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*Full Length Research Paper*

# Changing certain dietary cationic and anionic minerals: Impact on blood chemistry, milk fever and udder edema in buffaloes during winter

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**This study was conducted to examine the influence of varying dietary cation anion difference (DCAD) on acid base status, mineral dynamics, occurrence of milk fever and udder edema in Nili Ravi buffaloes in a randomized complete block design. Four iso-nitrogenous and iso-caloric diets having -22, -11, +11 and +22 meq/100 g DM DCAD were formulated and designated as high anionic (HA), low anionic (LA), low cationic (LC) and high cationic (HC), respectively. These diets were randomly allotted to 20 Nili Ravi buffaloes which were in their last two months of pregnancy. A linear increase in nutrient intake was recorded with increase in the DCAD level. Buffaloes fed with HA diet had higher nutrient digestibility than those fed with HC diet. Increased blood pH and serum $HCO_3$ were noticed in buffaloes fed with LC and HC diets. Serum ($Na^{+}$ + $K^{+}$) – ($Cl^{-}$ + $S^{--}$) was higher in buffaloes fed with LC and HC diets than those fed with LA and HA diets. Serum calcium and chloride increased with decreased DCAD level while serum magnesium, phosphorus and sulphur remained unchanged. Urine pH was also higher in buffaloes fed with LC and HC diets than those fed with LA and HA diets. Sodium, K, Mg and P balance increased with increased DCAD while its reverse was true for Ca and Cl balance. The concentration of progesterone tended to decrease while estrogen increased before and after parturition. However, their concentrations were more pronounced in buffaloes fed with LA and HA diets than those fed with LC and HC diets. One buffalo from each group fed withLC and HC diets had milk fever. Not a single case of milk fever was observed in buffaloes fed with LA and HA diets. Udder edema and mastitis were either absent or less severe in buffaloes fed with LA or HA diets while the problem was sever in buffaloes fed with LC and MC diets. This study revealed that feeding HA and LA diets prepartum can be a useful nutritional tool to minimize or prevent the incidence of milk fever and controlling udder edema.**

**Key words:** Cationic anionic diets, acid base status, hypocalcemia.

## INTRODUCTION

Early lactation is marked by numerous physiological and biological modifications aimed to ensure sustainable nutrient supply for lactogenosis and these alterations play significant role to shape the overall efficiency of milk and calf crops and thus profitability of dairy animals (Shahzad et al., 2007a). Despite considerable considerations of dairy nutritionists, high yielding dairy animals are subjected to various metabolic disorders like ketosis, fatty liver syndrome and hypocalcemia (milk fever), the last affects animal health status and productivity more adversely and bring heavy economic losses(Shahzad et al., 2008a). The subclinical form of milk fever affects about 50% of all high yielding dairy animals at calving and about 66% of those in their third lactation or greater (Beede et al., 1992). The principal cause for the occurrence of milk fever is the rapid entry of Ca from extracellular fluid pool to the mammary gland which fails to replenish the serum Ca by intestinal Ca absorption or

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**Abbreviations: DCAD,** Dietary cation anion difference; **HA,** high anionic; **LA,** low anionic; **LC,** low cationic; **HA,** high cationic.

bone Ca resorption. Low serum Ca reduces rumen function and also breeds problems like poor immunity and impaired nutrient supply for biosynthetic activities. It also increases the chances of occurrence of other disorders like ketosis, mastitis and uterine prolapse many folds (Curtis et al., 1984).

This disorder can be prevented by taking into account the measures that increase the rate of Ca entry into the extracellular fluid. Among different nutritional tools, mineral manipulation, that is, dietary cation anion difference (DCAD) has been researched more intensively to address the homeostatic regulation of blood calcium to minimize the fall of blood calcium during parturition, the most preventive principle of milk fever. A DCAD alteration alters acid base balance that ultimately enhances the effectiveness of tissue recognition ability of parathyroid hormone (Block, 1994; Wang and Beede, 1992), leading to increased Ca absorption from intestine and Ca mobilization from bones (Schonewille et al., 1994; Espino et al., 2003).

Sufficient scientific literature advocates the favorable effects of low or negative DCAD for preventing occurrence of milk fever in exotic dairy cows. However, more research is needed before specific recommendations can be made regarding optimal DCAD for preventing milk fever because a DCAD value is influenced by numerous factors, including feed intake, season, potential of diet to produce acid and concentrations of other fixed ions etc. Furthermore, direct implementation of the scientific information obtained from the studies executed on exotic dairy cows to the lactating buffaloes, a distinct breed that differs in physiological aspects and reared under different feeding regime and environmental conditions would not be a scientific approach. Therefore, this study was planned to examine the influence of DCAD on nutrients intake and their digestibilities, blood acid base status, hormonal profile, hypocalcaemia and udder edema in Nili Ravi buffaloes during winter, which is a normal breeding season in these animals.

## MATERIALS AND METHODS

The DCAD is the difference between milliequivalents of certain cations ($Na^+$, $K^+$) and anions ($Cl^-$, $S^{--}$) in the whole feed and is calculated with the following equation:

(Na + K) -(Cl + S) = meq/100 g DM…..(Tukker et al., 1992)

Four diets were formulated to have +220, +110, -110 and –220 meq/kg DM DCAD and were represented by high cationic (HC), low cationic (LC), low anionic (LA) and high anionic (HA), respectively. The DCAD levels were attained by using $CaCl_2$ and $NaHCO_3$. All diets were formulated to be iso-nitrogenous and iso-caloric using NRC (2001) values for energy and protein (Table 1). Twenty (20) dry pregnant Nili Ravi buffaloes were randomly allocated to four dietary treatments in a randomized complete block design, five buffaloes in each. Blocking was done according to the expected date of calving. The experiment was conducted during winter (October to January). All animals were in their last four months of pregnancy. First month of the trial was adjustment period while each week after every couple of week of remaining 3 months served as collection period.

The animals were kept on concrete floor in separate pens and no mechanical means were used to control the temperature. Average temperature and relative humidity during the trial were 12.5°C and 73% respectively. The diets were mixed daily and fed twice a day *ad libitum* but at 10% weighback during the collection periods. During every collection period, digestibility, mineral and nitrogen balance trials were conducted. Feed intake was recorded daily and representative samples were taken to analyze ADF, NDF and CP during collection periods. Digestibility was determined by using total collection method. During collection periods, complete collections of urine and faeces were made according to the procedure described by Nisa et al. (2004). The faeces of each animal were collected daily, weighed, mixed thoroughly and 20% of it was sampled and dried at 55°C. At the end of each collection period, dried faecal samples were composited by animal and 10% of the composited samples were taken for analysis. For urine collection, small special metal buckets fitted with plastic pipe were made to surround the vulva and plastic pipe. This plastic pipe ended in a large container (30 L). The urine excreted by each animal was acidified with 50% $H_2SO_4$ and 20% of it was sampled and preserved at -20°C. In the end of each collection period, the preserved urine samples were composited by animal after thawing and 10% of the composited sample was used for analysis. For urine pH, buffaloes were manually stimulated to urinate 3 times during each collection period and a sample of midstream urine was collected in a 30 ml container. Within 10 min of collection, pH was measured. Hand-held urine pH meters (Hach, Loveland, CO) were calibrated before each sampling with pH 7.0, 4.0 and 10 buffer solutions as the standard. Water intake was recorded by drinking water in buckets. A 250 ml water sample was taken in plastic bottle and frozen at –20°C till further analysis of minerals ($Na^+$, $K^+$, $Cl^-$, $S^{--}$, Ca, P and Mg).

Blood was collected twice at 6 and 12 h post feeding during each collection period. Blood samples (12 ml) were drawn from the jugular vein in two heparinized vacuum tubes. One tube was used for blood pH determination (Tucker et al., 1991a). Blood serum was harvested to analyze estrogen (E2), progesterone (P4), cortisol (ELISA method), serum $Na^+$, $K^+$, Ca, P and Mg (AOAC, 1990) and serum $HCO_3^-$ (Harold, 1976). Serum $Na^+$, $K^+$, Ca and Mg were analyzed via atomic absorption spectrophotometry (Model 4, Perkin-Elmer, Norwalk) and P was analyzed photometricaily via Spectronic 1001 (Milton Roy Co., Cincinnati, OH). Mineral balance ($Na^+$, $K^+$, $Cl^-$, $S^{--}$, Ca, P and Mg) was also determined by using equations as described by NRC (2001). Respiratory rate was counted once each period at 4.00 pm following arterial blood sampling. Two individuals counted the respiratory rate of each buffalo for 1 min. Counts exceeding 10% error were conducted a second time (West et al., 1991). Nitrogen balance was done by the difference of N consumed and the sum of fecal N plus urinary N excreted.

Milk fever was observed in buffaloes who exhibited clinical signs from 12 h before to 7 days after parturition. Buffaloes were considered affected by milk fever as serum Ca concentration decreased from 8.0 mg/dl on any day post calving, with or without signs of clinical paresis (Oetzel et al., 1988; Massey et al., 1993). Rating of udder edema was done according to Tucker et al. (1992b) while mastitis scoring was done according to scale devised by Faull and Hughes (1985).

### Statistical analysis

The data were analyzed using Randomized Complete Block Design. In case of any significance, means were separated by Duncan's Multiple Range Test (Steel and Torrie, 1984).

**Table 1.** Ingredients and chemical composition of DCAD diets for prepartum buffaloes.

| **Ingredient** | **DCAD[1] diet (meq/kg of DM)** | | | |
|---|---|---|---|---|
| | **HC** | **LC** | **LA** | **HA** |
| Wheat straw | 60 | 60 | 60 | 60 |
| Corn grain cracked | 8 | 8 | 8.5 | 8.5 |
| Molasses | 8 | 8 | 8.6 | 8.6 |
| Canola meal | 8.2 | 8.2 | 8.71 | 8.71 |
| Sunflower meal | 4.53 | 4.53 | 3.8 | 3.8 |
| Wheat bran | 8.35 | 8 | 5.84 | 5.11 |
| Urea | 1 | 1 | 1 | 1.1 |
| DCP[2] | 1.5 | 1.5 | 1.5 | 1.5 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| $NaHCO_3$ | 0.17 | 0 | 0 | 0 |
| $CaCl_2$ | 0 | 0.52 | 1.8 | 2.43 |
| | | | | |
| **Chemical composition** | | | | |
| $NE_L$/kg | 1.27 | 1.27 | 1.25 | 1.24 |
| CP[3] | 12.7 | 12.7 | 12.5 | 12.5 |
| NDF[4] | 52.4 | 52.3 | 51.3 | 50.9 |
| ADF[5] | 34.3 | 34.2 | 33.8 | 33.7 |
| NFC[6] | 25.8 | 25.7 | 25.8 | 25.4 |
| Ca | 0.7 | 0.9 | 1.3 | 1.5 |
| P | 0.6 | 0.6 | 0.6 | 0.6 |
| Na | 0.25 | 0.2 | 0.2 | 0.22 |
| K | 1.58 | 1.57 | 1.57 | 1.50 |
| Mg | 0.25 | 0.25 | 0.24 | 0.24 |
| Cl | 0.54 | 0.86 | 1.64 | 2.02 |
| S | 0.22 | 0.22 | 0.22 | 0.22 |
| DCAD (meq/kg DM) | 220 | 110 | -110 | -220 |

[1]Dietary cation anion difference {(Na + K) – (Cl + S)}. [2]Dicalcium phosphate.[3]Crude protein.[4]Neutral detergent fiber. [5]Acid detergent fiber. [6]Non-fermentable carbohydrate. HC, High cationic; LA, low cationic; HA, high anionic; LA, low anionic.

## RESULTS AND DISCUSSION

### Nutrients intake and digestibilities

The DMI increased in buffaloes fed on LC and HC diets.Average DMI of animals fed on HC, LC, LA and HA diets were 13.95, 13.20, 12.51 and 12.09 kg/day, respectively (Table 2). Maximum and minimum DMI was recorded in buffaloes fed on HC and HA diets, respectively. These findings were consistent with Moore et al. (2000), who reported that increasing DCAD resulted in significant increase in DMI. Similarly, West et al. (1991) also observed increased DMI at high DCAD level. Increased DMI with increase in the DCAD level might be attributed to increased blood $HCO_3$, acid base status of the animals and increased rumen pH at high DCAD level (Tucker et al., 1991). High DCAD tends to make the rumen pH alkaline which helps the ruminal cellulolytic microbes perform efficiently and the experimental diets in this study contain 60% wheat straw. Significant increase in DMI with increasing DCAD level has also been reported by others workers (Shahzad et al., 2008b; Shahzad et al., 2007b; Delaquis and Block, 1995b; Lemma et al., 1992; Tucker et al., 1988).

Anionic DCAD diets reduced DMI. The possible explanation of reduced DMI at low DCAD diets might be the acidogenic nature of these diets and inclusion of anionic salts. These salts, being unpalatable, might have reduced the palatability of the diet.

Digestibility of DM increased significantly in buffaloes fed on HA diet compared to those fed on LA, LC and HC diets. Average DM digestibility was 63.02, 63.64, 65.27, and 65.19% with HC, LC, LA and HA diets, respectively (Table 2). Minimum and maximum DM digestibility was observed in buffaloes fed on HC and HA diets, respectively. Similarly, NDF, ADF and CP digestibilities were also higher in buffaloes fed on HA diet compared to those fed on HC diet. The probable explanation of increased digestibility in animals fed on low DCAD level might increase rumen retention because of reduced feed intake

**Table 2.** Nutrients intake and digestibilities as influenced by varying DCAD in prepartum buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| DM[2] (kg/day) | 13.95 [a] | 13.20[ab] | 12.51[b] | 12.09[c] | 0.31 |
| Digestibilities % | 63.02[c] | 63.04[b] | 65.17[ab] | 65.39[a] | 0.41 |
| CP[3] (g/day) | 1771.65[a] | 1676.4[ab] | 1563.75[b] | 1511.25[c] | 52.21 |
| Digestibilities% | 71.01[c] | 71.09[b] | 72.16[ab] | 72.56[a] | 0.18 |
| NDF[4] (g/day) | 7309.8[a] | 6903.6[ab] | 6417.63[b] | 6153.8[c] | 235.51 |
| Digestibilities% | 60.8[c] | 60.1[b] | 61.52[ab] | 62.12[a] | 0.21 |
| ADF[5] (g/day) | 4784.8[a] | 4514.4[ab] | 4228.4[b] | 4074.33[c] | 141.41 |
| Digestibilities % | 61.10[c] | 61.11[b] | 61.88[ab] | 62.85[a] | 0.32 |

Means within the same row having different subscripts differ significantly ($P<0.05$).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.[2]Dry matter. [3]Crude protein. [4]Neutral detergent fiber. [5]Acid detergenfiber.

**Table 3.** Nitrogen balance as influenced by varying DCAD in prepartum buffaloes.

| Parameter | Diet[1] | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 283.46[a] | 268.22[b] | 250.20[c] | 241.80[d] | 7.12 |
| **Feces** | | | | | |
| Excretion(g/day) | 75.61[a] | 69.52[b] | 64.8[c] | 61.25[d] | 8.37 |
| Apparent absorption (g/day) | 207.85[a] | 198.70[b] | 185.4[c] | 180.55[d] | 7.65 |
| % of intake | 73.32 | 74.08 | 74.10 | 74.67 | 2.27 |
| **Urine** | | | | | |
| Excretion(g/day) | 58.7[a] | 57.05[ab] | 56.74[b] | 55.45[b] | 2.68 |
| % of intake | 28.24 | 28.71 | 30.60 | 30.71 | 0.76 |
| Balance(g/day) | 149.15[a] | 141.65[b] | 128.66[c] | 125.1[c] | 3.84 |

[a, b, c, d] Means in a row with different superscripts differ significantly ($P < 0.05$). [1]HC, LC, LA and HA stand for high cationic, low cationic, low anionic and high anionic diets, respectively.

A positive co-relation between digestibility and rumen retention time was also documented (Sarwar et al., 1996). However, in contrast to this findings, Delaquis and Block (1995a) reported that digestibility of nutrients remained unaltered by DCAD alteration. Tucker et al. (1991) also reported that DCAD has non-significant effect on ruminal fermentation pattern in dairy cows.

Nitrogen intake increased significantly with increased DCAD. The nitrogen intake was 283.46, 268.224, 250.2 and 241.8 g/day at HC, LC, LA and HA diets, respectively (Table 3). The maximum (283.46 g/day) and minimum (241.8 g/day) nitrogen intake was observed in buffaloes fed on HC and HA diets, respectively. Fecal and urinary N excretion increased with increasing DCAD levels (Table 3). There was significant increase in fecal and urinary N excretions in buffaloes fed on high DCAD diets compared with those fed on low DCAD diets. Increased N intake in buffaloes fed on high DCAD diet was due to increased DMI. Nitrogen balance was minimum (125.1 g/day) and maximum (149.15 g/day) in animals fed HA and HC diets, respectively (Table 3). In contrast to this study, Delaquis and Block (1995a) reported non-significant effect of DCAD on N balance in cows during late lactation. This may be because acid base disturbances due to DCAD were not enough in their study that can change N metabolism (May et al., 1986; Phromphetcharat et al., 1981; Welbourne, 1988).

## Acid base balance

Blood pH increased linearly with increase in DCAD level. The blood pH was maximum (7.43) and minimum (7.34) in buffaloes fed on HC and HA diets, respectively (Table 4). Similarly, $HCO_3^-$ was also increased with increase DCAD. Buffaloes fed on HC and HA diets had maximum (27.52 mmol/L) and minimum (23.12 mmol/L) blood $HCO_3^-$ respectively (Table 4).

The results of this study are consistent with those of Jackson et al. (1992) who reported that at low DCAD level

**Table 4.** Acid base balance and serum minerals as influenced by varying DCAD in prepartum buffaloes

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Blood pH | 7.432[a] | 7.425[ab] | 7.341[b] | 7.301[c] | 0.004 |
| $HCO_3$(mmol/L) | 27.52[a] | 26.01[ab] | 24.22[b] | 23.12[c] | 1.12 |
| Na (meq/L) | 144.84 | 144.16 | 143.91 | 143.85 | 0.76 |
| K(meq/L) | 4.24 | 4.24 | 4.23 | 4.22 | 0.38 |
| Cl(meq/L) | 98.12[c] | 99.75[b] | 101.12[ab] | 102.91[a] | 0.18 |
| S(meq/L) | 1.55 | 1.55 | 1.54 | 1.53 | 0.14 |
| Serum(Na+ K)– (Cl+S) (meq/L) | 49.41[c] | 47.10[b] | 45.48[a] | 43.63[a] | 1.05 |
| Ca (mg/dl) | 9.43[b] | 9.82[b] | 10.14[a] | 10.18[a] | 0.18 |
| Mg (mg/dl) | 2.61 | 2.63 | 2.65 | 2.64 | 0.13 |
| P(mg/dl) | 6.54 | 6.56 | 6.57 | 6.56 | 0.13 |
| Urine pH | 7.81[a] | 7.50[ab] | 6.47[b] | 5.95[c] | 0.35 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na +K) – (Cl + S)}.

blood pH and $HCO_3^-$ were low in dairy calves compared to those fed on diets containing high DCAD levels. Fredeen et al. (1988) also noticed significant depression in the $H^+$ concentration while concentration of $HCO_3^-$ increased with increasing DCAD from 0.7 to 90 meq/100 g of DM in pregnant and lactating goats. Blood $HCO_3^-$ response is inversely related to $H^+$ change that reflects the metabolic nature of the low DCAD diet. The phosphate and ammonia buffer system function for hydrogen ion excretion. Hydrogen ions combine with phosphate or ammonia after entering the renal tubules and a bicarbonate ion is formed that enters the extra-cellular fluids to further buffer acid in the extracellular fluids (Guyton, 2000).

Reduced blood pH and $HCO_3^-$ at low DCAD diets were also observed by Tucker et al. (1991). Similar results were reported by Moore et al. (2000) in prepartum and postpartum cows. This might be attributed to the acidogenic properties of low or negative DCAD diets. Among anionic salts, calcium chloride is mainly used as anionic source that has more acidifying properties than other salts and chloride is excreted in exchange of $Na^+$. When it is in excess, it is excreted with $HCO_3^-$ which reduced blood bicarbonate and increased $H^+$ that overcome the capacity of kidneys to excrete $H^+$ resulting in slight metabolic acidosis.

## Serum minerals

Serum Ca concentration increased linearly as the DCAD level decreased. It was maximum (10.98 mg/dl) in buffaloes fed on HA diet and minimum (9.43 mg/dl) in buffaloes fed on HC diet (Table 4). Increased serum Ca in buffaloes fed on LA and HA diet might be attributed to its higher dietary calcium concentration (1.3 to 1.5%) compared to all other DCAD diets. It may also be attributed to increased calcium absorption from the alimentary tract (Lomba et al., 1978) and calcium mobilization from bones (Joyce et al., 1997) due to mild metabolic acidosis, induced by this negative DCAD diet.

The results of this study are in line with Tucker et al. (1992) who reported that plasma Ca concentration increased linearly with decrease DCAD level in the diet. Feeding a low DCAD increased the flow of Ca through the readily exchangeable Ca pool (Takagi and Block, 1988) and enhanced the concentration of ionized Ca in the blood (Oetzel et al., 1988). Similar findings were also reported by Jackson et al. (1992) who observed increased serum Ca concentration in calves at low DCAD level (0 meq/100 g of DM) compared to those fed on diets containing high DCAD levels (21, 37 and 52 meq/100 g of DM). Tucker et al. (1991) also reported that low DCAD diets increased plasma Ca concentration in dairy cattle. This might be associated with increased Ca absorption (Verdaris and Evans, 1975) and increased Ca mobilization from bones (Fredeen et al., 1988) due to slight metabolic acidosis. A negative DCAD diet tends to increase the serum calcium, directly by calcium mobilization from bones and indirectly through increased absorption from the intestine due to increased synthesis of $1,25(OH)_2D_3$ (Block, 1994).

Serum $Na^+$ concentration increased linearly with increase in the DCAD levels. It was maximum (144.84) and minimum (138.57) in buffaloes fed on HC and HA diets, respectively (Table 5). The findings of this study are in line with Tucker et al. (1988) who reported increased plasma $Na^+$ at high DCAD levels. The plausible explanation of this might be increased uptake of Na at high DCAD level. Serum $K^+$ concentration remained unaffected (4.22 to 4.24 meq/L) by DCAD levels (Table 4). These results are consistent with those of Tucker et al. (1988) who observed non-significant effect of altering DCAD levels on plasma $Na^+$ and $K^+$ concentrations in lactating cows and calves, respectively. A linear increase in serum $Cl^-$ concentration was observed with decrease in

**Table 5.** Calcium balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 97.65$^{c}$ | 118.8$^{b}$ | 162.63$^{ab}$ | 181.35$^{a}$ | 10.11 |
| **Faeces** | | | | | |
| Excretion (g/day) | 56.51$^{c}$ | 67.36$^{b}$ | 85.12$^{ab}$ | 91.21$^{a}$ | 6.32 |
| Apparent absorption (g/day) | 41.14$^{c}$ | 51.44$^{b}$ | 77.51$^{ab}$ | 90.14$^{a}$ | 9.16 |
| **Urine** | | | | | |
| Excretion (mg/day) | 601.1$^{c}$ | 782.32$^{b}$ | 1521.1$^{ab}$ | 2019.23$^{a}$ | 21.11 |
| **Apparent retention (g/day)** | 40.54$^{c}$ | 50.66$^{b}$ | 75.99$^{ab}$ | 88.121$^{a}$ | 4.75 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

the DCAD levels. It was maximum (103.4 meq/L) in buffaloes fed on HA diet and minimum (98.3 meq/L) in buffaloes fed on HC diet. Similarly, Tucker et al. (1988) reported a linear increase in plasma $Cl^-$ concentration with decrease in the DCAD levels (+20 to -10 meq/kg of DM). This might be due to the fact that $CaCl_2$ supplementation in the diet increased $Cl^-$ concentration in the plasma (Tucker et al., 1991). Also observed similar effects of DCAD on plasma $Cl^-$ concentration in growing cattle.

Serum DCAD ($Na^+$ + $K^+$) – ($Cl^-$ + $S^{--}$) increased linearly with increasing DCAD level in the diets and these findings were in line with Tucker et al. (1988) and Hu and Murphy (2004). This was due to increased Cl content as DCAD level decreased in the diet. Blood $Cl^-$ and $HCO_3^-$ are inversely related to each other (Hu and Murphy, 2004). So, increased $Cl^-$ resulted in reduced blood pH and $HCO_3^-$.

Serum Mg remained unaffected by DCAD levels (Table 4). In contrast to this study, Jackson et al. (1992) reported a linear decrease in Mg concentration as the DCAD level increased. Serum P concentration remained unaltered at all levels of DCAD, which revealed the lack of marked effect of DCAD on P concentration in the blood. These results are in concordance with Delaquis and Block (1995a) who reported that plasma P was not affected by DCAD. No effect of DCAD was observed on serum $S^{--}$ concentration (Table 4). However, Delaquis and Block (1995b) reported that plasma $S^{--}$ concentration increased at low DCAD, which is due to the reason that $S^{--}$ is regulated renally, not intestinally that increased plasma S concentration.

## Urine pH

Urine pH increased linearly with increasing DCAD level in the diet. It was 7.81, 7.50, 6.47 and 5.95 with HC, LC, LA and HA diets, respectively (Table 4). Urine pH was highest (7.81) and lowest (5.95) in buffaloes fed on HC and HA diets, respectively. These results are consistent with Tucker et al. (1988) who reported that increasing DCAD levels in lactating cows increased urine pH. This increased urine pH at high DCAD levels might be due to decreased concentrations of $H^+$ and increased $HCO_3^-$ concentration (Fredeen et al., 1988; Tucker et al., 1988). West et al. (1991) reported a quadratic response in urine pH by increasing DCAD levels that indicated alkalinity of the diets. Increased urine pH at high DCAD diets may be associated with increased blood $HCO_3^-$ concentration and reduced serum $Cl^-$ concentration. Excretion of each $HCO_3^-$ carries $Na^+$ or other cations for renal rectification of alkalosis (Guyton, 2000). Reduced urine pH in low DCAD diets is associated with acidogenic properties of $CaCl_2$. Similar results were also observed by Merck (1983) and Tucker et al. (1988) who reported that decreased urine pH at low DCAD diets may be due to acidogenic properties of $CaCl_2$. Low DCAD diets resulted in increased $Cl^-$ concentration in the diet and increased urinary excretion of $Cl^-$. So much variation in urine pH is due to the animal 's' attempt to eliminate any change in the blood by excreting acidic or alkaline urine.

## Minerals balance

Fecal excretion of Ca increased as DCAD level decreased in the diet. It was minimum (58.8 g/day) and maximum (125.7 g/day) in buffaloes fed on HC and HA diets, respectively (Table 5). Similarly, urinary Ca also increased with decreasing DCAD. It was minimum (0.78 g/day) and maximum (1.16 g/day) in buffaloes fed on HC diet compared to those fed on HA diet (Table 5). It might be attributed to low Ca intake at high DCAD level. The findings of this study are consistent with other researchers (Fredeen et al., 1988; Gaynor et al., 1989; Takagi

**Table 6.** Phosphorus balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 83.7[a] | 79.2[b] | 75.06[b] | 72.54[b] | 1.78 |
| **Faeces** | | | | | |
| Excretion (g/day) | 68.52[a] | 63.41[ab] | 61.32[b] | 60.12[b] | 1.45 |
| Apparent absorption (g/day) | 15.18[a] | 15.79[a] | 13.74[b] | 12.42[b] | 0.78 |
| **Urine** | | | | | |
| Excretion (mg/day) | 37[c] | 42[b] | 49[ab] | 62[a] | 3.11 |
| **Apparent retention (g/day)** | 15.14 | 15.74 | 13.69 | 12.36 | 0.99 |

Means within the same row having different subscripts differ significantly ($P<0.05$).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

**Table 7.** Sodium balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 34.87[a] | 26.4[ab] | 25.04[b] | 26.6[b] | 8.47 |
| **Faeces** | | | | | |
| Excretion (g/day) | 6.41[a] | 4.41[ab] | 3.75[b] | 3.75[b] | 2.47 |
| Apparent absorption (g/day) | 28.46[a] | 21.99[b] | 21.27[b] | 22.65[b] | 5.19 |
| **Urine** | | | | | |
| Excretion (mg/day) | 16642[a] | 10522[b] | 11541[b] | 11521[b] | 2.67 |
| **Apparent retention (g/day)** | 11.82[a] | 11.47[ab] | 9.73[b] | 8.13[b] | 3.12 |

Means within the same row having different subscripts differ significantly ($P<0.05$).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

and Block, 1991) who reported that decreasing DCAD level in the diet resulted in increased urinary Ca. Increased urinary Ca is associated with its high plasma concentration, which is filtered through kidneys (Takagi and Block, 1988). The findings of this study were not consistent with Delaquis and Block (1995a) who observed non-significant difference in urinary Ca excretion with altering DCAD levels. This might be attributed to small difference in DCAD levels used in their study. The Ca balance was minimum (38.07 g/day) and maximum (54.49 g/day) in buffaloes fed on HC and HA diets respectively (Table 5).

Decreased excretion of fecal P was observed with decreasing DCAD level in the diet. It was maximum (68.14 g/day) and minimum (61.05 g/day) in buffaloes fed on HC diet and HA diets, respectively (Table 6). Similarly, urinary excretion of P was higher (0.061 g/day) in buffalos fed on HC diet compared to those (0.047 g/day) fed on HA diet (Table 6). The P balance was minimum (11.49 g/day) in buffaloes fed on HA diet and maximum (15.56 g/day) in buffaloes fed on HC diet (Table 6). The results of this study were in line with Delaquis and Block (1995) who reported that increasing DCAD level in the diet increased P absorption and balance in cows during late lactation.

Buffaloes fed on HC diet had higher intake and excretion of $Na^+$ and $K^+$ while these were lower in buffaloes fed on HA diet (Tables 7 and 8). Tucker et al. (1988) reported increased excretions of $Na^+$ and $K^+$ as DCAD increased from –10 to +20 meq/100 g of DM. This increased $Na^+$ and $K^+$ concentrations at high DCAD diets is due to their dietary concentrations as urinary composition is closely associated with diet composition. These findings are in line with West et al. (1992) who reported increased urinary $Na^+$ and $K^+$ concentration at high DCAD diets while its reverse was true for $Cl^-$ concentration. The minimum (9.5 vs. 134.11 g/day) and maximum (12.42 vs. 150.56 g/day) $Na^+$ and $K^+$ balances

**Table 8.** Potassium balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 220.41[a] | 207.24[ab] | 196.41[b] | 181.35[c] | 7.11 |
| **Faeces** | | | | | |
| Excretion(g/day) | 23.16[a] | 20.77[b] | 21.41[b] | 20.11[b] | 0.46 |
| Apparent absorption (g/day) | 197.25[a] | 186.47[ab] | 175[b] | 161.24[c] | 7.31 |
| **Urine** | | | | | |
| Excretion (mg/day) | 17912[a] | 15462[b] | 15460[b] | 15550[b] | 6130 |
| **Apparent retention (g/day)** , g/day | 179.34[a] | 171.01[ab] | 158.9[b] | 145.82[c] | 4.73 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

**Table 9.** Chloride balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake (g/day) | 75.33[c] | 113.52[b] | 205.16[ab] | 244.22[a] | 10.22 |
| **Faeces** | | | | | |
| Excretion (g/day) | 20.17[b] | 34.51[b] | 68.52 [a] | 81.12[a] | 8.13 |
| Apparent absorption (g/day) | 54.62 | 79.01 | 136.64 | 163.1 | 11.12 |
| **Urine** | | | | | |
| Excretion (mg/day) | 25123[a] | 40121[b] | 72541[b] | 92145[b] | 10.11 |
| **Apparent retention (g/day)** | 29.5[a] | 38.89[b] | 64.09[b] | 70.95[b] | 3.77 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

were observed with HA and HC diets respectively (Tables 7 and 8). At low DCAD, there was more excretion of $Cl^-$ (Table 9). This may be due to the fact that $CaCl_2$ was used to reduce the DCAD of the diet and at low DCAD diets; there was increased intake of $Cl^-$, which was manifested in urine and feces. These findings were in agreement with Tucker et al. (1988) and West et al. (1992) who reported decreased $Cl^-$ excretion at high DACD level and as the DACD reduced, $Cl^-$ concentration increased in the urine. Ammonium ions require $Cl^-$ and other anions for its excretion through urine to neutralize the blood. The minimum (26.36 g/day) and maximum (99.02 g/day) $Cl^-$ balance was observed with HC and HA diet, respectively (Table 9).

Urinary excretion of Mg increased with decreasing DCAD level in the diet (Table 10). It was maximum (3.98 g/day) and minimum (3.31 g/day) in buffaloes fed on HA and LC diets respectively. This might be attributed to increased Mg absorption at low DCAD diets. At high DCAD level, K inhibits Mg absorption (Greene et al., 1983; Schonewille et al., 1997). During metabolic acidosis, 25-$(OH)_2D_3$ is changed more effectively to its active form, which is 1,25 $(OH)_2D_3$ and releases more Mg in the urine (Chamberlain and Wilkinson, 1996). The findings of the present study contradicted Delaquis and Block (1995) who observed higher intake and excretion of Mg in animals offered high DCAD diets. However, in this study, the Mg balance remained unaltered across all diets (Table 10). Gaynor et al. (1989) observed an increase in urinary excretion of Mg as the DCAD level increased from 220 to 500 meq/kg of DM. Buffaloes fed on LC and HC diets had increased fecal and urinary excretion of $S^{--}$ compared to those fed on LA and HA diets (Table 11). This might be due to increased intakes at high DCAD levels. The $S^{--}$balance was improved in buffaloes fed on high DCAD diets than those fed on low

**Table 10.** Magnesium balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake(g/day) | 34.87$^{a}$ | 33.0$^{ab}$ | 30.02$^{b}$ | 29.02$^{c}$ | 1.03 |
| **Faeces** | | | | | |
| Excretion(g/day) | 24.53$^{a}$ | 22.11$^{ab}$ | 18.52$^{b}$ | 17.11$^{c}$ | 0.65 |
| Apparent absorption (g/day) | 10.34 | 10.89 | 11.50 | 11.91 | 0.57 |
| **Urine** | | | | | |
| Excretion (mg/day) | 3444$^{c}$ | 3651$^{b}$ | 4211$^{ab}$ | 5811$^{a}$ | 68.33 |
| **Apparent retention (g/day)** | 7.0 | 7.24 | 7.29 | 6.1 | 0.38 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}

**Table 11.** Sulpher balance as influenced by varying DCAD level in buffaloes.

| Parameter | DCAD[1] diet (meq/kg of DM) | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| Intake(g/day) | 30.69$^{b}$ | 29.04$^{b}$ | 27.52$^{a}$ | 26.6$^{a}$ | 1.01 |
| **Faeces** | | | | | |
| Excretion(g/day) | 14.91$^{b}$ | 13.65$^{b}$ | 12.55$^{a}$ | 11.8$^{a}$ | 1.21 |
| Apparent absorption(g/day) | 15.78$^{b}$ | 15.39$^{b}$ | 14.97$^{a}$ | 14.8$^{a}$ | 2.45 |
| **Urine** | | | | | |
| Excretion (mg/day) | 6133$^{c}$ | 5581$^{b}$ | 5014$^{a}$ | 4948$^{a}$ | 4.52 |
| **Apparent retention (g/day)** | 9.647 | 9.809 | 9.956 | 9.85 | 1.02 |

Means within the same row having different subscripts differ significantly (P<0.05).[1]Dietary cation anion difference {(Na + K) – (Cl + S)}.

DCAD diets (Table 11).

## Hormonal profile

The level of progesterone decreased few days before parturition (Table 12). It is a hormone that has vital role in pregnancy maintenance. It relaxes uterine smooth muscle and allows maintenance of pregnancy. Decrease in serum progesterone level few days before parturition is a normal phenomenon and is well documented (Willcox et al., 1983; Houdebine, 1985). Decrease in progesterone level results in smooth muscle contraction by activating the endogenous prostaglandin pathway (Csapo et al., 1973). It assists in the release of lysosomal phospholipase, which enhances prostaglandin production. It also inhibits the estradiol receptors and increases oxytocin receptors in the myometrium; Csapo et al., 1973).

Estradiol concentration increased after parturition to support lactogenesis (Table 12). It stimulates prolactin secretion from anterior pituitary gland and this process favours lactogenesis (Nagasawa, 1969; Tucker, 2000). Houdebine (1985) and Willcox et al. (1983) also reported increased estradiol before and after parturition. The onset of parturition is supported by changes in estrogen: progesterone (Challis, 1971; Liggins et al., 1973; Darne et al., 1987). Decreased progesterone and increased estradiol help in fetus expulsion (Salah, 1994).

Serum cortisol increased around parturition and this elevation was more pronounced in buffaloes fed on LA and HA diets (Table 12). The increased levels of cortisol at negative DCAD diets might be due to high $Cl^-$ content that induced metabolic acidosis. Espino et al. (2005) observed that serum cortisol level increased around parturition in sheep fed anionic diets compared to those

**Table 12.** Effects of different DCAD levels on estradiol (pg/ml), progesterone (ng/ml) and cortisol (ng/ml) concentrations at various intervals before and after parturition in buffaloes.

| Parameter | Days from parturition | | | | | |
|---|---|---|---|---|---|---|
| | -5 | 0[1] | 1 | 2 | 5 | 10 |
| **HC** | | | | | | |
| $E_2$ | 1.98 | 2.47 | 4.55 | 4.42 | 4.30 | 4.50 |
| $P_4$ | 1.25 | 1.33 | 0.23 | 0.67 | 0.51 | 0.43 |
| C | 12.20 | 15.54 | 14.67 | 13.18 | 11.55 | 10.20 |
| **LC** | | | | | | |
| $E_2$ | 1.74 | 2.25 | 4.52 | 4.29 | 4.42 | 4.823 |
| $P_4$ | 1.35 | 1.20 | 0.19 | 0.71 | 0.45 | 0.32 |
| C | 13.80 | 17.63 | 14.80 | 13.70 | 12.15 | 11.05 |
| **LA** | | | | | | |
| $E_2$ | 1.69 | 2.05 | 4.13 | 4.10 | 4.60 | 4.53 |
| $P_4$ | 1.22 | 0.95 | 0.31 | 0.61 | 0.25 | 0.37 |
| C | 16.63 | 21.48 | 17.5 | 14.63 | 12.90 | 12.10 |
| **HA** | | | | | | |
| $E_2$ | 1.76 | 2.12 | 4.30 | 3.97 | 4.01 | 4.62 |
| $P_4$ | 1.19 | 1.01 | 0.25 | 0.52 | 0.41 | 0.31 |
| C | 18.30 | 23.07 | 19.40 | 16.56 | 14.35 | 13.78 |

[1]Day of calving. HC, LC, LA and HA stand for high cationic, low cationic, low anionic and high anionic diets, respectively.

**Table 13.** Effect of varying levels of DCAD on serum calcium (mg/dl) concentration (mean $\pm$SD) at various intervals before and after parturition.

| DCAD (meq/kg of DM) | Day from parturition | | | | | |
|---|---|---|---|---|---|---|
| | -5 | 0[1] | 1 | 2 | 5 | 10 |
| -220 | 9.98 $\pm$ 0.14 | 9.88$\pm$0.14 | 9.88$\pm$0.17 | 10.21$\pm$ 0.11 | 10.11$\pm$0.19 | 10.52$\pm$0.18 |
| -110 | 9.99$\pm$ 0.06 | 9.79$\pm$0.13 | 9.84$\pm$0.18 | 10.11$\pm$ 0.17 | 10.12$\pm$0.22 | 10.41$\pm$0.17 |
| +110 | 9.74$\pm$ 0.08 | 9.69$\pm$0.11 | 9.42*(7.16[2])$\pm$ 0.14 | 9.58$\pm$0.18 | 9.34$\pm$0.16 | 9.77$\pm$ 0.12 |
| +220 | 9.42$\pm$ 0.06 | 9.33$\pm$0.12 | 9.21*(6.71[2])$\pm$0.14 | 9.68$\pm$0.15 | 9.36$\pm$0.12 | 9.54$\pm$ 0.12 |

1Day of calving. 2Serum Ca level in milk fever affected animal. *One from each of these buffaloes got milk fever (<8 mg/dl)

fed on cationic diets. Increased serum cortisol level around parturition has also been reported in cattle (Seren et al., 1977; Hudson et al., 1975; Heuwieser et al., 1987), goats and sheep. The elevation in cortisol is positively associated with phase of parturition and it might be due to increased need for glucocorticoids, which enhance mammary growth and favour lactation.

## Hypocalcemia

Incidence of milk fever was observed in buffaloes fed on high DCAD, that is, HC and LC diets, one from each group. Minimum (9.43 mg/dl) and maximum (10.78 mg/dl) serum Ca level was observed in HA and HC diets, respectively (Table 13). Milk fever affected animals had 7.16 and 7.43 mg/dl serum Ca level. Not a single milk fever case was observed in buffaloes fed on low DCAD that is, LA and HA diets. The findings of this study supported the findings of other researchers (Block, 1994; Lomba et al., 1978; Dishington, 1975; Ender et al., 1971) who reported occurrence of milk fever in animals fed on high DCAD diets. The probable reason of reduced milk fever by low DCAD feeding before parturition might be due to increased bone resorption (Bushinsky et al., 1985), renal production of 1,25$(OH)_2D_3$ (Block, 1993; Gaynor et al., 1989; Goff et al., 1991; Wang and Beede, 1992) or increased intestinal Ca absorption due to slight metabolic acidosis, induced by low DCAD diets (Fredeen et al., 1988). Reduction in milk fever by feeding low DCAD

**Table 14.** Impact of different DCAD levels on edema and mastitis in dry pregnant buffaloes.

| Day relative to calving | Diet[1] | | | | |
|---|---|---|---|---|---|
| | HC | LC | LA | HA | SE |
| -20 to –14 | 2.5[a] | 2.0[b] | 0 | 0 | 0.35 |
| -13 to –7 | 4.0[a] | 3.0[b] | 0 | 0 | 0.62 |
| -6 to –1 | 5.0[a] | 4.0[b] | 0 | 0 | 0.90 |
| 0 | 6.5[a] | 5.0[a] | 0 | 0 | 1.06 |
| 1 to 6 | 4.0[a] | 3.0[b] | 0 | 0 | 0.67 |
| 7 to 13 | 2.5[a] | 2.0[b] | 0 | 0 | 0.33 |
| 14 to 20 | 1.0[a] | 0.5[b] | 0 | 0 | 0.12 |
| Mastitis | 2.5[a] | 2.25[b] | 1.0[c] | 0 | 0.38 |

Udder edema (0= no problem, 10 = sever problem).Mastitis (0= no problem, 3 = sever problem). [a, b, c, d] Means in a row with different superscripts differ significantly ($P < 0.05$).[1]HC, LC, LA and HA stand for high cationic, low cationic, low anionic and high anionic diets, respectively.

during prepartum has also been reported by many workers (Tucker et al., 1992; Oetzel et al., 1988; Gaynor et al., 1989).

Tucker et al. (1991) reported increased plasma hydroxyproline, an indicator of bone resorption, in cows offered low DCAD diets during periparturient period. Feeding low DCAD diets increased Ca availability from bones directly or indirectly under the effect of PTH and 1,25-$(OH)_2O_3$ (Block, 1988). Metabolic alkalosis in buffaloes fed on high DCAD diet might have reduced PTH receptor recognition ability for efficient Ca mobilization from bones.

## Udder edema and mastitis

Udder edema was observed in buffaloes fed on HC and LC diets. The problem was more sever in buffaloes fed on HC compared to those fed on LC diets (Table 14). This might be attributed to the diuretic effect of $CaCl_2$ that was used to reduce the DCAD level. Our results were consistent with Nestor et al. (1988) who reported more chances for the occurrence of udder edema in those animals fed diets high in bicarbonate salts of sodium or potassium. Lema et al. (1992) pointed out that feeding $CaCl_2$ prepartum reduced the occurrence of udder edema in dairy heifers. Excess intake of $Na^+$ or $K^+$, in high DCAD diets, may be the major cause of udder edema (Al Ani and Vestweber, 1986; Vestweber and Al Ani, 1983). The results were also in agreement with previous studies (Randall, 1974; Conway et al., 1977; Jones et al., 1984) who reported that high DCAD before parturition is the major cause of udder edema. Restriction of high DCAD diets to pregnant heifers reduces the severity of udder edema.

A close relationship between udder edema and mastitis was observed. Mastitis problem was higher in those animals fed on LC and HC diets. This problem was also present in animals fed on LA diet but the severity was less in affected animals (Table 14). Mastitis was more pronounced in animals suffered from udder edema. Occurrence of such problems might be due to immune suppression in affected animals (Gunnink, 1984). Neutrophills are unable to ingest and kill bacteria and its ability of antibody production is impaired (Ishikawa, 1987; Kashiwazaki et al., 1985; Well et al., 1977). Immunoglobulins and conglutininare also reduce around parturition (Kehrli et al., 1990; Stable et al., 1991). The function of the mammary gland alters and it produces more colostrum that enhances iron, which is a prerequisite for bacterial growth (Todhunter et al., 1990). The animal fed high DCAD diet becomes hypocalcemic at parturition and it impairs smooth muscle contraction that is very vital to close teat sphinctor.

## Conclusion

The findings corroborate that lowering of DCAD in prepartum buffalo's induced slight metabolic acidosis even during winter as indicated by reduced serum $HCO_3$, blood and urine pH which helped to increase serum calcium leading to reduced occurrence of milk fever, udder edema and mastitis in buffaloes.

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