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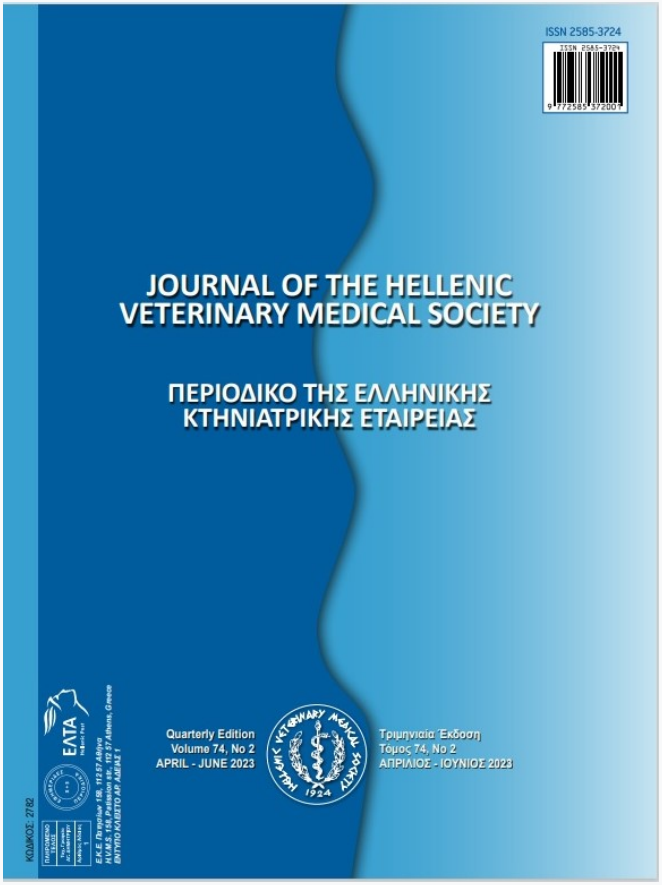

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The Effect of Variation in Dietary Cation-Anion Difference on Calcium Status, Blood Metabolites and Rumen Activity during the Transition Period of Holstein Dairy Cows

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ABSTRACT: A negative dietary cation-anion difference (DCAD) induces a compensated metabolic acidosis, stimulating calcium (Ca) absorption and mobilization before calving, thereby decreasing clinical and subclinical hypocalcemia postpartum. The study was designed to determine the effects of varying pre- and postpartum DCAD diets on serum total calcium, ionized calcium, blood and ruminal fluid metabolites, and milk production in prepartum and postpartum Holstein cows. Fifty-four multiparous dry Holstein cows n= 54, were enrolled in a completely randomized block experimental design at 29 days prior to expected parturition through 86 days in milk. A 3 x 2 factorial arrangement of treatments was utilized. Three DCAD levels were fed precalving (0, -120 and -200 mEq/kg DM), n=18 cows per treatment and two DCAD levels were fed post calving (+200 and +400 mEq/kg DM), n=27 cows per treatment. Prepartum urine pH was lower for cows fed -200 DCAD compared with those fed -120 or 0 DCAD. Postpartum urine pH was higher for cows fed +400 mEq/kg compared to cows fed +200 mEq/kg DCAD. Prepartum serum total calcium, ionized calcium, and hydroxyproline was highest for cows fed -200 DCAD compared to those fed -120 and 0 DCAD. Parathyroid hormone was highest for cows fed 0 DCAD compared to those fed -120 and -200 DCAD. Prepartum dry matter intake (DMI) was lower for -200 and -120 DCAD compared with 0 DCAD. Postpartum DMI was not different among treatments. Pre- and postpartum DCAD treatments did not affect total milk yield or milk fat, percentage of milk protein not affected by different pre-and postpartum DCAD levels. Prepartum anionic diets lowered urine pH and parathyroid hormone and raised serum hydroxyproline, resulting in improved Ca availability after parturition. Postpartum blood metabolites were unaffected in cows given positive DCAD (+200 and +400 mEq/kg DM). Calves born to cows fed low DCAD had no change in calf bieth weight. Colostrum amount and IgG concentrations were unaffected by treatments. No effects of pre-or postpartum DCAD treatments were observed for milk yield and fat-corrected milk. Feeding prepartum an acidogenic diet improved postpartum Ca status in multiparous Holstein cows.

***Keywords*:** acidogenic diet; colostrum; hydroxyproline; parathyroid hormone; multiparous; immunoglobulin-G

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INTRODUCTION

Parturition can cause physiological changes in dairy cows. Serum calcium (Ca) concentration deceases abruptly 2 to 3 days prior to calving due to the onset of lactation, resulting in inadequate availability of ionized Ca (Quiroz-Rocha et al., 2009) to meet the cow metabolic requirements. The disturbances in Ca homeostatic processes results in insufficient availability of ionized Ca (Horst et al., 1997). Hypocalcaemia is a clinical disease affecting 5% of lactating dairy cows (NAHMS, 2002), [National Animal Health Monitoring Service] or a subclinical disorder in 50% of the cows with more than 2 lactations (Horst et al., 2003). The Ca requirement for cows increases as pregnancy advances due to the developing fetus; however, around the time of parturition, the demand for Ca increases at least 2-fold due to colostrogenesis and subsequent milk production (House and Bell, 1993; Goff and Horst, 1997). The cation content of forages in the prepartum ration induces insensitivity to PTH signaling in the kidney and bone especially when a positive DCAD diet is fed (Liesegang et al., 2007; Goff and Koszewski, 2018). Alternatively, a dietary cation-anion difference (DCAD) strategy that is associated with the supplementation of anionic salts to create an acidogenic prepartum diet has been used to improve Ca homeostasis and, thus, to combat parturient paresis in periparturient cows (Goff et al.,2014). A negative DCAD diet causes compensated metabolic acidosis in cows to decrease urine pH and increase urinary Ca excretion (Leno et al., 2017). Compensated metabolic acidosis also directly affects Ca availability by increasing bone resorption and tissue responsiveness to hormonal signals (Liesegang et al., 2007; Rodriguez et al., 2016). Under conditions of a compensated metabolic acidosis prepartum, Ca is absorbed actively and passively from the rumen and small intestine, and mobilized from bone stores to be excreted through the urine to maintain homeostasis. This continuous Ca flux creates a supply of available Ca to be used at the tissue level at the initiation of lactation when urinary Ca excretion is conserved (Grünberg et al., 2011; Megahed et al., 2018). Irreversible Ca losses due to colostrum and milk synthesis at the start of lactation results in significant changes in Ca requirements and postparturient cows being unable to maintain normal blood Ca concentrations (Reinhardt et al., 2011). Although the daily requirement for calcium is just 30 g, DeGaris and Lean (2008) observed that blood Ca loss to milk can reach 50 g/d, with 15 g going to feces and urine loss and 15 g going to fetal development before calving. [we would say that the amount of Ca that can drainage for milk production is more that lost in feces and urine]. Another dietary approach for lowering urine pH to below 7.0 and increasing Ca availability is to provide additional dietary Ca in a partly acidogenic diet, however increasing dietary Ca has been found to raise urine pH, reversing or reducing the advantages of compensated metabolic acidosis (Goff and Koszewski., 2018). In the current study, our hypothesis was feeding prepartum acidogenic diet can increase the Ca availability in postpartum dairy cows, targeting a urine pH of 5.5 to 6.0, would allow for an increase in prepartum Ca flux (Lean et al., 2006) and improve rumen function in cows fed postpartum positive DCAD. Although positive DCAD diets are commonly fed during lactation, the potential interaction between prepartum and postpartum DCAD levels has received very limited attention in the literature. Thus, the main objective of our study was to evaluate the effects of interaction between a 3 levels of prepartum DCAD (0, -120 or -200 mEq/kg DM) and 2 levels of postpartum DCAD concentrations (+200 or +400 mEq/kg DM) on urine pH, IgG, PTH, hydroxyproline, Ca status, rumen metabolites, blood parameters and milk variables.

MATERIALS AND METHODS

Experimental design and cattle treatments

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

A field experiment was conducted from September 2021 to January 2022 at commercial dairy farm (located 76 km from Alexandria on the Cairo Desert Road). This facility is managed and controlled by the Ismailia governorate of Egypt.

Twenty nine days before expected parturition. Fifty-four multiparous Holstein cows (n=54) with range (1- 5) lactation number. Cows were randomly selected from the herd and were assigned by expected calving date, parity, and previous 305-day mature-equivalent production. 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 animal movements into and out of pens relative to calving date, sample collection, and animal care in a field location. Data were collected from 21 day prepartum and continued through 86 d postpartum. Cows were fed a total mixed ration (TMR) twice daily at 0700 and 1700 hours. Feed was manually pushed up twice daily, experimental diets were formulated to meet or exceed (NRC 2001) requirements (Table 1) and fed to provide a minimum of 3% feed refusal. The transition to the final lactation diet occurred over a 2-week period to avoid sub-acute ruminal acidosis SARA. Concentrate ingredients in the diet were mixed to form a grain mix prior to mixing in the total mixed ration (TMR). The grain mix and remaining ingredients were mixed in a mixer wagon used to deliver the TMR to cows; quantities of feed offered and refused were recorded daily. Cows were housed in tied barns housing system with a sand-bedded that contained fans and water fogger for cooling; cows were provided clear, clean, fresh drinking water. The water contained < 100 ppm nitrate, < 500 ppm sulfate, and < 1000 ppm total soluble salts with a pH of 7 to 8. Cows were milked three times daily (0800, 1600 and 2400 h) and milk was automatically recorded using an electronic milk meter fixed in each milking unit (Alpro, Deleval, Sweden).

Table 1. Ingredient composition of experimental diets formulated to differ in dietary cation-anion difference (DCAD).

	Precalving	Postcalving			
	0	-100	-180	+200	+400
Corn silage	39.35	38.94	38.68	32.07	32.07
Wheat straw	8.93	8.83	8.77		
Alfalfa hay				8.05	8.05
Corn, ground	19.69	19.49	19.35	25.08	25.08
Soybean meal, 44% CP	7.75	7.67	7.62	20.43	20.43
Beet pulp, dried	7.20	7.12	7.08		
Corn gluten feed				8.45	8.45
Sunflower meal, 36% CP	14.15	14.00	13.91		
Energizer-Gold[1]				0.85	0.85
OleoFat[2]				0.89	0.89
Limestone	1.06	1.11	1.14	0.54	0.54
Salt	0.30	0.30	0.30	0.41	0.41
Sodium bicarbonate				1	2.25
M&V Dry Cow premix[3]	0.31	0.30	0.30		
Magnesium oxide	0.23	0.22	0.22	0.19	0.19
Free feed silica[4]				0.19	0.19
Mycofix[5]				0.07	0.07
Diamond V Yeast XP[6]				0.07	0.07
Organic zinc				0.02	0.02
Potassium carbonate				0.27	0.55
Magnesium sulphate	0.15	0.45	0.82		
Dicalcium phosphate	0.22	0.22	0.22	0.09	0.09
M&V premix[7]				0.27	0.27
MegAnion[8]	0.37	0.53	0.60		
Calcium chloride	0.31	0.9	1.40		
DCAD mEq/kg DM[9]	0	-120	-200	+200	+400

[1]Calcium salts fatty acids 84.5% (IIFCO, Malaysia). [2]Fractionated fatty acids 99% (Nutrivet-Misr , Egypt). [3]Dry cow Mineral-vitamins premix contained each kg contains: Vit A 9,000,000 iu, vit D3 2,000,000iu, vit E 40,000mg, Mn 50,000 mg, Zn 50,000 mg, Fe 50,000 mg, Cu 10,000 mg, I 250 mg, Co150 mg, Se 250 mg $CaCo_3$ up to kg (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 contained each kg contains: VitA 9,000,000 iu, vitD3 2,000,000 iu, vitE 25,000 mg, Mn 50,000 mg, Zn 50,000 mg, Fe 50,000 mg, Cu 10,000 mg, I 100 mg, Co 200 mg, Se 200 mg $CaCo_3$ up to kg (El-Dakahlia Company, Egypt). [8]Origination (O2D Inc, Maplewood, MN, USA). Contains (%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. DCAD= (Na+K)-(Cl+S)= -8270mEq/kg DM. according to Goff(2018). [9]Dietary cation anion difference = DCAD = (Na+K) - (Cl+S).

The three treatment prepartum diets (Table 1) were formulated to contain 0, -120 and -200 mEq DCAD /kg DM. Eighteen cows were randomly assigned to each treatment. Immediately after calving cows in each prepartum treatment were reassigned to two levels of DCAD dietary treatments containing +200 and +400 mEq DCAD/kg DM (27 cows per treatment). The dietary DCAD concentrations were chosen to be consistent with previous published studies and to reflect values used in practice. Postpartum diets were formulated as shown in Table 2. Both Pre- and Postpartum diets were formulated using the Cornell Net Carbohydrate Protein System (CNCPS 2015 version 6.5, Cornell University, Ithaca, NY). Samples of individual feed ingredients, experimental diets were collected once each week, dry matter (DM) was determined using a forced-air drying oven set at 55°C for 48 hours. Samples were analyzed for crude protein (CP), ether extract (EE), ash, minerals (AOAC, 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Smith and Murphy, 1993). The analyses of feed ingredients were conducted by the Animal Reproductive Research Institute of the Ministry of Agriculture's Agriculture Research Center in Al-Harm, Egypt. Body weight measurements were recorded on 3 consecutive days at -21 d prepartum and 21, 44 and 86 DIM using digital scales. A BCS was assigned by one individual trained as described by Wildman et al., (1982) to maintain consistency throughout the trial. Immediately after calving, BW was measured and BCS was assigned once. The BW and BCS at -21 d prepartum were used as the initial BW and BCS.

Blood sample collection

Blood samples were taken from the coccygeal vein in plain vacutainer tubes and other tubes containing an (anti-coagulant) on days -14, -7, -2, 0, 2, 7, 14, and 21 relative to expected calving date. After centrifugation at 1600 X g for 15 minutes at 5°C, serum was collected and frozen at -80°C until analysis. The sampling time (roughly 1300 hour) corresponded to about 5 hours after the morning feeding. The analyses were conducted by the Animal Reproductive Research Institute of the Ministry of Agriculture's Agriculture Research Center in Al-Harm, Egypt. Serum samples were used for analysis of parathyroid hormone (PTH), hydroxyproline (OH-PRO), and

Table 2. Chemical composition of experimental diets formulated to differ in dietary cation-anion difference (DCAD), (mean + SD).

	Precalving	Postcalving			
	0	-120	-200	+200	+400
DM					
DM%	55 ± 4.4	56 ± 3.8	56 ± 3.6	54 ± 4.1	54 ± 4.2
CP	15.0 ± 0.7	14.8 ± 0.5	14.8 ± 0.8	17.5 ± 0.4	17.5 ± 0.6
Soluble protein, % of CP[1]	30.0 ± 0.6	31.0 ± 0.4	31.0 ± 0.5	26.0 ± 0.7	26.0 ± 0.9
Ether extract	3.00 ± 0.4	3.00 ± 0.6	3.00 ± 0.8	4.50 ± 0.9	4.50 ± 0.6
NDF	42.2 ± 1.7	41.9 ± 1.8	41.7 ± 1.9	28.0 ± 1.5	28.0 ± 1
NFC[2]	31.80 ± 1.4	31.80 ± 1.3	31.80 ± 1.6	40.80 ± 2	40.50 ± 1.9
TDN	70 ± 5.2	69 ± 4.8	69 ± 5.6	75 ± 5.7	74.8 ± 4.9
peNDF[3]	31.0 ± 1.2	31.0 ± 2	30.0 ± 1.7	23.0 ± 1.6	23.0 ± 1.5
NE_L, Mcal/kg[4]	1.51 ± 0.7	1.52 ± 0.6	1.51 ± 0.8	1.73 ± 0.9	1.71 ± 0.7
Ash	8.00 ± 0.4	8.9 ± 0.6	9.00 ± 0.7	9.20 ± 0.5	9.50 ± 0.8
Ca	0.99 ± 0.07	1.10 ± 0.08	1.20 ± 0.09	0.89 ± 0.1	0.88 ± 0.06
P	0.36 ± 0.02	0.37 ± 0.05	0.36 ± 0.04	0.41 ± 0.09	0.41 ± 0.07
Mg	0.38 ± 0.06	0.42 ± 0.09	0.48 ± 0.07	0.30 ± 0.1	0.30 ± 0.09
K	1.00 ± 0.08	1.00 ± 0.07	1.00 ± 0.05	1.20 ± 0.09	1.35 ± 0.06
Na	0.20 ± 0.04	0.20 ± 0.1	0.20 ± 0.03	0.43 ± 0.07	0.80 ± 0.08
Cl	0.51 ± 0.9	0.76 ± 0.2	1.05 ± 0.08	0.42 ± 0.07	0.42 ± 0.08
S	0.32 + 0.05	0.40 ± 0.06	0.40 ± 0.1	0.28 ± 0.04	0.28 ± 0.02
DCAD[5] (mEq/kg DM)	0 ± 3.1	-120 ± 3.6	-200 ± 4.1	+200 ± 5.2	+400 ± 5.5

[1]Soluble protein (%) = CP (%) − insoluble protein (%).
[2]NFC = 100 - [(NDF - neutral detergent insoluble CP) + CP + ash + fat
[3]Predicted by Cornell Net Carbohydrate and Protein System (v 6.55, Cornell University, Ithaca, NY
[4]Calculated from chemical composition (NRC, 2001).
[5]DCAD = (Na + K)- (S+Cl).

insulin which were assayed in 96-well plates using bovine ELISA kits validated for use in dairy cattle (Bioneovan Co., Keyuan Road, DaXing Industry Zone, Beeijing, China) following the manufacturer's instructions. The change in absorbance for unknown samples was compared with the standard curve to determine the concentrations. All colorimetric analyses were performed in triplicate, and coefficients of variation (inter- and intra-assay) for all assays were maintained below 10%. Total serum Ca (tCa) was determined colorimetrically according the manufacturer's instruction (RA-50 Chemistry Analyzer [Bayer, Germany]) using readymade chemical kits (CA 1210 Biodiagnostic Co., Cairo, Egypt). Ionized Ca (iCa) was determined by an ion-sensitive electrode of for serum (RapidLab 348, Bayer Diagnostics, Fernwald, Germany). Glucose was determined in whole blood immediately after collection by using a portable Free-Style Optium Neo reader with Free-Style Optium Neo blood glucose test strips (Abbott Diabetes Care Ltd, Range Road, UK), glucose measurement device and strips have been validated for use in dairy cattle.

Colostrum was collected, weighed, and sampled within one hour after parturition from the first milking and frozen at -20°C on the day of calving. IgG concentrations of colostrum were measured using the CAT number CA 1210 IgG ELISA test at 585 nm (Sunred co, China REF DZE201040108). Milk samples were collected at three consecutive milkings every 2 weeks, stored at -4°C until transportation to a commercial laboratory within 72 hour of collection for analysis. Samples were analyzed in the Animal Reproductive Research Institute of the Ministry of Agriculture's Agriculture Research Center in Al-Harm, Egypt, for chemical composition (milk protein, lactose, milk fat, total solid (TS) and solid-non-fat (SNF) using a pre-calibrated milk analyzer (LACTOSCAN LA, 8900 Zagora Bulgaria). Milk urea nitrogen (MUN) was analyzed using mid-infrared techniques (Method 972.16, AOAC International, 2006). Data from one cow in the -200/+400 DCAD treatment group was excluded from the data analysis because of mastitis. This cow was moved to the hospital herd and treated.

Ruminal fluid was collected during the last week before calving and 30 days postpartum by stomach tube and suction pump to avoid the contamination of the ruminal fluid with saliva secretions. These samples were taken three hours after feeding, after discarding the first few milliliters of the samples (100 mL). Ruminal fluid was filtered through two layers of cheesecloths. Ruminal fluid pH was measured immediately using a virtual pH meter Hanna gadgets pH, HANA instruments, Smithfield, R1, USA) and then 2 ml of fresh 25% meta phosphoric acid was added to 50 ml of strained ruminal fluid (Smith and Murphy 1993) and frozen at -20°C until analysis. Total volatile fatty acids (TVFAs) were determined using a steam distillation approach, as defined by Warner (1964) and ruminal fluid NH3-N was determined. (EA 920, Type 9512, Orion, Nidderau, Germany).

Urine samples were collected by manually stimulating the vulva on days -21, -14, -7, and -2 days prepartum, and at 2, 7, and 14 days postpartum, Urine samples were collected mid-stream and pH of the urine was determined immediately after collection using a portable pH meter (PHS-3C, Youke Instrument Co. Ltd., Shanghai, China). No individual clinical cases of metabolic diseases were observed in the current trial due to the effect of both pre- and postpartum treatments and the association with Ca disturbance before and after calving.

Statistical analysis

Experimental data were analyzed by ANOVA as a completely random design with individual cows as the experimental units. Results for the pre- and postpartum periods were analyzed separately. The MIXED procedure of SPSS V23 (https://www.ibm.com/eg-en/analytics/spss-statistics-software) was used for all analyses. Prepartum responses with a single measurement per cow were analyzed with the fixed effects of prepartum DCAD (0, -120, and -200 mEq/kg DM) and cow within treatment as the residual. Given that pens were not replicated in the present experiment, we recognize that unique effects associated with a particular pen could have influenced results; however, given the dynamic nature of a pen in an experiment of this type, any such effects would likely contribute to random error rather than fixed treatment effects. Responses with a single measurement [this is meaning the effect of treatments on different measures without the interaction between prepartum and postpartum treatments] per cow were analyzed with the fixed effects of prepartum level of DCAD (0, -120 or -200 mEq/kg DM), postpartum level of DCAD (+200 and +400 mEq/kg DM), day (-21, -14, -7, -2, 0, 2, 7, 14 and 21) and the interaction of prepartum or postpartum DCAD. Data with repeated measures within the experimental design were analyzed with the same mixed model described above and included the fixed effects of day and the interactions prepartum DCAD x day, postpar-

tum DCAD x day, prepartum DCAD x postpartum DCAD x day. Cow nested within prepartum DCAD and postpartum DCAD was a random effect in the model and accounted for. The repeated statement was included in all mixed models with repeated measurements with day specified as the repeated effect. Data from the preliminary period, it means the data were collected at the start of the trial such as body weight and body condition score, were included as covariates in the analysis of prepartum and postpartum effects. Significance was declared at $P \leq 0.05$, with a trend for variables with $P > 0.05$ and ≤ 0.10. Duncan's test was used to compare the differences among means.

RESULTS

No differences were observed due to prepartum, postpartum, or interaction of treatments in BW or BCS throughout the trial. Prepartum BW and BCS averaged (± SD) 736 ± 35 kg and 3.75 ± 0.46, respectively. Postpartum BW at calving and at 21 and 90 DIM averaged (± SD) 702.2 ± 21 kg, 660.9 ± 25 kg, and 658.3 ± 23 kg, respectively. Corresponding BCS at calving and at 21 and 86 DIM were (± SD) 3.50 ± 0.33, 3.45 ± 0.25, and 3.44 ± 0.55, respectively. Prepartum DMI was lowest for cows fed -120 and -200 compared with cows fed 0 DCAD, (12.6, 13.3 and 14.4 kg/d, respectively) ($P < 0.05$), whereas postpartum DMI was not affected by treatments, +200: 23.4 kg/d and +400: 23.6 kg/d.

Urine pH

The average prepartum urine pH of cows fed -200 mEq/ kg DM DCAD was lower compared with those fed 0 and -120 mEq/ kg DM DCAD (Table 3 and Figure 1 and Figure 2). There was a negative linear correlation between urine pH and iCa in cows fed prepartum DCAD (R = -0.74, R2 = 0.548; Figure 3).

Blood and ruminal metabolites

Prepartum serum metabolites are presented in Tables 3 and 4. Dietary DCAD and day had a significant ($P > 0.05$) effect on urine pH, tCa, and iCa (Figure 4 and Figure 5). Hydroxyproline tended to increase with negative DCAD for 0, -120, -200 and PTH was lower with increasing negative DCAD. There was a

Table 3. Urinary pH and blood metabolites measured prepartum for cows fed diets formulated to contain 0, -120 and -200 mEq/kg dietary cation-anion difference (DCAD).

Before calving DCAD					*P -value*		
	0	-120	-200	SEM	DCAD	day	DCAD x day
Urinary pH	7.50	7.02	5.93	0.057	0.001	0.001	0.099
Blood metabolites							
tCa mg/dL	8.38	8.85	9.40	0.048	0.001	0.001	0.170
iCa mg/dL	4.16^{c}	4.68^{b}	4.98^{a}	0.025	0.001	0.001	0.002
Glucose mg/dL	63.41	61.50	60.50	0.796	0.328	0.653	0.543

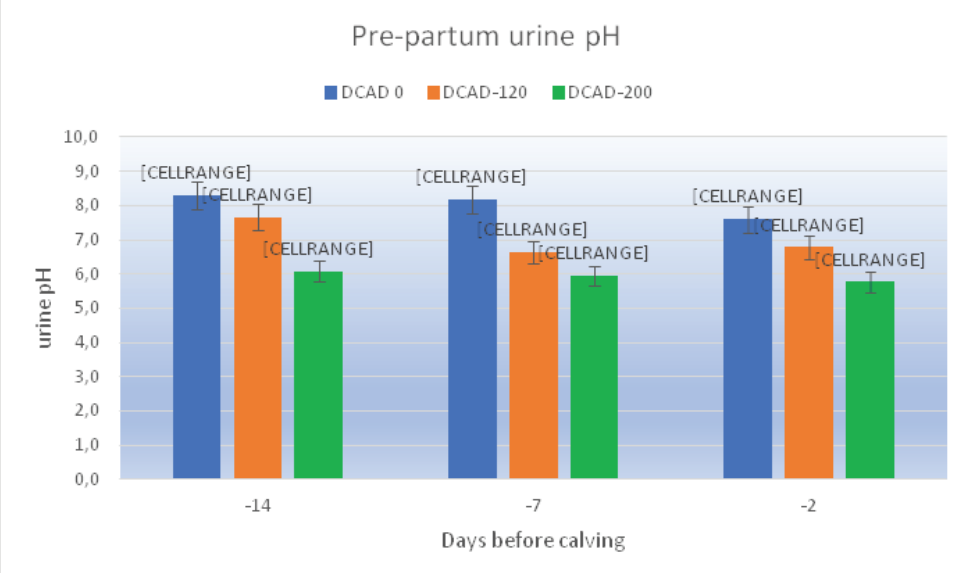

Fig. 1. Means (±SE) effect of prepartum DCAD for cows fed diets 0, -120 and -200 mEq/kg DM on urine pH concentrations.

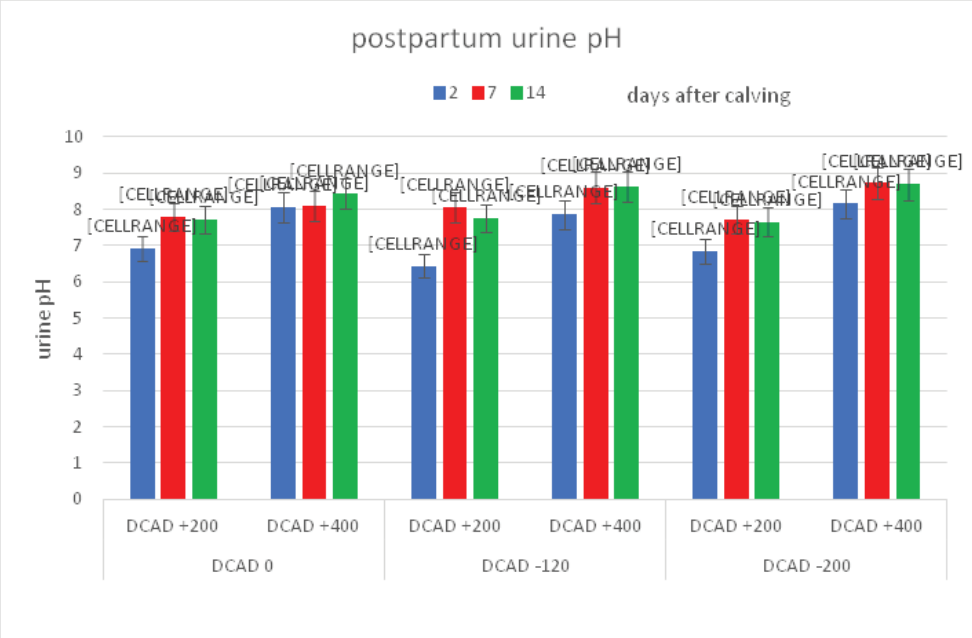

Fig. 2. Means (±SE) of urine pH concentrations for cows fed prepartum DCAD 0, -120 and -200 and postpartum DCAD+200 and +400 mEq/kg DM.

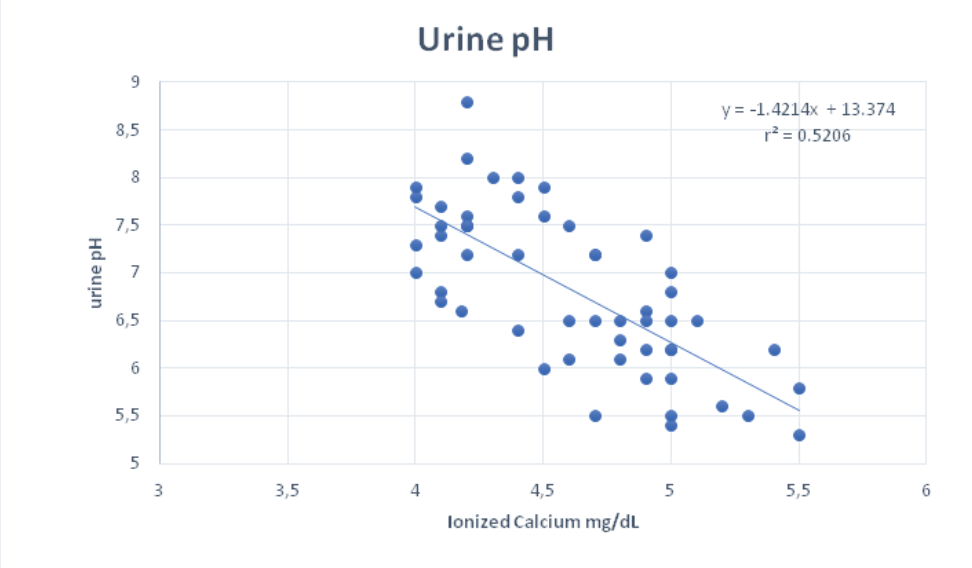

Fig. 3. Simple linear regression and negative linear correlation between urine pH and iCa (r = -0.74, r2 = 0.548) urine pH = (14.2 + -1.61*iCa) for cows fed prepartum DCAD (0, -120 and -200).

Table 4. Blood and ruminal metabolites prepartum for cows fed diets formulated to contain 0, -120 and -200 mEq/kg dietary cation-anion difference (DCAD) during experimental period.

	Before calving DCAD				
	0	-120	-200	SEM	*P - value*
Blood metabolites					
PTH pg/ml	62.37[a]	50.57[b]	34.66[c]	1.265	0.001
Hydroxyproline µg/ml	1.83[c]	2.27[b]	2.86[a]	0.043	0.001
Insulin, pmol/L	179.16	179.66	178.83	0.435	0.738
Ruminal metabolites					
pH	6.40	6.35	6.51	0.06	0.527
VFA, mM/L	101.33[c]	113.00[b]	122.33[a]	2.62	0.001
NH_3-N, mg/L	9.03	9.26	8.98	0.11	0.542

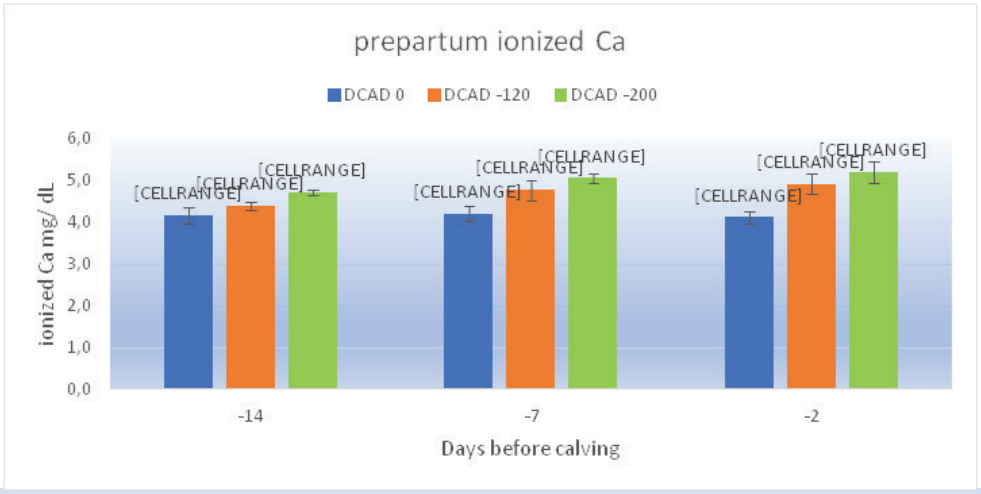

Fig. 4. Means (±SE) of ionized calcium concentrations and effect of prepartum DCAD for cows fed diets 0, -120 and -200 mEq/kg DM and day before calving.

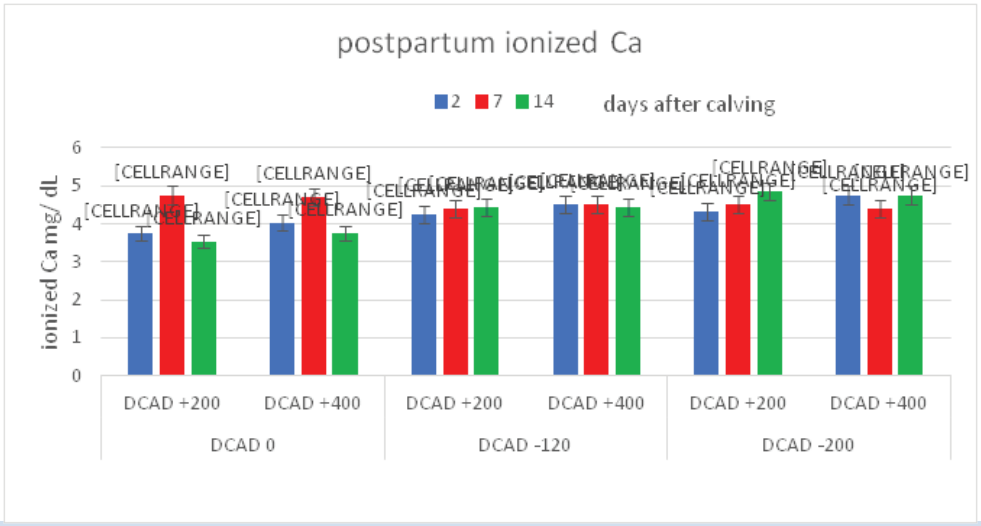

Fig. 5. Means (±SE) of ionized calcium concentrations for cows fed prepartum DCAD 0, -120, -200 and postpartum DCAD +200 and +400. There was no effect of and interaction between means for cows fed experimental diets $P > 0.05$.

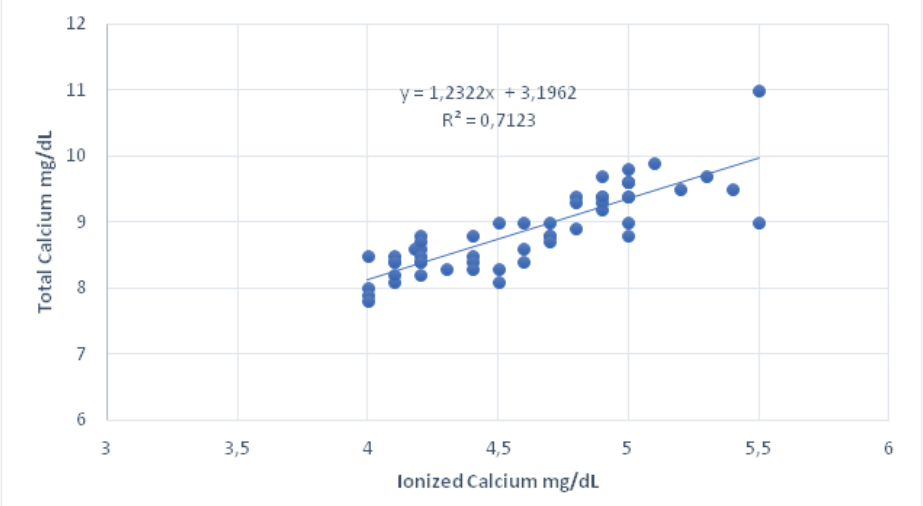

Fig. 6. Simple linear regression and positive correlation between tCa and iCa (R = 0.84, R2 = 0.712 $P < 0.001$) tCa = 3.2 + 1.23*iCa for cows fed prepartum DCAD (0, -120, -200) there is positive linear relationship tCa and iCa $p < 0.05$.

positive correlation between iCa and tCa (r = 0.84, P < 0.001; Figure 6). However, ruminal VFA concentrations were increased by decreasing prepartum DCAD level for (0, -120, -200). Ruminal pH and ruminal NH_3-N had non-significant differences among treatments (Table 4). Even though urine pH and PTH were lower (P < 0.05) for cows fed -200 compared to cows fed 0 and -120, tCa, iCa, hydroxyproline, and ruminal fluid VFA were greater (P < 0.05) for cows fed -200 DCAD. When compared to 0 and -120, feeding -200 DCAD resulted in lowering urine pH.

Postpartum urine pH was higher ($P < 0.001$) for cows fed +400 compared with those fed +200 DCAD postpartum. No interaction of perpartum and postpartum treatments were observed; but, interactions of prepartum DCAD x day ($P = 0.034$) and postpartum DCAD x day ($P = 0.003$) were observed. Postpartum concentrations of tCa and iCa were highest ($P < 0.001$) for cows fed -200, intermediate for -120, and lowest for those fed 0 prepartum DCAD. Concentrations of iCa tended ($P = 0.058$) to be higher for cows fed +400 compared to those fed +200 postpartum DCAD. No interaction of pre and postpartum DCAD were observed; but, interactions were observed for tCa and iCa due to prepartum DCAD and day ($P < 0.01$) and for iCa due to postpartum DCAD and day (P = 0.045). Prepartum and postpartum dietary treatments and interaction had no effect on blood glucose levels ($P > 0.05$) as shown in Table 5. Concentrations of PTH postpartum tended ($P = 0.064$) to be higher for cows fed 0 compared with -120 and -200 prepartum DCAD, but were not affected ($P > 0.10$) by postpartum DCAD. No differences ($P > 0.10$) were observed in postpartum concentrations of hydroxyproline and insulin among treatments.

Postpartum ruminal fluid pH was higher (P < 0.001) for cows fed +400 compared to those fed +200 postpartum DCAD. An interaction of pre- and postpartum DCAD (P = 0.016) was observed for total ruminal fluid VFA concentrations. No differences were observed in ruminal fluid NH_3-N concentrations among treatments.

Postpartum serum metabolites for tCa and iCa, ruminal fluid pH and VFA are presented in Tables 5 and 6. The interaction of prepartum DCAD 0, -120 and -200 mEq/kg DM x postpartum DCAD +200 and +400 mEq/kg DM, were affected by postpartum DCAD treatments after calving for ruminal fluid pH (P = 0.001). Ruminal fluid VFA was affected (P = 0.016) due to an interaction of treatments. This may be attributed to a high level of Na and K from adding sodium bicarbonate and potassium carbonate which could have increased rumen buffering in cows fed +400 DCAD than +200 DCAD. Whereas there was no effect of treatment or any interaction on concentrations of PTH, OH-PRO, insulin and ruminal ammoniaNH_3 (Tables 6). Postpartum urine pH was higher in cows fed DCAD +400 than fed +200 mEq/ kg DM (Table 6).

Table 5: Urinary pH and Ca concentrations for cows fed prepartum diets formulated to contain 0, -120 and -200 mEq/kg dietary cation-anion difference (DCAD) and postpartum diets formulated to contain +200 or +400 mEq/kg.

BEFORE	0		-120		-200					*P -value*				
AFTER	+200	+400	+200	+400	+200	+400	SEM	BEFORE	AFTER	day	BEFORE x AFTER	BEFORE x day	AFTER x day	BEFORE x AFTER x day
Urine pH	7.48	8.20	7.42	8.36	7.42	8.53	0.037	0.347	0.001	0.001	0.103	0.034	0.003	0.690
tCa mg/dL	7.98	8.09	8.64	8.47	8.92	9.19	0.054	0.001	0.511	0.001	0.247	0.003	0.587	0.086
iCa mg/dL	4.01	4.16	4.37	4.49	4.57	4.64	0.029	0.001	0.058	0.001	0.836	0.001	0.045	0.510

BEFORE= prepartum DCAD treatment (0, -120 and -200), AFTER= postpartum DCAD treatment (+200 and +400).

Table 6: Blood metabolites measured postpartum for cows fed prepartum diets formulated to contain 0, -120 and -200 mEq/kg dietary cation-anion difference (DCAD) and postpartum diets formulated to contain +200 or +400 mEq/kg.

BEFORE	0		-120		-200				*P* -value	
AFTER	+200	+400	+200	+400	+200	+400	SEM	BC	AFTER	BEFORE x AFTER
PTH pg/ml	38.15	39.10	36.44	36.25	31.07	35.50	0.828	0.064	0.317	0.514
Hydroxyproline µg/ml	1.67	1.76	1.63	1.81	1.80	1.73	0.027	0.669	0.252	0.198
Insulin, pmol/L	82.0	82.66	85.66	81.0	84.0	87.0	0.737	0.241	0.825	0.136
Ruminal Metabolites										
Ruminal fluid pH	6.73	7.33	6.66	7.30	6.56	7.36	0.05	0.828	0.001	0.644
VFA mM/L	120.33	130.00	132.66	117.00	119.66	131.00	1.78	0.993	0.627	0.016
Ruminal NH_3-N mg/dL	13.00	13.66	13.43	12.00	12.06	13.76	0.26	0.243	0.235	0.278

Table 7. Calf birth weight, colostrum yield and immunoglobulin-G (IgG) concentrations of cows fed on prepartum diets formulated to contain 0, -120 and -200 mEq/kg dietary cation-anion difference (DCAD).

Before calving DCAD mEq/kg DM	0	-120	-200	SEM	*P* -value
Colostrum IgG g/L	58.05	59.75	63.17	1.111	0.163
Calf birth weight kg	42.13	41.46	40.13	0.439	0.168
Colostrum milk kg	6.60	6.20	7.13	0.269	0.375

Table 8. Effect of prepartum and postpartum treatments on milk yield and it is composition for dairy cows fed experimental diets containing 3 prepartum and 2 postpartum DCAD levels

Precalving	0		-120		-200			*P*-value		
Postcalving Item	+200	+400	+200	+400	+200	+400	SEM	Pre-	Post-	Pre- x Post-
Milk yield, kg	33.25	34.64	34.64	34.81	33.87	33.97	0.596	0.607	0.490	0.719
[1]Fat-corrected milk 4%, kg/d	32.25	34.12	33.91	34.18	33.15	33.56	0.570	0.583	0.475	0.793
[2]Fat-corrected milk, 3.5%, kg/d	34.86	36.88	36.66	36.95	35.84	36.28	0.616	0.583	0.475	0.793
[3]SCM, kg/d	32.78	34.50	34.52	34.59	33.66	33.85	0.574	0.576	0.467	0.784
[4]FPCM, kg/d	34.99	36.90	36.85	36.99	36.03	36.25	0.614	0.553	0.493	0.782
[5]ECM kg/d	35.88	37.83	37.78	37.92	36.94	37.16	0.629	0.552	0.494	0.782
Milk fat,%	3.8	3.90	3.86	3.88	3.86	3.92	0.012	0.169	0.868	0.656
Fat yield, kg	1.26	1.35	1.33	1.35	1.30	1.33	0.022	0.566	0.468	0.844
Milk protein,%	3.4	3.40	3.45	3.40	3.45	3.39	0.012	0.346	0.812	0.583
Protein yield, kg	1.13	1.17	1.19	1.18	1.15	1.15	0.020	0.451	0.568	0.739
Milk lactose,%	4.88	4.85	4.82	4.85	4.84	4.83	0.013	0.310	0.675	0.782
Lactose yield, kg	1.62	1.68	1.67	1.68	1.63	1.64	0.028	0.541	0.477	0.716
Total solids, %	12.85	12.92	12.92	12.90	12.92	12.91	0.027	0.812	0.523	0.970
Total solids yield, kg	4.27	4.47	4.47	4.49	4.37	4.38	0.075	0.584	0.470	0.762
Solids-non-fat,%	9.05	9.02	9.06	9.02	9.06	8.99	0.023	0.185	0.510	0.961
Solids-non-fat yield, kg	3.00	3.12	3.13	3.13	3.06	3.05	0.053	0.595	0.474	0.728
Milk urea nitrogen, mg/dL	11.82	11.57	12.38	12.47	12.13	11.88	0.213	0.772	0.947	0.697

[1]fat-corrected milk, 4% = (0.4 x milk yield) + (15 x fat yield),
[2]fat-corrected milk, 3.5% = (0.4324 x milk yield) + (16.218 x fat yield),
3solid-corrected milk, kg = (12.3 x fat yield) + (6.56 x solid-not-fat yield) - (0.0752 x milk yield),
[4]fat-protein-corrected milk, kg = (12.82 x fat yield) + (7.13 x protein yield) + (0.323 x milk yield),
[5]energy-corrected milk, kg = (0.327 x milk yield) + (12.95 x fat yield) - (7.65 x protein yield)

Colostrum yield and quality and milk production

Colostrum yield, IgG and calf birth weight were not affected by experimental diets as shown in Table 7. No differences were observed of insulin, glucose, IgG, colostrum yield and insulin due to cows being fed the experimental diets.

There were no significant effects of DCAD on milk yield, fat corrected milk, solids corrected milk, MUN, total solids, solid not fat energy-corrected milk, milk fat and protein percentages among cows fed the pre- or postpartum treatments (Table 8).

Discussion

No clinical or subclinical hypocalcemia was observed prepartum or postpartum as plasma iCa and tCa concentrations never declined below 1.76 mmol/L or 7 mg/dL, respectively. Feeding a -180 mEq/ kg DM DCAD diet prepartum reduced urine pH which is consistent with previous reports (Santos et al., 2019). The decline observed in our trial is slightly greater than reported in most trials at this DCAD concentration. Anionic salts have been reported to prevent hypocalcemia in multiparous cows at or near calving (Horst et, 1997; Moore et., 2000; Block, 1984). In the current study, cows consuming an acidogenic diet increased serum iCa and tCa concentrations. As a result, limits were set for iCa and tCa at 48 and 48 hours before and after calving for iCa, respectively. The current study is contrary with the results of Moore et al. (2000), who found no change in postpartum Ca concentrations in cows fed an anionic diet. In our current trial, serum Ca reached a nadir 2 d postpartum comparable with previous reports by Joyce et al. (1997) and Romo et al. (1991). Acidogenic diets raise serum Ca concentrations by increasing calcium mobilization from bone, as evidenced by enhanced serum hydroxyproline, a marker of bone resorption, which agrees with Goff et al. (1991), and is mediated by increased serum PTH concentrations (Horst et al., 1997). It was hypothesized that as blood acidity increases, tissue

response to PTH increased (Romo et al., 1991; Horst et al., 1997). Because hydroxyproline is a marker of bone resorption (Abu Damir et al., 1994), greater serum hydroxyproline concentrations suggest that cows were better equipped to respond to Ca homeostasis challenges around the time of calving because of their increased ability to mobilize calcium. Our results are similar with previous studies (Block 1984; LeClerc et al., 1989) in which hydroxyproline concentrations increased as the dietary DCAD decreased. Cows fed the -180 prepartum DCAD had lower serum PTH concentrations 2 d after calving ($P < 0.001$) than those fed either 0 or -120 DCAD.

The negative DCAD (-200 mEq/ kg DM) diet resulted in a lower urine pH, which was consistent with (Van Soest et al., 1991; Santos et al., 2019). Anionic salts have been proven to prevent hypocalcemia in multiparous cows at or near calving (Horst et,1997; Moore et., 2000; Block, 1984).

Cows fed DCAD diets (-200 and -120 mEq/kg DM) had lower serum PTH concentrations than cows fed the 0 DCAD diet. The blood PTH levels are usually inversely proportional to iCa concentrations (Jonsson et al., 1980). The rationale for the higher concentrations of blood PTH in cows fed 0 and -120 DCAD compared to those fed -200 DCAD is that the parathyroid gland is particularly sensitive to any decrease in blood Ca levels (it responds to changes in ionized Ca levels, but we always measure total Ca which is going to be about 2 times the ionized Ca). Each cow had a normal serum Ca concentration in her blood. A young calf's total Ca level might be 10.5 mg/dl, while an older cow's level might be 9.0 mg/dl. When blood Ca falls below the normal level for that animal, the gland secretes more parathyroid hormone. The PTH concentrations in the blood will increase 10 to 20-fold. If a cow is fed anions correctly, the increase is only transitory and returns to baseline levels by day 2 or 3 (Goff et al., 1989). The target tissues react to PTH and work on bone and kidney cells to bring blood Ca levels back to normal. Although the PTH concentrations increased when blood Ca falls in cows not fed anions, the cow's blood and urine are more alkaline, and tissues are resistant to the effects of PTH. This is due to the PTH receptor failing to recognize the hormone effectively. As a result, blood calcium levels do not rise rapidly or at all, and the parathyroid gland secretes significant amounts of hormone for a prolonged length of time. The PTH concentrations in the blood of cows with milk fever are extraordinarily high; however, this does not help them maintain adequate calcium levels (Goff et al., 2004).

The current study agrees with Apper-Bossard et al. (2010) who reported no differences of ruminal fluid pH and NH_3. The rumen has a strong buffer system to maintain a stable rumen environment by keeping any sudden rise or fall in ruminal fluid pH within a normal range (Tucker et al., 1992). The negative DCAD diets had no negative effect on ruminal content fermentation since the VFAs rose and the ruminal fluid pH did not change considerably. Given the difficulty of interpreting ruminal fluid VFA concentrations, which vary substantially depending on sampling site and rumen dilution rate due to rate of passage and total rumen contents, we would avoid attempting to assign a direct influence of DCAD on ruminal fluid VFA. Because rumen dilution rate and rate of passage can be altered by osmotic changes generated by additional anions with negative DCAD, the apparent increases in VFA concentration with negative DCAD in cows fed -120 and -200 mEq/kg DM were difficult to understand.

Feeding a negative DCAD diet to dairy cows in late gestation had no effect on colostrum yield and IgG concentration. This is in line with previous findings (Weich et al., 2013; Diehl et al., 2018; Lopera et al., 2018). This is consistent with the findings of Weich et al. (2013) who reported no difference in birth weight between calves born to cows fed a diet containing -160 mEq/kg DM (41.1 kg) and calves born to cows fed +120 mEq/kg DM (41.1 kg) (44.6 kg). Similarly, Diehl et al. (2018) found that feeding a diet containing -200 mEq/kg DM for 28 days before to delivery had no influence on calves' birth weight when compared to a control diet containing -30 mEq/ kg DM.

Colostrum quantity and composition were unaffected by lowering DCAD in prepartum diets from about +130 to -130 mEq/kg DM. Brix levels are closely connected with colostrum IgG concentrations (Martinez et al., 2012), and Brix readings of 21% are a good indicator for colostrum high enough IgG concentration to transfer passive immunity, as observed in the current study (50 g IgG/L; Quigley et al., 1998).

Results for production were similar to previous studies (Weich et al., 2013; Balbir et al., 2017; Silva 2015) that reported feeding negative DCAD prepartum did not affect postpartum milk yield. Moore et al. (2000) fed anionic salts and observed no depression on milk yield from 7 to 70 days in milk. In contrast

to our findings, Beede et al. (1991) observed that addition of anionic salts to the prepartum diet increased milk yield in the following lactation by 3.6%. Our results are not consistent with those of others (Roche et al., 2005; Ju et al., 2007a, b) who have reported a positive association between DCAD and milk fat test. Several studies have shown that addition of dietary rumen buffers such as $NaHCO_3$ and K_2CO_3 increase milk fat percent, especially when depressed milk fat occurs (Hu et al., 2007a, b). West et al. (1992) reported an increase in the milk fat percent by increasing DCAD, without any adverse impact on milk yield. Conversely, some studies have reported an increase in milk production without changes in milk fat percent (Tucker et al., 1991; West et al., 1991). No difference in percentage of lactose associated with altered DCAD agrees with other studies (West et al., 1992; Tucker et al 1988). Martinez et al. (2018) evaluated daily milk production and weekly milk fat and protein content of 79 cows and did not find an effect of DCAD treatment effect on milk yield, ECM, or true protein content, but they reported approximately 0.23 percentage points higher fat in the first 49 postpartum when cows were fed negative DCAD prepartum. Our results differ from those of Leno et al. (2017) who compared three DCAD levels (+183, +59 and -74 mEq/kg of DM) and noted an increase in milk yield and ECM in the first 3 weeks postpartum by decreasing prepartum DCAD. Our results agree with those of Santos et al. (2019), who found in multiparous cows that fat content was not different between DCAD groups, but milk protein content also not affected by feeding an acidogenic diet prepartum.

CONCLUSION

Anionic salts supplements were effective at acidifying prepartum diets based on urine pH, which is an indicator of compensated metabolic acidosis and will increase mobilization of iCa status. Feeding anionic diets improve Ca availability for cows after parturition and maintain Ca homeostatic system which increase extracellular Ca pool from resorption Ca bone, reabsorption intestinal Ca. Calf birth weight, IgG and amount of colostrum were unaffected by treatments before calving, whereas we found increasing percent-ages of milk protein, total solid and solid-not-fat in cows given DCAD (-120 and -200 mEq/kg DM vs. 0 mEq/kg DM). No effects were observed in postpartum treatments or the interaction between pre- and postpartum DCAD on blood measures, milk yield, and fat corrected milk. Levels of glucose, PTH, OH-PRO were within normal ranges and had no detrimental effect on cow productive performance.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest**.**

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