Influence of Dietary Cation-Anion Differences (DCAD) on Nutrient Intake, Milk Yield and Metabolic Parameters of Transition Buffaloes

Harneet Kour[1], Ravinder Singh Grewal[1,*], Prahlad Singh[2], Jaspal Singh Lamba[1], Jasmine Kaur[1] and Shashi Nayyar[3]

[1]*Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (Punjab), India*

[2]*Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (Punjab), India*

[3]*Department of Veterinary Physiology and Biochemistry, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (Punjab), India*

Abstract: The study was divided into 2 phases. During 1st phase, 20 pre-partum buffaloes were divided into two groups, given -100 (high negative; HN) and -50 (low negative; LN) mEq/kg of dry matter (DM) diets. In the second phase, +200 (low positive; LP) and +400 (high positive; HP) mEq/kg of DM diets were formulated for postpartum buffaloes. Buffaloes that received LN DCAD were divided into 2 groups (LN-LP and LN-HP), and those that received HN DCAD were also divided into 2 groups (HN-LP and HN-HP). In the first phase, the group fed the HN DCAD diet had significantly lesser DM, nutrient intake, and urine pH ($p<0.05$) but higher ME intake and ADF digestibility. Also, higher serum phosphorus levels ($p<0.05$), fewer cases of hypocalcemia and ketosis, and a relatively smaller decrease in body weight were reported with the HN group. In the second phase, DM, organic matter, and NDF intakes were higher ($p<0.05$) in HN-HP and LN-LP groups, whereas ME intake and nutrient digestibility were higher in HN-LP and LN-HP ($p<0.05$). Daily milk, solid not fat (SNF), fat, protein, lactose yields, and plasma total protein, urea, and cholesterol concentrations were the highest in the HN-HP group ($p<0.05$), but the highest fat content and fat yield were recorded in the HN-LP group ($p<0.05$). Better efficiency of milk production and the lowest cases of hypocalcemia and ketosis were observed for both groups. In conclusion, for transition buffaloes, the HN and LN DCAD (-100 and +200 mEq/kg of DM, respectively) diets showed a beneficial impact.

Keywords: Buffalo, dietary cation-anion balance, milk yield, hypocalcaemia, ketosis and transition period.

INTRODUCTION

The success of buffalo husbandry lies in ensuring the proper and optimal reproductive rhythm of individual animals in the herd, especially during the transition period. During this period, buffaloes are mostly susceptible to ketosis and parturient paresis, which are still major diseases that lead to a decrease in milk production during lactation [1]. For good management during the transition period, the dietary cation-anion difference (DCAD) concept was introduced to prevent the occurrence of major metabolic diseases and for optimum nutrient utilization. Its importance can be determined by the fact that it is ranked 3rd in homeostatic priorities [2]. It is represented by the formula ($[Na^+] + [K^+]) – ([Cl^-] + [S]) =$ mEq/100gm of DM.

Feeding negative DCAD during prepartum is associated with compensated metabolic acidosis [3, 4]. This is evidenced by reduced plasma bicarbonate, lower urinary pH, and higher urinary net acid excretion in dairy cattle, which was, in turn, successful in preventing milk fever and other metabolic diseases. In contrast, supplementing positive cations during prepartum can result in metabolic alkalosis, reduced tissue responsiveness to parathyroid hormone, and reduced ability to mobilize calcium [5]. In the postpartum period, the animal produces a lot of metabolic acids. During early lactation, a high amount of fat is mobilized, and respiration is increased, leading to increased blood acid load. The cations act as a buffer to compensate for high blood acid load, especially in high milk-producing animals [6]. The number of studies on buffaloes is very low. Therefore, the present study is planned to examine the influence of varying levels of negative or positive DCAD on the performance of transition buffaloes concerning metabolic health and nutrient intake.

MATERIAL AND METHODS

Ethics Procedures, Estimation of Cations and Anions, and Diets

This experiment was approved by the Institutional Animal Ethics Committee (IAEC) during the proceedings of the 56th meeting of IEAC, GADVASU,

*Address correspondence to this author at the Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (Punjab), India;
E-mail: ravigrewal71@yahoo.co.in

held at Ludhiana on 12.12.2020. The present study was conducted in the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The prepartum and postpartum ration was formulated and balanced according to ICAR [7], and the estimation of cations and anions in the total mixed ration (TMR) was done by Punjab Biotechnology Incubator (PBTI), Mohali. The ingredients and chemical composition of the experimental total mixed ratio (TMR) in pre- and postpartum periods are presented in Table **1**.

Animals, Diets, and Experimental Design

In the study, 20 Murrah buffaloes, 4 weeks before calving (prepartum), were used. The grouping of animals was done following a completely randomized block design. Three parameters were selected for the grouping of animals, i.e., average body weight (630 vs. 670 kg), 1st, 2nd, and 3rd lactation milk yield (1211.4 vs. 1217.5 kg), and parity (1st, 2nd, or 3rd). This was done to avoid pre-experimental variation.

The study was carried out in two phases, the prepartum and postpartum periods. During the prepartum period, animals were randomly allocated to two groups (each group having 10 buffaloes) fed negative DCAD diets, i.e., low negative (LN) and others fed high negative (HN). The DCAD of the LN and HN diets had -50 and -100 mEq/kg of DM, respectively, adjusted using the equation: [(%Na/0.023 + %K/0.039) - (%Cl/0.0355 + %S/0.016)]. During the postpartum period, the buffaloes fed on the HN and LN DCAD diet in the prepartum period were separately allocated into two positive DCAD diets (LP and HP). The DCAD of the LP and HP diets had +200 and +400 mEq/kg of DM, respectively, adjusted using the same equation. Based on prepartum and postpartum feeding of anions and cations at two levels, four groups (each group having 5 buffaloes) were formed after calving (LN-LP, LN-HP, HN-LP, and HN-HP) to know the cumulative effect of all the four combinations of cations and anions. Also, the effect of feeding cations at two different levels (LN and HN group) was also assessed

Table 1: Prepartum and Postpartum Ratio Composition

Ingredients	Chemical composition		
Prepartum ration	**Parts (per 100 kg)**	**Prepartum ration**	**Parts (per 100 kg)**
Maize	24	De-oiled rice bran	13
Bajra	5	Rice bran	10
Mustard cake	17	Wheat bran	10.1
Cotton seed meal	5	Mineral mixture	1.7
Soya meal	7	Salt	1
Guar Korma	3		
Postpartum ration	**Parts (per 100 kg)**	**Postpartum ration**	**Parts (per 100 kg)**
Maize	23	Yeast saccharide	0.15
Bajra	5	Bypass fat	1.5
Barley	8	Methionine	0.07
Mustard cake	13	Choline	0.5
Cotton meal	5	Lysine	0.15
Cotton seed	5	Toxin binder	0.15
Soya meal	10.5	Biotin	0.02
Full fat soya	3	Niacin	0.1
Guar Korma	5	Vitamin E	0.1
Rice bran	5	DCP	0.7
Wheat bran	5	DDG	0.5
Mineral mixture	1	Limestone powder	0.7
Salt	1.5	Urea	0.8
Soda bicarbonate	0.8		

The roughage portion was made up of mixed silage and wheat straw in a 70:30 ratio.

individually without considering the prepartum treatment. Such distinction was made to ascertain the effect of just feeding positive cations on milking animals. By doing this, we can verify the effect of anions, cations, and a combination of anion-cation mixture as well. The TMR was similar; only dietary cation-anion levels were altered by adding ammonium chloride (NH_4Cl) with a molecular weight of 53.5 and an equivalent weight of 53.5, and disodium hydrogen phosphate (Na_2HPO_4) with a molecular weight of was used to achieve positive DCAD levels of the postpartum diet. The calculated DCADs of TMR samples obtained on different prenatal and postnatal days and the amounts of $NH_4Cl$and Na_2HPO_4 added to reach the final or required DCAD level are presented in Table **2**.

Dry Matter Intake, Nutrient Intake, and Digestibility

A feeding trial of 90 days (30 for prepartum and 60 for postpartum) was conducted on 20 transition buffaloes. Before the termination of the feeding trial, a 5-day digestion trial was conducted after a period of adaptation of 3 days by direct method separately in prepartum as well as after calving. For the digestion trial, 5 animals from the prepartum and postpartum groups were selected on the basis of previous criteria. Samples of feed, feces, and orts were analyzed for total ash, ether extract, N [8], and cell wall constituents [9].

Blood, Urine, and Milk Analysis

Blood was collected on -25 and -10 days before calving in prepartum animals and on +10 and +25 days after calving in postpartum animals to monitor changes in various blood parameters with different levels of DCAD. A total of 11 blood parameters were analyzed, which were glucose, cholesterol, total protein, albumin, calcium, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), urea, creatinine, phosphorus, and non-esterified fatty acids (NEFA). Plasma samples were analyzed using Erba diagnostic kits in a semi-autoanalyzer. NEFA was estimated using an ELISA sandwich kit specific for quantitative detection of bovine non-ester fatty acid (NEFA). Urine samples were also collected on -25, -10 days, +10 and +25 days. It was estimated using phenomena of hydrogen-ion activity in water-based solutions by electric pH, indicating its acidity or alkalinity expressed as pH. The chemical composition of Murrah buffalo milk, i.e., fat (%), Solid not fat (SNF), total protein, and lactose, was analyzed by using the milk analyzer "Lactoscan LA" from Milktronic Ltd, Bulgaria.

Daily Milk Yield Record

After successful calving, all the animals were milked daily, and a record of the daily yield was kept. The record for milk yield of 20 buffaloes was recorded daily

Table 2: Calculated (CDCAD) and Final Dietary Cation-Anion Difference (FDCAD) of Total Mixed Ration (TMR) on Different Days before (DBP) and after (DFP) the Parturient and dose of Anionic (NH_4Cl) and Cationic (Na_2HPO_4) Chemicals Added to TMR

Sample no.	DBP	CDCAD*	NH_4Cl added to TMR	FDCAD of TMR
1	-31 days	+40.00	LN= 58 g	-50.34
			HN= 90 g	-100.17
2	-25 days	+32.00	LN= 53 g	-50.54
			HN= 85 g	-100.30
3	-10 days	+38.76	LN= 57 g	-50.02
			HN= 89 g	-99.86
Sample no.	**DFP**	**CDCAD**	**Na_2HPO_4 added to TMR**	**FDCAD of TMR**
5	0 day	+140.94	LP= 50 g	+199.64
			HP= 150 g	+317.04
6	+20 days	+245.47	LP= 10 g	+257.21
			HP= 132 g	+400.44
7	+40 days	+236.09	LP= 10 g	+247.83
			HP= 140 g	+400.45

*DCAD (meq/kg DM) = (%Na/0.023 + %K/0.039) – (%Cl/0.0355 + %S/0.016).

for up to 60 days from the day of calving for each animal.

Statistical Analysis

Data were analyzed by using SPSS [10] software using ANOVA with interaction, as described by Ridgman [11]. The experimental design used was a completely randomized block design. One-way ANOVA (LSD, Tukey's-b, and Duncan) was used for the analysis of postpartum results (with 4 groups: LN-LP, LN-HP, HN-LP, HN-HP). The independent t-test was used for analyzing prepartum as well as postpartum data (with two groups: LP & HP, LN & HN). The analysis of mean differences was tested using Duncan's multiple-range tests. The accepted level of significance was $p<0.05$.

RESULTS

Dry Matter Intake, Nutrient Intake, and Digestibility in the Prepartum Period

In the prepartum period, the dry matter intake (DMI), nutrient intake, and digestibility of the LN and HN DCAD diets are given in Table **3**.

There was a significant difference in DMI, organic matter (OM) intake, crude protein (CP) intake, ether extract (EE) intake, and CP digestibility ($p<0.05$), which was found to be the highest in the LN group during the 5-day digestion trial. However, acid detergent fiber (ADF) digestibility and metabolic energy (ME) intake were higher in the HN group ($p<0.05$).

Blood Parameters in the Prepartum Period

The average results of blood analysis of -50 (LN) and -100 (HN) DCAD group collected on -25 and -10 days (before calving) are given in Table **4**.

There was a significant difference in levels of inorganic phosphorus, which was observed more in the HN group compared to the LN group ($p<0.05$). Other parameters were high in the HN group, except calcium and NEFA, which were higher in the LN group, but the results were not significant ($p>0.05$).

Urine pH and Body Weight in the Prepartum Period

Monitoring urine pH is an effective indicator of extracellular fluid acid–base balance. The results of the effect of negative DCAD on urine pH and body weight recorded on -25 days and -10 days are given in Table **5**.

There was a significant difference in average urinary pH in the two groups fed different levels of

Table 3: The Nutrient Intake and Digestibility in Buffaloes Fed the Diets (LN and HN Diets) Used in the Prepartum Period

Parameter	LN	HN	SEM	P- value
Nutrient intake, kg/d				
DM	11.17^{b}	9.72^{a}	0.334	0.03
OM	10.00^{b}	8.68^{a}	0.310	0.03
CP	1.52^{b}	1.29^{a}	0.041	0.01
NDF	6.24	5.27	0.195	0.12
ADF	3.24	2.85	0.107	0.69
EE	0.27^{b}	0.24^{a}	0.006	0.03
ME*	1.56^{a}	1.66^{b}	0.031	0.04
Digestibility of Nutrients, %				
DM	64.20	63.70	0.743	0.81
OM	67.76	66.86	1.288	0.64
CP	73.93^{b}	69.68^{a}	0.831	0.03
NDF	53.86	55.63	1.846	0.51
ADF	38.40^{a}	45.74^{b}	2.286	0.03
EE	77.90	76.17	0.671	0.54

[a,b]Means with different superscripts within a row differ significantly ($P<0.05$).
*ME unit= MJ/kg DMI.

Table 4: The Blood Parameters in Buffaloes Fed Diets (LN and HN Diets) Used in the Prepartum Period

Parameters	LN	HN	SEM	P-value
Glucose (mg/dl)	62.75	68.13	1.975	0.75
Cholesterol (mg/dl)	80.21	87.86	5.990	0.30
Total Protein (g/dl)	5.04	5.57	0.276	0.10
Albumin (g/dl)	1.87	2.04	0.135	0.92
Calcium (mg/dl)	13.32	9.94	1.477	0.12
SGPT (IU/L)	29.78	26.93	2.237	0.18
SGOT (IU/L)	112.09	112.09	6.626	0.37
Urea (mg/dl)	33.33	32.90	1.963	0.42
Creatinine (mg/dl)	2.41	2.27	0.141	0.23
Phosphorus (mg/dl)	4.20^{a}	5.98^{b}	0.571	0.04
NEFA (mEq/L)	0.31	0.30	1.956	1.00

^{a,b}Means with different superscripts within a row differ significantly (P<0.05).

Table 5: The Urine pH and Body Weight in Buffaloes Fed the Diets (LN and HN Diets) Used in the Prepartum Period

Parameters	LN	HN	SEM	P-value
Urine pH				
-25 days	7.73^{b}	7.02^{a}	0.108	0.03
-10 days	7.39^{b}	6.95^{a}	0.136	0.03
Average	7.56^{b}	6.99^{a}	0.121	0.02
Body Weight (kg)				
-25 days	628.00	639.50	23.945	0.52
-10 days	607.00	622.90	21.944	0.72
Change	-21.00	-16.60	-	-
Calf Weight	32.20	30.60	1.420	0.32

^{a,b}Means with different superscripts within a row differ significantly (P<0.05).

negative DCAD diets. It was observed that the group fed HN DCAD had lesser urine pH compared to LN DCAD. Both the negative DCAD groups had normal calving with timely expulsion of the placenta with no significant difference in calf weight. In a part of this study where the Body conditions score (BCS) was evaluated based on the 7 skeletal checkpoints method by Anitha *et al*. [12], the results were satisfactory and within range. The BCS of both groups was found to be in the range of 4.025 – 4.037, which was optimum according to their stage [13].

Incidences of Milk Fever and Hypocalcemia in the Prepartum Period

The results of probable cases of hypocalcemia or milk fever as per serum calcium and phosphorus levels are given in Table **6**.

Table 6: Cases of Hypocalcemia/Milk Fever in Buffaloes Fed the Diets (LN and HN Diets) Used in the Prepartum Period

Negative DCAD	Cases with Ca <7.5 mg/dl	Cases with P <2mg/dl
LN	2	4
HN	3	1

The blood parameter analysis done on -25 and -10 days of calving revealed that none of the buffalo in any group had serum calcium level <5.92 mg/dl.

Incidence of Ketosis in the Prepartum Period

Probable cases of ketosis as per serum NEFA (mEq/L) and glucose (mg/dl) levels are given in Table **7**.

Table 7: Cases of Ketosis in Buffaloes Fed the Diets (LN and HN Diets) Used in the Prepartum Period

Negative DCAD	NEFA >0.3 mEq/L	Glucose <55 mg/dl
LN	3	2
HN	1	0

It was observed that 30% of the buffaloes fed LN DCAD level had serum NEFA concentration of more than 0.3 mEq/l. On the other hand, buffaloes fed HN DCAD had only one case (10%) of serum NEFA concentration above 0.3 mEq/l.

Dry Matter Intake, Nutrient Intake, and Digestibility in the Postpartum Period

The DMI, nutrient intake, and digestibility of the diets (LN-LP, LN-HP, HN-LP, and HN-HP diets) used in the postpartum period are given in Table **8**.

There was a significant difference in the nutrient intake and digestibility among the four groups, with the highest nutrient intake in the HN-HP group and variable digestibility during the 5-day digestion trial. The highest level of DMI and OMI was observed in the HN-HP and LN-LP groups, respectively, and ME intake was highest for the HN-LP and LN-HP groups, respectively (p<0.05). Dry matter digestibility was highest in the LN-HP group, and CP% was highest in HN-LP and LN-HP. However, there was no significant difference in nutrient intake during the whole trial.

Blood Parameters in the Postpartum Period

The relative effect of varying levels of DCAD fed to four groups of transition buffaloes on various blood parameters is given in Table **9**.

It was observed that total protein, urea, and cholesterol varied significantly among groups (p<0.05) found highest in the HN-HP group (i.e., animals that were fed HN during prepartum and HP during postpartum). Other parameters like glucose, albumin, SGOT, and phosphorus were found to differ non-significantly (p>0.05), but the highest values were found in HN-HP, whereas calcium was found highest in group LN-LP, i.e., animals that were fed LN during prepartum and LP during postpartum.

Urine pH and Body Weight in the Postpartum Period

The results of the relative effect of varying levels of DCAD on urine pH and body weight recorded on +10 and + 25 days are given in Table **10**.

Table 8: The Nutrient Intake and Digestibility in Buffaloes Fed Varying Levels of Cations and Anions in Diets (LN, HN in Prepartum and LP, HP in Postpartum)

Parameter	LN-LP	LN-HP	HN-LP	HN-HP	SEM	P-value
Nutrient intake, kg/d						
DM	13.16^{b}	11.86	11.49^{a}	13.92^{b}	0.160	0.01
OM	12.04^{b}	10.44^{a}	10.09^{a}	12.68^{b}	0.151	0.01
CP	1.90	1.92	1.83	1.90	0.248	0.15
NDF	7.80^{b}	6.85^{a}	6.50^{a}	8.40^{c}	0.111	0.02
ADF	3.64^{b}	5.89^{a}	5.66^{a}	3.96^{b}	0.124	0.03
EE	0.36	0.40	0.41	0.56	0.050	0.40
ME*	0.90^{a}	1.05^{b}	1.09^{b}	0.86^{a}	0.110	0.02
Digestibility of Nutrients, %						
DM	63.68^{ab}	65.21^{b}	60.43^{a}	61.40^{ab}	0.559	0.03
OM	67.20	67.25	62.02	64.04	0.706	0.05
CP	73.32^{a}	78.90^{b}	78.00^{b}	70.44^{a}	0.693	0.02
NDF	58.96	59.38	53.37	55.96	0.914	0.15
ADF	41.00^{a}	57.45^{b}	58.33^{b}	32.52^{a}	2.01	0.01
EE	77.48^{ab}	79.27^{ab}	81.03^{b}	74.76^{a}	0.733	0.02

^{a,b}Means with different superscripts within a row differ significantly (P<0.05).
*ME unit= MJ/kg DMI.

Table 9: The Blood Parameters of Buffaloes Fed Varying Levels of Cations and Anions in Diets (LN, HN in Prepartum and LP, HP in Postpartum)

Parameters	LN-LP	LN-HP	HN-LP	HN-HP	SEM	P-value
Glucose (mg/dl)	64.01	65.18	66.44	71.80	1.325	0.16
Cholesterol (mg/dl)	69.98^{a}	71.06^{a}	84.83^{ab}	98.17^{b}	3.889	0.03
Albumin (g/dl)	2.62	2.04	2.75	3.10	0.188	0.25
Total protein (g/dl)	4.96^{ab}	4.013^{a}	4.57^{a}	6.10^{b}	0.233	0.01
Calcium (mg/dl)	15.25	12.17	12.42	10.09	0.720	0.09
SGPT (IU/L)	35.61	32.86	31.97	34.65	1.586	0.85
SGOT (IU/L)	138.50	122.02	133.90	142.89	3.820	0.25
Urea (mg/dl)	43.68^{bc}	30.07^{a}	32.53^{ab}	46.89^{c}	1.970	0.03
Creatinine (mg/dl)	2.24	2.25	2.09	2.42	0.076	0.49
Phosphorus (mg/dl)	5.39	4.13	2.89	7.07	0.604	0.08
NEFA (mEq/L)	0.27	0.25	0.24	0.28	0.025	0.20

^{a,b}Means with different superscripts within a row differ significantly (P<0.05).

Table 10: The Urine pH and Body Weight in Buffaloes Fed Varying Levels of Cations and Anions in Diets (LN, HN in Prepartum and LP, HP in Postpartum)

Parameters	LN-LP	LN-HP	HN-LP	HN-HP	SEM	P-value
Urine pH	8.31	8.14	7.99	8.17	0.062	0.35
Body Weight (kg)	573.50	586.50	558.50	554.50	12.38	0.80

It was observed that urine pH and body weight were less significantly different between groups (p>0.05). However, the lowest urine pH was reported in the HN-LP group.

Milk Production and Composition in the Postpartum Period

Results of daily milk yield and composition as affected by varying levels of DCAD on milk yield and milk composition of a transition buffalo were recorded during the period of 8 weeks. The average effect of varying levels of DCAD on milk yield and milk composition of lactating buffaloes is given in Table **11**, and the change in the trend of weekly milk yield is given in Figure **1**.

There was a significant difference in daily milk yield, fat %, fat yield, protein, protein yield, and various other components of milk (p<0.05) except lactose% and

Table 11: The Milk Yield and Milk Composition of Buffaloes Fed Varying Levels of Cations and Anions in Diets (LN, HN in Prepartum and LP, HP in Postpartum)

Parameters	LN-LP	LN-HP	HN-LP	HN-HP	SEM	P-value
Daily milk yield (kg)	9.00^{a}	10.06^{b}	9.87^{b}	10.37^{b}	0.092	0.01
Fat Yield (kg)	0.71^{a}	0.71^{a}	0.79^{b}	0.78^{b}	0.007	0.01
SNF Yield (kg)	0.92^{a}	1.02^{b}	1.01^{b}	1.04^{b}	0.009	0.01
Protein Yield (kg)	0.34^{a}	0.37^{b}	0.37^{b}	0.38^{b}	0.003	0.01
Lactose Yield (kg)	0.51^{a}	0.57^{b}	0.56^{b}	0.58^{b}	0.005	0.01
Fat %	7.98^{b}	7.22^{a}	8.02^{b}	7.45^{ab}	0.029	0.01
SNF %	10.27	10.18	10.24	10.18	0.013	0.08
Protein %	3.81^{b}	3.76^{a}	3.80^{b}	3.79^{b}	0.004	0.01
Lactose %	5.73	5.75	5.76	5.72	0.005	0.20
Density %	34.54^{b}	34.07^{a}	34.33^{b}	33.93^{a}	0.036	0.01
Milk kg/kg DMI	0.64^{a}	0.68^{ab}	0.71^{b}	0.71^{b}	0.007	0.01

*The figures with different superscripts in a row differ significantly (P<0.05).

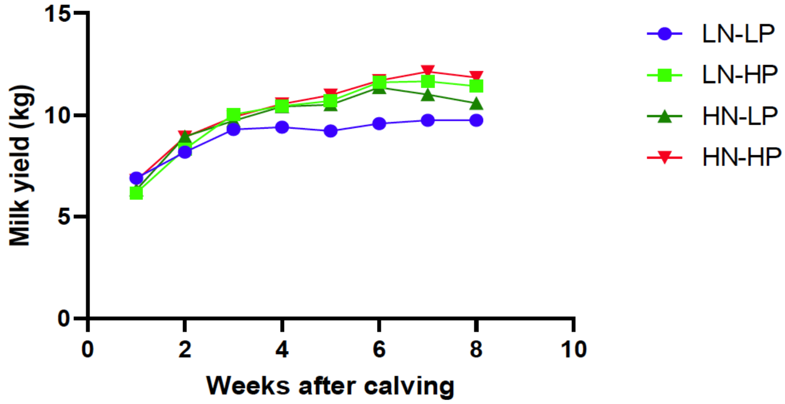
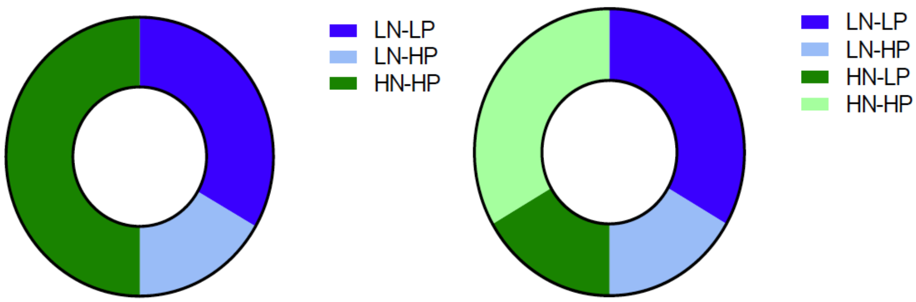


Figure 1: Weekly milk yield as influenced by varying levels of DCAD.



a) Sub-clinical hypocalcemia b) Clinical hypocalcemia

Figure 2: Prevalence of **a**) subclinical and **b**) clinical hypocalcemia in buffaloes fed varying levels of cations and anions in diets (LN, HN in prepartum and LP, HP in postpartum).

SNF%. The highest milk yield, SNF yield, protein yield, and lactose yield were recorded in group HN-HP, but the highest fat%, fat yield, and lactose yield were recorded in the HN-LP group. Better efficiency of milk production was observed with both groups (HN-HP and HN-LP).

Incidence of Milk Fever and Hypocalcaemia in the Postpartum Period

The result of the relative effect of DCAD on blood Ca and P status and preventing DCAD is given in Figure **2.**

From the results, it can be concluded that the HN-LP diet, i.e., HN DCAD during prepartum and LP during postpartum is effective in preventing hypocalcemia as well as sub-clinical hypocalcemia.

Incidence of Ketosis in the Postpartum Period

It was observed that minimum cases of ketosis/fatty liver were reported in two groups, i.e., LN-HP and HN-LP, shown in Figure **3**.

It was observed that minimum cases of ketosis/fatty liver were reported in two groups, i.e., LN-HP and HN-LP.

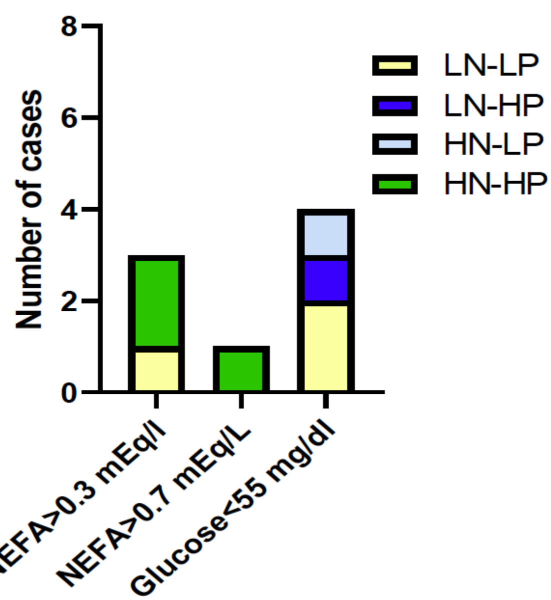


Figure 3: Prevalence of ketosis in buffaloes fed varying levels of cations and anions in diets (LN, HN in prepartum and LP, HP in postpartum).

Table 12: Nutrient Intake and Digestibility of Nutrients in Buffaloes Fed Diets (LP and HP) in Postpartum Period Digestion Trial

Parameter	LP	HP	SEM	P-value
Nutrient intake, kg/d				
DM	12.68[a]	13.33[b]	0.215	0.04
OM	11.48	12.04	0.205	0.06
CP	1.96	2.00	0.001	0.15
NDF	7.42[a]	7.95[b]	0.148	0.01
ADF	4.21	4.51	0.158	0.22
EE	0.37	0.51	0.072	0.16
ME intake (MJ/kg DMI)	0.96	0.92	0.017	0.13
Digestibility of Nutrients, %				
DM	62.75	62.48	0.795	0.81
OM	65.72	64.95	0.958	0.59
CP	74.66	72.86	0.915	0.19
NDF	57.36	56.93	1.297	0.81
ADF	45.95	39.64	2.752	0.11
EE	78.49	76.04	1.011	0.09

[a,b]Means with different superscripts within a row differ significantly (P<0.05).

Dry Matter, Nutrient Intake, and Digestibility in the Postpartum Period (Irrespective of Prepartum Treatment)

Results of average DMI and nutrient intake recorded during the 5-day digestion trial in postpartum animals given LP and HP DCAD diets without considering them are given separately in Table **12**.

The perusal of data revealed that during the 5-day digestion trial, the dry matter intake (DMI) and NDF intake were higher in the HP group compared to the LP group. The difference in nutrient intake between the two groups was also significant. However, when the data of the whole postpartum period was compared for the two groups, there was no significant difference in the nutrient intake and digestibility.

Blood Parameters in the Postpartum Period (Irrespective of Prepartum Treatment)

The average results of blood analysis done in the LP and HP group collected on +10 and +25 days (after calving) are given in Table **13**.

There was a significant difference in blood calcium, total protein, and phosphorus levels between the two groups ($p<0.05$). The highest blood calcium was

Table 13: The Blood Parameters of Buffaloes Fed Diets (LP and HP) in the Postpartum Period

Parameters	LP	HP	SEM	P-value
Glucose (mg/dl)	65.23	69.69	1.771	0.48
Cholesterol (mg/dl)	77.40	91.73	5.280	0.34
Total Protein (g/dl)	4.76[a]	5.63[b]	0.281	0.21
Albumin (g/dl)	2.68	2.98	0.271	0.43
Calcium (mg/dl	13.83[b]	10.17[a]	0.897	0.04
SGPT (IU/L)	33.79	33.89	2.167	0.22
SGOT (IU/L)	136.23	136.00	5.109	0.83
Urea (mg/dl)	38.11	41.73	2.544	0.72
Creatinine (mg/dl)	2.16	2.40	0.073	0.12
Phosphorus (mg/dl)	4.14[a]	7.48[b]	0.825	0.32
NEFA (mEq/L)	0.26	0.28	0.987	0.89

[a,b]Means with different superscripts within a row differ significantly (P<0.05).

Table 14: The Urine pH and Body Weight of Buffaloes Fed Diets (LP and HP) in the Postpartum Period

Parameters	LP	HP	SEM	P-value
Urine pH				
+10 days	7.97	7.92	0.908	0.83
+25 days	8.33	8.38	0.088	0.83
Average	8.15	8.15	0.089	0.96
Body Weight (kg)				
+10 days	574.5	579.0	23.12	0.56
+25 days	557.5	562.0	21.10	0.23
Change	-17.00	-17.00	-	-

[a,b]Means with different superscripts within a row differ significantly (P<0.05).

reported in the group fed LP TMR, whereas the highest concentration of total protein and phosphorus was found in the group fed HP TMR.

Urine pH and Body Weight in the Postpartum Period (Irrespective of Prepartum Treatment)

The results of feeding positive DCAD on urine pH and body weight are given in Table **14**.

It was found that urine pH increased linearly with an increase in DCAD compared to levels observed with negative DCAD feeding, but the difference was reported to be non-significant between groups (p>0.05).

Milk Production and Composition in the Postpartum Period (Irrespective of Prepartum Treatment)

Results of average milk yield and composition recorded as affected daily by LP and HP DCAD levels are given in Table **15** and Figure **4**.

There was a significant difference in daily milk yield and various components of milk fat between the two groups fed different levels of cations. Daily milk yield, protein yield, SNF yield, and lactose yield are significantly higher in the HP group as compared to the LP group (p<0.05) with little difference in fat yield. The trend of weekly milk yield as influenced by two levels of positive DCADs is given in Figure **5**.

Incidence of Milk Fever and Hypocalcaemia in the Postpartum Period (Irrespective of Prepartum Treatment)

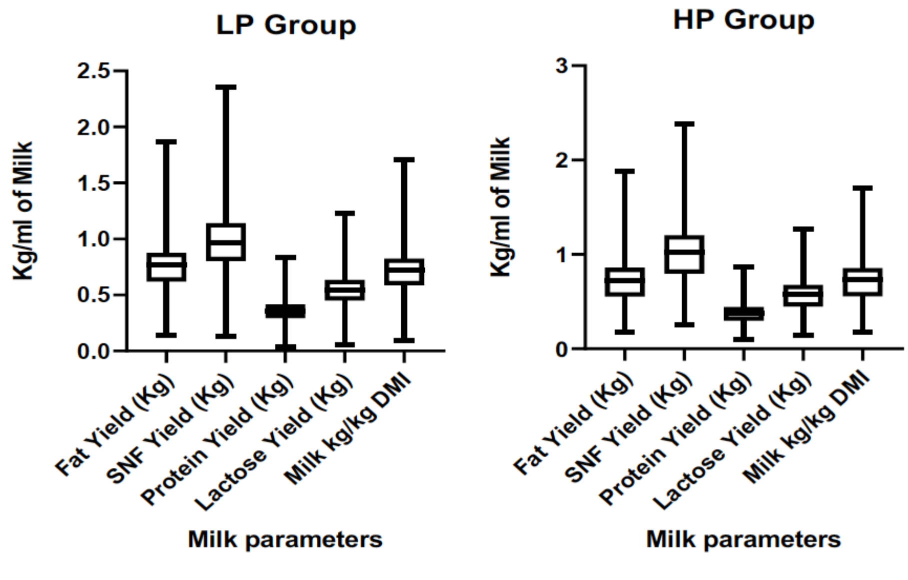
The probable cases of sub-clinical hypocalcemia as per serum calcium and phosphorus levels are given in Table **16**.

They revealed that animals fed HP TMR had no case of hypocalcemia or milk fever compared to LP TMR, but sub-clinical hypocalcemia was reported higher in HP TMR than LP TMR. However, phosphorus levels varied less significantly.

Table 15: The Milk Yield and Composition of Buffaloes Fed Diets (LP and HP) in the Postpartum Period

Parameters	LP	HP	SEM	P-value
Daily Milk Yield (kg)	9.42^a	10.19^b	0.093	0.01
Fat %	8.01^b	7.33^a	0.029	0.01
SNF %	10.28^b	10.19^a	0.013	0.01
Protein %	3.79^b	3.75^a	0.004	0.01
Lactose %	5.74^b	5.71^a	0.005	0.04
Fat Yield (kg)	0.75	0.75	0.007	0.87
SNF Yield (kg)	0.96^a	1.03^b	0.009	0.01
Protein Yield (kg)	0.35^a	0.38^b	0.003	0.01
Lactose Yield (kg)	0.54^a	0.58^b	0.005	0.01
Density	34.46^b	34.02^a	0.036	0.01
Milk kg/kg DMI	0.71	0.73	0.006	0.06
ME intake MJ/kg milk	1.16	1.08	0.005	0.15

[a,b]The figures with different superscripts in a row differ significantly (P<0.05).



a b

Figure 4: Effect of DCAD on milk parameters, **a**) LP DCAD on milk parameters, **b**) HP DCAD on milk parameters.

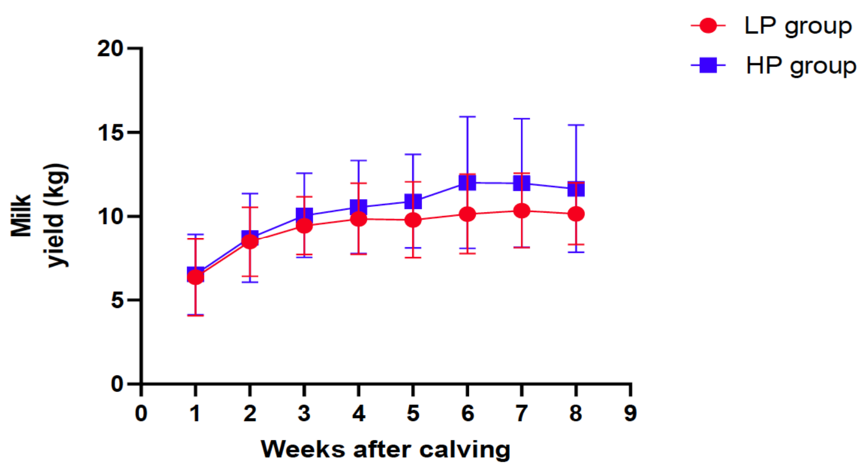


Figure 5: Effect of positive DCAD on weekly milk yield (LP and HP group).

Table 16: Cases of Hypocalcemia/Milk Fever of Buffaloes Fed Diets (LP and HP) in the Postpartum Period

Positive DCAD	Cases with Ca <8.6 mg/dl	Cases with Ca <7.2 mg/dl	Cases with P <2 mg/dl
LP	2	1	4
HP	5	0	3

Table 17: Cases of Ketosis of Buffaloes Fed Diets (LP and HP) in the Postpartum Period

Positive DCAD	NEFA >0.7 mEq/L	Glucose <55 mg/dl
LP	0	3
HP	1	1

Incidence of Ketosis in the Postpartum Period (Irrespective of Prepartum Treatment)

The effect of a postpartum-positive DCAD diet on the incidence of ketosis is given in Table **17**.

It was observed that no case of higher plasma NEFA concentration was reported for the group-fed LP TMR, and only one case was reported for the group-fed HP TMR. Although, few cases were reported with low glucose levels, which may be indicative of low energy reserves.

DISCUSSION

Nutrient Digestibility

The reason for low intake with the HN diet might be due to unpalatability and slight metabolic acidosis caused by anionic salt supplementation. Anionic salts decrease the feed intake when added in higher quantities. Shahzad *et al.* [14], El-Mashed *et al.* [15], and Martins *et al.* [16] reported similar results. In our previous experiment of this study, an *in vitro* digestibility trial [17] was conducted before the animal trial to analyze the effect of adding anions on *in vitro* rumen fermentation patterns and degradability. It was found that HN DCAD had better *in vitro* degradability and other parameters compared to LN and the control group [18].

On the other hand, it was found that the positive DCAD increased DMI as they are more palatable (compared to anions) and helped in overcoming negative energy. We also observed no deleterious effect of cations in our *in vitro* experiment [18]. The significant difference in DMI and nutrient intake between the four groups highlighted the fact that giving different levels of anions and cations can alter the feeding behavior in buffaloes.

Blood Parameters

The level of phosphorus is important in preventing and diagnosing milk fever in buffaloes, but calcium is important in cattle. In the present study, buffaloes that were fed high negative salt in prepartum had better plasma phosphorus levels than the group that was fed low anionic levels. It was also found that all the parameters except calcium and NEFA were high in the group fed HN TMR, unlike cattle in which serum parameters decreased with an increase in anion concentration. The levels of SGPT and SGOT are elevated in buffaloes with parturient paresis, which was observed for the group fed higher cation levels during postpartum. Shahzad *et al.* [19], Leno *et al.* [20], and Melendez *et al.* [21] observed similar results. In the current study, a high level of phosphorus was contributed by the type of salt used in the postpartum study, i.e., Disodium hydrogen phosphate.

Urine pH and Body Weight

The anions are responsible for achieving optimum urine pH in prepartum buffaloes, associated with calcium mobilization caused by mild metabolic acidosis [14, 22]. The kidney can efficiently eliminate excess anions from the blood; thus, the addition of anionic salts induces a sharp reduction in urinary pH [23, 24]. After calving, the urine pH increased linearly when the level of disodium hydrogen phosphate was increased. Hu *et al.* [25], Shahzad *et al.* [2], and Apper Bossard *et al.* (2010) [26] also observed similar results.

Milk Yield and Composition

The group that was fed higher levels of cations and anions had higher milk and milk component yield, which was reported in previous studies [20, 27, 28]. However, some studies showed no effect of varying levels of DCAD on milk yield [22, 23, 25]. If only two levels of cations were compared, results of daily milk yield, protein yield, SNF yield, and lactose yield were higher in the HP group as compared to the LP group. Shahzad *et al.* [14] and Iwaniuk *et al.* [29] reported similar results. Other levels of cations and anions can be explored to determine their effect on milk yield and its composition.

Hypocalcaemia and Milk Fever

Calcium falls below 7.5 mg/dl, and phosphorus decreases to less than 2 mg/dl in hypocalcemia [30]. In our study group, fed HN DCAD levels in the prepartum period were effective in preventing hypocalcemia or milk fever compared to TMR with LN DCAD levels. A similar result was reported by Seifi *et al.* [22], Shahzad *et al.* [14], and Martinez *et al.* [30]. If early postpartum blood serum Ca is less than 8.6 mg/dl, it is considered subclinical hypocalcemia and is recognized as unacceptable periparturient health disorder [4]. The reason for less calcium level with a high positive DCAD diet can be the state of pseudo-hypo-parathyroidism caused by metabolic alkalosis in dairy cows. In our study, the HN-HP group had maximum cases of subclinical hypocalcemia, and no cases were reported for the HN-LP group.

Ketosis and Fatty Liver

Ketosis and fatty liver are characterized by increased plasma levels of NEFA, ketone body concentration (>35 mg/dL), and low plasma glucose (<55 mg/dl) [21]. Normal NEFA levels in dairy bovine are <0.3 mEq/L during 3 weeks to 3 days before calving and <0.7 mEq/L for fresh cows. The feeding of cations and anions is directly associated with the palatability of the feed; as anions decrease palatability, and cations increase it, thus increasing the feed intake as well. However, the relation between altering DCAD in the diet and its effect on blood NEFA levels and glucose levels is still unclear. More research is needed in this area. In previous studies, a decrease in the incidence of ketosis has been reported with the use of a negative DCAD (anionic) diet in the prepartum period [21, 28].

CONCLUSIONS

The varying levels of DCAD had many positive effects on the health and performance of transition buffaloes. The results of the present study indicated that in the first phase (prepartum) of the study, -100 mEq/kg of DM (HN) DCAD level was better for the prepartum stage (90 g/head/day of ammonium chloride) and in the second phase (postpartum) +200 mEq/kg of DM (LP) DCAD level is better in postpartum stage (12 g/head/day of disodium hydrogen phosphate) for buffaloes in many aspects. However, more research is required with respect to the dose of cations and anions given in the diet. The rumen microbial profile can also be explored for a better understanding of the interaction between rumen fauna and DCAD.

FUNDING/ACKNOWLEDGMENT

We are extremely thankful to ICAR for providing funding and support for our research under AICRP-approved EFC on Nutrition and Reproduction under the topic "Improvement of feed resources and nutrient utilization in raising animal production" for the period 2017-20.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest, and the manuscript does not contain clinical studies or patient data.

REFERENCES

[1] Youssef MA, El-Khodery SA, El-deeb WM, El-Amaiem WE. Ketosis in buffalo (Bubalus bubalis): clinical findings and the associated oxidative stress level. Trop Anim Health Pro 2010; 42: 1771-7. https://doi.org/10.1007/s11250-010-9636-9

[2] Shahzad MA, Sarwar M. Influence of varying dietary cation anion difference on serum minerals, mineral balance and hypocalcemia in Nili Ravi buffaloes. Livest Sci 2008a; 113(1): 52-61. https://doi.org/10.1016/j.livsci.2007.02.013

[3] Charbonneau E, Pellerin D, Oetzel GR. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. J Dairy Sci 2006; 89(2): 537-48. https://doi.org/10.3168/jds.S0022-0302(06)72116-6

[4] Shire JA, Beede DK. DCAD revisited: Prepartum use to optimize health and lactational performance. Proceedings of the Southwest Dairy Nutrition and Management Conference 2013; 1-11.

[5] Goff JP, Liesegang A, Horst RL. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. J Dairy Sci 2014; 97(3): 1520-8. https://doi.org/10.3168/jds.2013-7467

[6] Razzaghi A, Aliarabi H, Tabatabaei MM, Saki AA, Valizadeh R, Zamani P. Effect of dietary cation-anion difference during prepartum and postpartum periods on performance, blood and urine minerals status of holstein dairy cow. Asian-Australas J Anim Sci 2012; 25(4): 486. https://doi.org/10.5713/ajas.2011.11325

[7] ICAR. Handbook of Animal Husbandry (4th ed.). Department of Agricultural Research and Education, Government of India, New Delhi 2013; p. 43. ISBN: 9788171641369, 8171641369.

[8] AOAC. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists; 2007. Gaithersburg.

[9] Robertson JB, Van Soest PJ. The detergent system of analysis and its applications to human foods. The Analysis of Dietary Fiber in Food 1981; 123: 158.

[10] SPSS. Statistical Packages for Social Sciences Version 21.0. SPSS Inc. Chicago, IL, USA 2016.

[11] Ridgman WJ. Statistical Methods, 8th edn, Snedecor GW, Cochran WG. Statistical Methods. 8th Edn IOWA State University Press. Ames, Iowa, USA. 1994. xx+ 503 pp. Ames: Iowa State University Press (1989). $44.95 (hard covers). ISBN 0 8138 1561 6. J Agric Sci 1990; 115(1): 153. https://doi.org/10.1017/S0021859600074104

[12] Anitha A, Rao KS, Suresh J, Moorthy PS, Reddy YK. A body condition score (BCS) system in Murrah buffaloes. Buffalo Bull 2011; 30(1): 79-96.

[13] Kour H, Grewal TS, Lamba JS, Kaur J. Effect of varying levels of dietary cation-anion difference (DCAD) on body condition score of transition buffaloes. Haryana Vet 2022; 61(SI-2): 141-143.

[14] Shahzad MA, Sharif M, Nisa M, Sarwar M, Khalid MF, Saddiqi HA. Changing certain dietary cationic and anionic minerals: Impact on blood chemistry, milk fever and udder edema in buffaloes during winter. Afr J Biotechnol 2011; 10(62): 13651-63. https://doi.org/10.5897/AJB10.1832

[15] El-Mashed SM, Metwally HM, Salama AM, Gado HM. Effect of dietary cation–anion differences (DCAD) on rumen fermentation, digestibility and milk production in Friesian dairy cows. Egyptian Journal of Nutrition and Feeds 2015; 18(2 Special): 105-15. https://doi.org/10.21608/ejnf.2015.104429

[16] Martins CM, Arcari MA, Welter KC, Gonçalves JL, Santos MV. Effect of dietary cation–anion difference on ruminal metabolism, total apparent digestibility, blood and renal acid–base regulation in lactating dairy cows. Animal 2016; 10(1): 64-74. https://doi.org/10.1017/S1751731115001548

[17] Menke KH, Steingass H. Estimation of energetic feed value obtained by chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev 1988; 28: 07-55.

[18] Kour H, Grewal TS, Lamba JS, Kaur J, Nayyar S. Effect of Varying Dietary Cation-Anion Difference (DCAD) on *in vitro* Nutrient Digestibility. J Anim Res 2021; 11(04): 01-04. https://doi.org/10.30954/2277-940X.04.2021.26

[19] Shahzad MA, Sarwar M. Influence of altering dietary cation anion difference on milk yield and its composition by early lactating Nili Ravi buffaloes in summer. Livest Sci 2008b; 113(2- 3): 133-43. https://doi.org/10.1016/j.livsci.2007.03.002

[20] Leno BM, Ryan CM, Stokol T, Kirk D, Zanzalari KP, Chapman JD, Overton TR. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. J Dairy Sci 2017; 100(6): 4604-22. https://doi.org/10.3168/jds.2016-12221

[21] Melendez P, Bartolomé J, Roeschmann C, Soto B, Arevalo A, Möller J, Coarsey M. The association of prepartum urine pH, plasma total calcium concentration at calving and postpartum diseases in Holstein dairy cattle. Animal 2021; 15(3): 100148. https://doi.org/10.1016/j.animal.2020.100148

[22] Seifi HA, Mohri M, Farzaneh N, Nemati H, Nejhad SV. Effects of anionic salts supplementation on blood pH and mineral status, energy metabolism, reproduction and production in transition dairy cows. Res Vet Sci 2010; 89(1): 72-7. https://doi.org/10.1016/j.rvsc.2010.01.013

[23] Babir M, Atif FA, Rehman AU. Effect of pre-partum dietary cation-anion difference on the performance of transition Sahiwal cattle. JAPS: Journal of Animal & Plant Sciences 2017; 27(6).

[24] Zimpel R, Poindexter MB, Vieira-Neto A, Block E, Nelson CD, Staples CR, Thatcher WW, Santos JE. Effect of dietary cation-anion difference on acid-base status and dry matter intake in dry pregnant cows. J Dairy Sci 2018; 101(9): 8461-75. https://doi.org/10.3168/jds.2018-14748

[25] Hu W, Kung Jr L, Murphy MR. Relationships between dry matter intake and acid–base status of lactating dairy cows as manipulated by dietary cation–anion difference. Anim Feed Sci Technol 2007; 136(3-4): 216-25. https://doi.org/10.1016/j.anifeedsci.2006.09.013

[26] Apper-Bossard E, Faverdin P, Meschy F, Peyraud JL. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regulation in dairy cows receiving 2 contrasting levels of concentrate in diets. J Dairy Sci 2010; 93(9): 4196-210.

[27] Rodrigues RO, Cooke RF, Rodrigues SM, Bastos LN, de Camargo VF, Gomes KS, Vasconcelos JL. Reducing prepartum urine pH by supplementing anionic feed ingredients: Effects on physiological and productive responses of Holstein× Gir cows. J Dairy Sci 2018; 101(10): 9296-308. https://doi.org/10.3168/jds.2018-14660

[28] Lean IJ, Santos JE, Block E, Golder HM. Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows: A meta-analysis. J Dairy Sci 2019; 102(3): 2103-33. https://doi.org/10.3168/jds.2018-14769

[29] Iwaniuk ME, Weidman AE, Erdman RA. The effect of dietary cation-anion difference concentration and cation source on milk production and feed efficiency in lactating dairy cows. J Dairy Sci 2015; 98(3): 1950-60. https://doi.org/10.3168/jds.2014-8704

[30] Martinez N, Risco CA, Lima FS, Bisinotto RS, Greco LF, Ribeiro ES, Maunsell F, Galvão K, Santos JE. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J Dairy Sci 2012; 95(12): 7158-72. https://doi.org/10.3168/jds.2012-5812

Received on 04-11-2023 Accepted on 19-12-2023 Published on 18-01-2024

https://doi.org/10.6000/1927-520X.2024.13.02