EFFECT OF PRE-PARTUM SUPPLEMENTATION OF VITAMIN E TO MURRAH BUFFALOES ON METABOLIC ADAPTATION AND GROWTH PERFORMANCE OF CALVES


Dairy Cattle Physiology Division
National Dairy Research Institute, Karnal-132 001, India

Received: 01-05-2012
Accepted: 05-12-2012

ABSTRACT

Present study was undertaken in buffalo neonates born to vitamin E (dl-alpha-tocopherol acetate) -supplemented and non-supplemented Murrah buffaloes. Calves from vitamin E-supplemented buffaloes [n= 10; VeC (vitamin E-supplemented calves)] and non-supplemented buffaloes [n= 10; CC (control calves)] constituted the treatment and control groups respectively. Sampling of blood was done on day 0 (before colostrum feeding), 1, 3, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112 and 126 post birth and analyzed for various energy metabolites, hormones, enzymes and growth parameter. Glucose registered a rise at 24h in response to colostrum feeding and was significantly different (P< 0.01) between groups and age. Precolstral plasma NEFA levels were high (P< 0.05) in calves of both the groups at birth. The levels declined significantly (P< 0.01) after colostrum feeding at 24h in VeC and CC. Plasma thyroidal hormones irrespective of the treatment were highest at birth and gradually declined. Alkaline phosphatas e and LDH differed significantly (P< 0.05) between the two groups of calves. Plasma insulin, AST and ALT did not differ between the groups. Birth weight and average daily gain in VeC was significantly higher (P< 0.01) as compared to CC. Present study revealed that the new born calves were physiologically immature at birth and pre-partum supplementation of vitamin E results in improved growth, metabolic and endocrine profile of calves.

Key words: Buffalo calves, Colostrum, Growth, Hormones, Metabolites, Vitamin E.

INTRODUCTION

The neonatal period represents a critical stage in development of physiological functions. During this period, morphological and functional changes are necessary in newborn calves for their survival during extra uterine life (Egli and Blum, 1998). Colostrum intake by calves within the first 24 h of life is needed not only for an adequate immune status (Singh et al., 2013), but also to produce the additional important and favorable effects on metabolic and endocrine traits for viability (Hadorn et. al., 1997). The colostrum intake immediately after birth might have prolonged positive effects on plasma glucose status during first week of life. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization. Greater the extent of negative energy balance, more NEFA will be released from body fat resulting in higher concentration of NEFA in the blood. Sharma et al.(1985) reported high values for insulin during first week of life and thereafter gradually declined until two months of age in male buffalo calves. ALP is released into the blood during injury and normal activities like bone growth and pregnancy. Retskii (2005) recorded peak alkaline phosphatase activity in newborn calves observed immediately after birth. Serum alkaline phosphatase appeared to be from intestinal sources and/or colostrum could be stimulating the intestinal alkaline phosphatase activity (Fay et al., 1981). Vitamin E has been reported to be an immunopotentiator, which not only provides additional immunity to dairy animals when fed peripartum but also has been reported to enhance physiological maturity and calf viability (Amer and Hashem, 2008). Sikka et al. (2002) and Amer and Hashem (2008) observed increased body weight gain, growth rate and viability in the calves born from buffaloes supplemented with minerals and

*Corresponding author's e-mail: dranil02@gmail.com
vitamins. Keeping in view the dynamics of energy metabolites, metabolic hormones and enzymes in newborns the present study was designed to explore the effect of pre-partum supplementation of vitamin E on metabolic status and growth performance of buffalo neonates.

**MATERIAL AND METHODS**

The present study was conducted on twenty Murrah buffaloes in advance state of pregnancy from October 2009 until May 2010. These buffaloes randomly divided into two groups (10 in each group). Control group were fed diet as practiced for pregnant buffaloes in National Dairy Research Institute Karnal dairy farm. The treatment group supplemented with 2000 IU vitamin E (dl-alpha-tocopherol acetate) mixed with moistened concentrate and fed individually in the morning per day with normal diet. Vitamin E supplementation was initiated 30 days prior to expected date of parturition and was continued until parturition. Calves born from these buffaloes (both the groups) were removed from their dams before colostrums ingestion and housed in calf’s pen separately in to two groups after weighing and ear tagging. The colostrums of the same dam were fed to the newborn within 2 hours of birth, which was designated as 0 h and followed subsequent feedings at an interval of 12 h for five days. Calves were fed via nipple bottle and amount of colostrum was supplied as per the body weight of the calf. After five days, all the calves were fed whole milk @ 1/10th of their body weight up to one month (in two equal feedings). The second month, whole milk was reduced to 1/15th and skim milk was provided @ 1/25th of their body weight. Concentrate mixture and some green fodder was offered after three months.

Approximately 10 ml blood was drawn in sterile heparinised and non-heparinised vacutainer tubes from each calf by jugular venipuncture on day 0 (Before colostrum feeding), followed by day 1, 3, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112 and 126 post birth at 6.00 AM in the morning. Plasma and serum was analyzed for energy metabolites (Glucose and NEFA), hormones (Insulin, \(T_3\) and \(T_4\)) and enzymes (ALP, LDH, AST and ALT). Plasma glucose was estimated by using GOD-POD kit (Span Diagnostics Ltd., India) and NEFA was estimated by extraction method (Chloroform: Methanol, 49:49:2) as described by Shipe et al. (1980). Insulin, \(T_3\) and \(T_4\) were determined by RIA method. Serum enzymes were estimated by using kinetic assay kits (pNPP-AMP) for Alkaline phosphatase, (Optimised DGKC) for Lactate dehydrogenase, (Modified UV) for Aspartate aminotransferase (AST) and Alanine amino transferase (ALT) procured from Span Diagnostics Ltd., India.

**Statistical analysis:** The data obtained were subjected to analysis of variance using Harvey’s Least-Squares analysis by computer software package. The general mathematical model was-

\[ Y_{ijk} = \mu + T_i + D_j + e_{ijk} \]

Where, \(Y_{ijk}\) is the response for serum variable, \(\mu\) is the overall mean, \(T_i\) is treatment effect, \(D_j\) is days effect, and \(e_{ijk}\) is the residual with the usual assumptions for errors.

**RESULTS AND DISCUSSION**

Effect of ante-partum supplementation of vitamin E to buffaloes on energy substrates of buffalo calves

**Glucose:** Precocolstral plasma glucose levels were significantly low in calves of both the groups at birth with mean values 57.68± 4.38 mg/dl in CC and 51.29± 4.38 mg/dl in VeC respectively. The levels increased significantly (\(P< 0.01\)) after colostrum feeding at 24h in both the groups. Glucose levels were significantly affected (\(P< 0.01\)) by ante-partum supplementation of vitamin E in VeC as compared to CC. The levels also varied significantly with age (\(P< 0.01\)) in VeC and CC born from ante partum supplemented and non-supplemented mothers. In CC, the glucose concentration remained elevated (\(P< 0.01\)) until day 14 post births while in VeC higher glucose level sustained for up to 28 days (figure 1).

In present study, neonates were born with low glucose levels (hypoglycemic), first colostrum feeding increased the blood glucose level, these finding are in agreement with the finding of Pandita and Madan (2010) in buffalo calves, Kurz and Willett (1991) in cattle calves. Rauprich (2000) reported a rise in glucose concentration from day 1 to day 3 in calves fed colostrum for 3 days followed by milk replacer. Other studies in calves (Grutter and Blum, 1991; Hadorn et al., 1997; Hammon and Blum, 1998) have also reported a rise in pre-prandial plasma glucose concentration. Kurz and Willett (1991) found a substantial increase in glucose concentration in cattle calves from birth to 24h. Piccione et al. (2010) did not find any effect on...
glucose levels during first week and first month in neonatal calves. In contrast, Kurz and Willett (1991) found a substantial increase in glucose concentration in cattle calves from birth to 24h. Thus, ingestion of colostrum or milk replacer was directly correlated with a corresponding rise in plasma glucose. Since all the collections were made at 6.00A.M. in the morning prior to feeding, differences in the levels up to day 28 in experimental and day 14 in control demonstrated calves ability to adapt to extra-uterine conditions for homeostasis. Other studies in calves (Grutter and Blum, 1991; Hadorn et al., 1997; Hammon and Blum, 1998) have also reported a rise in postprandial plasma glucose concentration.

**Non-Est erified Fatty Acids (NEFA):** Precolostral plasma NEFA levels were high (P< 0.05) in calves of both the groups at birth with means amounting to 148.48± 4.45 and 142.24± 4.45 in CC and VeC respectively. The levels declined significantly (P< 0.01) after colostrum feeding at 24h in treated group with mean levels 126.14± 4.45 and 133.41± 4.45 in control group. The concentration further declined on day3 in all the calves of both the groups and remained almost similar afterwards until day 126 post birth. Statistical analysis indicated that levels were significantly affected (P< 0.01) in both the groups (figure 2).

Most of the reports have indicated high values of NEFA declining substantially after first colostrum (Vermorel et al., 1989; Baumrucker and Blum, 1994; Hadron et al., 1997). Others (Hammon and Blum, 1998; Lents et al., 1998) also found high concentrations of NEFA after birth followed by a rapid decline after the first meal. Blum et al. (1997) found an increase in plasma non-esterified fatty acid (NEFA) if colostrum was withheld for the first 24 h after birth than in calves fed colostrum immediately after birth. Since the energy requirements are greatly altered after birth, the calves tend to mobilize fat reserves for meeting energy demands (Lents et al., 1998) and when these calves receive nutrients for meeting their energy demands from colostrum or milk, the level reduces in plasma (Piccioni et al., 2010).

**Effect of ante-partum supplementation of Vitamin E to buffaloes on Endocrine function of buffalo calves**

**Thyroidal Hormones:** Thyroidal hormone (Triiodothyronine and thyroxine) concentration was highest at birth and registered a gradual decline afterwards. The levels were not affected by colostrum feeding in both the groups. The mean level of T$_3$ in CC at birth was 6.24± 0.42 ng/ml. The concentration thereafter declined significantly (P< 0.01) and steadily reached to 2.28± 0.42 ng/ml on day 7 post birth. Later on, the values were nearly identical to adult buffaloes. The experimental calves (VeC) though registered a similar pattern of decline, but concentration remained elevated for almost two months as against 7 days in CC. Statistical analysis indicated that T$_3$ levels were significantly affected.
Previous reports have also shown plasma thyroidal levels to be declining significantly with age (Khurana and Madan, 1984; Grongnet et al., 1985; Sharma et al., 1985). The levels, however, were not influenced by feeding different amount of colostrum, or by delaying colostrum feeding or by fasting (Blum and Hammon, 2000) as found in present investigation also. In contrast, Grongnet et al. (1985) however, reported that levels were influenced by quantity and timings of colostrum feeding. The levels in calves were also not influenced by ante-partum supplementation of vitamin E to buffaloes. Sikka et al. (2002) have also recorded similar observation in newborn buffalo calves.

**Insulin:** Plasma insulin levels were highest at birth in all the calves and registered a massive decline until day 28. Afterwards the levels were almost undetectable. The pattern of decline was identical for both CC and VeC (figure 5).

Hugi et al. (1997) reported that secretory capacity of insulin was not fully developed in neonatal calves. Insulin level in the present investigation did not alter in response to colostrum intake or ante-partum supplementation of vitamin E. Colostral insulin seemed not to be intestinally absorbed even if administered in pharmacological amounts (Grutter and Blum, 1991) in the calves. Hammon et al. (2000)
demonstrated that the plasma insulin concentration was markedly decreased in calves if colostrum feeding was delayed for 12 to 24 h.

Effect of ante-partum supplementation of Vitamin E to buffaloes on enzymatic function of buffalo calves

Alkaline Phosphatase: Serum alkaline phosphatase activity was significantly low in calves in both the groups at birth with mean activity amounting to 27.89±2.16 and 32.54±2.16 IU/L in CC and VeC respectively. The levels increased significantly after colostrum feeding at 24h with mean levels amounting to 35.69±2.16 & 44.87±2.16 IU/L in CC and VeC respectively. Statistical analysis indicated that levels were significantly affected (P<0.05) by ante-partum supplementation of vitamin E. The levels also varied significantly with age (P<0.01) in calves born from ante partum supplemented and non-supplemented mothers (figure 6).

Present study demonstrated low alkaline phosphatase activity prior to feeding, increased significantly after colostrums intake at 24h. The data agrees with earlier reports for cattle (Kurz and Willett, 1991) and buffalo calves (Pandita and Madan, 2010), where the activity was shown to increase significantly in response to colostrum feeding. Kurz and Willett (1991) reported lowest alkaline phosphatase activity immediately after birth enhancing to peak levels by 6h in response to colostrums feeding within 1h followed by a decline until 24 hour. Zanker et al. (2001) demonstrated that plasma ALP transiently increased after colostrum intake and was higher in calves fed colostrum within the first 12h of life than in those fed later after birth. Pandita and Madan (2010) found an increase in serum ALP concentration in buffalo neonates in response to colostrum intake at 24h after birth as compared to precolostral concentration. Kurz and Willet (1991) attributed these hiked levels to be the components of colostrum or intestinal sources and colostrums may be stimulating the intestinal ALP activity. In contrast, Retskii (2005) recorded peak alkaline phosphatase activity in newborn calves immediately after birth. Boyd (1989) was unable to correlate serum alkaline phosphatase activity with other colostrum constituents. Fay et al. (1981) found considerable activity in both duodenum and ileum of newborn calves that never received colostrum. Thus, serum alkaline phosphatase appeared to be from intestinal sources and/or colostrum could be stimulating the intestinal alkaline phosphatase activity. Comparatively higher ALP activity in calves born to supplemented buffaloes were related to higher birth weight of these calves and higher rate of gain in these calves.

Lactate dehydrogenase: Serum lactate dehydrogenase activity was significantly low in calves in both the groups at birth with mean activity 84.95±13.13 and 78.54±13.13 IU/L in CC and VeC respectively. The levels increased significantly after colostrum feeding at 24h in both the groups with
mean levels as 100.98± 13.13 IU/L (control calves) and 98.78± 13.13 IU/L (experimental) in male and female calves respectively. The activity remained significantly elevated (P< 0.01) afterwards also in both the groups. Statistical analysis indicated that levels were significantly affected (P< 0.01) by ante partum supplementation in CC and VeC. The levels did not differ significantly with age (P> 0.01) in both male and female calves born from ante partum supplemented and non-supplemented mothers (figure 7).

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that converts pyruvic acid into lactic acid. This enzyme was reported to be very high in first colostrum which was subsequently reduced to low levels in mature milk (Zanker, 1997). Cavallina et al. 2003 (unpublished data cited by Antonio Borghese, 2005) reported low values (1325 IU/l) for buffalo calves. Piccioni et al. (2010) did not observe significant effect of days of life on LDH activity during first week or first month of life. A transient increase in plasma LDH after intake of first colostrum in neonatal calf indicated that this enzyme was absorbed from colostrum (Zanker, 1997).

Alanine aminotransferase and Aspartate aminotransferase activity

Serum alanine aminotransferase activities in both the groups at birth prior to colostrum feeding were low with mean values amounting to 11.85± 2.46 (control calves) and 12.37± 2.46 IU/L (experimental calves). Serum aspartate aminotransferase averaged 13.08± 1.93 (control calves) and 12.19± 1.93 IU/L (experimental calves). Though the activity increased after colostrum feeding in both the groups the difference was statistically not significant. Afterwards the activity remained static for both of these enzymes. Statistical analysis indicated that activity of these enzymes were not significantly affected (P< 0.01) by ante partum supplementation of vitamin E in both (figure 8-9).

Slight but no significant increased activity of AST and ALT after colostrums intake was found in present investigation. In contrast, Kurz and Willett (1991) found an increase in AST activity with first feeding with levels increasing from 23.0± 2.0 to 80.0± 7.0 IU/L upon feeding of colostrums after 1h suggesting that this enzyme was a component of colostrum. Pauli (1983) found low levels of AST in colostrum thus suggesting that the source of serum AST was from the intestinal brush border. Piccioni et al. (2010) reported a significant effect of days of life on AST levels but not on ALT during the first week or first month of life. Retskii (2005) found significantly higher aspartate aminotransferase activity after colostrum intake in the calves but alanine aminotransferase was within normal limit.

Effect of ante-partum supplementation of Vitamin E to buffaloes on growth performance of calves: The supplemented calves had higher birth
weight as compared to control calves. Difference in birth weight in supplemented calves and control calves was about 3.0 kg. These calves registered a significant increase \((P<0.01)\) in body weight with age. The total weight gain and average daily gain \((ADG)\) was higher in supplemented calves \((P<0.01)\) as compared to control calves (figure 10).

Earlier it has been reported that limited or complete failure of Immunoglobulin transfer can result in suboptimal or diminished growth (Parreno et al., 2004). A close association between body weight gain and acquisition of passive immunity has been reported previously in buffalo calves (Sikka et al., 1996, 1997; Amer and Hashem, 2008). Burdick et al. (2009) associated weight gain in calves with serum Ig concentration. However, Nicola et al. (1996) did not find any improvement in body weight gain of calves born from vitamin E and selenium supplemented cows. Present data is in conformity to the previous observations made with respect to birth weights of the calves (Amer and Hashem, 2008; Sikka et al., 1996, 1997) where a significant increase in birth weight has been recorded in calves born to vitamin E supplemented buffaloes. In the present study, not only the body weights but also the ADG were higher in supplemented calves.

In conclusion, data of this study demonstrate that the newborn calves were metabolically immature at birth and ante-partum administration of vitamin E not only enhanced metabolic and endocrine status but also enhanced growth and ADG in buffalo calves.

**REFERENCES**


