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Effect of prepartum dietary-cation anion difference on performance, blood mineral and metabolite concentrations in Holstein dairy cows

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Summary

Dietary cation-anion difference (DCAD) initiates a compensatory metabolic acidosis, which improves calcium (Ca) absorption and mobilization before calving and minimizes clinical and subclinical hypocalcaemia after calving. The goal of this study was to evaluate how mineral concentrations, blood metabolites, parathyroid hormone (PTH), and productive performance were affected by prepartum dietary cation-anion difference (DCAD mEq [(Na + K – Cl + S)]/kg of dry matter (DM)) in postpartum dairy cows. Forty-eight multiparous Holstein cows ranging from 2 to 5 lactations with an average body weight of 685 ± 10 kg (mean ± SD) were allocated in a randomized block design with three prepartum diets differing in DCAD (0, –100, and –180 mEq/kg DM). All cows were fed the same postpartum diet and kept on trial for 66 days. Cows fed –180 and –100 mEq DCAD had greater prepartum non-esterified fatty acid (NEFA) concentrations than cows fed 0 mEq DCAD. Cows fed –180 mEq DCAD had greater serum Ca concentrations than cows fed –100 and 0 mEq DCAD. PTH concentrations were greater in cows fed 0 mEq DCAD than in cows fed –100 or –180 mEq DCAD. Cows fed 0 mEq DCAD had higher milk protein and solid-not-fat (SNF) levels than cows fed –100 or –180 mEq DCAD. Treatment and interaction had no effect on milk yield, 3.5% fat-corrected milk (FCM), energy-corrected milk (ECM), milk fat, total solids (TS), lactose, milk urea nitrogen (MUN), magnesium (Mg), sodium (Na), chloride (Cl), potassium (K), beta hydroxybutyric acid (BHBA), and liver or kidney functions. We concluded that adding anionic salts to dairy cows' diets (–100 and –180 mEq DCAD) improved postpartum Ca availability and reduced clinical and subclinical hypocalcaemia while having no effect on milk yield or fat-corrected milk.

Keywords: anionic salts, hypocalcaemia, metabolic acidosis, milk urea nitrogen, parathyroid hormone

At the onset of lactation, metabolism changes to provide cows nutrients needed for mammary milk synthesis. Cows unable to alter their energy metabolism rapidly enough for milk synthesis either produce less milk or are susceptible to metabolic disorders (4). The transition period is defined by (33) as three weeks prior to and following parturition. This period of the dairy cow's lactation cycle is regarded as most important due to a higher risk of metabolic disease, which impacts milk production and fertility in later lactations (30). Hypocalcaemia affects the majority of mature cows during or immediately after calving (28). Hypocalcaemia has a negative impact on cow health and herd profitability, as it increases the risk of metabolic, lowers milk supply, reduces reproductive efficiency, and increases culling risk for cows early in lactation (7, 34, 40). An acidogenic diet has been used in prepartum dairy cow diets to prevent or reduce the severity of hypocalcaemia around parturition (15, 25). Anionic supplementation increases prepartum calcium (Ca) mobilization and tissue sensitivity to parathyroid hormone (PTH) (21).

During early lactation, body fat reserves are transported into the bloodstream as non-esterified fatty acids (NEFA), which contributes to meeting the overall cow energy needs. The liver eliminates a considerable portion of NEFA through partial oxidation into ketone bodies, such as beta hydroxybutyric acid (BHBA), or by re-esterifying them into triglycerides (26). According to previous studies, there is a relationship

between metabolite concentrations and milk yield, with higher ketone body concentrations in milk during early lactation being associated with a decrease in milk production (13). Serum BHBA elevation in the first week after calving was associated with early and overall lactation milk losses (14). Addition of prepartum anionic supplements, especially inorganic forms, has been associated with a reduced prepartum dry matter intake (DMI) and subsequent early lactation milk loss (43). Cows with hypocalcemia produce less milk and are more likely to suffer from mastitis, a displaced abomasum, and retained placenta, among other metabolic diseases (10). Subclinical hypocalcaemia reduces DMI and energy metabolism (35). The objective of our study was to investigate the influence of reducing the prepartum dietary cation-anion difference (DCAD) on early lactation blood mineral concentrations, energy metabolites, and milk production, and thus to achieve a better understanding of the metabolic response to prepartum acidogenic diets and blood Ca concentrations in transition cows. We hypothesized that supplementing anionic salts to the dairy cow diet that induced a tremendous metabolic acidosis would improve the Ca status of peripartum cows and reduce the incidence of clinical and subclinical hypocalcemia, as well as increase milk yield and it is composition in dairy cows after parturition.

Material and methods

This experiment was conducted according to the guidelines of Kafrelsheikh University and approved by the local experimental animal care committee, Faculty of Agriculture, Kafrelsheikh University, Egypt (id 4/2016 EC). All precautions were taken to decrease the risk of injury and disease throughout the trial period.

Cow selection and management. A field trial was conducted from December 2020 to March 2021 at a private dairy farm containing 1500 milking cows (located 80 kilometers from Cairo on the Ismailia Desert Road), which belongs to the Ismailia governorate of Egypt. Multiparous Holstein cows (n = 48) were selected from the herd 28 days before expected parturition and blocked by expected calving date, parity, and previous 305-d mature-equivalent production. Selected cows averaged 3.5 ± 0.20 lactations (mean ± SD; range: 2-5 lactations) and had an average body weight of 685 ± 10 kg (mean ± SD) and body condition score of 3.75 ± 0.89 (mean ± SD, 1-5 scale) at enrollment. The number of cows per treatment used in the experiment was based on previous research in the literature indicating a sample size sufficient to provide power to detect significant differences in the primary variables of interest, while also providing a minimal range in calving date (approximately 2 weeks) across treatments and the ability to manage the logistics of sample collection and animal care in a field location. Blood samples were collected weekly starting at day 22 prepartum and continued through day 66 postpartum. Prepartum diets were formulated to provide 3 DCAD levels (0, –100, or –180 mEq DCAD/kg DM), and postpartum diet provided DCAD level (+250 mEq/kg DM). The dietary DCAD concentrations were chosen to be consistent with previously published studies and to reflect values used in practice.

Cows were housed in an open yard with a shed area about one third of the total space area. Each cow had a square of about 40 m^2 in yard space, with sand bedding, and was dry due to exposure to direct sun light. Cows were provided with free access to clean fresh water and were fed to achieve the formulated dry matter intake (DMI) based on recommendations from (38). The water was of good quality according to water quality criteria for livestock given by (38), with < 100 ppm nitrate, < 500 ppm sulfate, 7 to 8 pH, and < 1000 ppm total soluble salts (TSS).

Cows were milked three times daily (at 8:00, 16:00 and 24:00 h), and milk flow was automatically recorded by an electronic milk meter fixed in each milking unit in the parlor, with data transported through a network to a computer system computing the average milk yield per cow per day per month (Alpro-herd management system, Delaval, Sweden).

Dietary treatments. The composition of diets is shown in Table 2. The individual intake was not measured. Determining of DMI is a challenge in conducting a field study with intensive sampling at a commercial dairy, and DMI data could not be analyzed statically. The average DMI for different treatments in the prepartum diet was 13, 13.1, and 13.2 kg/day for the 0, –100, and –180 mEq/kg DM diets, respectively. In the postpartum period, DMI averaged 22.4 kg DM/day for the +250 mEq/kg DM.

Cows were fed in groups with a total mixed ration (TMR) twice daily, at 7:00 and 17:00 h. Three prepartum treatment diets (Tab. 1) were formulated (0, –100, and –180 mEq DCAD/kg DM), with 16 cows randomly assigned to each treatment. In both pre- and postpartum periods, the open-yard pens used to house the cows were adjacent to each other, with the same size and stocking density, and were managed under identical conditions according to standard operating procedures at the farm by a single individual member of the research team. After calving, during two weeks of transition feeding, cows were provided a lactation diet formulated to contain +250 mEq DCAD/kg DM and were fed their respective postpartum diets with a total mixed ration (TMR) for the remainder of the trial. The prepartum and postpartum diets were formulated as shown in Tables 1 and 2, using CNCPS V 6.5 (8), Cornell Net Carbohydrate and Protein System (Cornell University, Ithaca, NY) ration evaluator to meet or exceed NRC recommendations from (38). Dry matter was determined using a forced-air drying oven set at 55°C for 48 h. The diets were analyzed for crude protein (CP), ether extract (EE), crude ash, calcium, phosphorus, sodium, potassium, magnesium, chloride, sulfur (1), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (44). Total digestible nutrients (TDN), net energy for lactation (NE_L) and non-fiber carbohydrates (NFC) were calculated according to NRC (38). The analyses of feed ingredients were carried out in the Animal Reproductive Research Institute of the Ministry of Agriculture's Agriculture Research Center in Al-Harm, Egypt. The chemical composition of the experimental diets is shown in Table 2.

Blood sample minerals and hormone procedures. Blood samples were collected weekly from day 14 before to

Tab. 1. Composition of experimental diets formulated to differ in dietary cation-anion difference (DCAD)

Ingredients	Prepartum DCAD			Postpartum DCAD
	0	–100	–180	+250
Corn silage	39.35	38.94	38.68	32.07
Wheat straw	8.93	8.83	8.77	
Alfalfa hay				8.05
Corn, ground	19.69	19.49	19.35	25.08
Soybean meal, 44% CP	7.75	7.67	7.62	20.43
Beet pulp, dried	7.20	7.12	7.08	
Corn gluten feed				8.45
Sunflower meal, 36% CP	14.15	14.00	13.91	
Energizer-Gold[1]				0.85
OleoFat[2]				0.89
Limestone	1.06	1.11	1.14	0.54
Salt (NaCl)	0.30	0.30	0.30	0.41
Sodium bicarbonate				1.15
M&V Dry Cow premix[3]	0.31	0.30	0.30	
Magnesium oxide	0.23	0.22	0.22	0.19
Free feed silica[4]				0.19
Mycofix[5]				0.07
Diamond V Yeast XP[6]				0.07
Organic zinc glycinate				0.02
Potassium carbonate				0.27
Magnesium sulphate	0.15	0.45	0.82	
Dicalcium phosphate	0.22	0.22	0.22	0.09
M&V premix[7]				0.27
MegAnion[8]	0.37	0.51	0.54	
Calcium chloride	0.31	0.82	1.06	
DCAD mEq/kg DM[9]	0.0	–100	–180	+250

Explanations: [1]Calcium salts fatty acids 84.5% (IIFCO, Malaysia); [2]Fractionated fatty acids 99% (Feedico, El-Sadat City, Egypt); [3]Dry cow mineral-vitamins premix composition (per kg): Vitamin A 9,000,000 IU, Vitamin D3 2,000,000 IU, Vitamin E 40,000 mg, Mn 50,000 mg, Zn 50,000 mg, Fe 50,000 mg, Cu 15,000 mg, I 250 mg, Co 150 mg, Se 250 mg (El-Dakahlia Company, Egypt); [4]Silica mycotoxin binder (Avitasa, Spain); [5]Enzymatic mycotoxin binder biology (Biomin GmbH, Austria); [6]Diamond V (Cedar Rapids, IA, USA); [7]Dairy cow Mineral-Vitamins premix composition (per kg): Vitamin A 12,000,000 IU, Vitamin D3 2,500,000 IU, Vitamin E 35,000 mg, Mn 80,000 mg, Zn 100,000 mg, Fe 50,000 mg, Cu 20,000 mg, I 300 mg, Co 400 mg, Se 300 mg $CaCo_3$ up to kg (El-Dakahlia Company, Egypt); [8]MegAnion (Origination O2D Inc, Maplewood, MN, USA) composition (%DM): 81.5% CP, 3.8% ADF, 7.5% NDF, 0.52% EE, 0.26% Ca, 0.49% P, 2.5% Mg, 1.6% K, 0.07% Na, 23.7% Cl, and 3.1% S; [9]Dietary cation-anion difference: DCAD = (Na + K) – (Cl + S) = –8270 mEq/kg DM according to Goff (19)

Tab. 2. Chemical composition of experimental diets formulated to differ in dietary cation-anion difference (DCAD)

Components	Prepartum DCAD			Postpartum DCAD
	0	–100	–180	+250
DM%	56	57	57	53
CP	15.0	14.8	14.8	17.5
Soluble protein,% of CP[1]	30.0	31.0	31.0	26.0
Ether extract	3.00	3.00	3.00	4.50
NDF	42.2	41.9	41.7	28.0
NFC[2]	31.8	31.8	31.8	40.8
TDN	70.0	69.0	69.0	75.0
peNDF[3]	31.0	31.0	30.0	23.0
NE_L, Mcal/kg[4]	1.51	1.52	1.51	1.73
Ash	8.00	8.9	9.00	9.20
Ca	0.99	1.10	1.20	0.89
P	0.36	0.37	0.36	0.41
Mg	0.38	0.42	0.48	0.30
K	1.00	1.00	1.00	1.23
Na	0.20	0.20	0.20	0.56
Cl	0.51	0.70	0.80	0.34
S	0.32	0.40	0.48	0.34
DCAD[5] (mEq/kg DM)	0.0	–103.8	–181.9	+250.6

Explanations: DM – dry matter; CP – crude protein; NDF – neutral detergent fiber; NFC – non-fiber carbohydrates; TDN – total digestible nutrients; peNDF – physically effective neutral detergent fiber; NEL – net energy for lactation; Ca – calcium; P – phosphorus; Mg – magnesium; K – potassium; Na – sodium; Cl – chloride; S – sulfur; DCAD – dietary cation-anion difference; [1]Soluble protein (%) = CP (%) – insoluble protein (%). Calculated based on the CNCPS (8) model; [2]NFC = 100 – [(NDF – neutral detergent insoluble CP) + CP + ash + fat)], calculated according to NRC (38); [3]Predicted by Cornell Net Carbohydrate and Protein System v 6.55 (Cornell University, Ithaca, NY), calculated according to Mertens (36); [4]Calculated from chemical composition NRC (38); [5]DCAD = (Na + K) – (S + Cl)

21 days after calving via coccygeal venipuncture into either sodium heparin or no anticoagulant vacutainer serum tubes. Sampling time (approximately 13:00 h) corresponded to approximately 5 h after morning feeding. Plasma and serum were separated after centrifugation at 2000 × g for 20 min at 5°C, and frozen at –80°C until analysis. Analyses were performed in Veterinary Diagnostic Lab of Animal Reproductive Research Institute, Agriculture Research Center, Ministry of Agriculture, Al-Harm, Cairo, Egypt. Serum was analyzed for parathyroid hormone (PTH), and insulin concentrations were determined using a bovine ELISA kit (No. 18, Keyuan Road, DaXing Industry Zone, Beijing, China). Plasma/Serum concentrations of Ca, P, Mg, Na, K, Cl, urea, and creatinine were determined colorimetrically using an RA-50 chemistry analyzer (Bayer, China) according to the manufacturer's instructions and readymade commercial chemical kits (CAT. No. CA 1210, PH 1710, MG 1610, SO 1910, PT 1820, CL 1211, UR 2110 and CR 1251 Biodiagnostic co. Egypt). Serum total protein and albumin were determined colorimetrically (RA-50 chemistry analyzer, Bayer, China) using readymade commercial chemical kits (CAT. No. TO 2020, AB 1010 Biodiagnostic co. Egypt). Globulin was calculated mathematically (globulin = total protein – albumin). Glucose and BHBA were measured

immediately in whole blood using a portable Free-Style Optium Neo reader with blood glucose and BHB ketone test strips (Abbott Diabetes Care Ltd, Range Road, UK). The glucose measurement device has been validated for use in dairy cattle. Plasma NEFA was determined according to the procedures of Johnson and Peters (1993) using a C kit from Wako Chemicals USA Inc. (Richmond, VA). Concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using an automated biochemistry analyzer (Olympus AU 400; Olympus Corp., Tokyo, Japan).

Milk samples. Milk samples were collected at 3 consecutive milkings every 2 weeks until day 60 after calving. They were mixed together, and approximately 200 to 250 mL were stored at 4°C until transportation to a commercial laboratory (Animal Research Centre) within 72 h of collection for analysis. Samples were analyzed for chemical composition (milk protein, lactose, milk fat, and solid-not-fat [SNF]) using a pre-calibrated milk analyzer (LACTOSCAN LA, 8900 Zagora Bulgaria). Total solids (TS) content was calculated by adding fat content to SNF. Milk urea nitrogen (MUN) was analyzed by mid-infrared techniques (method 972.16) (2).

Statistical analysis. The trial followed a completely random design with a cow as the experimental unit. All data were analyzed by ANOVA, and results for the pre- and postpartum periods were analyzed separately. Data were analyzed by mixed models according to the MIXED procedure of SPSS V23 (https://www.ibm.com/eg-en/analytics/spss-statistics-software). Prepartum responses with a single measurement per cow were analyzed with the fixed effects of prepartum DCAD (0, –100, or –180 mEq/kg DM) and cow within treatment as the residual. Data with repeated measures within the prepartum period were analyzed by the same mixed model with the addition of the fixed effects of day and day × prepartum treatment interaction. Responses with a single measurement per cow in the postpartum period were analyzed with the fixed effects of the prepartum level of DCAD (0, –100, or –180 mEq/kg DM) and postpartum level of DCAD (+250 mEq/kg DM). For repeated measures, sampling day and interactions between prepartum DCAD and sampling day were added to the model. The repeated statement was included in all mixed models with repeated measurements, with day specified as the repeated effect. Data from the preliminary period were included as covariates in the analysis of prepartum effects. Significance was assumed at $P \leq 0.05$. Duncan's test was used to compare differences between means.

Results and discussion

Blood metabolites. Serum metabolites are shown in Tables 3 and 4. The consumption of anionic salt diets had an effect on concentrations of serum Ca, NEFA, tended to have greater with increasing negative DCAD for 0, –100, –180, $P < 0.05$, for the treatment and day of sample as shown. Prepartum plasma concentration of NEFA as show (Tab. 3), there is effect of treatment and day, but there were no effects for interaction $P > 0.05$, but postpartum plasma NEFA, there is effect of treatment and day but no effect for interaction DCAD × day, $P > 0.05$. As shown in Tab. 3, there was no hypocalcaemia in either pre- or postpartum cows, since serum Ca never decreased below 8 mg/dL for cows fed 0, –100, or –180 mEq DCAD. The elevated serum Ca level in cows consuming –180 and –100 mEq DCAD diets, stated effect of interaction DCAD × day, compared to the lowest level for 0 DCAD diet, could be attributed to moderate metabolic acidosis generated by anionic salt supplementation. Phosphorus concentrations in blood increased at the time of calving (day 0) (8.79 0.490 mg/dL) and day 2 postpartum (8.77 0.499 mg/dL) due to the effect of day. Treatment and DCAD × day interaction had no effect on

Tab. 3. Prepartum and postpartum mineral concentrations and blood metabolites for cows fed diets formulated to contain 0, –100, and –180 mEq/kg DM

Minerals and blood metabolites	DCAD			SEM	P-value		
	0	–100	–180		DCAD	Day	T × day
Prepartum							
Ca mg/dL	8.38^{c}	8.85^{b}	9.40^{a}	0.048	0.001	0.001	0.170
P mg/dL	6.23	6.20	6.26	0.032	0.103	0.820	0.011
Mg mg/dL	2.85	2.86	2.85	0.014	0.981	0.284	0.980
Na mEq/L	142.12	142.19	141.66	0.137	0.945	0.575	0.377
K mEq/L	4.94	4.90	4.93	0.012	0.276	0.825	0.675
Cl mEq/L	104.58	105.22	103.66	0.305	0.124	0.869	0.930
Glucose mg/dL	63.41	61.00	60.50	0.633	0.653	0.328	0.543
BHB mmol/L	0.525	0.566	0.575	0.019	0.539	0.399	0.656
NEFA µmol/L	231.66^{b}	256.00^{a}	275.83^{a}	4.378	0.001	0.001	0.181
Postpartum							
Ca mg/dL	8.59^{c}	8.89^{b}	9.27^{a}	0.042	0.001	0.001	0.001
P mg/dL	7.62	8.01	7.59	0.089	0.096	0.001	0.477
Mg mg/dL	2.83	2.84	2.88	0.011	0.155	0.069	0.065
Na mEq/L	143.28	142.99	142.88	0.213	0.729	0.029	0.975
K mEq/L	5.38	5.37	5.36	0.033	0.976	0.139	0.937
Cl mEq/L	104.85	105.22	104.32	0.255	0.356	0.916	0.970
Glucose mg/dL	61.90^{a}	59.40^{b}	60.66^{b}	0.607	0.247	0.001	0.025
BHB mmol/L	0.827	0.767	0.827	0.016	0.229	0.888	0.270
NEFA µmol/L	788.66^{b}	790.10^{b}	854.20^{a}	9.072	0.005	0.001	0.118

Explanations: DM – dry matter; DCAD – dietary cation-anion difference; T – time of sample taking; Ca – calcium; P – phosphorus; Mg – magnesium; Na – sodium; K – potassium; Cl – chloride; BHB – beta hydroxybutryrate; NEFA – non-esterified fatty acids

Tab. 4. Prepartum and postpartum liver and kidney functions and blood hormones for cows fed diets formulated to contain 0, –100, and –180 mEq/kg DM

Parameters	DCAD			SEM	P-Value
	0	–100	–180		
Prepartum					
AST u/L	73.80	73.88	71.66	0.451	0.108
ALT u/L	30.76	31.59	30.80	0.280	0.276
Total protein g/mL	6.39	6.40	6.50	0.040	0.474
Albumin g/dL	3.14	3.11	3.13	0.023	0.921
Globulin g/dL	3.25	3.28	3.37	0.033	0.335
Urea mg/dL	32.00	31.61	3.11	0.178	0.154
Creatinine mg/mL	1.21	1.21	1.20	0.005	0.530
Insulin pmol/L	179.16	178.66	179.66	0.416	0.738
PTH pg/mL	48.45	31.28	39.46	1.873	0.001
Postpartum					
AST u/L	71.84	71.47	69.02	0.707	0.218
ALT u/L	28.25	28.36	28.21	0.249	0.973
Total protein g/dL	6.50	6.51	6.65	0.033	0.138
Albumin g/dL	3.13	3.25	3.82	0.177	0.250
Globulin g/dL	3.37	3.25	3.82	0.173	0.385
Urea mg/dL	29.16	28.8	29.24	0.165	0.541
Creatinine mg/dL	1.24	1.21	1.22	0.010	0.513
Insulin pmol/L	82.33	83.33	85.50	0.799	0.269
PTH pg/mL	51.80	50.57	47.38	1.232	0.339

Explanations: DM – dry matter; DCAD – dietary cation-anion difference; PTH – parathyroid hormone; AST – aspartate aminotransferase; ALT– alanine aminotransferase

mineral concentrations of Mg, Na, K, and Cl in either pre- or postpartum. DCAD supplementation and interaction had no effect on liver and renal functions, such as ALT, AST, BHBA, albumin, total protein, urea, and creatinine (Tab. 3, 4).

DCAD and endocrine effects. Parathyroid hormone (PTH) was higher in cows fed prepartum 0 mEq DCAD compared with cows fed –100 or –180 mEq DCAD, whereas insulin hormone was not altered by DCAD treatments in either pre- or postpartum dairy cows. No differences in PTH were observed in cows fed postpartum. Our results are consistent with those from (11), who investigated the effects of reducing DCAD in prepartum diets (–100 and –180 mEq/kg DM), which included increased parathyroid hormone sensitivity in cows (18). Goff et al. (2014) found that an increased renal production of $1,25(OH)_2$ vitamin D3 (17, 22) increased the responsiveness of target Ca resorption from bone (6, 20, 33), and higher blood Ca concentrations were associated with an increased responsiveness of target Ca resorption from bone (6, 20, 33, 39, 41).

DCAD and energy metabolites. The concentrations of NEFA in blood have been used to estimate energy balance (31). Prepartum cows fed –100 or –180 mEq DCAD/kg DM had greater plasma NEFA concentrations, which is consistent with previous findings. According to previous research, plasma NEFA concentrations begin to rise from the last 2 weeks of gestation (45) to the last 2 to 3 days (32) before parturition. Plasma NEFA concentrations peak around calving and subsequently begin to decline as feed intake increases after calving (24, 29, 32). Greater concentrations of NEFA were detected on the day of calving in cows fed –180 mEq DCAD/kg DM compared with cows fed 0 or –100 mEq DCAD/kg DM because of the reduced dry matter intake in cows fed low DCAD diets. No differences due to treatment, day, or interaction were observed in blood BHBA. There were no effects of treatment or DCAD × day interaction on blood glucose levels prior to calving ($P > 0.05$) in cows given DCAD 0, –100 and –180 mEq/kg DM. We detected a significant effect of DCAD treatments and DCAD × day interaction ($P < 0.05$) on blood glucose postpartum (Tab. 3).

DCAD and productive performance. There were no significant effects of DCAD on milk yield, 3.5% fat-corrected milk (FCM), milk urea nitrogen, total solids, energy-corrected milk (ECM) or milk fat% resulting from pre- or postpartum treatments and no interactions between pre- and postpartum treatments ($P > 0.05$) (Tab. 5). Our results are in line with previous studies (3, 34, 46, 42), in which prepartum negative DCAD feeding did not affect postpartum milk yield. Moore et al. (37) fed anionic salts and observed no

Tab. 5. Milk yield, FCM 3.5%, and milk composition for cows fed diets formulated to contain 0, –100, and –180 mEq/kg DM

Prepartum	DCAD			SEM	P-Value		
	0	–100	–180		DCAD	parity	DCAD × parity
Milk yield kg/d	38.00	38.92	39.57	1.008	0.837	0.994	0.757
FCM 3.5 kg/d[1]	39.17	40.08	40.92	1.079	0.843	0.988	0.853
ECM kg/d[2]	40.00	40.56	41.53	1.131	0.889	0.998	0.817
Fat %	3.69	3.67	3.71	0.024	0.547	0.186	0.254
Protein %	3.23	3.11	3.14	0.028	0.428	0.186	0.034
Lactose %	4.70	4.68	4.68	0.025	0.654	0.050	0.748
Total Solid %	12.56	12.21	12.39	0.520	0.133	0.071	0.055
SNF %	8.87	8.53	8.68	0.049	0.129	0.021	0.232
MUN mg/dL	11.71	12.42	11.57	0.441	0.669	0.060	0.108

Explanations: SNF – solid-not-fat; MUN – milk urea nitrogen; DM – dry matter; DCAD – dietary cation-anion difference; [1]3.5% fat-corrected milk (FCM) = (0.432 × kg of milk yield) + (16.218 × kg of fat); [2]Energy-corrected milk (ECM) = (0.327 × kg of milk yield) + (12.95 × kg of fat) + (7.65 × kg of true protein), corrected to 3.5% fat and 3.2% milk protein

decrease in milk yield from day 7 to day 70 in milk. In contrast to our findings, Beede et al. (5) observed that the addition of anionic salts to the prepartum diet increased milk yield in the following lactation by 3.6%. Increased milk protein (Tab. 5) may be attributed to interaction between DCAD and parity (P = 0.034), but increased solid-not-fat (SNF) percentages in cows fed 0 mEq DCAD/kg DM compared with cows fed –100 or –180 mEq DCAD/kg DM were related to the effect of prepartum DCAD ($p < 0.05$) (Tab. 5).

The main hypothesis of our study was that feeding a negative DCAD prepartum diet would result in an improved postpartum Ca status, with the advantages increasing as prepartum DCAD decreased. Thus, it was expected that cows fed a reduced prepartum DCAD would have a higher blood Ca and milk production. This fundamental hypothesis was supported by linear increases in postpartum blood Ca with decreasing prepartum DCAD. Feeding acidifying diets to dairy cows causes a reduction in blood pH, resulting in an acidic extracellular pH, which accelerates osteoclastic bone resorption, leading to greater blood serum Ca concentrations (16). Parathyroid hormone (PTH) activity, blood Ca and P concentrations, and $1,25(OH)_2$ vitamin D3 govern bone Ca and P resorption. Bone resorption is increased by diets with negative DCAD, and P concentrations in blood are expected to rise along with Ca concentrations. Other mechanisms, such as improved P absorption in the digestive tract, could contribute to higher P concentrations (27). PTH, which is released at times of Ca stress, increases P excretion in the kidneys and saliva, which can be detrimental to maintaining appropriate blood P concentrations. This is one reason why cows with hypocalcemia have a low P concentration. PTH may increase blood P concentration by stimulating bone mineral resorption and increase the efficiency of intestinal phosphate absorption by stimulating the kidneys to release 1,25-dihydroxyvitamin D. PTH, on the other hand, is produced in response to hypocalcaemia, not hypophosphatemia. In our present study, this could be related to the fact that when bone mobilization begins, hydroxyapatite, which is rich in calcium and phosphorus, contributes to an increase in these minerals in the blood.

Plasma NEFA concentrations. The increased prepartum non-esterified fatty acids due to the detrimental effect of anionic salts on dry matter intake, although BHBA indicates that the short-term energy balance was not affected by treatments or interactions. This would be predicted in the absence of any metabolic challenge, which usually happens at or shortly after calving, unless DMI dropped dramatically, resulting in a sustained negative energy balance that was not visible based on BHBA.

Parathyroid hormone. The target tissues respond to PTH and work on bone and kidney cells to restore blood Ca levels back to normal. The PTH concentration rises when blood Ca falls in cows not fed anions that have more alkaline blood and urine, although tissues are resistant to the effects of PTH. We believe that the PTH receptor does not correctly detect the hormone. As a result, blood calcium levels do not rise rapidly or at all, and the parathyroid gland secretes considerable amounts of the hormone for a prolonged period of time. PTH concentrations in the blood of cows with milk fever are extremely high, but this does not help them maintain adequate calcium levels, since tissues do not recognize it (21).

Milk composition. This study discovered some interesting effects of treatment on milk protein metabolism. Throughout the postpartum period, cows fed decreasing DCAD showed a linear decline in milk protein percentage and MUN content. One possible link between DCAD feeding and protein metabolism is the need for extra nitrogenous compounds to assist the excretion of acid as ammonium in urine, particularly when urine pH decreases below 6.3 (9), as was the case in cows fed negative DCAD. In line with (12), we found that the milk protein percentage was lower for cows fed diets with –100 and –180 mEq DCAD/kg DM compared to cows fed 0 mEq DCAD/kg DM, but further research is needed to better define the effects of the prepartum DCAD concentration on the milk protein content.

In conclusion, feeding prepartum anionic salts to dairy cows improved Ca availability at calving for cows fed –100 and –180 mEq/kg DM compared with cows fed 0 mEq/kg DM. Postpartum serum Ca concentrations increased significantly with decreasing prepartum DCAD. Reducing DCAD had no observable effect on prepartum Mg, Na, K, Cl, productive performance, insulin, BHBA, or glucose in the early lactation period. Prepartum plasma concentrations of NEFA were increased in cows fed –180 mEq DCAD compared to cows fed 0 and –100 mEq DCAD. Negative DCAD diets in prepartum dairy cows are caused by higher blood P concentration near parturition, which can be attributed to mineral resorption.

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