EFFECT OF L-CARNITINE SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF BUFFALO COWS

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SUMMARY

Thirty apparently healthy pregnant multiparous buffalo cows were used starting from two weeks prepartum until 120 days postpartum. They were divided according to their parity and body condition score into two groups. The first group (control, n=10) was fed basal ration while the second group (L-carnitine, n=20) was fed on the same ration supplemented with about 5 gm/head/day of L-carnitine. It was found that, L-carnitine supplementation led to a significant improvement in body condition score, dry matter as well as energy intakes in addition to significant increase in milk production during early lactation. Serum concentrations of keton bodies, bilirubin and AST during the first month of lactation were all significantly reduced in the L-carnitine group compared control. Whereas, serum concentrations of cholesterol and insulin hormone were significantly increased by supplementation. Time intervals to first ovulation as well as first estrus were significantly ($P<0.011$) decreased in buffalo cows receiving L-carnitine supplementation (41.50±7.04 and 62.20±2.27 days, respectively) compared to the non-supplemented control (58.00±2.72 and 75.83±3.90 days, respectively). Similarly, time interval to first service and conception (days open) was significantly ($P<0.01$) reduced by L-carnitine supplementation. The recorded values were 95.83±5.58 and 119.67±3.98 days, respectively in the control group compared with 74.35±1.78 and 81.60±3.44 days, respectively in the L-carnitine group. Number of services per conception was also decreased ($P<0.01$) in buffalo cows receiving L-carnitine (1.40±0.11) compared to the non supplemented control (2.00±0.01).

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 (Hegazy, 1993, Hegazy *et al*., 1995 and 1996 and De Vries *et al.*, 1999).

L-Carnitine is a water-soluble vitamin-like naturally occurring substance (Blum, 1994). It is present in the tissues of all animals, especially in muscle and liver. It is biosynthesized from protein-bound lysine and methionine in the liver. Recently, L-carnitine has been demonstrated to act as a carrier in the transport of activated long chain fatty acids across the membrane of the mitochondria (Scholte *et al*., 1996). Moreover, its role in detoxifying excess intracellular acyle groups and in the production of metabolic energy from a variety of other substances such as fatty acids, pyruvate and ketone bodies add to it importance (Blum, 1994). Also, it stimulates lipolysis and thermogenesis, and acts as a receptor and storage substance for activated fatty acids. In this capacity; it is essential for fat and energy metabolism.

Trials have indicated that, L-carnitine has beneficial effects in both productive and reproductive performance of monogastric species including sows (Harmeyers, 1993 and Freemaut *et al*., 1993), mares (Then and Leibertseder, 1994) and poultry (Leibertseder and Then, 1994). In contrast to monogastric species the energy supply of ruminants

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is based almost entirely on oxidation of short and long chain fatty acids rather than carbohydrates. L-carnitine is therefore, even more important for ruminant than monogastric species. Noseir and EIAmrawi (2000) found an improvement effect of L-carnitine supplementation on fertility of normal and subfertile rams.

The aim of this work was to investigate the effect of L-carnitine supplementation on both productive and reproductive performance of buffalo cows during early lactation.

MATERIALS AND METHODS

Thirty apparently healthy pregnant multiparous buffalo cows were used starting from two weeks prepartum until 120 days postpartum. They were divided according to their parity and body condition score (Surinder *et al.*, 1987) into two groups. The first group (control, n=10) was fed *ad libitum* on a balanced total mixed rations (Table 1) according the feeding standard of buffaloes (Ranjhan and Pathak, 1979). The second group (L-carnitine, n=20) was fed on the same ration supplemented with 5 gm/head/day of L-carnitine (Carniking, Lonza Ltd., Basel, Switzerland). L-carnitine was premixed with mineral and vitamin mixtures before being added to the ration. Monthly samples were taken from the ration for proximate analysis according to AOCA (1984). Unconsumed amounts of TMR were recorded daily for calculation of dry matter (DM) and TDN intake. Body condition scoring was carried out by the same person every month. Milk yield as well as fat% of each animal were recorded weekly.

One week after calving, each buffalo cow was examined rectally twice weekly to detect both uterine and ovarian changes. Estrus was checked twice daily by a prone fertile bull.

Animals coming in heat after 45 days postpartum were naturally serviced and examined rectally 45 days later. Blood samples were taken twice weekly to measure serum progesterone (P4) hormone starting from 2 weeks postpartum until conception. Progesterone was qualified by radioimmunoassay kit supplied by Diagnostic System Laboratories, USA. First ovulations were verified by occurrence of two consecutive observed P4 values > 1.0 ng/ml serum.

Table 1. Ingredients as well as chemical constituent of total mixed rations offered to buffalo cows until 120 days of lactation.

Ingredients	Kg/head/day
Ground com	4.80
Rice bran	3.70
Cotton seed meal	4.00
Mineral mixture	0.034
Vitamin mixture	0.017
Lime stone	0.23
Common salt	0.11
Berseem hay	4.00
Wheat Straw	2.75
Chemical constitutents(on DM basis)	
Dry matter (Kg)	17.00
Crude protein(%)	14.00
Total digestible nutrients(%)	67.00
Calcium(%)	0.70
Phosphorus(%)	0.35

Serum samples taken during the first month postpartum were used for calorimetric determination of ketone bodies (Pawan, 1958) as well as for bilirubin, cholesterol and AST (Aspartate amino-transferase) using kits supplied by Sclavo, Italy. Insulin hormone was determined by radioimmunoassay kit supplied by Diasari, Italy. All data were statistically analyzed according to Snedecor and Cochran (1981).

RESULTS

Response in body condition score, DM and TDN intake as well as milk yield during the first 120 days postpartum are presented in Table 2. Body condition score loss during the first month of lactation was significantly ($P<0.05$) less in buffalo cows receiving L-carnitine than control. DM and TDN intake were significantly greater with L-carnitine administration during the first 2 months of postpartum period. Milk yield (7% fat corrected milk) was significantly ($P<0.01$) higher during the first 120 days of lactation in buffalo cows receiving L-carnitine when compared with control group.

Table 3 showed that serum concentrations of ketone bodies, bilirubin and AST during the first month of lactation were all significantly reduced in the L-carnitine group compared to the control. Whereas serum concentrations of cholesterol and insulin hormone were significantly increased by supplementation.

It is worth mentioning that, two buffalo cows (untabulated data) from each group (2/10, 20% in the control group compared with 2/20, 10% in the L-carnitine group) were suffering from anestrum until the end of the experiment (at 120 days postpartum). The data of such animals was not included in the calculation of the following reproductive traits.

Table 4. represent the effect of L-carnitine supplementation in the time interval from parturition to complete uterine involution, first ovulation, first estrus, first service and days open as well as on number of services per conception. It was found that uterine involution did not significantly vary with L-carnitine supplementation. Time intervals to first ovulation as well as first estrus were significantly ($P<0.01$) decreased in buffalo cows receiving L-carnitine supplementation compared to the non supplemented controls.

Time interval to first service and conception (days open) were significantly ($P<0.01$) reduced by L-carnitine supplementation. Number of services per conception was also decreased ($P<0.01$) in buffalo cows receiving L-carnitine compared to non supplemented controls.

DISCUSSION

Body condition score loss during the first month of lactation was significantly less in buffalo cows receiving L-carnitine than in controls. Similar results were recorded in sows by Freemaut *et al.* (1993) and Harmeyers (1993). A cardinal feature of dairy cows and buffaloes, particularly high-yielding animals, in the first month of lactation is the development of negative energy balance because feed intake is insufficient to meet the nutrient demand for both maintenance and lactation (Hegazy, 1993 and NRC, 2001). As a result of this energy deficiency, buffalo cows mobilize body reserves for milk production and lose condition and weight. The positive effect of L-carnitine supplementation on body condition in the present study is probably a result of more efficient use of fatty acids, promoting glyconeogensis and formation of propionate in the rumen, preventing ketosis as well as ensuring a sustained release of energy for the animal.

The improvement in milk yield in L-carnitine supplemented sows was previously recorded by Freemaut *et al.* (1993) who suggested that the positive effects were probably a result of better energy utilization. Experimental evidence produced by recent research suggests that L-carnitine not only promotes mitochondrial oxidation of fatty acids but also fulfils other biochemical functions. Such functions include acetyl buffers, maintenance of sufficient mitochondrial concentrations of Co-enzyme A under conditions of anaerobic generation of energy, stimulation of both the tricarboxylic acid cycle and the outward transport of ATP from the mitochondria during intensive muscle work (Scholte *et al.*, 1996).

Table 2. Effect of L-carnitine supplementation on performance of buffalo cows.

Items	Control	L-carnitine	Probability
Body condition score change (unit):			
0-30 days postpartum	-0.56 ± 0.03	-0.31 ± 0.03[b]	P<0.05
30-60 days postpartum	+0.31 ± 0.05	+0.43 ± 0.05	NS
60-120 days postpartum	+0.51 ± 0.09	+0.49 ± 0.06	NS
Dry matter intake (Kg/day):			
0-30 days postpartum	12.70 ± 0.38[a]	13.63 ± 0.31[b]	P<0.08
30-60 days postpartum	14.00 ± 0.28[a]	15.10 ± 0.27[b]	P<0.05
60-120 days postpartum	16.45 ± 0.32	16.56 ± 0.21	NS
Total digestible energy intake (Kg/day):			
0-30 days postpartum	8.51 ± 0.26[a]	9.13 ± 0.21[b]	P<0.08
30-60 days postpartum	9.38 ± 0.25[a]	10.11 ± 0.18[b]	P<0.05
60-120 days postpartum	11.02 ± 0.21	11.10 ± 0.14	NS
Daily milk yield (7% FCM#Kg)			
0-30 days postpartum	7.40 ± 0.31[a]	9.64 ± 0.34[b]	P<0.01
30-60 days postpartum	11.83 ± 0.31[a]	13.80 ± 0.35[b]	P<0.01
60-120 days postpartum	9.40 ± 0.34[a]	11.40 ± 0.34[b]	P<0.01

Within rows, means followed by different letters were significantly different at corresponding P.
7% fat corrected milk.

Table 3. Effect of L-carnitine supplementation on serum concentration of some metabolites during the first month of lactation of buffalo cows.

Parameters	Control	L-carnitine	Probability
Keton bodies (mg%)	2.46 ± 0.11[a]	1.46 ± 0.06[b]	P<0.01
Cholesterol (mg%)	81.70 ± 2.71[a]	93.90 ± 1.50[b]	P<0.01
Bilirubin (mg%)	2.69 ± 0.10[a]	1.55 ± 0.05[b]	P<0.05
AST (IU/ml)	69.10 ± 1.80[a]	52.20 ± 1.74[b]	P<0.01
Insulin (IU/ml)	12.90 ± 0.30[a]	15.40 ± 0.25[b]	P<0.05

Within rows, means followed by different letters were significantly different at corresponding P.

Table 4. Effect of L-carnitine supplementation on postpartum reproductive traits of buffalo cows.

Traits	Control	L-carnitine	Probability
Uterine involution (day)	31.00 ± 1.41	28.45 ± 0.90	NS*
First ovulation (day)	8.00 ± 2.72[a]	41.50 ± 7.04[b]	P<0.01
First detected estrus (day)	75.83 ± 3.90[a]	62.20 ± 2.27[b]	P<0.01
First service (day)	95.83 ± 5.58[a]	74.35 ± 1.78[b]	P<0.01
Days open (day)	119.67 ± 3.98[a]	81.60 ± 3.44[b]	P<0.01
Number of services/conception	2.00 ± 0.01[a]	1.40 ± 0.11[b]	P<0.05

Within rows, means followed by different letters were significant at corresponding P.
*Non significant

The results of the present paper show that there were significant elevations in serum ketone bodies, bilirubin, and AST levels in control groups. These alterations in blood composition reflect the metabolic consequence of the high priority accorded milk production of dairy cows in early lactation when appetite is limited and the hormonal environment may predispose to mobilization of 'adipose tissue reserves (Baird, 1982). Baumgartner and Bauman, (1996) suggested that a metabolism overloaded with ketone bodies and their precursor (acetate and butyrate) results in a marked increase in acetyl-CoA and a reduction in free CoA, thereby severely inhibiting the generation of energy through the tricarboxylic acid cycle. This metabokic bottleneck might be significantly alleviated through the increased availability of L-carnitine, which is known to act as a buffer for the acetyl group of acetyl-CoA. Lactating ruminants are unable to make additional supplies of carnitine available. However, the demand for L-carnitine in this situation is disproportionally increased as a result of the secretion of large quantities of L-carnitine in the milk and urine. Nevertheless, a specific ratio of acetyl coenzyme A to free CoA is an essential precondition for normal mitochondrial energy metabolism. Adequate supplies of L-carnitine make the generation of energy more efficient and promote of the transport of ATP from the mitochondria to the cystosol. As a result the metabolism of stored fat (lipolysis) is stimulated to a lesser extent and the energy utilization of ketone bodies improve. Harmeyers (1993) suggested that L-carnitine supplementation increases ketogenesis in the liver, stimulating keton body oxidation by peripheral tissue at the same time. This is coupled with a decrease of ketone body plasma concentration. Moreover, in subclinical ketosis the L-carnitine excretions increase still further (Baumgartner and Bauman, 1996). L-carnitine supplementation in the feed can compensate for any reduction in L-carnitine in dairy cows as a result of metabolic processes.

Concerning insulin level, Bassett *et al*. (1971) reported that peripheral concentrations of insulin are directly proportional to level of feed intake in ruminants. The L-carnitine group in the present study consumed more DM than the control group. Low insulin makes ovarian follicles less responsive to GnRH stimulation (Cornfield *et al*., 1990). Poretsky and Kalin (1987) suggested that insulin affects ovarian tissues similarly to pituitary gonadotropins; these actions include direct effects on steroidogenic receptors number, modulation of gonadotropin receptor number, non-specific enhancement of cell viability and synergism with other gonadotropins. They added that insulin augmented the effect of FSH in stimulating granulosa cell proliferation and enhanced progesterone from small and large follicles.

The reported increase in serum plasma bilirubin in the control group in the current study can result from increased input of bilirubin into plasma or from a decrease in removal from the circulation. It is thought that free fatty acids that increase in early lactation due to the metabolic stress of negative energy balance compete with bilirubin for binding sites on the hepatic transport system and reduced hepatic uptake of bilirubin (Naylor *et al*., 1980). Also, the elevation of the enzyme AST in the control group may increase damage or disruption of the hepatic cell membrane.The reduced cholesterol concentration in the serum of control buffalo cows reflects reduced synthesis and / or secretion of cholesterol by the liver. A condition was previously recorded in cows suffering from fatty liver during early lactation without a concurrent increase in BCS (Reid *et al*., 1983). This may due to reduced hepatic cholesterol synthesis (Brumby *et al*., 1975).

The improvement effect of L-carnitine in buffalo cows fertility is in agreement with previous report, of improved fertility in sows by Freemant *et al.* (1993) and Harmeyers (1993) and in rams by Noseir and ElAmrawi (2000). Recovery or 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 DM 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.

From this work, it can be concluded that, L-carnitine can be successively used for dairy buffalo cows during early lactation to enhance their productive and reproductive status.

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RESEARCH ABSTRACTS

FEEDING AND NUTRITION

A. Kannan, Sajjan Siha and K.R. Yaday. *Department of Animal Nutrition, CCS Haryana Agricultural University, Hisar –125 004, India.* Effect of replacing mustard cake with sunflower cake on milk yield and composition in Murrah buffalo. *Indian Journal of Animal Nutrition* (2003), **20**(2) : 198-201.

Twelve lactating Murrah buffaloes with similar calving period were divided into three groups of four each based on body weight and milk yield in a completely randomized design. The buffaloes were fed iso-nitrogenous and iso-caloric concentrate mixtures containing deoiled sunflower cake replacing mustard cake at 0, 50 and 100 % protein source (T1, T2 and T3) to elucidate the effect on milk yield and milk composition. In addition, weighed quantity of wheat straw (*ad-libitum*) and green oat (20 kg) were fed as per Ranjhan (1998) requirements. The feed intake and milk yield were recorded daily while the milk composition parameters were analysed at weekly intervals. Though the daily dry matter intake was similar, intake of DM per kg milk production was found to be significantly ($P<0.05$) higher at 100% replacement. The daily milk yield as well as 6% FCM and SCM yields did not differ due to dietary variations. Similar trend was observed with respect to milk composition in terms of fat, protein, total solids and SNF. This resulted in comparable cost of