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Nutrient intake, acid base status and growth performance of growing male buffalo calves fed varying level of dietary cation anion difference

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Abstract

Influence of +110, +220 and +330 mEq/kg of dry matter (DM) dietary cation anion difference (DCAD) on growth performance of growing male buffalo calves was examined in a randomized complete block design. Three DCAD diets were randomly allotted to three groups, ten calves in each group. A linear increase in nutrient intake was recorded with increased DCAD level. However, digestibilities of nutrients remained unaltered across all diets. Calves fed +330 DCAD diet had higher nitrogen balance than those fed +110 DCAD diet. Blood pH and serum HCO_3 increased with increased DCAD level. Serum chloride was high in calves fed +110 DCAD diet, while serum (Na+K)−(Cl+S) increased linearly with increased DCAD level. Serum calcium increased with decreased DCAD level while serum magnesium and phosphorus remained unaffected. Ca balance remained unaltered by calves fed varying level of DCAD. Urine pH increased with increased DCAD level. Calves fed +220 and +330 DCAD diets gained more weight than those fed +110 DCAD diet. In conclusion, increased DCAD level not only increased dry matter intake but also weight gain in growing buffalo calves.

Keywords: DCAD; Buffalo calves; Nitrogen and calcium balance; Growth

1. Introduction

Dietary minerals are integral part of all biological functions in animal body. They play a key role in transfer of fluids from cell to cell, tissue to tissue and organ to organ which ultimately play a vital role to fulfill the diverse needs of production, growth and reproduction of animals. In animal, body transfer of fluids is important because of the electrical potential, produced by the difference of positively and negatively charged particles, especially minerals. The minerals may be cation (positively charge) and anion (negatively charge) and the difference between these cations (like Na, K) and anions (like Cl, S) is referred as dietary cation anion difference (DCAD) or dietary cation anion balance (DCAB) and the equation mostly used to know the difference is (Na+K)−(Cl+S) mEq/Kg dry matter (DM) (Tucker et al., 1992).

The DCAD affects the acid base status which plays a key role in body physiology (Sanchez, 2003). Any change in DCAD induces certain changes in blood chemistry for example if DCAD decreases; it causes an increase in blood H^+ and decrease in blood HCO_3, blood pH and urine pH (Block, 1994; Spanghero, 2004). The reduction in blood HCO_3 and urine pH works as a compensatory mechanism (Block, 1994; Sanchez et al.,

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1997). Alteration in blood pH affects insuline secretion and its effectiveness (Schade et al., 1981; Robertson, 1987) and growth hormone (Challa et al., 1993). Alteration in DCAD also affects the dry matter intake (DMI) and growth in calves (Fettman et al., 1984; Jackson et al., 1992; Jackson and Hemken, 1994). It is assumed that increased DMI in calves fed high DCAD diet might be due to increased buffering capacity due to alkalogenic nature of high DCAD diet (Block, 1994).

In tropical and subtropical, growing calves are well known victims of high temperature and humidity which reduce not only the DMI but also growth performance and ultimately profitability of the enterprise. Feeding high DCAD diet to growing calves might be an important nutritional tool to improve acid base status which may increase growth rate through increased DMI during hot summer. However, scientific information regarding alteration of DCAD on performance of male buffalo calves is limited. Therefore, the present study was planned to determine the influence of varying level of DCAD on acid base status, nutrients intake, Ca and nitrogen balance and growth rate for growing buffalo male calves.

2. Materials and methods

The experiment was planned to determine the effects of varying level of DCAD on acid base status and growth performance of growing *Nili Ravi* male buffalo calves. It was conducted at Animal Nutrition Research Center, Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan.

The DCAD is the difference between milliequivalents of cation (Na, K) and anions (Cl, S) in the whole feed. The most common equation (Tucker et al., 1992; Roche et al., 2003, 2005) used for the said purpose was as under:

$$\mathrm{DCAD} = (\mathrm{Na} + \mathrm{K}) - (\mathrm{Cl} + \mathrm{S})\ \mathrm{mEq/kg\ DM}.$$

Three diets were formulated to have +110, +220 and +330 mEq/kg DM DCAD. The +110, +220 and +330 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). Thirty *Nili Ravi* male buffalo calves of about 12 months of age, were randomly allocated to three dietary treatments in a randomized block design, ten calves in each group.

Calves were housed on concrete-floored separate pens and no mechanical means were used to control the house temperature. The first 20 d were adaptation period while last 10 d of each month were collection period.

Table 1
Ingredients and chemical composition of DCAD diets for growing buffalo calves

	DCAD[a] (mEq/kg of DM)		
	+110	+220	+330
Ingredient			
Wheat straw	50.0	50.0	50.0
Corn grain cracked	11.0	11.0	11.0
Molasses	7.0	7.0	8.45
Wheat bran	9.55	9.70	6.70
Sunflower meal	12.0	12.0	12.0
Canola meal	6.0	6.0	6.0
Vegetable oil	1.5	1.5	2.0
Urea	1.0	1.0	1.2
DCP[b]	1.0	1.0	1.0
Salt	0.50	0.50	0.50
$CaCl_2$	0.45	0	0
$NaHCO_3$	–	0.30	1.15
Chemical composition			
ME (Mcal/kg)	2.24	2.24	2.24
CP[c]	14.0	14.0	14.1
NDF[d]	48.3	48.1	47.10
ADF[e]	31.4	31.4	31.0
NFC[f]	27.8	27.8	27.6
Ca	0.72	0.56	0.56
P	0.58	0.58	0.55
Na	0.29	0.37	0.60
K	1.49	1.5	1.51
Mg	0.28	0.28	0.27
Cl	0.92	0.67	0.64
S	0.22	0.22	0.22

[a] Dietary cation anion difference {(Na+K)−(Cl+S)}.
[b] Dicalcium phosphate.
[c] Crude protein.
[d] Neutral detergent fiber.
[e] Acid detergent fiber.
[f] Non-fermentable carbohydrate.

The diets were mixed daily and fed twice (0300 and 1400 h) a day *ad libitum* but at 10% weighback during collection period. The experiment lasted for 120 d.

Feed intake was recorded daily and their representative samples were taken for analysis. The calves were weighed weekly. Faeces were collected daily, dried at 55 °C, bulked and mixed at the end of each collection period. Digestibility was determined by using total collection method (Sarwar et al., 1996). During collection periods, complete collections of urine and faeces were made according to the procedure described by Williams et al. (1984). The faeces of each calf 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 composted by calf and 10% of the composted samples were taken for analysis. For urine collection, small special metal

Table 2
Effect of varying level of DCAD on nutrient intake and digestibility in growing buffalo calves

	DCAD[1] diets (mEq/kg of DM)			
	+110	+220	+330	SE
DM[2] (kg/d)	4.87[b]	5.77[a]	5.93[a]	0.31
Dig.%	62.55[b]	62.44[b]	62.11[b]	0.17
CP[3] (g/d)	681.8[b]	807.8[a,b]	836.13[a]	64.11
Dig.%	71.77[b]	71.67[b]	71.55[b]	0.11
NDF[4] (g/d)	2352.2[b]	2775.4[a,b]	2793.03[a]	197.5
Dig.%	60.66[b]	60.32[b]	60.30[b]	0.12
ADF[5] (g/d)	1529.18[b]	1811.8[a,b]	1838.3[a]	113.2
Dig.%	54.32[b]	54.77[b]	54.87[b]	0.21

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 detergent fiber.

buckets fitted with plastic pipe were used. This plastic pipe ended in a large container (30 l). The urine excreted by each calf was acidified with 50% H_2SO_4 and 20% of it was sampled and preserved at −20 °C (Nisa et al., 2004). At the end of each collection period, the preserved urine samples were composted by calf after thawing and 10% of the composted sample was used for analysis. Within 10 min of urine collection, pH was measured. Hand-held urine pH meters (Hach, Loveland, CO) were calibrated before each sampling with a pH 7.0 buffer solution as the standard.

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 which was immediately placed on crushed ice for blood pH analysis within 2 h (Tucker et al., 1991). Blood serum was harvested to analyze serum Na, K, Cl, Ca, P, Mg, and S (AOAC, 1990) and serum HCO_3 (Harold, 1976). Serum Na, K, Ca, Cl, S and Mg were analyzed via atomic absorption spectrophotometry (model 4, Perkin-Elmer, Norwalk) and P was analyzed photometrically via Spectronic 1001 (Milton Roy Co., Cincinnati, OH). The nitrogen and calcium balance was determined using the equations as described by NRC (2001).

2.1. 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).

3. Results

3.1. Nutrient intake and digestibility

Maximum (5.93 kg/d) and minimum (4.87 kg/d) DMIs were recorded in calves fed +330 and +110 mEq/kg DCAD diets, respectively (Table 2). Calves fed +330 consumed 21.76% more feed than those fed +110 mEq/kg DCAD diet. Calves fed +110 and +220 consumed 4.87 and 5.77 kg/d, respectively. However, DMI in calves fed +220 and +330 mEq/kg DCAD diets remained unaltered.

A constant increase in crude protein (CP) and neutral detergent fiber (NDF) intake was observed with increased DCAD level of diet. Calves fed +330 and +110 mEq/kg DCAD diets consumed maximum (836.13 g/d) and minimum (681.8 g/d) CP, respectively. Calves fed +110 and +220 mEq/kg had 681.8 and 807.7 g/d CP intake, respectively. Similar trend was noticed for N intake (Table 3). Increase in nitrogen balance was also noticed with increasing the DCAD level of diet. The NDF intake was maximum (2793.03 g/d) and minimum (2352.2 g/d) in calves fed +330 and +110 DCAD diets, respectively. Similar trend was noticed for acid detergent fiber (ADF) intake. The digestibilities of DM, NDF and CP remained unchanged by DCAD alteration (Table 2).

3.2. Blood pH and HCO_3

The blood pH was maximum (7.391) in calves fed +330 while minimum (7.321) in those fed +110 DCAD diets, respectively (Table 4). Calves fed +110 and +220 mEq/kg DCAD diets had 7.321 and 7.379 blood pH, respectively. A constant increase in blood HCO_3 was noticed with increased DCAD diet (Table 4). The

Table 3
Effect of varying level of DCAD on nitrogen balance in growing buffalo calves

	DCAD[1] diets (mEq/kg of DM)			
	+110	+U220	+330	SE
Nitrogen intake, g/d	109.1[b]	129.25[a,b]	133.8[a]	5.59
Faecal nitrogen, g/d	30.24[b]	36.44[a]	37.85[a]	1.31
% of intake	27.72	28.19	28.29	1.32
Apparent absorption, g/d	78.86[b]	92.81[a,b]	95.95[a]	8.33
% of intake	72.28	71.80	71.71	1.31
Urinary nitrogen, g/d	9.43[b]	11.83[a,b]	11.90[a]	1.77
Apparent retention, g/d	69.43[b]	80.98[a,b]	84.05[a]	8.16
% of intake	63.64	62.65	62.82	1.02
Nitrogen balance, g/d	39.67[b]	48.27[a,b]	49.75[a]	3.21
% of intake	36.36	37.45	37.18	0.19

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

Table 4
Effect of varying level of DCAD on blood pH and mineral picture of growing buffalo calves

	DCAD[1] diets (mEq/kg of DM)			
	+110	+220	+330	SE
Blood pH	7.321[c]	7.379[b]	7.391[a]	0.003
Urine pH	7.46[c]	7.88[b]	8.01[a]	0.29
HCO_3 mmol/l	23.52[c]	26.31[b]	28.81[a]	1.0
Na, mEq/l	122.2	122.6	123.4	0.17
K, mEq/l	4.46	4.48	4.81	0.1
Cl, mEq/l	94.62[a]	93.55[b]	92.11[b]	1.0
S, mEq/l	1.47	1.48	1.49	0.1
Serum (Na+K)−(Cl+S), mEq/l	30.57[c]	32.05[b]	34.61[a]	1.10
Ca, mg/dl	9.46[a]	9.22[b]	9.01[b]	0.12
Mg, mg/dl	2.32	2.30	2.30	0.15
P, mg/dl	7.31	7.30	7.31	0.09

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

maximum (28.81 mmol/l) and minimum (23.52 mmol/l) HCO_3 were recorded in calves fed +330 and +110 DCAD diets, respectively.

3.3. Serum minerals

Serum Ca increased with decreased DCAD level. Calves fed +110 and +330 DCAD diets had maximum (9.46 mg/dl) and minimum (9.01 mg/dl) serum Ca, respectively (Table 4. Serum Cl also followed the similar trend. Calves fed +110 and +330 DCAD diets had maximum (94.62 mEq/l) and minimum (92.11 mEq/l) serum Cl, respectively. Serum phosphorus, sodium and potassium remained unaltered by altering DCAD.

Table 5
Effect of varying level of DCAD on Ca balance in growing buffalo calves

	DCAD[1] diets (mEq/kg of DM)			
	+110	+220	+330	SE
Intake, g/d	35.04[b]	32.31[a,b]	33.21[a]	3.41
Faecal excretion, g/d	19.5[a]	17.9[b]	18.7[b]	2.47
% of intake	55.65	55.40	56.31	0.45
Apparent absorption, g/d	15.54[a]	14.41[b]	14.51[b]	3.37
% of intake	44.35	44.59	43.69	0.74
Urine excretion, mg/d	383[a]	353[b]	351[b]	16.22
% of intake	1.09	1.09	1.06	0.06
Apparent retention, g/d	15.16[a]	14.06[b]	14.16[b]	2.01
% of intake	43.26	43.51	42.63	1.32

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

Table 6
Effect of varying level of DCAD on growth performance of growing calves

	DCAD[1] diets (mEq/kg of DM)[2]			
	+110	+220	+330	SE
Weight gain, g/d	625[b]	672[a,b]	698[a]	22.5
FCR[3]	0.13	0.12	0.12	0.01

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 intake per kg live weight gain.
[3]Feed conversion ratio.

A slight increase in serum Mg was noticed with decreased DCAD level (Table 5). A linear increase in serum DCAD (Na+K−Cl+S) was noticed with increased DCAD level of diet. Calves fed +330 had higher serum DCAD (34.61) than those (30.57 mEq/l) fed +110 mEq/kg DCAD diet. Serum sulphur was not altered by altering the DCAD level of diet.

3.4. Urinary pH

A linear increase in urine pH was observed with an increased DCAD level. Urine pH decreased with decreased DCAD level (Table 4). Minimum (7.46) and maximum (8.01) urine pH were noticed in calves fed +110 and +330 DCAD diets, respectively.

3.5. Calcium balance

Urinary Ca increased with decreased DCAD diet (Table 5). Maximum (383 mg/d) and minimum (351 mg/d) urinary Ca excretions were recorded in calves fed +110 and +330 DCAD diets, respectively.

3.6. Growth performance

An increased weight gain was observed with an increased DCAD level of diet in growing buffalo calves (Table 6). Calves fed +330 and +110 DCAD diets had maximum (698, g/d) and minimum (625, g/d) weight gains, respectively. Feed conversion ratio remained unchanged in calves fed varying level of DCAD.

4. Discussion

4.1. Nutrient intake and digestibility

Increased DMI by calves fed high DCAD might be attributed to increased blood HCO_3, acid base balance (Sanchez and Beede, 1994) and rumen pH (Tucker et al.,

1991). The higher HCO_3 not only increased ruminal buffering capacity but also ruminal fluid dilution and flow of undegraded starch (Sanchez and Beede, 1994). Similar findings were reported by other workers (West et al., 1991; Delaquiz and Block, 1995; Chan et al., 2005; Apper-Bossard et al., 2006). However, in contrast to present findings, Fredeen et al. (1988a) reported unaltered DMI by goats fed increased DCAD level. The plausible reason for their findings might be the very high DCAD level (450 to 900 mEq/kg) compared to the present study (+110 to +330 mEq/kg).

Reduced DMI in calves fed +110 DCAD may be attributed to poor palatability of diet due to anionic salt ($CaCl_2$). Calcium chloride, being unpalatable, might have reduced the feed consumption. Tucker et al. (1988) also reported decreased feed intake in cows fed $CaCl_2$. Decreased DMI with reduced DCAD level of diet had also been reported by West et al. (1991, 1992).

Higher N balance in calves fed +220 and +330 DCAD diets might be due to increased DMI than those fed +110 DCAD diet. However, Delaquiz and Block (1995) reported unaltered N balance due to DCAD alteration. This contrast might be attributed to small differences in N intake (463.9 versus 439.09 g/d) due to DCAD alteration. It is speculated that although narrow DCAD range altered the acid base status of the lactating cows yet it was not sufficient to alter the N balance (May et al., 1986; Welbourne et al., 1988).

4.2. Blood pH and HCO_3

Reduction in blood pH with reduced DCAD level might be attributed to increased Cl content of diet. Calves fed +110 DCAD diet had high Cl (0.92%) due to anionic salt ($CaCl_2$). The Cl absorption takes place in the posterior segment of intestine, when it was in excess of Na, in exchange for HCO_3 to maintain electrical neutrality, resulting in reduced blood HCO_3 and increased H^+ concentration. Eventually, the higher DCAD increased the blood HCO_3 and reduced H^+, the reverse was true for low DCAD diet (Block, 1994). 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 HCO_3 ion is formed that enters the extracellular fluids to further buffer acid in the extracellular fluids (Guyton, 1976). Low DCAD diet (high Cl) might have overcome the ability of kidneys to excrete sufficient hydrogen ion to maintain a constant blood pH, resulting in a slight systemic acidosis. A high DCAD diet tends to have high blood pH due to more HCO^-_3 production and H^+ excretion (Tucker et al., 1992; Spanghero, 2004). These findings were consistent with West et al. (1991) who reported a decreased blood pH (7.32) in cows fed low DCAD diet compared to those (7.42) fed high DCAD diet. However, the increased pH was within the normal range (Stewart, 1983). Roche et al. (2005) also observed an increased blood HCO_3 with increased DCAD level.

4.3. Serum minerals

Increased serum Ca in calves fed +110 DCAD diet might be attributed to increased calcium absorption from the alimentary tract (Lomba et al., 1978) and increased calcium mobilization from bones (Joyce et al., 1997) due to mild metabolic acidosis, induced by this low DCAD diet. Slight metabolic acidosis, induced by low DCAD diet, increased the recognition ability of receptor tissues not only for parathyroid gland but also for $1,25(OH)_2D_3$. Thus, low DCAD might have increased serum Ca directly from calcium mobilization from bones and indirectly through increased absorption from the intestine due to increased synthesis of $1,25(OH)_2D_3$ (Block, 1994). It is also supported by Gaynor et al. (1989) who observed higher plasma hydroxyproline, an index of bone resorption, in cows fed diet rich in anions (Cl or S). Increased plasma Ca in sheep fed low DCAD compared to those fed high DCAD diet has also been reported by Espino et al. (2003).

Increase in serum Na and decrease in serum Cl as DCAD increased were anticipated because of increased dietary concentrations of these minerals as DCAD increased and decreased, respectively. Decreased serum Cl with increased DCAD level is also supported by Roche et al. (2003) who observed a linear reduction in plasma Cl with increased DCAD diet. Jackson et al. (2001) also reported higher (96.7 mEq/l) plasma Cl in calves fed 0 mEq/kg than those (94.3 mEq/l) fed 200 mEq/kg DCAD diet.

Slight variation in serum Na (122.2–123.4 mEq/l) and K (4.46–4.81 mEq/l) might be attributed to dietary alteration of these minerals as excess dietary Na and K were excreted through kidney (Hu and Murphy, 2004). Similar results were reported by West et al. (1991) who stated that increased DCAD level (−116 to 312 mEq/kg) didn't affect the serum sodium (141.64, 142.50 mEq/l) and potassium (4.91, 4.70 mEq/l) concentrations. Slight reduction in serum sulphur with increased DCAD level might be because of dietary concentration. Moreover, S balance is regulated renally not intestinally thus increased intake, increased the blood serum S (Krijgsheld et al., 1979). These findings are in concordance with Delaquiz and Block (1995).

A linear increase in serum DCAD (Na+K−Cl+S) in the present study was also supported by other researchers (West et al., 1991; Tucker et al., 1988; Hu and Murphy, 2004) who observed a non-significant change in serum Na or K but an increased serum Cl with decreased DCAD level. The inverse relation between blood Cl and HCO_3 concentration has been demonstrated in an imperial model by Hu and Murphy (2004). Moreover, Cl is absorbed in exchange of HCO_3 to maintain the neutrality of body which results in decreased HCO_3 and blood pH (Block, 1994).

4.4. Urinary pH

Increased urinary pH with increased DCAD level might be attributed to higher blood HCO_3 and lower urine net acid excretion, implying that the acid load of the animals decreased rapidly as DCAD increased (Hu and Murphy, 2004; Spanghero, 2004). The alteration in urine pH that reflects alteration in blood pH and kidneys minimize this change by making the urine pH alkaline, by excreting more HCO_3 and conserving H^+, or acidic, by excreting more H^+ and conserving more HCO_3 (Roche et al., 2003). Waterman et al. (1991) also reported increased anions (Cl or S) or decreased cations (Na and K) that reduced urine pH sharply. Moreover, a reduced urine pH with increased dietary anions (Cl and S) had been reported by many workers (Jackson et al., 1992; Jackson and Hemken, 1994; Pehrson et al., 1999; Spanghero, 2004). Urine pH had been used as an indicator of metabolic acid or alkali load (Sanchez et al., 2002). Increased urine pH (8.09) was recorded in dairy calves fed 200 DCAD diet compared to those (6.80) fed 0 DCAD diet (Jackson et al., 2001). However, urine pH has a threshold limit of as low as 4.5, induced by a low or negative DCAD diet (Roche, 1999). An increased urinary pH is considered an indicator of blood pH, implying that the acid load of the lactating cows decreased dramatically as cation anion difference increased (Hu and Murphy, 2004; Pehrson et al., 1999).

4.5. Calcium balance

Increased urinary Ca excretion in calves fed +110 diet might be due to slight metabolic acidosis, induced by low DCAD diet. This metabolic acidosis might have increased Ca resorption from bones and intestinal Ca absorption (Schonewille et al., 1994 and Roche et al., 2003) due to increased synthesis of $1,25(OH)_2D_3$ (Goff et al., 1991). Acidosis maintains a high Ca flux through the exchangeable pool without affecting the pool size (Fredeen et al., 1988b). The reduced urinary Ca excretion in calves fed high DCAD diets might be due to gradual vanishing effect of metabolic acidosis. Ruminant kidneys are highly sensitive to blood acid base status and increase the excretion of Ca during acidosis, independent of the hormonal action usually associated with Ca metabolism (Stacy and Wilson, 1970). These results are in concordance with West et al. (1992) who observed an increased urinary Ca: creatinine (0.30 versus 0.09) excretion with increased (120 versus 465 mEq/kg) level of DCAD. Alteration in fecal Ca excretion might be attributed to dietary concentration of this mineral. Moreover, increased Ca absorption in calves fed +110 DCAD diet also supported the assumption that metabolic acidosis induced by low DCAD diet might have increased $1,25(OH)_2D_3$ synthesis which increased Ca absorption. In contrast to these findings, Schonewille et al. (1994) reported similar amount of Ca absorbed in non-pregnant dry cows when extra Ca supplemented in a Cl rich diet was fed. Calcium intake was almost 1.8 times higher when extra Ca was supplemented in cows fed anionic diet but amount of Ca absorbed was similar. This difference may be attributed to the fact that they used non-pregnant dry cows while in present study, growing calves were used, the latter has different Ca dynamics due to growth and development. Above all this controversy, it is more important to produce slight metabolic acidosis in order to regulate increased Ca absorption than Ca level.

4.6. Growth performance

Increased weight gain in calves fed +220 and +330 DCAD diets might be attributed to increased DMI. Reduced DMI in calves fed +110 DCAD might be attributed to slight metabolic acidosis, induced by low DCAD level. During growth, metabolic activities take place at a rapid rate resulting in more CO_2 production, which tends to make the cellular environment acidic because CO_2 acts as an acid (carbonic acid) after combining with water. This slight acidic situation doesn't let the cell and its organelles to work its maximum capacity and might have reduced cellular activities resulting in poor growth rate in calves fed +110 DCAD diet. While a high DCAD, being alkalogenic, makes the cellular environment slight alkalosis, reduced the extent of cellular acidity produced by CO_2, generated by metabolic activities and thus allow the cell to work in its maximum (Block, 1994). Similar findings were reported by Jackson et al. (1992) who observed quadratic increase in the average daily gain in growing calves fed high DCAD diets. This may be due to quadratic increase in DMI with increased

DCAD level of diet. Fettman et al. (1984) studied the effect of Cl supplementation in ration of dairy cows. They reported increased weight gain in calves fed 0.10% Cl diet compared to those 0.45% Cl diet. The increased weight gain in calves fed low Cl (0.10%) diet was due to increased feed consumption. Wheeler and Hruska (1981) also reported an increased weight gain in steers fed 100 mEq/kg of DM DCAD diet due to increased feed consumption. Jackson and Hemken (1994) observed that calves fed 13 mEq/100 g DM diet gained 0.14 kg/d more weight than those fed diets containing −18 mEq/100 g of DM. Average daily gain was higher in calves fed 130 mEq/kg DM DCAB diet. Decreased growth rate in calves fed low DCAD diet was due to metabolic acidosis induced by low DCAD diet. Moreover, when the acid balance of the diet is deviated toward acidosis apart from the homeostatic welfare, most metabolic pathways cannot work optimally and thus they are more involved in homeostatic regulation than growth (Mongin, 1980).

In conclusion, growing buffalo calves fed +220 and +330 DCAD diets gained more weight than those fed +110 DCAD diet.

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