



## Effect of Dietary Cation-Anion Difference during Prepartum and Postpartum Periods on Performance, Blood and Urine Minerals Status of Holstein Dairy Cow

A. Razzaghi\*, H. Aliarabi<sup>1</sup>, M. M. Tabatabaei<sup>1</sup>, A. A. Saki<sup>1</sup>, R. Valizadeh and P. Zamani<sup>1</sup>

Department of Animal Science, Ferdowsi University of Mashhad, P.O.Box 91775-1163, Mashhad, Iran

**ABSTRACT :** Twenty four periparturient cows were used to determine the effects of DCAD on acid-base balance, plasma and urine mineral concentrations, health status, and subsequent lactation performance. Each group of 12 cows received either a diet containing -100 DCAD or +100 DCAD for 21 d prepartum. Both anionic and cationic groups were divided into two groups, one received a +200 DCAD and the other +400 DCAD diet for 60 d postpartum. Prepartum reduction of DCAD decreased DMI, urinary and blood pH, urinary concentrations of Na or K and increased plasma and urinary Ca, Mg, Cl and S. Also cows fed -100 DCAD diet consumed the most dry matter in the first 60 d after calving. Postpartum +400 DCAD increased milk fat and total solid percentages, urinary and blood pH and urinary Na and K concentrations, but urinary Ca, P, Cl and S contents decreased. Greater DMI, FCM yields were observed in cows fed a diet of +400 DCAD than +200 DCAD. No case of milk fever occurred for any diets but feeding with a negative DCAD diet reduced placenta expulsion time. In conclusion, feeding negative DCAD in late gestation period and high DCAD in early lactation improves performance and productivity of dairy cows. (**Key Words :** Dietary Cation-anion Difference, Lactation, Acid-base Balance, Calcium, Health Status, Dairy Cow)

### INTRODUCTION

Besides tremendous changes in energy and protein flux around the time of calving, periparturient cows also experience large changes in mineral element dynamics (Horst et al., 1997). A key component of mineral metabolism in these cows is preventing hypocalcemia, which reduces dry matter intake (Hansen et al., 2003) and increases the risk of metabolic disorders (Curtis et al., 1983).

Hypocalcemia is a particular concern in the newly calved cow, where the sudden demand for calcium at the onset of lactation severely tests the calcium homeostatic capabilities of the animal (Goff, 2008). Hypocalcemia increases the risk of cows getting other diseases. Hypocalcemia is a predisposing factor for dystocia, prolapsed uterus, retained placenta, and early metritis (Grohn et al., 1989; DeGaris and Lean, 2008). Dietary

cation-anion difference has a role in animal productivity and health via its influence on the acid-base balance and calcium metabolism in the animal that often become 'broken' in dairy cows (Sanchez, 2003). Reducing DCAD by increasing dietary acidity or employing anionic salts has been efficacious and cost effective in the prophylaxis of hypocalcemia (Chan et al., 2006). High concentrations of dietary anionic salts cause an influx of negatively charged ions systemically, leading to increased hydrogen ion concentration to maintain electroneutrality. Increased hydrogen ion concentration induces a mild metabolic acidosis. Acidogenic diets are hypothesized to increase bone resorption, blood Ca and intestinal Ca absorption (Horst et al., 1997).

The prevalence of hypocalcemia is as high as 70% for multiparous cows, although only 8% exhibited clinical hypocalcemia (Beede, 1995) that lowers the 16% yearly milk yield (Block, 1984). Feeding low DCAD during the 3 to 4 wk before calving had beneficial effects on systemic acid-base status, calcium metabolism, prepartum health and also postpartum productive performance (Horst et al., 1994). However, feeding negative DCAD to periparturient dairy cows proved a useful nutritional practice, it enhanced blood

\* Corresponding Author : Ali. Razzaghi. Tel : +98-511-8788494, Fax : +98-511-8788494, E-mail : arazzaghi88@gmail.com

<sup>1</sup> Department of Animal science, Bu-Ali Sina University, Hamedan, Iran.

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calcium and postpartum milk production (Block, 1984; Beede et al., 1992; Moore et al., 2000). The recent advances in mineral nutrition demonstrated that cows fed higher DCAD level produced more milk during early lactation (Sanchez, 2003; Hu and Murphy, 2004; Hu et al., 2007a). The potential effect of DCAD on lactating dairy cows has also been explored, and results indicates that DCAD and production are related possibly through acid-base regulation (Sanchez and Beede, 1994; Hu and Murphy, 2004). About 11% dry matter intake (DMI) and 9% milk yield were increased in early lactating cows fed +200 vs. -100 DCAD diet (Tucker et al., 1988).

In addition, manipulating DCAD might benefit lactating dairy cows immediately after calving to about 50 d postpartum (Chan et al., 2005; Hu et al., 2007b).

The objectives of this study were to determine the interaction effects of prepartum and postpartum diets with different levels of DCAD on plasma and urine concentration of mineral elements, acid-base balance, calcium homeostasis, DMI, milk production and health status in dairy cows.

## MATERIALS AND METHODS

### Experimental design and animal care

The trial consisted a 21 d prepartum phase followed by a 63 d postpartum phase. Twenty-four multiparous Holstein cows were randomly allocated to a high DCAD (+100 mEq/kg DM; n = 12) or low DCAD (-100 mEq/kg DM; n = 12) 3 wk before calving. Anionic salt was added to reduce DCAD. After calving, the cows in each group were reallocated to receive dietary DCADs of +200 or +400 mEq/kg DM (6 cows per group) creating a split-plot in time design with a 2x2 factorial arrangement. All diets were formulated according to NRC (2001) recommendations for periparturient and early lactation Holstein cows (DCAD = (Na+K+0.15 Ca+0.15 Mg)-(Cl+0.6 S+0.5 P)/kg of DM) (Table 1).

Cows were housed in individual pens and milked three times a day at 04:00, 12:00 and 20:00 h. Water was available *ad libitum*. DCAD was altered using CaCl<sub>2</sub>, NH<sub>4</sub>Cl, MgSO<sub>4</sub> and CaSO<sub>4</sub> or K<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> salts in diets. Feed given to cow at 0900 and 2100 h before calving and at 0500, 1400 and 2100 h after calving.

### Sample collection and analysis

Feed intake of each cow was recorded on d 15, 10, 5 and 2 prepartum and on weekly intervals postpartum. The prepartum or postpartum diet samples, were composited for analysis of DM, CP, EE, Ca, and P (AOAC, 1990) and feed NDF and ADF were analyzed according to the method initially described by Van Soest et al. (1991). The Na, K and

**Table 1.** Ingredients and chemical composition of diets for dairy cows in pre and post-partum periods

Ingredients (% DM)	DCAD (mEq/kg DM)			
	-100	+100	+200	+400
	Prepartum		Postpartum	
Alfalfa	40.07	39.90	23.96	23.82
Corn silage	28.98	28.54	14.36	14.29
Concentrate mixture <sup>1</sup>	28.18	29.68	-	-
Beet pulp	1.13	1.67	-	-
Anionic salts <sup>2</sup>	1.64	0.21	-	-
Concentrate mixture	-	-	61.68 <sup>3</sup>	61.89 <sup>4</sup>
Nutrient levels (% DM)				
NE <sub>L</sub>	1.31	1.33	1.58	1.57
CP	13	13.2	18.4	18.5
NDF	48.7	48.3	34.8	34.3
ADF	27.2	27.5	19	18.9
Ca	0.76	0.48	0.82	0.82
Mg	0.31	0.23	0.29	0.29
P	0.25	0.26	0.41	0.41
Na	0.21	0.21	0.49	0.78
K	1.19	1.20	1.14	1.42
Cl	0.85	0.56	0.53	0.53
S	0.42	0.22	0.24	0.24
DCAD <sup>5</sup> (mEq/kg DM)	-108	+106	+206	+404

<sup>1</sup> Concentrate composition (%): barley, 20.5; corn, 20.5; wheat, 4.5; wheat barn, 28.5; cotton meal, 19; soybean meal, 6; premix 1, 1.

<sup>2</sup> Ingredients: NH<sub>4</sub>Cl, CaCl<sub>2</sub>, MgSO<sub>4</sub>, CaSO<sub>4</sub> (each 0.41% for anionic diet).

<sup>3</sup> Composition (for 61.68%): barley, 13; wheat, 3.78; corn, 12.04; wheat barn, 8.71; cotton meal, 12.04; soybean meal, 7.55; fat calcium soaps, 1.72; NaHCO<sub>3</sub>, 0.68; CaCO<sub>3</sub>, 1; MgO, 0.18; salt, 0.49; premix 2, 0.49.

<sup>4</sup> Composition (for 61.89%): barley, 12.51; wheat, 2.87; corn, 11.74; wheat barn, 8.30; cotton meal, 12.33; soybean meal, 8.01; fat calcium soaps, 1.71; NaHCO<sub>3</sub>, 1.77; K<sub>2</sub>CO<sub>3</sub>, 0.49; CaCO<sub>3</sub>, 1; MgO, 0.18; salt, 0.49; premix 2, 0.49.

<sup>5</sup> Actually determined dietary cation-anion difference (Na+K+0.15 Ca+0.15 Mg)-(Cl+0.6 S+0.5 P).

Mg contents were measured using atomic absorption spectrophotometry (GBC-3000, Australia), Chlorine was assessed via a potentiometer; P and S were detected using spectronic (Spectronic 6300, Australia).

A total of 4 urine samples were collected about 4 h after the morning feeding on d 2 and 12 prepartum and postpartum. Midstream urine was collected in plastic containers and Urine samples (40-ml for each cow) were stored at -20°C till later assay of mineral contents. Likewise, ten-ml of blood samples were taken from subcutaneous abdominal vein, using heparinized plastic syringes, 4 h after the morning feeding on d 2 and 12 pre-calving; on d 0 at calving; and on d 2 and 12 postpartum to obtain plasma via centrifugation at 3,000 rpm for 20 min. The plasma was

stored at -20°C until later analyzed for minerals. Urine and blood pH on d 2 and 12 prepartum and postpartum was measured immediately after sampling using a hand-held pH meter (HANNA-210, Italy).

Clinical hypocalcemia was considered if the cow was recumbent and plasma total Ca concentration was <5.5 mg/dl, and occurrence of subclinical hypocalcemia was established by blood Ca concentrations below 7.5 mg/dl at any time during the experimental period (Goff and Horst, 1997). Retained placenta was recorded when the placenta membrane remained in the uterus for 24 h or longer postpartum (Kelton et al., 1998). Udder edema was recorded if the udder became swollen and the teats became flushed. Mastitis, metritis, endometritis and ketosis were monitored by Veterinarian. Milk production was recorded on d 15, 30, 45 and 60. Milk samples from three milkings were collected relative to production on 15 and 30 DIM and analysed for fat, protein, lactose, SNF and TS concentration using Milko-scan (Foss-605, Denmark).

### Statistical analysis

The general linear model procedure of SAS software system (2004) was used to analyze data. All data associated with prepartum cows were analyzed according to the model of a completely randomized design:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where  $\mu$  = overall mean;  $T_i$  = effect of treatment I ( $i = 1, 2$ );  $e_{ij}$  = error term.

All data related to postpartum cows, except Ca and DMI were analyzed with the GLM procedure (SAS, 2004) according to the model of a split-plot in time design with a 2×2 factorial arrangement:

$$Y_{ijl} = \mu + A_i + B_j + AB_{ij} + E_{a_{ijk}} + T_l + AT_{il} + BT_{jl} + ABT_{ijl} + E_{b_{ijl}}$$

Where  $\mu$  = overall mean;  $A_i$  = effect of prepartum DCADs;  $B_j$  = effect of postpartum DCADs;  $AB_{ij}$  = effect of interaction between DCAD levels in pre-calving and postpartum;  $E_{a_{ijk}}$  = main error;  $T_l$  = effect of time;  $AT_{il}$  = effect of interaction between time and prepartum DCADs;  $BT_{jl}$  = effect of interaction between time and postpartum DCADs;  $ABT_{ijl}$  = effects of interaction between time and prepartum and postpartum DCADs;  $E_{b_{ijl}}$  = sub-error.

The model used in this study for plasma calcium on d 0 at calving and on d 2 and 12 postpartum and dry matter intake on first 9 wk postpartum was (interactions between treatment and time were significance):

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Where  $\mu$  = overall mean;  $A_i$  = effect of prepartum DCADs;  $B_j$  = effect of postpartum DCADs;  $AB_{ij}$  = effect of interaction between DCAD levels in pre-calving and postpartum;  $e_{ijk}$  = error term.

The health status was processed by Chi-square test. Duncan's multiple range test was used to examine means. A statistically significant difference was noted unless  $p < 0.05$ .

## RESULTS

### Fluid acid-base balance

Urinary and blood pH values were lowest ( $p < 0.01$ ), in prepartum cows receiving DCAD of -100 mEq/kg DM and highest in cows receiving +100 mEq/kg DM (Table 2). Dairy cows fed the +200- and +400- DCAD postpartum showed the lowest and highest urine and blood pH values respectively ( $p < 0.01$ ). However, urine and blood pH of lactating cows were not affected by prepartum DCAD diet level (Table 6).

### Plasma mineral concentration

Feeding -100 DCAD diet resulted in higher ( $p < 0.01$ ) plasma Ca in periparturient cows than the positive DCAD diet (+100 mEq/kg DM). A similar trend was recorded for plasma Mg, Cl and S concentrations. Alteration in prepartum DCAD did not affect the plasma P, Na and K concentrations significantly (Table 2).

Plasma Ca was significantly affected by time, with the nadir on d 1 relative to the higher values on d 2 and 12 postpartum (Table 3). At calving, greater plasma Ca was observed for -100 DCAD diet compared to +100 DCAD diet ( $p < 0.01$ ), but no significant difference in postpartum DCAD levels was observed for plasma Ca on d 2 and 12. Increased plasma Na and K concentrations ( $p < 0.01$ ) were noticed in lactating dairy cows fed +400 DCAD compared to those fed +200 DCAD concentration (Table 4). In early lactation, plasma Mg, P, Cl and S contents did not differ due to prepartum and postpartum DCAD alteration.

### Urine mineral concentration

Concentrations of Ca, Mg, Cl and S in urine were higher ( $p < 0.01$ ) for cows fed -100 DCAD than cows fed +100 DCAD on d 2 and 12 prepartum (Table 2). However, cows fed +100 DCAD had higher Na and K contents in urine than cows fed -100 DCAD at d 2 and 12 pre-calving.

A significant increase ( $p < 0.01$ ) in urinary Ca, P, Cl and S was recorded in cows fed +200 compared to +400 DCAD diet (Table 5). Urinary K and Na contents tended to decrease with decreasing DCAD in early lactation. Mg concentration remained unaltered due to DCAD alteration across all diets. Urine mineral contents were not affected by dietary prepartum DCAD levels in early lactation cows.

**Table 2.** The effects of DCAD on fluid acid-base balance, plasma and urinary mineral concentrations and DMI in periparturient dairy cows

	DCAD <sup>1</sup> (mEq/kg DM)				SEM
	-100		+100		
	Day (pre-calving)				
	2	12	2	12	
<b>Urinary<sup>2</sup></b>					
pH	6.66 <sup>b</sup>	6.69 <sup>b</sup>	7.77 <sup>a</sup>	7.81 <sup>a</sup>	0.04
Calcium (mg/dl)	16.23 <sup>a</sup>	16.22 <sup>a</sup>	9.07 <sup>b</sup>	9.06 <sup>b</sup>	0.43
Magnesium (mg/dl)	28.68 <sup>a</sup>	28.67 <sup>a</sup>	18.55 <sup>b</sup>	18.53 <sup>b</sup>	0.21
Phosphorus (mg/dl)	1.61	1.61	1.53	1.52	0.04
Sodium (mEq/L)	94.68 <sup>b</sup>	94.67 <sup>b</sup>	125.39 <sup>a</sup>	125.37 <sup>a</sup>	0.59
Potassium (mEq/L)	43.49 <sup>b</sup>	43.48 <sup>b</sup>	46.17 <sup>a</sup>	46.17 <sup>a</sup>	0.19
Chloride (mEq/L)	246.24 <sup>a</sup>	246.23 <sup>a</sup>	143.01 <sup>b</sup>	143.00 <sup>b</sup>	5.31
Sulfur (mEq/L)	180.76 <sup>a</sup>	180.77 <sup>a</sup>	154.11 <sup>b</sup>	154.12 <sup>b</sup>	1.51
<b>Blood<sup>3</sup></b>					
pH	7.36 <sup>b</sup>	7.37 <sup>b</sup>	7.40 <sup>a</sup>	7.39 <sup>a</sup>	0.004
Calcium (mg/dl)	9.07 <sup>a</sup>	9.23 <sup>a</sup>	8.18 <sup>b</sup>	8.29 <sup>b</sup>	0.001
Magnesium (mg/dl)	2.21 <sup>a</sup>	2.23 <sup>a</sup>	2.04 <sup>b</sup>	2.08 <sup>b</sup>	0.13
Phosphorus (mg/dl)	6.48	6.49	6.47	6.48	0.03
Sodium (mEq/L)	136.99	137.31	138.22	138.36	0.86
Potassium (mEq/L)	4.08	4.10	4.13	4.16	0.03
Chloride (mEq/L)	94.69 <sup>a</sup>	94.69 <sup>a</sup>	93.56 <sup>b</sup>	93.56 <sup>b</sup>	0.21
Sulfur (mEq/L)	1.54 <sup>a</sup>	1.55 <sup>a</sup>	1.48 <sup>b</sup>	1.48 <sup>b</sup>	0.06
<b>Days to calving</b>					
	15	10	5	2	SEM
Dry matter intake (-100)	12.02	11.32	9.73	9.05	0.08
Dry matter intake (+100)	12.47	12.57	10.95	9.98	0.11

<sup>a-b</sup> Means within a row with different superscripts are different ( $p < 0.05$ ).

<sup>1</sup> Calculated dietary cation-anion difference ( $(\text{Na} + \text{K} + 0.15 \text{ Ca} + 0.15 \text{ Mg}) - (\text{Cl} + 0.6 \text{ S} + 0.5 \text{ P})$ ).

<sup>2</sup> Samples were collected on d 2 and 12 prepartum. <sup>3</sup> Samples were collected on d 2 and 12 prepartum.

**Table 3.** The effect of dietary cation-anion difference on plasma calcium concentration postpartum

Calcium*		Day		
		1	2	12
<b>Group<sup>1</sup></b>				
	1	8.28 <sup>a</sup>	9.12	9.30
	2	8.27 <sup>a</sup>	9.08	9.29
	3	7.79 <sup>b</sup>	9.10	9.29
	4	7.80 <sup>b</sup>	9.05	9.28
SEM		0.08	0.16	0.13
Balance 1 <sup>2</sup>	-100	8.27 <sup>a</sup>	9.10	9.29
	+100	7.79 <sup>b</sup>	9.08	9.28
Balance 2 <sup>3</sup>	+400	8.034	9.11	9.29
	+200	8.033	9.07	9.28
SEM		0.05	0.11	0.09

\* Calcium (mg/dl).

<sup>1</sup> In all of tables; Group 1: cows that received prepartum diet with -100 DCAD and postpartum diet with +400 DCAD, group 2: cows that received prepartum diet with -100 DCAD and postpartum diet with +200 DCAD, group 3: cows that received prepartum diet with +100 DCAD and postpartum diet with +400 DCAD, group 4: cows that received prepartum diet with +100 DCAD and postpartum diet with +200 DCAD.

<sup>2</sup> In all of tables; prepartum dietary cation-anion difference levels (-100 and +100 mEq/kg DM).

<sup>3</sup> In all of tables; postpartum dietary cation-anion difference levels (+400 and +200 mEq/kg DM).

**Table 4.** The effect of dietary cation-anion difference on plasma minerals concentrations in postpartum period

Group		Mineral*					
		Na	K	Mg	P	Cl	S
1		143.56 <sup>a</sup>	4.55 <sup>a</sup>	2.31	6.48	93.07	1.42
2		138.71 <sup>b</sup>	4.24 <sup>b</sup>	2.28	6.42	93.44	1.43
3		143.56 <sup>a</sup>	4.55 <sup>a</sup>	2.28	6.48	93.08	1.42
4		138.71 <sup>b</sup>	4.23 <sup>b</sup>	2.26	6.43	93.44	1.42
SEM		0.39	0.04	0.05	0.07	0.50	0.03
Balance 1	-100	141.61	4.40	2.29	6.45	93.26	1.43
	+100	141.13	4.39	2.12	6.64	93.26	1.42
Balance 2	+400	143.56 <sup>a</sup>	4.55 <sup>a</sup>	2.29	6.48	93.07	1.42
	+200	138.71 <sup>b</sup>	4.24 <sup>b</sup>	2.27	6.42	93.44	1.43
SEM		0.27	0.03	0.03	0.05	0.35	0.02

\* Na (mEq/L), K (mEq/L), Mg (mg/dl), P (mg/dl), Cl (mEq/L), S (mEq/L).

**Table 5.** Effect of dietary cation-anion difference on urine minerals concentrations in postpartum period

Group		Mineral*						
		Na	K	Ca	Mg	P	Cl	S
1		102.44	232.24 <sup>a</sup>	3.43 <sup>b</sup>	23.95	2.36 <sup>b</sup>	52.23 <sup>b</sup>	218.54 <sup>b</sup>
2		95.82	203.30 <sup>b</sup>	4.73 <sup>a</sup>	27.74	3.06 <sup>a</sup>	57.92 <sup>a</sup>	235.51 <sup>a</sup>
3		102.58	234.22 <sup>a</sup>	3.46 <sup>b</sup>	24.13	2.37 <sup>b</sup>	51.93 <sup>b</sup>	218.52 <sup>b</sup>
4		97.03	203.29 <sup>b</sup>	4.72 <sup>a</sup>	27.94	3.15 <sup>a</sup>	57.72 <sup>a</sup>	235.50 <sup>a</sup>
SEM		2.5	1.62	0.29	1.9	1.6	1.46	3.55
Balance 1	-100	98.86	217.77	4.08	25.85	2.71	55.07	227.01
	+100	99.80	218.76	4.09	26.03	2.76	54.83	227.02
Balance 2	+400	102.51 <sup>a</sup>	233.23 <sup>a</sup>	3.45 <sup>b</sup>	24.04	2.36 <sup>b</sup>	52.08 <sup>b</sup>	218.53 <sup>b</sup>
	+200	96.15 <sup>b</sup>	203.30 <sup>b</sup>	4.73 <sup>a</sup>	27.84	3.10 <sup>a</sup>	57.82 <sup>a</sup>	235.51 <sup>a</sup>
p value								
SEM		1.77	1.15	0.21	1.34	1.13	1.03	2.51

\* Na (mEq/L), K (mEq/L), Ca (mEq/L), Mg (mEq/L), P (mg/dl), Cl (mEq/L), S (mEq/L).

**Table 6.** Effect of dietary cation-anion difference on blood and urine pH, milk production and milk compositions in postpartum period

Group		B-pH <sup>1</sup>	U-pH <sup>2</sup>	Milk yield	4% FCM	Fat	Protein	SNF	TS	Lactose
1		7.45 <sup>a</sup>	8.32 <sup>a</sup>	31.71	30.93 <sup>a</sup>	4.01 <sup>a</sup>	2.94	8.40	13.22 <sup>a</sup>	4.63
2		7.42 <sup>b</sup>	8.26 <sup>b</sup>	30.08	26.58 <sup>bc</sup>	3.62 <sup>ab</sup>	2.82	8.35	11.40 <sup>c</sup>	4.63
3		7.44 <sup>a</sup>	8.33 <sup>a</sup>	30.37	28.45 <sup>ab</sup>	3.81 <sup>a</sup>	3.04	8.43	12.50 <sup>b</sup>	4.59
4		7.42 <sup>b</sup>	8.27 <sup>b</sup>	29.54	24.41 <sup>c</sup>	3.16 <sup>b</sup>	2.88	8.36	11.82 <sup>c</sup>	4.62
SEM		0.0003	0.006	0.61	1.04	0.17	0.11	0.15	0.21	0.005
Balance 1	-100	7.43	8.29	30.89	28.75 <sup>a</sup>	3.81	2.88	8.38	12.31	4.63
	+100	7.43	8.30	29.96	26.54 <sup>b</sup>	3.48	2.96	8.40	12.16	4.61
Balance 2	+400	7.45 <sup>a</sup>	8.33 <sup>a</sup>	31.04	29.69 <sup>a</sup>	3.91 <sup>a</sup>	2.99	8.42	12.86 <sup>a</sup>	4.61
	+200	7.42 <sup>b</sup>	8.26 <sup>b</sup>	29.81	25.49 <sup>b</sup>	3.39 <sup>b</sup>	2.85	8.36	11.61 <sup>b</sup>	4.62
SEM		0.0002	0.004	0.43	0.73	0.12	0.08	0.10	0.15	0.05

<sup>a-c</sup> Means within a column with different superscripts are significantly different (p<0.05). <sup>1</sup> pH blood. <sup>2</sup> pH Urinary.

### Dry matter intake and lactation performance

Cows fed -100 DCAD had lower ( $p < 0.01$ ) DMI during 15, 10, 5 and 2 d prior to parturition (Table 2). Cows fed +400 DCAD consumed more DM than those fed +200 DCAD at 2, 4, 5 and 9 wk postpartum (Table 7). In addition, cows fed -100 DCAD prepartum had higher DMI at wk 1 postpartum (Table 7) and 4% FCM yield (Table 6) than cows fed +100 DCAD ( $p < 0.01$ ). Milk yield did not differ among treatments. However, milk fat and total solid percentages and 4% FCM yield increased for cows fed +400 DCAD in early lactation period.

### Health status

Milk fever case was not detected in this study. Four episodes of hypocalcemia were observed in cows fed +100 DCAD diet, but no hypocalcemia occurred in the cows fed -100 DCAD diet. There were no significant differences in the occurrence of retained placenta, metritis, endometritis, ketosis, mastitis and udder edema due to DCAD treatments. However, the prevalence of disorders tended to be lower in the group of cows fed -100 DCAD diet than the positive DCAD diet (Table 8).

## DISCUSSION

### Fluid acid-base balance

The kidney can efficiently eliminate excess anions from the blood, thus addition of anionic salts induces a sharp reduction in urinary pH. The negative DCAD diet may have overcome the capacity of kidneys to excrete sufficient  $H^+$  to maintain a constant blood pH, resulting in a slight systemic acidosis (Tucker et al., 1992). It is well documented (Joyce et al., 1997; Pehrson et al., 1999; Shahzad et al., 2008) that increased dietary anions (Cl and S) decreased the urine pH. Monitoring the pH of urine may be considered a sensitive method for assessing the acid-base balance in extracellular

**Table 8.** The effect of dietary cation-anion difference on health status

Prepartum	DCAD* (mEq/kg DM)			
	-100		+100	
Postpartum	+200	+400	+200	+400
Milk fever	0/6	0/6	0/6	0/6
Hypocalcemia	0/6	0/6	2/6	2/6
Retained placenta	0/6	0/6	3/6	1/6
Udder edema	0/6	0/6	1/6	3/6
Ketosis	0/6	0/6	1/6	0/6
Mastitis	1/6	0/6	3/6	4/6
Metritis	0/6	0/6	2/6	0/6
Endometritis	0/6	0/6	2/6	0/6
No. of total disorder	1	0	14	10

\* Represents dietary cation-anion difference ( $Na+K+0.15 Ca+0.15 Mg - (Cl+0.6 S+0.5 P)$ ).

fluids (Seifi et al., 2004). Results of the current study showed an incidence of a mild metabolic acidosis as reported by Vagnoni and Oetzel (1998). Similar results have been observed in previous studies for dairy cows (West et al., 1991; Moore et al., 2000; Charbanuae et al., 2006). Thus, DCAD has been shown to be associated with fluid acid-base balance. Spanghero (2004) found that DCAD was associated with urinary pH ( $r^2 = 0.81$ ) as also noticed by Liesegang et al. (2007). A higher urine pH has also been reported with increased cation (Na or K) of diet (Waterman et al., 1991). Alteration in urine pH reflected alteration in blood pH and kidneys played a vital role to minimize this change by making the urine pH alkaline, by excreting more  $HCO_3^-$  and conserving  $H^+$  (Roche et al., 2003).

### Plasma mineral concentration

A slight variation in prepartum plasma Na and K might be attributed to dietary alteration of these minerals as excess

**Table 7.** The effect of dietary cation-anion difference on dry matter intake in postpartum period

Group		Week								
		1	2	3	4	5	6	7	8	9
1		14.36 <sup>a</sup>	15.00 <sup>a</sup>	16.78	18.57 <sup>a</sup>	20.25 <sup>a</sup>	21.35	22.42 <sup>ab</sup>	23.11	23.61 <sup>a</sup>
2		14.30 <sup>a</sup>	14.43 <sup>b</sup>	16.35	18.25 <sup>b</sup>	19.47 <sup>b</sup>	20.88	21.96 <sup>b</sup>	22.86	23.10 <sup>b</sup>
3		13.18 <sup>b</sup>	14.91 <sup>a</sup>	16.76	18.65 <sup>a</sup>	19.93 <sup>ab</sup>	21.22	22.78 <sup>a</sup>	23.06	23.58 <sup>a</sup>
4		13.20 <sup>b</sup>	14.45 <sup>b</sup>	16.45	18.12 <sup>b</sup>	19.52 <sup>b</sup>	21.23	22.63 <sup>ab</sup>	23.06	23.00 <sup>b</sup>
SEM		0.16	0.12	0.14	0.08	0.18	0.20	0.24	0.12	0.14
Balance 1 <sup>1</sup>	-100	14.33 <sup>a</sup>	14.71	16.57	18.41	19.85	21.12	22.19 <sup>b</sup>	22.99	23.36
	+100	13.91 <sup>b</sup>	14.68	16.61	18.38	19.72	21.22	22.71 <sup>a</sup>	23.06	23.29
Balance 2 <sup>2</sup>	+400	13.77	14.96 <sup>a</sup>	16.77 <sup>a</sup>	18.61 <sup>a</sup>	20.09 <sup>a</sup>	21.28	22.60	23.09	23.60 <sup>a</sup>
	+200	13.75	14.44 <sup>b</sup>	16.40 <sup>b</sup>	18.18 <sup>b</sup>	19.49 <sup>b</sup>	21.05	22.30	22.96	23.05 <sup>b</sup>
SEM		0.88	0.01	0.02	0.01	0.03	0.29	0.23	0.32	0.01

<sup>1</sup> Prepartum dietary cation-anion difference levels (-100 and +100 mEq/kg DM).

<sup>2</sup> Postpartum dietary cation-anion difference levels (+400 and +200 mEq/kg DM).

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 DM) did not affect the plasma sodium and potassium concentrations significantly.

The increased plasma Ca level for cows consuming -100 DCAD diet compared to those receiving +100 DCAD diet might be due to mild metabolic acidosis induced by negative DCAD concentration. Bones act as a major reservoir of buffers for acid-base control of body fluids. When animals are placed on acidifying diets, the blood pH decreases. Frick et al. (2009) concluded that an acidic extracellular pH increases osteoclastic bone resorption which may result in increased plasma Ca concentrations.

The nadir of plasma Ca observed on d 1 may be due to the highly increased blood Ca demand for colostrum production. Kume et al. (2001) reported negative retention after calving. Similar findings were reported by Charbonneau et al. (2006); Lean et al. (2006) and Moore et al. (2000). The increased plasma Mg concentration in cows fed -100 DCAD may result from their higher Mg intake in close-up diets. Similar results were reported by Joyce et al. (1997); Lean et al. (2006) and Li et al. (2008). There was no significant effect of prepartum DCAD levels on serum phosphorus which is consistent with other reports (Shahzad et al., 2008; Wu et al., 2008). An increase in plasma chloride and sulphur concentrations by decreasing the DCAD level to -100 mEq/kg of DM is also supported by Roche (1999) and Tucker et al. (1988) who reported a linear increase in plasma Cl and S concentration with decreased DCAD level and it may be due to dietary concentrations in their experiments. In early lactation, there was no difference between treatments regarding Ca, Mg, P, Cl and S contents of plasma. This finding is supported by Delaquis and Block (1995a) who reported that mineral concentrations were not significantly affected by dietary DCAD levels. Increased plasma Na and K concentrations in cows fed +400 mEq/kg DM are consistent with the findings of Roche et al. (2005) who observed a linear increase in plasma Na and K concentrations of early lactating cows as ration DCAD increased.

#### **Urine mineral concentration**

The findings of the present study for urine mineral concentrations were in line with Tucker et al. (1988; 1992) who reported increased excretions of Na and K as the DCAD level increased from -20 to 10 mEq/100 g of DM, while Cl and S excretions decreased. This increase in Na and K concentrations with high DCAD diet is due to the fact that high DCAD diet contains higher contents of these minerals and urine composition is closely associated with diet composition (West et al., 1991).

The present findings are also consistent with West et al. (1992) who reported increased urinary Na and K concentrations at high DCAD levels while its reverse was true for Cl concentration. Although, dietary concentrations of Cl and S were similar between treatments, their urinary concentrations were higher in cows fed +200 mEq/kg of DM. This is in line with Delaquis and Block (1995b) who recorded increased urinary S in early lactating cows with decreased DCAD level. High dietary Cl content of cows fed -100 DCAD diet caused the kidneys to excrete more Cl in urine to maintain normal blood pH but in spite of that a slight metabolic acidosis was experienced, as was evident from altered prepartum blood pH. Similar findings were reported by West et al. (1991; 1992) who reported increased urinary Cl by decreasing the DCAD level. In pre-calving, increased urinary S by decreasing the level of DCAD might be attributed to dietary concentrations. So, more S intake may result in more plasma and urinary concentrations and vice versa (Takagi and Block, 1991). Increased urinary Ca excretion in cows fed -100 DCAD might be due to a slight metabolic acidosis, induced by negative DCAD diet. The pre-calving reduction in systemic pH was associated with an increased urinary output of Ca and this metabolic acidosis might have increased Ca resorption from bones and intestinal Ca absorption (Roche et al., 2003) due to increased synthesis of  $1,25(\text{OH})_2 \text{D}_3$  (Goff et al., 1991).

It is also reported that ruminants' kidneys are highly sensitive to blood cation-anion difference and increase the excretion of Ca during acidosis, independent of the hormonal action usually associated with Ca metabolism (Stacy and Wilson, 1970). Increased urinary Mg in cows fed -100 DCAD diet might be attributed to improved absorption because Mg absorption increased as DCAD level decreased. Moreover, increased urinary Mg concentration might be an indirect indicator of improved  $1,25(\text{OH})_2 \text{D}_3$  synthesis, because Mg is utilized when  $25(\text{OH}) \text{D}_3$  is converted to its active form  $1,25(\text{OH})_2 \text{D}_3$ , and during slight metabolic acidosis this process works more efficiently and thereby releasing more Mg in urine (Chamberlain and Wilkinson, 1996). Similar findings are reported by Gaynor et al. (1989); Lean et al. (2006); Oetzel et al. (1988); and Wu et al. (2008). In prepartum, urinary P concentration was unaffected by either DCAD treatments or time. This is supported by finding of Delaquis and Block (1995a) and Joyce et al. (1997). Postpartum, urinary P increased in cows fed +200 DCAD diet compared to those fed +400 DCAD diet, which is in agreement with Delaquis and Block (1995b) who reported increased urinary P at low DCAD compared to those fed high DCAD diets.

#### **Dry matter intake and lactation performance**

Decreased DMI in cows fed negative DCAD diet during

the days prior to parturition might be attributed to low rumen pH demonstrated by Tucker et al. (1991). The finding confirms results of previous researches (Block, 1984; Chan et al., 2006; Charbannuae et al., 2006) that showed anionic salts in a TMR decrease DMI prepartum. Vagnoni and Oetzel (1998) speculated that the reduction in feed intake for non-lactating dairy cows is likely caused by the acidogenic response to anion supplementation, rather than inherent poor palatability of supplemental acidogenic salts. This was supported by the observation that the more acidogenic mixtures caused the greatest feed intake depression of nonlactating dairy cows in a comparison among several different anion sources.

Increased DMI by cows fed +400 DCAD might be attributed to increased blood  $\text{HCO}_3^-$ , acid-base balance (Sanchez and Beede, 1994) and rumen pH (Tucker et al., 1991). Increased DMI with increasing the DCAD has also been reported by many researchers (Tucker et al., 1991; West et al., 1991; Hu et al., 2007:2007a). However, the present results did not agree with Chan et al. (2005) who reported that increasing DCAD from 20 to 50 mEq/100 g of DM had no effect on DMI in cows from 0 to 42 d postpartum. Buffer addition increased feed consumption in some studies (Kilmer et al., 1981). Moreover, by wk 1 postpartum, cows fed -100 DCAD diet had higher DMI than cows fed +100 DCAD diet. Improvements in Ca status apparently overcome any detrimental effects of reduced prepartum DMI (Joyce et al., 1997). Milk yield did not differ among treatments. Similar results have been observed in previous studies for dairy cows (Apper-Bossard et al., 2006; Hu et al., 2007b; Wildman et al., 2007; Wu et al., 2008).

Increased milk fat and TS percentages and also 4% FCM yield in cows fed +400 DCAD diet may be attributed to additional ruminal buffering provided by +400 DCAD diets. These results are consistent with those of others (Roche et al., 2005; Hu et al., 2007a) that have demonstrated a positive relationship between DCAD and milk fat concentration. Numerous studies have shown that addition of dietary buffers such as  $\text{NaHCO}_3$  and  $\text{K}_2\text{CO}_3$  increase milk fat percentage, especially when depressed milk fat occurred (Hu et al., 2007a). The effect of buffers on milk fat production is probably mediated via the rumen. Milk fat percentage is positively related to ruminal pH (Allen, 1997). Higher rumen pH has been reported to decrease the concentration of trans fatty acids in the rumen (Wildman et al., 2007). West et al. (1992) reported an increase in the milk fat percentage by increasing DCAD, without any effect on milk yield. Conversely, some studies have reported an increase in milk yield without changes in milk fat percentage (Tucker et al., 1991; West et al., 1991). Unaltered protein, lactose and SNF percentages due to

alteration in DCAD are in concordance with other studies (Tucker et al., 1988; West et al., 1992).

### Health status

There were no cases of milk fever in this study. Present results showed the expulsion time of placenta after parturition was lower in animals fed anionic diets which confirmed the results of Goff and Horst (1998). The results of Joyce et al. (1997) showed, in cows fed low DCAD diets, incidence of retained placenta was zero, but Hu et al. (2007a) observed diets with low DCAD did not alter the incidence of metabolic disorders. Overall, the reduced DCAD diet could improve periparturient dairy cow health.

### IMPLICATIONS

The results of this study clearly show the role of DCAD in regulating DMI and blood acid-base status. Supplementation of diets in the last 3 wk prepartum with anionic salts mixture at a rate sufficient to decrease DCAD dietary may benefit blood calcium homeostasis and tend to increase 4% FCM production and DMI in first week during postpartum and also improve health status. Thus, an input of -100 DCAD at close-up period followed by +400 DCAD at early lactation rations is recommended. Changes in urinary and blood pH and excretion of minerals were consistent with changes in DCAD. Further larger-scale and longer-term trials are needed to confirm the data presented here.

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