levels to buffaloes.

Gestation periods for treated groups were relatively less than that control group, presumably due to amelioration in fetal growth imposed by increased metabolism.

Group (G2) of buffaloes showed a lower values of calving interval than that obtained in group (G1), and both groups were less than non- treated group (G0), Table (6). In Egyptian buffaloes, calving interval varied from 471 to 585 (**Mahdy *et al.*, 2001 and El-Moghazy, 2003). Youssef (1992)** attributed the variations in calving interval to delay of resumption of ovarian activity (31.6%), delay of time at which buffaloes displays it's the first postpartum heat (10.3%), longer service period (57.2%) and gestation length (0.9%).In addition, management practices, particular level and type of feeding during late pregnancy and early postpartum period are largely responsible for increasing calving interval and this could be corrected by supplementation of L-carnitine. This results were agreement with **Noseir *et al.* (2003)** they found that L-carnitine supplementation did not significantly affect uterine involution. But, time intervals to first ovulation, first estrus, time interval to first service and conception and number of services per conception were significantly (P<0.01) decreased in buffalo cows receiving L-carnitine supplementation compared to control. **Scholz *et al.* (2014)** found that L-carnitine supplementation improves fertility in the

cows. Whereas there was no difference in days from calving to first insemination, there was a trend for a lower insemination index, increases the conception rate, lower level on service to pregnancy and a decreased significantly days open. **Pirestani *et al.* (2011)** found that L-carnitine supplementation reduced open days, calving interval to first service that due to reduction of milk parameters such as ketone bodies and blood parameters such as cholesterol and triglyceride and then it was caused decrease ketosis and fatty liver disease. With the decreasing ketosis, it is induction of first estrus after calving and decreased the open days will result to reduce the calving to first service. Also, they adding that combined choline + L-carnitine was significantly affecting ($p \leq 0.05$) on services per pregnancy, decreasing significantly days open and lower significantly ($p \leq 0.05$) calving interval to first service and calving interval to first visible estrus than the other groups. **Bayoumy (2010)** found that supplementation of (2 g L-carnitine in oral dose/cow/day) to Friesian cows significantly ($P<0.05$) reduced postpartum first estrus interval, postpartum first service interval, service period length, number of service per conception, days open and conception rate compared with control group. The improvement in fertility of buffaloes in this study can be due to L-carnitine supplementation has been used to supporting the energy need and regulates metabolic processes appearing in late pregnancy period. On the other hand, **Khaled and Ismail (2006)** found that the differences between groups (control, L-carnitine and L-carnitine + fat) were not significant in relation to the onset of estrus. The mean duration of the induced estrus period for control group was not significantly longer compared to supplemented groups. Characteristics of the estrous cycle indicated that intervals to reach the maximum concentration of P4 and P4 concentration ≥ 1 ng/ml were relatively shorter in group G2 than that in other groups (Table7). This finding may reveal stimulatory effect of L-carnitine supplementation for enhancement of ovulation and shortly reaching the luteal phase hence reducing the service period length. However, mean concentration of P4 was less in G2 when compared with that of G1.

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Table (7). Characteristics of ovulation, conception and ovarian hormones in the different experimental groups in buffalo cows.

Items	Treatments						Sig.
	G0		G1		G2		
Interval to maximum P4 (day)	14.6^{a}		13.1^{b}		12.0^{c}		**
Value of maximum P4 (ng/ml)	$2.8^{b}\pm0.1$		$4.2^{a}\pm0.2$		$4.8^{a}\pm0.2$		**
Interval to reach P4 ≥1 ng/ml (day)	$5.3^{a}\pm0.2$		$4.5^{b}\pm0.3$		$4.1^{b}\pm0.2$		**
1st ovulation (day)	76.0^{a}		66.0^{b}		56.0^{c}		**
Progesterone(P4) ng/ml	$0.48^{c}\pm0.13$		$0.89^{b}\pm0.45$		$1.19^{a}\pm0.63$		**
Oestrogen (EST 17β) pg/ml	$24.88^{b}\pm5.97$		$32.28^{a}\pm8.70$		$32.76^{a}\pm9.71$		**
P4/EST ratio	$0.05^{a}\pm0.02$		$0.04^{b}\pm0.01$		$0.04^{b}\pm0.03$		**
	Response of treatment						
	No.	%	No.	%	No.	%	
Heat	4	57.14	7	100	7	100	
Ovulation	3	42.86	5	71.43	6	85.71	
Conception at first insemination	3	42.86	5	71.43	6	85.71	

Means bearing different superscripts within the same raw are significantly different ($P < 0.05$).

It was noticed that most of buffaloes in the present study approximately ovulated after uterine involution. Meanwhile, number of services per conception ranged between 1.29-2.14 being relatively less in L-carnitine treated groups. The delay in resumption of ovarian activity and manifestation of estrus in lactating buffaloes because of low rate of gonadotropine release (**El-Gaafrawy, 2000**) or surrounding environmental conditions (**Gilad *et al.*, 1993**) may be a limiting factor affecting NS/C. Characteristics of ovulation for the experimental groups of the present study were in accordance with those reported by **Gordon (1996), Darwash *et al.* (1997)** denoting that P4 concentrations in buffaloes reach its maximum level around day 14-15th of estrous cycle then decline to the minimum level on the days 19-24th of estrous.

Table (7) showed that in control group (G0) four buffaloes came in heat after 54 days after delivery, three of them ovulated and conceived. In the second group (G1), seven animals manifested

oestrus after 48 days and were ovulated while five of them got pregnant. In the third group (G2) all animals exhibited oestrus sings within 41days, six of them were ovulated and all will pregnant. Results revealed a higher pregnancy rate in G2 (85.71%) than that observed in groups G1 (71.43%) and G0 (42.86%). This may in relation to level of L-carnitine supplementation. The marked improvement in pregnancy rate of buffaloes supplemented with L-carnitine compared with untreated group is in agreement with the findings of **Scholz *et al.* (2014)**, they found that conception rate and pregnant rate were significantly improved in the L-carnitine supplemented group as compared to the control.

Noseir *et al.* (2003) come back the improvement effect of L-carnitine in buffalo cows fertility to improvement in energy balance from its nadir towards a positive state may provide an important signal for initiation of ovarian activity. This signal includes an increase in glucose, insulin and insulin-like growth factor-1 and decrease in free fatty acids. Moreover, initiation of cycling requires adequate dry matter and body reserves. If these condition are met, LH pulse and frequency will increase, insulin will also increase associated with an increase in number and affinity of LH receptors leading to first ovulation 10-14 days after the negative balance nadir. LH is a peptide hormone that requires sufficient amounts of ATP for its formation (**Lehninger, 1982**). In the present study there was a significant increase in serum cholesterol in the L-carnitine supplemented group. Most of the female sex hormones that controls reproduction (e.g. estrogen and progesterone) are steroid hormones, and all steroid hormones are ultimately made from a single precursor, cholesterol, which in turn is made from acetyl-CoA (**Lehninger, 1982).** In the same line, **Stevenson and Call (1983)** suggested that the conception rate in lactating cows was related to the number of ovulatory cycles preceding insemination. Hence the reestablishment of ovulatory cycles early after parturition assures multiple estrous cycles prior to the recommended breeding period and in this manner influences the conception rate. Also, **Noseir and El-Amrawi (2000)** found that L-carnitine supplementation improved fertility in rams.

(Table 8) showed that length of estrous cycle was differ between groups. Treated groups showed the minimum values followed by untreated group. There was no effect of L-carnitine supplementation on the diameter of the largest and second largest follicules in treated groups, but there was a differences between treated and untreated groups. Furthermore, there was no effect of L-carnitine supplementation on the number of class 1, 2, and 3 follicles within each group. But G2 was showed the highest size than thatG1 and G0. However, the total number of follicles tended to be greater for G2 than for G1 and G0, respectively. The CL diameter followed the same pattern, whereas G2 was greater than that other groups.

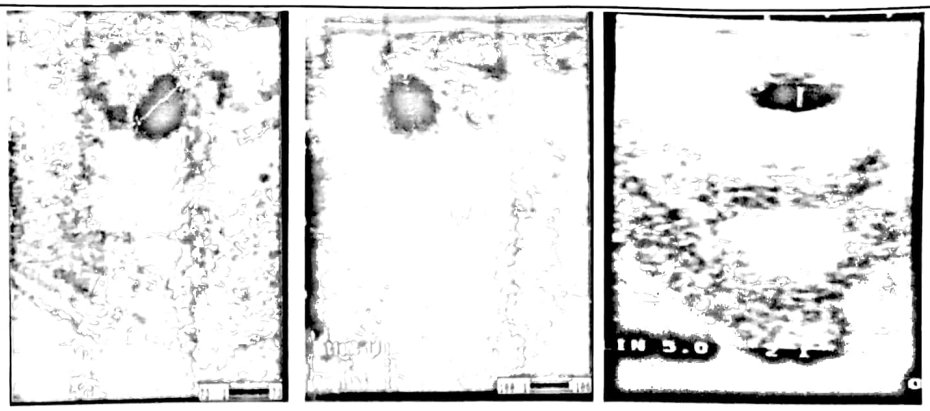
Table (8) Effect of L-carnitine supplementation on size and number of follicles in buffalo cows.

Items	Treatments			Sig.
	G0	G1	G2	
Length of oestrous cycle (d)	22.8^{a}	20.7^{b}	19.5^{c}	**
Follicle diameter (mm)[y]				
Largest (F1)	11.2^{b}	13.4^{a}	13.9^{a}	**
Second largest (F2)	6.7^{b}	9.3^{a}	9.5^{a}	
Size classes of follicles (number)[y]				
3.0 to 5 mm	0.81	0.96	1.09	
5.0 to 10 mm	0.82	0.96	1.33	
≥□10.0 mm	0.88	1.05	1.28	
Total number of follicles [y]	2.51^{c}	2.97^{b}	3.70^{a}	**
Corpus luteum (CL) diameter, mm	20.0^{c}	21.3^{b}	22.1^{a}	**

Y Mean between d 14 and 21 of the oestrous cycle.

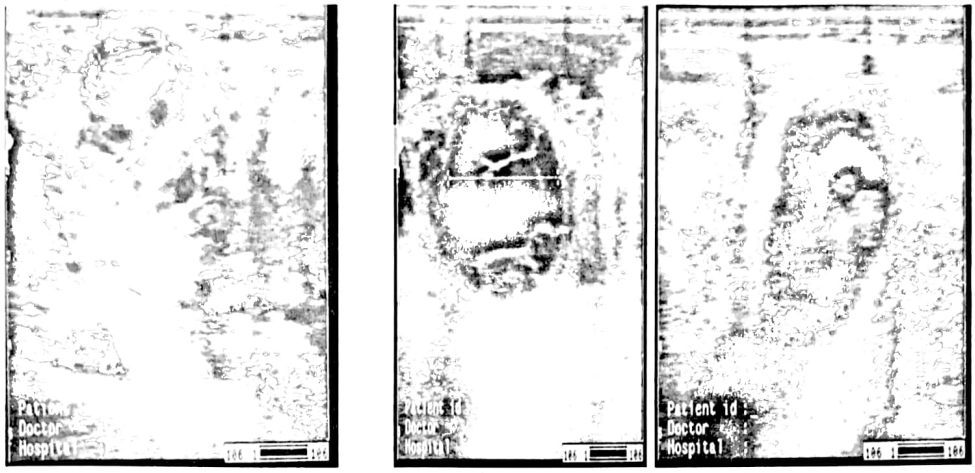
a, b Means within a row with a different letters differ ($P < 0.05$).

Through previous data L-carnitine groups showed the better conception rate compared to untreated group could result from L-carnitine enhancement prostaglandin synthesis and fertilization of the ova. This results were agreement with **Khaled and Ismail (2006)** found that the number and diameter of ovarian follicles were significantly increased in dietary treated groups than control. Also, the number of CL was significantly greater in L-carnitine plus calcium salts of fatty acids (CSFA) than control and L-carnitine supplemented groups.



G2 G1 G0

Figure (1). Represent the ultrasonography of ovarian follicle diameter for (G2, G1 and G0), respectively.



a b c

Figure (2). Represent the ultrasonography of presence of complete CL(a), dominant follicle(b) and incidence of pregnancy(c) in G2.

Conclusion

This study tried to disclose changes in blood parameters and reproductive performance of buffaloes that received L-carnitine. Our results demonstrated that supplemental L-carnitine affected selected biochemical parameters such as cholesterol, urea and glucose which are indicators of energy metabolism. It can be concluded that, L-carnitine can be successively used for buffalo cows during late pregnancy and early lactation to enhance their reproductive status and health.

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الملخص العربى

دراسة تأثير إضافة ل- كارنيتين في مرحلة نهاية الحمل على الأداء الإنتاجي والتناسلي للجاموس

تأثير اضافة ل-كارنيتين على بعض مكونات الدم والاداء التناسلي لإناث الجاموس1-

أسامة مصطفى المالكي1 - خالد إبراهيم إبراهيم زيدان2

قسم بحوث تربية الجاموس1- قسم بحوث تغذية الحيوان2معهد بحوث الانتاج الحيواني-مركز البحوث الزراعية- دقي - جيزة-مصر

تهدف الدراسة الى تقييم اضافة ل-كارنيتين على بعض مكونات الدم والاداء التناسلي لإناث الجاموس في نهاية مرحلة الحمل . شملت الدراسة عدد واحد وعشرين جاموسة (3-4 مواسم) قسمت إلى ثلاث مجاميع (7 حيوانات بكل منهما):

- المجموعة الاولى (G0) لم تتلقى اي اضافات وعوملت كمجموعة ضابطة (كنترول).
- المجموعة الثانية (G1) عوملت بإضافة 5 جم ل- كارنيتين / راس / يوم.
- المجموعة الثالثة (G2) عوملت بإضافة 7جم ل- كارنيتين / راس / يوم. استمرت الدراسة لمدة خمسة اشهر تم خلالها معاملة كلا المجموعتين (G1 و G2) ب ل- كارنيتين قبل الولادة بشهرين واستمرت الى ثلاثة أشهر بعد الولادة خلال الفترة التجريبية، وضعت الحيوانات تحت الملاحظة الدقيقة في أواخر مرحلة الحمل، كذلك نقلت الحيوانات الى الامكان المخصصة للولادة قبل عملية الولادة بيوم. تم جمع عينات الدم أسبوعيا عبر الوريد الوداجي من جميع الحيوانات خلال الفترة التجريبية. وتم ملاحظة حوث الشياع لجميع الحيوانات كذلك تم تسجيل عدد الأيام المفتوحة (DO) و عدد التلقيحات اللازمة للإخصاب (NSPC). وأشارت النتائج إلى أن الجاموس المعامل بإضافة مستويات مختلفة من ل-كارنيتين اظهر تحسن بالنسبة لمكونات الدم في فترة ما بعد الولادة باستثناء التراى جلسريد. كذلك فإن تركيز كلا من البروتينات الكلية والكريتاتين و الألانين اميلو ترانسفيراز في فترة ما قبل الولادة لم تتتأثر معنويا بإضافة ل- كارنيتين . اظهرت الدراسة ايضا ان اضافة ل-كارنتين ادى الى تحسن علامات الشبق و خصوصا الاعتلاء والاستجابة لعملية التدليك ونزول المخاط المهبلي وكثافة النشاط الشبق. اما فيما يتعلق بالأداء التناسلي، فقد سجلت فروق ذات دلالة معنوية بين المجموعات في بعض المقاييس مثل NSPC، CI، الزمن اللازم لسقوط المشيمة، والزمن اللازم لعودة الرحم داخل الحوض، إغلاق عنق الرحم وتماثل قرني الرحم ووقت التبويض. لستنتج من ذلك ان اضافة ل- كارنيتين في مرحلة الحمل المتأخرة ومرحلة الحليب المبكر يحسن من النواحي التمثيلية والتناسلية لإناث الجاموس.