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Influence of Varying Dietary Cation-Anion Difference on Blood Metabolites of Holstein Dairy Cows

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HEH wrote the first draft, collected samples, and executed the field trial. AMMM placed the experimental design of the trial. EMAR analysed the raw data of the experiment. NME edited the manuscript. MNN reviewed the draft.

Key words
Parathyroid hormone, Total calcium, Ionized calcium, Hydroxyproline, Acidogenic

ABSTRACT

A negative dietary cation-anion difference (DCAD) motivates a compensated metabolic acidosis, stimulating calcium (Ca) absorption and mobilization before calving and decreasing clinical and subclinical hypocalcemia postpartum. The objective of this trial was to determine the effects of varying pre- and postpartum DCAD, mEq [(Na + K - Cl + S)]/ kg of dry matter on blood total Ca, ionized iCa, blood, and rumen metabolites, and endocrine status in prepartum and postpartum phases. Forty-eight multiparous Holstein cows range lactation 1-5 with an average body weight 706 kg ±7.29 SD were enrolled in a nested block design trial at 29 d prior anticipated parturition through 90 d in milk. A factorial arrangement of treatments -3 DCAD concentrations Tr1. (0.0, -100 or -180 mEq/kg DM) and -2 postpartum DCAD concentrations Tr2. (+250 or +350 mEq/kg DM). Prepartum urine pH was lower for cows fed -180 DCAD compared with -100 or 0.0 DCAD. Postpartum urine pH was higher for cows fed +350 mEq/kg compared with those fed +250 mEq/kg DCAD. Prepartum serum tCa, iCa, hydroxyproline (OH-PRO) was highest for -180 DCAD compared with both -100 and 0.0 DCAD. Parathyroid hormone was highest for 0.0 DCAD compared with -100, -180 DCAD. After calving there is a significant effect of tCa and iCa of prepartum treatment and we didn't obtain any effect due to interaction between prepartum x postpartum treatment. Feeding an acidogenic diet improved Ca postpartum status.

INTRODUCTION

The successful preventing hypocalcemia in a positive dietary cation-anion difference (DCAD) diet relies on reducing dietary Ca concentration to below requirements to stimulate Ca mobilization from labile bone stores and absorption from the diet maintenance for an individual animal can be as low as 25 g/d (Crenshaw *et al*., 2011). This nutritional strategy has fallen out of favor due to high cation-containing forages and an insensitivity to Parathyroid hormone (PTH) signaling found in kidney and bone PTH receptors when a positive DCAD diet is fed (Liesegang *et al*., 2007; Goff and Koszewski, 2018). Alternatively, supplementing anionic salts to create an acidogenic diet prepartum has been used to improve Ca homeostatic before calving (Goff *et al*., 2014). Acidogenic diets formulation strategy causes compensated metabolic acidosis in blood of prepartum cows, decreasing urine pH and increasing urinary Ca excretion (Leno *et al*., 2017). Compensated metabolic acidosis also directly impacts Ca availability by increasing bone Ca mobilization and tissue responsiveness to hormonal signals (Liesegang *et al*., 2007; Rodriguez *et al*., 2016). Under circumstances of prepartum compensated metabolic acidosis, Ca is absorbed actively and passively from the rumen and small intestine and mobilized from bone stores to be excrected through the urine to maintain Ca homeostasis. This continuous Ca flux creates a supply of available Ca to be used at the initiation of lactation when urinary Ca excretion is conserved (Grünberg *et al*., 2011; Megahed *et al*., 2018). The objective of our study was to evaluate the effects 3 levels of DCAD prepartum (0.0, -100

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or -180 mEq/kg DM) and 2 levels of DCAD postpartum (+250 or +350 mEq/kg DM), dietary strategy on urine pH, IgG, PTH, hydroxyproline, Ca status and blood parameters.

MATERIALS AND METHODS

Management procedures of experimental animals

The field experiment was carried out on a private dairy farm in Egypt, which is approximately 80 kilometers from Cairo on the Ismailia desert road. Forty-eight multiparous Holstein cows with 3 to 4 wk of expected parturition were selected from the herd. Cows were fed twice daily at 700 and 1700 h. Prepartum diets were formulated to provide 0.0 mEq DCAD/kg dry matter (DM) as a control, -100 mEq/kg DM or -180 mEq DCAD /kg DM. Immediately after calving cows in each prepartum treatment were split and fed a lactation diet formulated to contain either +250 or +350 mEq DCAD/kg DM throughout the remainder of the trial. Cows were housed in an open yard with a shed area and milked three times daily 08:00, 16:00 and 24:00 h. Prepartum and postpartum diets were formulated using the Cornell net carbohydrate protein system (CNCPS version 6.5, Cornell University, Ithaca, NY) (Table I). Dietary ingredients were analyzed for DM, CP, EE, Ash, minerals (AOAC 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Van-Soest *et al*., 1991), TDN, NEL and NFC were calculated according to NRC (2001).

Blood samples and measurement

Blood samples were collected from coccygeal vein on -14, -7, -2, 0, 2, 7, 14 and 21 d relative to predicted calving. Blood was collected into vacutainer tubes and serum was separated after centrifugation at 1,600 × g for 15 min at 5 °C, and frozen at −80°C until analysis. Sampling time (approximately 1300 h) corresponded to approximately 5 h after morning feeding. The analyses were performed in laboratory of Animal Reproductive Research Institute, Agriculture Research Center, Ministry of Agriculture, Al-Harm, Egypt. Blood serum samples were used for analysis of PTH and hydroxyproline (OH-PRO) were determined using bovine ELISA kit (Keyuan Road, DaXing Industry Zone, Beijing, China), tCa was determined calorimetrically according to the manufacture's instruction (RA-50 Chemistry Analyzer (Bayer) using readymade chemical kits, (CA 1210 Biodiagnostic co. Egypt). iCa was determine by an ion-sensitive electrode of blood serum (RapidLab 348, Bayer Diagnostics, Fernwald, Germany). Colostrum was collected, weighed, and sampled immediately from first milking directly after parturition and frozen at −20°C. Concentrations of IgG were determined by calorimetrically at 585 nm CAT no CA 1210 IgG ELISA assay (Sunred co, China REF DZE201040108). Midstream urine samples were collected prepartum on days -21, -14, -7 and -2 d, after calving were measured at 2, 7 and 14 d, (Fig. 1) by manual stimulation of the vulva and were measured for urine pH immediately after collection by using a portable pH meter (PHS-3C, Youke Instrument Co. Ltd., Shanghai, China).

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

Ingredients (% DM)	Prepartum DCAD			Postpartum DCAD	
	0	-100	-180	+250	+350
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	-	-
Energier-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	-	-	-	1.15	1.88
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	0.44
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	0.51	0.54	-	-
Calcium chloride	0.31	0.82	1.06	-	-
DCAD mEq/kg DM[9]	0.0	-100	-180	+250	+350

[1]Calcium salts fatty acids 84.5% (IIFCO, Malaysia). [2]Fractionated fatty acids 99% (El- Sadat City, Egypt). [3]Dry cow Mineral-vitamins premix contained each 3kg contains: Vit A 9,000,000iu, vit D3 2,000,000iu, vit E 40,000mg, Mn 50,000mg, Zn 50,000mg, Fe 50,000mg, Cu 15,000mg, I 250mg, Co150mg, Se 250mg (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 3kg contains: VitA 12,000,000iu, vitD3 2,500,000iu, vitE 35,000mg, Mn 80,000mg, Zn 100,000mg, Fe 50,000mg, Cu 20,000mg, I 300mg, Co400mg, Se 300mg CaCo3 up to 3kg (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) according to Goff and Koszewski (2018).

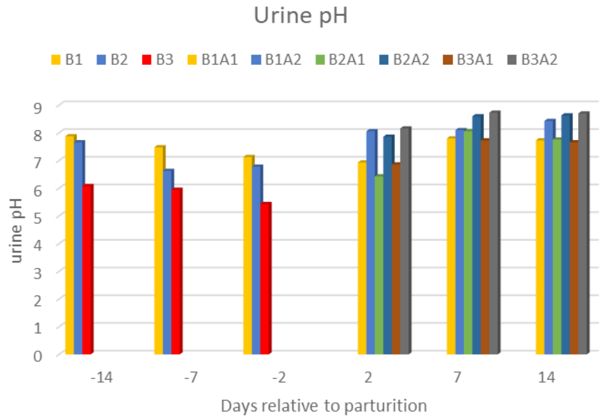

Fig. 1. Urine pH (Means±SE) for cows fed prepartum DCAD (Tr1) 0.0, -100, -180 (BC1, BC2, BC3) and postpartum DCAD (Tr2) +250 and +350 (AC1, AC2) showing effect of interaction (BC1AC1, BC1AC2, BC2AC1, BC2AC2, BC3AC1, BC3AC2), there is effect of prepartum DCAD (Tr1) between means for cows fed 0.0, -100, -180 (7.5, 7.02 and 5.82), $P < 0.005$, there is effect of time sample -2, -7 (6.45 and 6.68) and -14 (7.21) $P < 0.05$ and the interaction Tr1 x T, $P = 0.247$. postpartum there is effect of treatment Tr2 x T time of sample at 14, 7 urine pH (8.15 and 8.17), 2 days after calving pH (7.38), P> 0.05, also there is interaction after calving Tr1 x T time of sample p. 0.05 but there is no interaction after calving from Tr1 x Tr2 x T, P = 0.690.

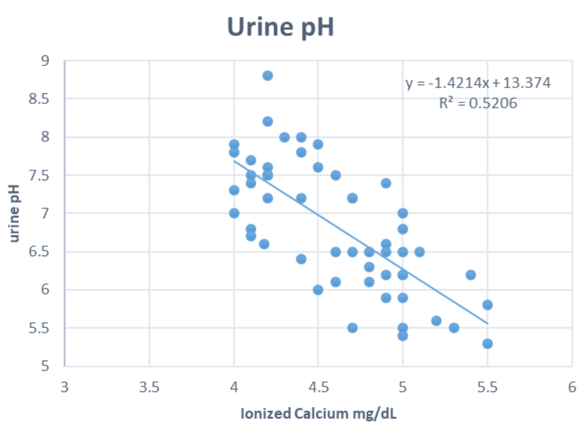

Fig. 2. Simple linear regression and negative linear correlation between urine pH and iCa (R = -0.74, R^2 = 0.548) urine pH = (14.2 + -1.61*iCa) for cows fed prepartum (Tr1) DCAD (0.0, -100, -180) there is negative linear relationship urine pH and iCa $p < 0.05$.

Statistical analysis

Statistical analysis of experimental data was carried out through the SPSS V23 (https://www.ibm.com/eg-en/analytics/spss-statistics-software).

RESULTS

The composition of diets are shown in Table II and average DM intake in different treatments of prepartum diet DCAD 0.0, -100, or -180 mEq/kg DM (13, 13.1, 13.2 kg DM/day) and postpartum DCAD +250 or +350 mEq/kg DM (22.4 and 22.6 kg DM/day), respectively. Prepartum urine pH, tCa and iCa are presented in (Table III). Cows fed on prepartum DCAD have a significant with higher concentrations of tCa, iCa due to 0.0, -100 and -180 mEq/kg DM (Figs. 3, 4). The Hydroxyproline increased with increasing negative DCAD, while urine pH and PTH decreased in cows fed -180 than 0.0 and -100 mEq/kg DM (Fig. 1, Tables III, V). Postpartum urine pH, tCa and iCa (Table VI) were affected due to 0.0, -100 and -180 mEq/kg DM, +250 and +350 mEq/kg DM, (Table VI). No differences were observed in serum PTH, OH-PRO and Insulin in postpartum due to 0.0, -100 and -180 mEq/kg DM, +250 and +350 mEq/kg DM or interaction (Table VII). There is no effect of (0.0, -100 and -180 mEq/kg DM) on calf birth weight, IgG and colostrum yield (Table IV).

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

Ingredients (% DM)	Prepartum DCAD			Postpartum DCAD	
	0	-100	-180	+250	+350
CP	15.0	14.8	14.8	17.5	17.5
Soluble protein (% of CP[1])	30.0	31.0	31.0	26.0	26.0
Ether extract	3.00	3.00	3.00	4.50	4.50
NDF	42.2	41.9	41.7	28.0	28.0
NFC[2]	31.80	31.80	31.80	40.80	40.50
TDN	70.0	69.0	69.0	76.0	75.0
peNDF[3]	31.0	31.0	30.0	23.0	23.0
NEl (Mcal/kg[4])	1.51	1.52	1.51	1.73	1.71
Ash	8.00	8.9	9.00	9.20	9.50
Ca	0.99	1.10	1.20	0.89	0.88
P	0.36	0.37	0.36	0.41	0.41
Mg	0.38	0.42	0.48	0.30	0.30
K	1.00	1.00	1.00	1.23	1.27
Na	0.20	0.20	0.20	0.56	0.76
Cl	0.51	0.70	0.80	0.34	0.34
S	0.32	0.40	0.48	0.34	0.33
DCAD[5] (mEq/kg DM)	0.0	-103.8	-181.9	+250.6	+351.2

Values calculated using CNCPS v6.55 (2015). [1]Soluble protein (%) = CP (%) − insoluble protein (%). [2]NFC = 100 – [(NDF – neutral detergent insoluble CP) + CP + ash + fat)] Calculated according to (NRC, 2001). [3]Predicted by Cornell Net Carbohydrate and Protein System (v 6.55, Cornell University, Ithaca, NY) Calculated according to Mertens (1997). [4]Calculated from chemical composition (NRC, 2001). [5]DCAD = (Na + K)– (S+Cl).

Table III. Urinary pH and blood calcium measured prepartum for cows fed diets formulated to contain 0, -100 and -180 mEq/kg dietary cation-anion difference (DCAD).

Item	Prepartum DCAD (Tr1)			SEM	DCAD	P -value	
	0.0	-100	-180			day	DCAD x day
Urinary pH	7.50	7.02	5.93	0.057	0.001	0.001	0.099
tCa (mg/dL)	8.38	8.85	9.40	0.048	0.001	0.001	0.170
iCa (mg/dL)	4.16	4.68	4.98	0.025	0.001	0.001	0.002

Table IV. Blood metabolites prepartum for cows fed diets formulated to contain 0, -100 and -180 mEq/kg dietary cation-anion difference (DCAD) during experimental period.

Blood metabolites	Prepartum DCAD (Tr1) (mEq/kg DM)			SEM	P value
	0	-100	-180		
PTH pg/ml	62.37a	50.57b	34.66c	1.265	0.001
Hydroxyproline µg/ml	1.83c	2.27b	2.86a	0.043	0.001
Insulin, pmol/L	179.16	179.66	178.83	0.435	0.738

[ab]Means in the same row with different superscripts differ ($P < 0.05$).

Table V. Calf birth weight, colostrum yield and Immunoglobulin-G (IgG) concentrations of cows fed diets formulated to contain 0, -100 and -180 mEq/kg dietary cation-anion difference (DCAD).

	Prepartum DCAD (Tr1) (mEq/kg DM)			SEM	P -value
	0.0	-100	-180		
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

DISCUSSION

DCAD and ionized calcium status

Both tCa and iCa had a positive connection at 48 h before and 48 h following parturition, respectively (Figs. 3, 4, 5). Figure 5 reveals a favourable association between iCa and tCa (r = 0.84, P 0.00) using simple linear regression. There was no evidence of hypocalcemia in either pre- or postpartum cows, as blood calcium levels were normal at 8-12 mg/dL and never went below 2 mmol/L or 8 mg/dL for all cows administered DCAD (Tables III, V). Serum Ca reached its nadir during the calving period or 1 and 2 d after parturition due to effect of treatment, day and interaction DCAD x day (Fig. 3), similar to previous reports (Romo *et al*., 1991; Abu Damir *et al*., 1994). Acidogenic diets are hypothesized to increase serum Ca by increasing Ca mobilization from bone as indicated by elevated serum hydroxyproline which is indicator of bone resorption as shown in Table IV which agrees with (Goff *et al*., 1991) mediated through increased serum PTH concentration (Horst *et al*., 1997). Tissue responsiveness to PTH was postulated to increase with greater blood acidity (Goff *et al*., 1991; Horst *et al*., 1997).

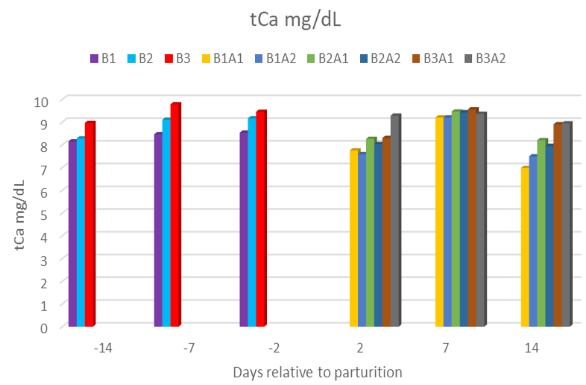

Fig. 3. Means of tCa concentrations for cows fed prepartum and effect of (Tr1) DCAD 0.0, -100, -180 (BC1, BC2, BC3. $P < 0.001$) and effect of postpartum (Tr2) DCAD +250 and +350 (B1A1, B1A2, B2A1, B2A2, B3A1, B3A2. $P = 0.511$), day $P < 0.001$ and interaction Tr1 x Tr2 x day P = 0.086, during experimental period.

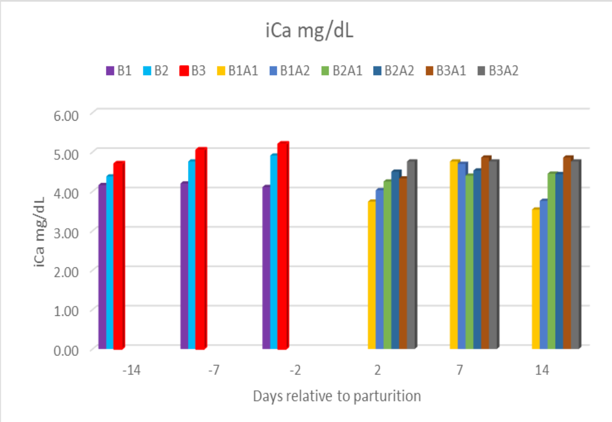

Fig. 4. Means serum iCa concentrations for cows fed prepartum and effect of Tr1 DCAD 0.0, -100, -180 (BC1, BC2, BC3. $P < 0.001$) and effect of postpartum Tr2 DCAD +250 and +350 (B1A1, B1A2, B2A1, B2A2, B3A1, B3A2. $P = 0.511$), day $P < 0.001$ and interaction Tr1 x Tr2 x day $P = 0.510$, during experimental period.

Table VI. Urinary pH and Ca concentrations for cows fed prepartum diets formulated to contain 0, -100 and -180 mEq/kg dietary cation-anion difference (DCAD) and postpartum diets formulated to contain 250 or 350 mEq/kg.

Tr1	0		-100		-180		P -value							
Tr2	250	350	250	350	250	350	SEM	Tr1	Tr2	day	Tr1 x Tr2	Tr1 X day	Tr2 x day	Tr1 X Tr2 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

Tr1= prepartum DCAD treatment (0.0, -100 and -180), Tr2= postpartum DCAD treatment (+250 and +350).

Table VII. Blood metabolites measured postpartum for cows fed prepartum diets formulated to contain 0, -100 and -180 mEq/kg dietary cation-anion difference (DCAD) and postpartum diets formulated to contain 250 or 350 mEq/kg.

Tr1	0		-100		-180		P-value			
Tr2	+250	+350	+250	+350	+250	+350	SEM	Tr1	Tr2	Tr1x Tr2
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

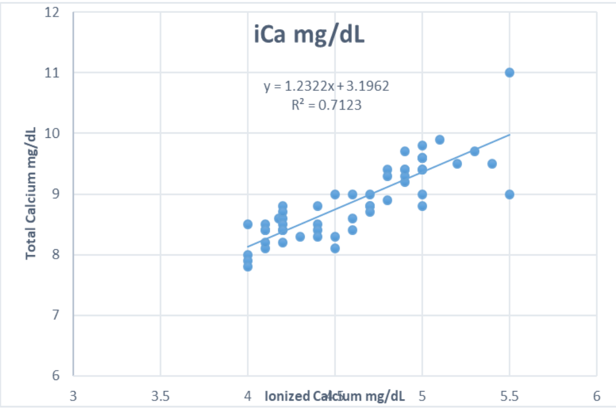

Fig. 5. Simple linear regression and positive correlation between tCa and iCa (R = 0.84, R^2 = 0.712 P < 0.001) tCa = 3.2 + 1.23*iCa for cows fed prepartum (Tr1) DCAD (0.0, -100, -180) there is positive linear relationship tCa and iCa p < 0.05.

Parathyroid hormone and iCa

Cows fed prepartum DCAD diets -180 and −100 mEq/kg DM had lower serum PTH concentrations compared with cows fed on 0.0 mEq/kg DM DCAD diet (Table IV). Serum PTH concentrations usually are negatively correlated with serum iCa concentrations (Jonsson *et al.*, 1980). The blood PTH level increased 10-20 fold. If a cow is fed anions, the rise is temporary and falls by day 2 -3 to baseline levels (Goff *et al.*, 1989). The target tissues respond to the PTH and it works on bone and kidney cells to restore blood Ca to the normal level. In cows not fed anions, that have more alkaline blood and urine- the PTH concentrations increased when blood Ca falls, but the tissues are resistant to the effects of PTH. We think that PTH receptor is not properly recognizing the hormone. As a result of blood Ca does not rise rapidly or at all and the parathyroid gland continues to secrete large amounts of hormone for a longer period. Cows with milk fever will have extremely high blood levels of PTH- but it is not helping them maintain normal Ca because the tissues do not recognize it (Goff *et al.*, 2014).

Urine pH

Prepartum DCAD treatments and sample day had a significant effect on urine pH decrease (Fig. 1, Table III), as well as a negative association between urine pH and iCa. Increasing urine pH can lead to a decrease in iCa concentrations (r = -0.74, P 0.001), (Fig. 2). The urine pH was lower in the negative DCAD (-18 mEq/100 g of DM), which was consistent with (Goff *et al.*, 2014; Santos *et al.*, 2019; Leno *et al.*, 2017; Moore *et al.*, 2000). In contrast, Moore *et al.* (2000) reported no differences in postpartum Ca metabolism in cows given an anionic diet. The reason for increasing urine pH after calving due to (+250 or +350 mEq/kg DM) and day (Fig. 1) is due to high levels of sodium bicarbonate and potassium carbonate for raising DCAD and rumen buffering because lactating cow diet contains higher levels of concentrates, thus increasing DCAD postpartum to avoid ruminal acidosis.

Colostrum yield, IgG and calf BW

There were no effects on IgG, Colostrum yield, or calf birth weight (Table V), demonstrating that giving nutritional anions to dairy cows in late gestation had no impact on colostrum supply or IgG. Other studies have reached the same result as we have (Lopera *et al*., 2018; Weich *et al*., 2013; Diehl *et al*., 2018; Martinez *et al*., 2018). Lowering DCAD of prepartum meals from about +130 to -130 mEq/kg DM had no effect on the yield or quality of colostrum (Martinez *et al*., 2018). This is consistent with the findings of Weich *et al*. (2013), who observed no differences in birth weight between calves from cows fed a diet containing -160 mEq/kg DM (41.1 kg) and calves fed +120 mEq/kg DM (41.1 kg) (44.6 kg).

CONCLUSIONS

Prepartum anionic diets raised hydroxyproline and lowered urine pH and parathyroid hormone, resulting in better Ca availability after parturition and Ca concentrations at normal levels on calving day. Calf birth weight, IgG, and colostrum yield were unaffected by cows given a negative DCAD approach (-100 and -180 mEq/kg DM). Postpartum blood metabolites were unaffected in cows given positive DCAD (+250 and +350 mEq/kg DM).

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Statement of conflict of interest

The authors have declared no conflict of interest.

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