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Effect of Prepartum Dietary Cation Anion Difference on Blood Mineral and Metabolite Concentrations and Lactation Performance of Holstein Dairy Cows

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Abstract

Dietary cation-anion difference (DCAD) drives a compensated metabolic acidosis, which increases calcium (Ca) uptake and mobilisation before calving and reduces clinical and subclinical hypocalcemia postpartum. This approach is frequently employed in conjunction with dietary Ca restriction, which has traditionally been utilised to mobilise Ca prepartum in order to prepare cows for lactation. Supplemental dietary Ca in conjunction with a negative DCAD formulation that does not restore compensated metabolic acidosis may be helpful. The goal of this study was to see how mineral concentrations, blood metabolites, endocrine state, and lactation 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 Holstein cows entering 1-5 lactation with an average body weight of 685 kg 10 SD (n= 48) were used in a randomised block with a three-treatment arrangement to offer three prepartum diets with different DCAD (0, -100, and -180 mEq/kg DM). Cows were kept on trial for a total of 66 days after calving. Cows given -180 and -100 DCAD had greater prepartum NEFA concentrations than cows fed 0.0 DCAD. Cows fed (-180 DCAD) had greater serum Ca concentrations than cows fed (-100, 0.0 DCAD) owing to impact therapy. Because of the influence of the day, phosphorus content was greater at 0 and 2 days postpartum. PTH levels were greater in cows fed (0.0 DCAD) than in cows fed (-100, -180 DCAD). There are no changes in therapy or interaction DCAD x day on blood BHB, Glucose, Mg, Na, K, Cl between prepartum and postpartum. Milk protein was significant owing to the interaction DCAD x L, and solid not fat (SNF) was significant due to the impact of L. Milk yield, fat corrected milk (FCM 3.5%), ECM, fat, total solid, lactose, and milk urea nitrogen were not affected by treatment or interaction (MUN). We discovered that supplementing nursing cows' diets with anionic salts can enhance Ca availability postpartum and reduce clinical and subclinical hypocalcemia.

Keywords: β-Hydroxybutyrate; None-esterified fatty acids; Parathyroid hormone; Energy corrected milk; Lactation no; Milk urea nitrogen; Solid-not fat

Introduction

Grummer defined the transition period as the three weeks preceding and following parturition. Due to a higher risk of metabolic illness that impacts output and fertility in later lactations, this phase is designated as the most essential of the dairy cow's lactation. Most cows experience hypocalcemia during or shortly after close calving. Hypocalcemia has a detrimental influence on cow health and herd profitability because it raises the risk of metabolic and viral disease, lowers milk supply, lowers reproductive efficiency, and raises the risk of culling cows early in lactation [1]. During the prepartum period, an acidogenic diet has been utilised to prevent or minimise the severity and duration of hypocalcemia around parturition. Increased calcium mobilisation prepartum and restoring tissue sensitivity to parathyroid hormone (PTH) stimulation are two mechanisms of action linked with feeding an acidogenic diet prepartum. The commencement of lactation changes metabolism to provide the nutrients required for milk synthesis to the mammary tissues. Cows that are unable to change their energy metabolism quickly enough for milk synthesis either produce less milk than they should or are susceptible to metabolic problems. During early lactation, body fat reserves are transported into the bloodstream as NEFA, which contributes to overall energy needs. By metabolising NEFA into ketone bodies like BHBA or re-esterifying them into triglycerides, the liver eliminates a considerable proportion of NEFA from the bloodstream. Previous research has found a correlation between metabolite concentrations and milk output. Increased ketone body concentrations in milk during early lactation have been linked to a decrease in milk production. Early and overall lactation milk losses were linked to increases in serum BHBA in the first week after calving. Increased blood BHBA levels in the second week after calving were linked to early lactation milk loss but higher total milk output. Lower prepartum DMI caused by the addition of anions commonly comes from decreased palatability, but it could also be a response to metabolic acidosis induced by anionic supplementation. Whatever mechanism anionic diets use to have their negative effect on prepartum DMI, falling DMI might have a negative contradictory effect on metabolism [2]. Cows with hypocalcemia produce less milk and are more likely to have mastitis, a misplaced abomasum, and a retained placenta, among other metabolic issues. Subclinical hypocalcaemia reduces DMI and energy metabolism. The purpose of this study was to see what influence lowering the DCAD of the prepartum diet had on mineral status, energy metabolites, and lactation performance. We expected that prepartum DCAD would enhance Ca status and decrease negative energy balance, resulting in higher DMI and milk production in the postpartum period.

Materials and Methods

Cows and Diets

The field experiment of this study was conducted from December to March 2021 at private dairy farm, Ismailia Desert Road, which belongs to Ismailia governorate of Egypt. Forty-eight multiparous

Holstein cows within 3 to 4 wk of expected parturition were selected from the herd and blocked with 3 treatments 16 cows each treatment by expected calving date, parity, and previous 305-d mature-equivalent production [3]. Cows had completed 1-5 lactation and average 685 kg ±10 SD body weight. Data were collected beginning 22 d prepartum through 66 d postpartum. Cows were fed twice daily at 7:00 and 17:00 hours. Prepartum diets were formulated to provide 0.0 mEq DCAD/kg DM as a control, -100 mEq/kg DM or -180 mEq DCAD/kg DM.

Immediately after calving cows fed a lactation diet formulated to contain +250 mEq DCAD/kg DM throughout the remainder of the trial. Cows were housed in an open yard and milked three times daily 08:00, 16:00 and 24:00 h. Prepartum and postpartum diets were formulated as show using the Cornell Net Carbohydrate and Protein System (CNCPS version 6.5, Cornell University, Ithaca, NY) ration evaluator [4]. Dry matter was determined using a forced-air drying oven set at 55°C for 48 h. Dietary ingredients were analyzed for CP, EE, Ash, minerals (AOAC 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin, TDN, NEL and NFC were calculated according to NRC, 2001 (Tables 1 and 2).

Prepartum 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	7.2	7.12	7.08	
Corn gluten feed				8.45
Sunflower meal, 36% CP	14.15	14	13.91	
Energizer-Gold1				0.85
OleoFat2				0.89
Limestone	1.06	1.11	1.14	0.54
Salt	0.3	0.3	0.3	0.41
Sodium bicarbonate				1.15
M and V Dry Cow premix3	0.31	0.3	0.3	
Magnesium oxide	0.23	0.22	0.22	0.19
Free feed silica4				0.19
Mycofix5				0.07
Diamond V Yeast XP6				0.07
Organic zinc				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 and V premix7				0.27
MegAnion8	0.37	0.51	0.54	
Calcium chloride	0.31	0.82	1.06	
DCAD mEq/kg DM9	0	-100	-180	250

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

Prepartum DCAD				
	0	-100	-180	250
DM%	56	57	57	53
CP	15	14.8	14.8	17.5
Soluble protein, % of CP1	30	31	31	26
Ether extract	3	3	3	4.5
NDF	42.2	41.9	41.7	28
NFC2	31.8	31.8	31.8	40.8
TDN	70	69	69	78
peNDF3	31	31	30	23
NEl, Mcal/kg4	1.51	1.52	1.51	1.73
Ash	8	8.9	9	9.2
Ca	0.99	1.1	1.2	0.89
P	0.36	0.37	0.36	0.41
Mg	0.38	0.42	0.48	0.3
K	1	1	1	1.23
Na	0.2	0.2	0.2	0.56
Cl	0.51	0.7	0.8	0.34
S	0.32	0.4	0.48	0.34
DCAD5 (mEq/kg DM)	0	-103.8	-181.9	250.6

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

Blood Samples Minerals and Hormones procedures

Blood samples were collected from *via* coccygeal venipuncture on -14, -7, -2, 0, 2, 7, 14 and 22 d relative to predicted calving and were collected *via* coccygeal venipuncture from cows into vacutainers containing either sodium heparin and plain vacutainer serum tubes. Sampling time (approximately 1300 h) corresponded to approximately 5 h after morning feeding [5]. Plasma and serum were separated after centrifugation at 2000 × g for 20 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 Veterinary Diagnostic Lab of Animal Reproductive Research Institute, Agriculture Research Center, Ministry of Agriculture, Al-Harm, Egypt. Blood serum samples were used for analyzed sifors of parathyroid hormone (PTH) and insulin were determined using bovine ELISA kit (No. 18, Keyuan Road, DaXing Industry Zone, Beijing, China) [6]. Concentrations of Ca, P, Mg, Na, K, Cl, urea and creatinine were determined colorimetrically using a RA-50 chemistry analyzer (Bayer, address) according to the manufacture's instruction (RA-50 chemistry analyzer (Bayer, China) using 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). Total protein and albumin were determined colorimetrically (RA-50 chemistry analyzer (Bayer, China) using a commercial readymade chemical (kits), (CAT. No. TO 2020, AB 1010 Biodiagnostic co. Egypt). Globulin was mathematically calculated (globulin = total protein – albumin). Glucose and BHB were measured immediately in whole blood by using a portable Free-Style Optium Neo reader with blood glucose and ketone test strips (Abbott Diabetes Care Ltd, Range Road, UK). Plasma NEFA was measured 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 [7].

Milk samples

Milk samples were collected from 3 consecutive milking every two weeks. Samples from individual cows were composited (200 to 250 mL) stored at 4°C until analysis within 72 h after collection concentrations of (milk fat, protein, lactose, TS, and SNF and milk urea nitrogen (MUN)] using pre-calibrated ultrasonic milk analyzer [LACTOSCAN LA, 8900 Zagora BULGARIA). Milk urea nitrogen (MUN) was analyzed using mid-infrared techniques (method 972.16, AOAC International, 2006). The current experiment was conducted according to the guidelines of Kafr El-Sheikh University and approved by the local experimental animal care committee, Faculty of Agriculture, Kafr El-Sheikh University, Egypt (id 4/2016 EC). All precautions were taken to reduce suffering throughout the trial period [8].

Statistical analysis

Statistical analysis of experimental data was carried out through the SPSS V23 (https://www.ibm.com/eg-en/analytics/spss-statistics-software). Software package by analyzing the data through one-way ANOVA. The effect of treatment (0.0, -100 and -180 mEq/kg), period (- 14, - 7, - 2, 0, 2, 7. 14 a nd 2 1) , treatment x period interaction was determined by two- way Analysis of variance (ANOVA). The obtained results were analyzed statistically of block design according to using general linear models procedure adapted by SPSS V23. Prepartum and postpartum data were analyzed separately Significance was declared at $P< 0.05$, and a trend was declared when $P \geq 0.05$ and <0.1.

Results

Cows fed on DCAD have a significant effect on concentrations of serum Ca, NEFA, tended to have higher with increasing negative DCAD for 0.0, -100, -180, Means ±SD (8.38 ±0.289, 8.85 ±0.499, 9.40 ±0.557 mg/dL), $P < 0.05$, for the treatment and day of sample as shown but there is no interaction effect on Ca concentration. Postpartum Ca there significant effects of treatment and day of sample and interaction DCAD x day $P < 0.05$ as presented, (8.59 ± 1.04, 8.89 ± 0.741, 9.27 ± 0.513 mg/dL). Prepartum plasma concentration of NEFA as show, means (231 ± 26.48, 256 ± 27.83, 275.83 ± 39.18 µmol/L), there is effect of treatment and day as shown (Figure 4) but there is no effect for interaction $P > 0.05$, but postpartum plasma NEFA means ±SD (788.66 ±218.56, 790.66 ±181.50, 854.2 ± 203.577 µmol/L), there is effect of treatment and day but there is no effect for interaction DCAD x day, $P >0.05$. Serum phosphorus concentration there is effect of interaction DCAD x day as shown, means ±SD (6.23 ± 0.196, 6.207 ± 0.271, 6.36 ± 0.279 mg/dL), $P <0.05$, postpartum phosphorus there is effect of day but neither DCAD nor interaction DCAD x day were affected on Phosphorus, $P > 0.05$. While concentration of PTH was higher in cows fed prepartum 0.0 DCAD compared with cows fed -100 and -180 DCAD. Means for diets 0.0, -100 and -180 DCAD (48.45 ± 4.11, 31.28 ± 3.83, 39.46 ± 2.45 pg/ml) $P <0.05$, in postpartum PTH no effect were observed of DCAD treatment. There is no effect prior to calving of treatment or interaction DCAD x day on blood glucose $P > 0.05$, but after calving the effect on blood glucose was obvious of interaction DCAD x day $P < 0.05$, means glucose for diets 0.0. -100 and -180 DCAD (61.90 ±6.13, 59.40 ± 6.10, 60.66 ±7.89 mg/dL). Milk protein as presented was affected by interaction of DCAD x L lactation number and treatment $P<0.05$ (3.10 ± 0.206, 3.22 ± 0.158, 3.21 ± 0.146 %). We were observed effect of lactation on milk lactose (4.62 ±0.110, 4.74 ±0.196, 4.75 ± 0.144 %) and SNF (8.54 ±0.312, 8.90 ± 0.401, 8.81 ± 0.225 %) as shown. Mineral concentrations of Mg, Na, K, Cl not changed by treatment and interaction DCAD x day in both prepartum and postpartum. Liver and kidney functions like ALT, AST, Albumin, Total protein, urea and creatinine not affected by treatment and interaction. Blood metabolites like Insulin, BHB also didn't change by DCAD treatment or interaction $P>0.05$ [9-15].

Mineral Status

There was no incidence of hypocalcemia in both cows pre and postpartum, as serum Ca never fell below the 8 mg/dL for cows fed (0.0, -100, -180 DCAD) as shown. The increased serum Ca level as shown in described effect of interaction DCAD x day for cows consuming -180 and -100 DCAD diet compared to the lowest level for 0.0 DCAD diet might be due to mild metabolic acidosis has been induced by anionic salt supplementations. Bones act as a major storage of buffers for acid-base regulation of body fluids. When dairy cows are placed on acidifying diets, the blood pH drops. Frick et al. concluded that an acidic extracellular pH activate osteoclastic bone

resorption which may result in higher plasma Ca concentrations. We noticed that P concentrations in blood increased at the time of calving 0 d (8.79 ±0.490 mg/dL) and 2 d postpartum (8.77 ±0.499 mg/dL) due to the effect of day as presented in this is compatible with several of the individual studies that had significant increases in blood P concentrations at calving. Bone P resorption is controlled by PTH activity, blood concentrations of Ca and P, and by $1,25(OH)_2$ vitamin D3. If increased bone resorption is stimulated by the diets with lower DCAD, it is likely that P concentrations in the blood would raise along with Ca concentrations. However, other mechanisms, such as a likely increased absorption of P in the intestinal tract, could contribute to the increased P concentrations [16-25].

Discussion

Plasma NEFA Concentrations

Peak plasma NEFA concentrations occur around the time of calving as shown, start decline as feed intake increases after calving [26-30]. In our present trial higher concentration of NEFA was in the day of calving in cows fed -180 compared with 0.0 and -100 mEq/kg DM of DCAD this due to low dry matter intake in cows fed low DCAD diets. No difference were observed in blood BHB as shown due to treatment, day or interaction.

Reason of increasing prepartum non-esterified fatty acid due to negative effect of anionic salts on reduction of dry matter intake, whereas BHB indicate that short-term energy balance was not altered among treatments or interaction [31-39]. This would be expected in the absence of any metabolic challenge that typically occurs at or immediately after calving unless DMI declined significantly to cause an extended negative energy balance that was not apparent based on BHB. Compared with NEFA prepartum concentrations, all values were higher postpartum as shown due effect of DCAD and day, which would be expected [40-50].

Parathyroid Hormone

The effects of lowering DCAD in prepartum diets (-100 and -180 mEq/kg DM) have been reviewed, and include increased parathyroid hormone sensitivity in cows; increased renal output of 1,25(OH)2 vitamin D3; and increased responsiveness of target Ca resorption from bone; and higher plasma ionised Ca concentrations (Figure 1).

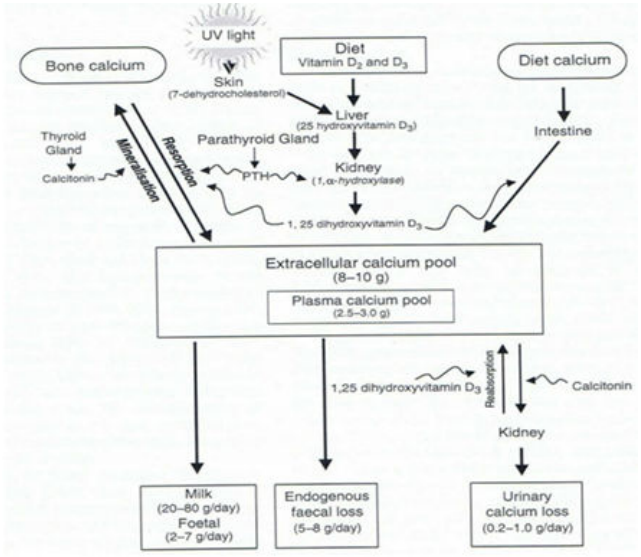

Figure 1: Model of the tissues and hormones associated with calcium homeostasis a cow 500 kg (McNeil et al 2002).

Prepartum DCAD					P -value		
DCAD	0	-100	-180	SEM	T	Day	T x day
Ca mg/dL	8.38c	8.85b	9.40a	0.048	0.001	0.001	0.17
P mg/dL	6.23	6.2	6.26	0.032	0.103	0.82	0.011
Mg mg/dL	2.85	2.86	2.85	0.014	0.981	0.284	0.98
Na mEq/L	142.12	142.19	141.66	0.137	0.945	0.575	0.377
K mEq/L	4.94	4.9	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	0.93
Glucose mg/dL	63.41	61	60.5	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	231.66b	256.00a	275.83a	4.378	0.001	0.001	0.181
Postpartum							
Ca mg/dL	8.59c	8.89b	9.27a	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	0.97
Glucose mg/dL	61.90a	59.40b	60.66b	0.607	0.247	0.001	0.025
BHB mmol/L	0.827	0.767	0.827	0.016	0.229	0.888	0.27
NEFA µmol/L	788.66b	790.10b	854.20a	9.072	0.005	0.001	0.118

Table 3: Prepartum and postpartum Mineral concentrations and blood metabolites for cows fed anionic salts diets (0.0, -100 and -180 mEq/kg DM) during experimental diets.

Milk Production and Composition

According to West (1992), boosting DCAD increased milk fat % without affecting milk production. On the other hand, other studies have found that milk output has increased without a change in milk fat %.

Other investigations have shown no variation in lactose % owing to changes in DCAD [51-55]. According to Beede et al., adding anionic salts to the prepartum diet improved milk production by 3.6 percent in the subsequent lactation, which contradicts our findings. Moore (2000) gave anionic salts to cows and found no difference in milk production between 7 and 70 DIM. Martinez et al., examined daily milk production and weekly milk fat and protein content in 79 cows and found no effect of DCAD treatment on milk yield, ECM, or true protein content.

But they did find that cows fed negative DCAD prepartum had approximately 0.23 percentage points more fat in the first 49 postpartum days [56-60]. Who evaluated three dairy cow diets (DCAD of +183, +59, or 74 mEq/kg of DM) and found that decreasing prepartum DCAD increased milk production and ECM in the first three weeks postpartum. We concur with who showed that whereas fat content did not differ across DCAD groups in multiparous cows, milk protein content rose when an acidogenic diet was fed prepartum.

Our results disagree with a meta- analysis that characterized improved milk yields (+1.7 kg/d) and increased FCM (+1.1 kg/d) in multiparous cows (Table 3).

The composition of diets are shown and average dry matter 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 (22.5 kg DM/day) respectively.

Serum metabolites are presented in (Tables 4 and 5).

Prepartum	**DCAD**				
		-100	-180	SEM	P -Value
AST u/L	73.8	73.88	71.66	0.451	0.108
ALT u/L	30.76	31.59	30.8	0.28	0.276
Total protein g/dL	6.39	6.4	6.5	0.04	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	31.61	3.11	0.178	0.154
Creatinine mg/dL	1.21	1.21	1.2	0.005	0.53
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	**DCAD**				
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	6.5	6.51	6.65	0.033	0.138
Albumin g/dL	3.13	3.25	3.82	0.177	0.25
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	0.01	0.513
Insulin pmol/L	82.33	83.33	85.5	0.799	0.269
PTH pg/ml	51.8	50.57	47.38	1.232	0.339

Table 4: Prepartum and postpartum liver, kidney functions and blood hormones for cows fed anionic salts diets (0.0, -100 and -180 mEq/kg DM) during experimental diets.

DCAD					**P -Value**		
Item	0	-100	-180	SEM	DCAD	L	DCAD x L
Milk yield kg/d	38	38.92	39.57	1.008	0.837	0.994	0.757
1FCM 3.5 kg/d	39.17	40.08	40.92	1.079	0.843	0.988	0.853
2ECM kg/d	40	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 %	3.23a	3.11b	3.14b	0.028	0.428	0.186	0.034
Lactose %	4.7	4.68	4.68	0.025	0.654	0.05	0.748
Total Solid %	12.56	12.21	12.39	0.52	0.133	0.071	0.055
SNF %	8.87a	8.53b	8.68a	0.049	0.129	0.021	0.232
MUN mg/dL	11.71	12.42	11.57	0.441	0.669	0.06	0.108

Table 5: Milk yield, FCM3.5% and milk composition for cows fed anionic salts diets (0.0, -100 and -180 mEq/kg DM) during experimental diets.

DCAD had no significant impact on milk output, FCM 3.5 percent, ECM, or milk fat percent related to treatment or interaction DCAD x L, as indicated, P>0.05.

This could be related to genetic potential or the production of a slightly acidic environment in the rumen by a lower DCAD diet. We agree with results [16].

Found that prepartum negative DCAD feeding had no effect on postparturient milk productionIncreased milk protein could be attributable to the effect of interaction DCAD x L, P=0.034, whereas increased SNF percentages in cows fed 0.0 vs. -100 and -180 mEq/kg DCAD diets could be attributed to the effect of DCAD, P=0.021 [60-65].

Others have established a favourable connection between DCAD and milk fat test, however our findings contradict [18]. Adding dietary rumen buffers
such $NaHCO_3$ and K_2CO_3 to the diet has been demonstrated in multiple trials to enhance milk fat percentage, especially when milk fat is low (Figures 2 and 3).

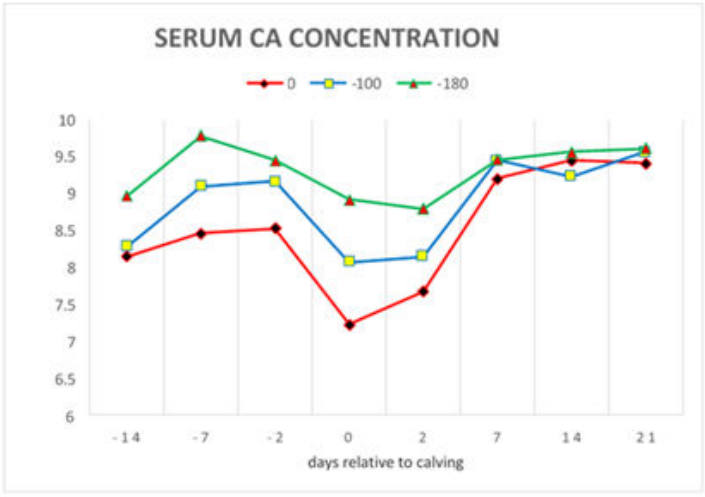

Figure 2: Ca concentrations in different days before calving -14 to -2 day and postpartum from 0 to 21 day for cows fed DCAD diets (0.0, -100 and -180 mEq/dL), there is significant effect of DCAD and interaction between DCAD x day on Ca level, $p < 0.05$

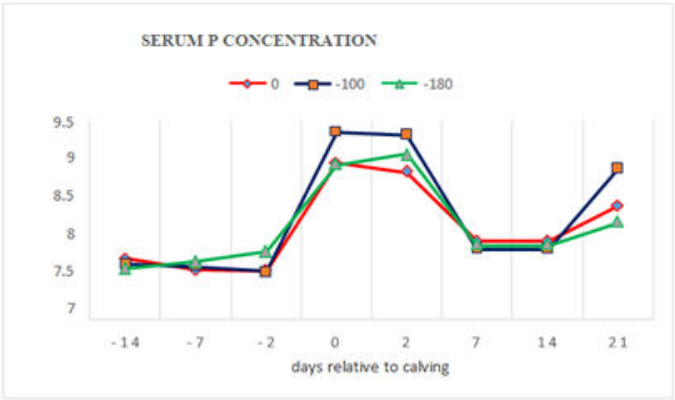

Figure 3: Phosphorus concentrations in different days before calving -14 to -2 day and postpartum from 0 to 21 day for cows fed DCAD diets (0.0, -100 and -180 mEq/dL), there is significant effect of day of sample $p < 0.05$ but there is no interaction effect between DCAD x day on P concentrations, $p > 0.05$.

Blood NEFA concentrations have been used as an estimate of energy balance [66]. The plasma NEFA concentration was higher prepartum cows fed DCAD -100 and -180 mEq/dL we are in line with Previous studies have shown that plasma NEFA concentrations begin to increase from the last 2 wk of gestation to the last 2 to 3 d before parturition (Figures 4 and 5).

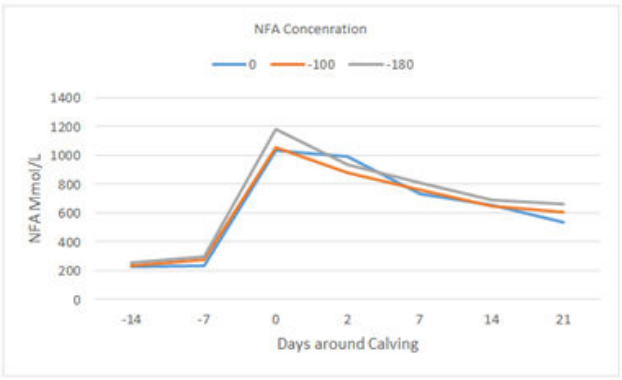

Figure 4: plasma NEFA µmol/L concentrations in different days before calving -14 to -2 day and postpartum from 0 to 21 day for cows fed DCAD diets (0.0, -100 and -180 mEq/dL), there is significant effect of day of sample $p < 0.05$ but there is no interaction effect between DCAD x day on P concentrations, $p > 0.05$

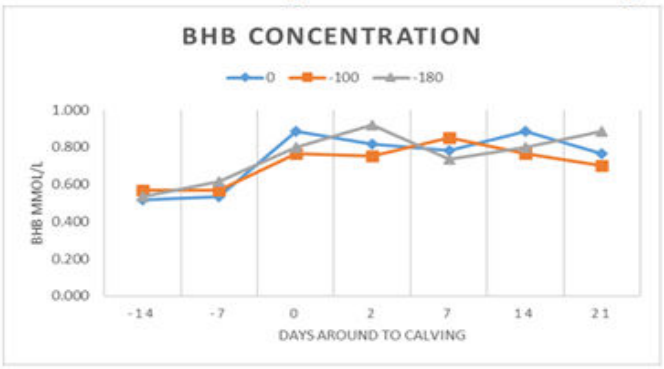

Figure 5: Blood BHB concentrations in different days before calving -14 to -2 day and postpartum from 0 to 21 day for cows fed DCAD diets (0.0, -100 and -180 mEq/dL), there is significant effect of day of sample $p < 0.05$ but there is no interaction effect between DCAD x day on P concentrations,

Conclusion

Feeding anionic salts in prepartum dairy cows improves Ca availability probably induced a similar state of compensated metabolic acidosis in cows fed -100 and -180 mEq/kg DM. Postpartum serum Ca concentrations raised significantly with different prepartum DCAD. Reducing the DCAD of the prepartum was not affect on Mg, Na, K, Cl, lactation performance, Insulin, BHB, and Glucose in the early postpartum period. Prepartum plasma concentrations of NEFA were increased in cows fed -180 DCAD than in cows fed 0.0 and -100

DCAD. These results indicate that effective of diets contain anionic salts to induce metabolic acidosis in prepartum dairy cows.

Conflict of Interest

The Authors declare there is no conflict of interest.

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