Research Article

DOI: 10.48165/ijar.2021.42.2.6

The Indian Journal of Animal Reproduction

The official journal of the Indian Society for Study of Animal Reproduction

Year 2021, Volume-42, Issue-2 (Dec)

ACS Publisher www.acspublisher.com

ISSN 0970-2997 *(Print)*

Plasma Biochemical Profile of Murrah Buffaloes (*Bubalus bubalis*) Fed Peripartum Positive Dietary Cation Anion Difference Diet

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ABSTRACT

A comparative study was done to evaluate peripartum Dietary Cation-Anion Difference (DCAD) feeding in buffaloes. Murrah buffaloes (n=25) were randomly divided into three groups: Group 1 (n=10), Group 2 (n=10) and Group 3 (n=5, control). Buffaloes in Group 1 and Group 2 were fed DCAD levels of -100 mEq 100 g^{-1} of dry matter (DM) starting 30 days prepartum. During the postpartum period and upto 30 days, Group 1 was fed on DCAD levels of +300 mEq 100 g^{-1} and Group 2 was fed DCAD levels of +400 mEq 100 g^{-1}. Group 3 (control) buffaloes were fed standard diet without any cationic or anionic salts. Blood sampling was carried out on day -7, 0, 20, 26, 32, 38, 45, 52 and 54 of calving. PUN after 26 days postpartum was significantly low ($P< 0.05$) in buffaloes fed on two different DCAD levels than the control group. In Group 2, CK activity was significantly higher ($P< 0.05$) as compared to group 1 and group 3 across 38 and 54days postpartum. In Group 3, creatinine was significantly higher ($P< 0.05$) than group 1 across day 20, 52 and 54 postpartum. Glucose levels in Group 1, 2 and 3 buffaloes ranged from 67.55±2.84 to 99.44±8.00 $mgdl^{-1}$, 67.00±2.97 to 110.87±8.49 $mgdl^{-1}$ and 68.00±5.34 to 93.4±10.74 mg dl^{-1}, respectively. Glucose levels were significantly higher ($P< 0.05$) in Group 3 than group 1 and group 2 across 38, 52 and 54 day postpartum. Total protein concentration in Group 1, 2 and 3 buffaloes ranged from 6.65±0.36 to 9.61±0.33 gdl^{-1}, 6.46±0.32 to 10.06±0.56 gdl^{-1} and 9.02±0.41 to 10.06±0.46 gdl^{-1}, respectively. Total protein levels were significantly high ($P< 0.05$) in Group 1 and 3 as compared to group 2 across 7days prepartum. On day 54 postpartum, total protein concentration were significantly high ($P< 0.05$) in Group 3 as compared to Group 1 and 2. It was concluded that creatinie kinase activity increased and plasma urea nitrogen decreased with the feeding of high cationic diets. Total protein levels were lower in low cationic fed buffaloes.

Key words: Dietary Cation Anion Difference, Plasma Urea Nitrogen, Creatine Kinase, Creatinine, Glucose, Total Protein, Buffaloes.

How to cite: Arora, A., Singh, P., Dhindsa, S.S., Grewal, R., Singh, N., Honparkhe, M., & Malik, V.S. (2021) Plasma Biochemical Profile Of Murrah Buffaloes (Bubalus bubalis) Fed Peripartum Positive Dietary Cation Anion Difference Diet. *The Indian Journal of Animal Reproduction, 42*(2), 31–38. https://doi.org/10.48165/ijar.2021.42.2.6

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Received 22-12-2022; Accepted 26-01-2023

INTRODUCTION

Murrah buffalo is a high milk yielder as compared to other breeds of buffalo. Murrah buffalo average milk yield is around 2500-3600 litre per lactation, is resistant to most of the diseases. Murrah buffalo adapts to most of the Indian climatic conditions. Buffaloes occasionally show silent heat. Dairy nutrition plays a vital role in many body functions and milk yield. Nutrients in the diet, are required for optimum production, health and reproduction. Carbohydrates, fats, proteins and vitamins are generally provided in the diet but usually minerals are ignored. The importance of minerals has been widely reported as these play a vital role in various metabolic functions.

Transition period begins is earmarked from the last 3 weeks of gestation to 3 weeks of early lactation. It is the period of hormonal and metabolic alternations in the dairy animals. Transition is a critical phase where management needs to be more focused. Most of the fetal growth and proliferation of mammary cells occur during the last few weeks of gestation which leads to an increase in nutrient demands. Poor management during this phase affects the health, production and reproduction of dairy animals.

Dietary Cation-Anion Difference (DCAD) level of feed is one of the emerging nutritional interventions to prevent metabolic disorders during the transition period. DCAD is the difference of sum of cations and anions in the diet. DCAD diet strategy alters the acid-base balance and mineral homeostasis. Calcium mineral homeostasis is mainly associated with PTH sensitivity of bones and kidneys and this sensitivity is dictated by acid-base balance.Supplementation of anionic salts reduces the prepartum dry matter intake and energy status (Moore *et al*., 2000). Dietary manipulation of cations and anions levels is an appropriate intervention to alter the acid-base balance. Altering the acid-base balance, calcium homeostatic mechanism gets improved and prevents hypocalcemia. DCAD levels are expressed in mEq Kg^{-1} of Dry matter (DM) or mEq 100 g^{-1} of DM. DCAD is calculated by the equation;

$$\textit{DCAD (mEq 100 } g^{-1} \textit{of DM)} = [(\textit{percent } K^{+} \textit{divided by } 0.039) + (\textit{percent } Na^{+} \textit{divided by } 0.023)] - [(\textit{percent } Cl^{-} \textit{ divided by } 0.0355) + (\textit{percent } S^{-} \textit{ divided by } 0.016)]$$

Above equation incorporates only major ions, other ions also influence the acid base balance. DCAD reduces the incidence of milk fever and various other periparturient diseases and improves the future performance of dairy animals. Low DCAD diet induces metabolic acidosis and cows fed low DCAD diets had higher ionized calcium during prepartum and at the time of calving (Lopera *et al*., 2018).

DCAD diet has its influence on metabolism. High cationic levels in diet are associated with high protein degradation. Plasma urea nitrogen (PUN) indicates protein degradation (Wildman *et al*., 2007). Creatinine levels indicate kidney function and protein degradation. Higher protein degradation is associated with increased PUN (Wildman *et al*., 2007) and creatinine levels (Diehl *et al*., 2018). DCAD diet had no influence on creatinine concentration in goats (Farooq *et al*., 2014) Diets rich in potassium had no influence on serum glucose (Harrison *et al*., 2012) level in early lactating cows. DCAD diet had no influence on the glucose levels in healthy dairy cows (Lean *et al*., 2019). High anionic diets increase the glucose levels during prepartum period in dairy cows (Martinez *et al*., 2018). Sufficient literature was not available to correlate the variation of biochemical parameters with level of DCAD feeding in dairy buffaloes. This specified a need to study the biochemical parameter alterations with the different levels of DCAD feeding. The objective of this study was to evaluate the effect of DCAD feeding on metabolic and biochemical parameters in postpartum buffaloes.

MATERIALS AND METHODS

Selection and Grouping of animals

Healthy (n=25) dairy Murrah buffaloes (BCS ≥ 3) maintained under optimum housing, feeding and management health practices were incorporated in the study. All buffaloes were randomly allocated to three groups;

Group 1: n=10; fed DCAD diet levels of -100 mEq 100 g^{-1}ofDM starting day 30 prepartum followed by +300 mEq 100 g^{-1} of DM upto 30 days postpartum daily.

Group 2: n=10, fed DCAD diet levels of -100 mEq 100 $g^{-1}$of DM starting day 30 prepartum followed by +400 mEq 100 g^{-1} of DM upto 30 days postpartum daily.

Group 3: (Control)n=5, fed DCAD diet levels of 187 mEq 100 g^{-1} of DMstarting day 30 prepartum followed by 203.22 mEq 100 g^{-1} of DM upto 30 days postpartum daily.

DCAD diet preparation

a) **Prepartum period:**

Composition of Close up ration fed to buffaloes at dairy farm is given in Table 1.

- DCAD from concentrate = (Na^+%/0.023 + K^+%/0.039) - (Cl^-%/0.0355 +S^-%/0.016)
  = (5.75/0.023 +11.15/0.039) − (6.80/0.0355 + 2.83/0.016)=167 mEq 100^{-1} g of DM

Fodder sample was sent to Punjab Biotechnology Incubator Agri-Food Testing Laboratory, Mohali (Punjab) for element analysis.

- DCAD from green fodder = 20 mEq 100^{-1} g of DM
- Total DCAD = DCAD from concentrate + DCAD from green fodder
  = 167 + 20 mEq 100^{-1} g of DM
  = 187 mEq 100^{-1} g of DM

To attain DCAD level of -100 mEq 100^{-1} g of DM, 153.8 g of ammonium chloride (NH_4Cl) was added in the total mixed ration (TMR).

b) **Postpartum period:**

Composition of milking ration fed to dairy buffaloes is given in Table 2.

Table 1: Composition of Close up Ration (of DM) fed to the advanced pregnant buffaloes

Sr.	Ingredient	Quantity (Kg)	Sodium (%)	Potassium (%)	Chloride (%)	Sulphur (%)
1	Salt	10	4	-	6	-
2	LSP	0	-	-	-	-
3	Mustard cake	170	0.119	2.39	0.063	1.24
4	Cotton meal	50	0.035	0.82	0.035	0.2
5	Soda	5	1.36	-	-	-
6	Barley	0	0	-	-	-
7	Deoiled rice bran	130	0.039	2.04	0.117	0.247
8	Rice bran	100	0.03	1.57	0.09	0.19
9	Wheat bran	101	0.040	1.33	0.16	0.21
10	Bajra	50	0.0005	0.235	0.03	0.055
11	Guar korma	30	0.051	0.273	0.042	-
12	Maize	240	0.048	1.008	0.192	0.24
13	Soya	70	0.028	1.484	0.07	0.238
14	Full fat soya	0	-	-	-	-
15	Mineral mixture	17	-	-	-	-
16	DCP	0	-	-	-	-
17	DDG	0	-	-	-	-
18	Maize oil cake	0	-	-	-	-
19	AD_3	0.05	-	-	-	-
20	Yea sac	1.5	-	-	-	-
21	Bioplex	1	-	-	-	-
22	Nutrical	15	-	-	-	-
23	Methionine	0.3	-	-	-	-
24	Lysine	1	-	-	-	-
25	Toxin binder	1	-	-	-	-
26	Biotin	0.2	-	-	-	-
27	Niacin	1	-	-	-	-
28	Choline	10	-	-	-	-
29	Vitamin E	1	-	-	-	-
Total		1000	5.75	11.15	6.80	2.831

Table 2: Milking Ration composition (% of DM) fed to early lactating buffaloes

Sr.	Ingredient	Quantity (Kg)	Sodium (%)	Potassium (%)	Chloride (%)	Sulphur (%)
1	Salt	15	6	-	9	-
2	LSP	7	-	-	-	-
3	Mustard cake	130	0.091	1.83	0.052	0.949
4	Cotton meal	50	0.035	0.82	0.035	0.2
5	Soda	8	2.18	-	-	-
6	Barley	80	0.016	0.44	0.104	0.096
7	Cotton seed	50	-	-	-	-
8	Rice bran	50	0.015	0.78	0.045	0.095
9	Wheat bran	50	0.02	0.66	0.08	0.105
10	Bajra	50	-	-	-	-
11	Guar korma	50	0.085	0.455	0.07	-
12	Maize	230	0.046	0.966	0.184	0.23
13	Soya	105	0.042	2.22	0.105	0.357
14	Full fat soya	30	0.003	0.597	0.012	0.093
15	Mineral mixture	10	-	-	-	-
16	DCP	7	-	-	-	-
17	DDG	50	-	-	-	-
18	Yea sac	1.5	-	-	-	-
19	Bypass fat	15	-	-	-	-
20	Methionine	0.7	-	-	-	-
21	Lysine	1.5	-	-	-	-
22	Toxin binder	1.5	-	-	-	-
23	Biotin	0.2	-	-	-	-
24	Niacin	1	-	-	-	-
25	Choline	5	-	-	-	-
26	Vitamin E	1	-	-	-	-
27	Urea	8	-	-	-	-
Total		1000	2.53	8.79	0.687	2.125

Table 3: Quantity of anionic and cationic salt added in the TMR of buffaloes

Group	Prepartum		Postpartum	
	DCAD level (mEq 100 g-1 DM)	NH4Cl in TMR(g)	DCAD level (mEq 100 g-1 DM)	Na2HPO4 in TMR(g)
1	-100	153.8	+300	68.93
2	-100	153.8	+400	140.15
3	187	0	203.22	0

- DCAD from concentrate = (Na^+/0.023 + K^+/0.039) - (Cl^-/0.0355 +S^-/0.016)
  = (2.53/0.023 +8.79/0.039) – (0.687/0.0355 + 2.125/0.016)
  = 183.22 mEq 100^{-1} g of DM
- DCAD from green fodder
  = 20 mEq 100^{-1} g of DM
- Total DCAD
  = DCAD from concentrate + DCAD from green fodder
  = 183.22 + 20 mEq 100^{-1} g of DM
  = 203.22 mEq 100^{-1} g of DM

To attain DCAD level of 300 and 400 mEq 100^{-1} g of DM, 68.93 g and 140.15 g of disodium hydrogen orthophosphate (Na_2HPO_4) was added in the TMR, respectively.

DCAD levels and addition of salts

Amount of anionic and cationic salts added to achieve the desired DCAD levels in the diet are given in Table 3

Blood sampling

Jugular vein blood (10-12 ml) sample was collected in a heparinized vial and plasma was separated and stored at -20 ^{0}C.Biochemical parameters viz.PUN, CK, creatinine, glucose and total protein were analyzed by VITROS DT-11 Chemistry system (Ortho-Clinical Diagnostics, Johnson and Johnson, SA) using standard kits (Vitro-Ortho-Clinical Diagnostics, Mumbai).Briefly, VITROS DT slide method was performed using the VITROS DT slides and the VITROS Chemistry Products DT calibrator kit on VITROS DT 60/DT60 2 Chemistry Systems. VITROS DT slide is multilayered, analytical element coated on a polyester support. A drop of sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Slide is analyzed by computer analysis inbuilt in VITROS DT Chemistry System.

Statistical analysis

Data obtained was subjected to the statistical analysis by using IBM-SPSS version 26 software. The parameters were tested for normality using Sharpiro-Wilk test. The treatment effects and evaluation parameters were tested by repeated measures analysis of variance with the effect of individual buffaloes within the period. Mean ± Standard Error of Mean (SEM) values have been reported for fixed effects. Values for significance were considered at 5 percent (P < 0.05).

RESULTS AND DISCUSSION

Plasma urea nitrogen (PUN) concentration

Plasma Urea Nitrogen (PUN) in Group 1, 2 and 3 buffaloes ranged from 16.5 ± 0.88 to 18.3 ± 0.68 mg dl^{-1}, 16.0 ± 0.40 to 17.8 ± 0.47 mg dl^{-1} and 15.4 ± 0.79 to 22.0 ± 0.86 mg dl^{-1}, respectively (Table 4). PUN was significantly higher (*P*< 0.05) in Group 3than Group 1 and 2across day 26, 38, 52 and 54 postpartum. On 32 and 45 day postpartum, PUN was significantly low (*P*< 0.05) in Group 2 as compared to Group 1 and 3. PUN concentration was significantly high (*P*< 0.05) in Group 3 (19.4 ± 0.34mg dl^{-1}) as compared to Group 1 (17.5 ± 0.25 mg dl^{-1}) and 2 (16.6 ± 0.27 mg dl^{-1}). In another study, the high levels of DCAD in diet reduced the protein degradation resulting in low PUN levels (Wildman *et al.*, 2007).

Table 4: Plasma Urea Nitrogen (PUN) levels (mg dl^{-1}, Mean ± SEM) in buffaloes

Day of calving	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=5)	*P* value (*P*< 0.05)
-7	16.5 ± 0.88^{a}	16.0 ± 0.93	17.4 ± 1.18^{b}	0.57
0	16.8 ± 0.59^{b}	16.7 ± 0.62	15.4 ± 0.79^{b}	0.14
20	18.1 ± 0.44^{b}	17.8 ± 0.47	18.0 ± 0.59^{b}	0.88
26	18.0 ± 0.50^{2a}	17.1 ± 0.53^{2}	20.0 ± 0.67^{1a}	0.003
32	18.3 ± 0.38^{1a}	16.0 ± 0.40^{2}	19.6 ± 0.51^{1a}	0
38	17.7 ± 0.37^{2b}	16.7 ± 0.39^{2}	20.6 ± 0.50^{1a}	0
45	18.3 ± 0.68^{1a}	16.3 ± 0.73^{2}	20.6 ± 0.92^{1a}	0.002
52	17.4 ± 0.64^{2a}	16.2 ± 0.68^{2}	22.0 ± 0.86^{1a}	0
54	16.6 ± 0.48^{2b}	17.0 ± 0.51^{2}	21.0 ± 0.64^{1a}	0

Creatine Kinase (CK) activity

Creatine Kinase (CK) activity in Group 1, 2 and 3 buffaloes ranged from 175.22 ± 15.4 to 241.44 ± 21.9 U L^{-1}, 192 ± 23.26 to 575.75 ± 162.05 U L^{-1} and 187.6 ± 92.75 to 319 ± 50.06 U L^{-1} respectively. CK activity was significantly higher (*P*< 0.05) in Group 2 compared to Group 1 and 3 across days 38 and 54 postpartum. CK activity of Group 2 was significantly higher (*P*< 0.05) than Group 1 over the period of sampling. The reduction of postpartum CK activity was observed after feeding the high anionic diets during prepartum period (Diehl *et al.*, 2018). CK activity obtained for three groups are given in Table 5.

Table 5: Plasma Creatine Kinase (CK) activity (U L^{-1}, Mean ± SEM)

Day of calving	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=5)	P value (P< 0.05)
-7	204 ± 69.13	303.37 ± 73.3	187.6 ± 92.75^{b}	0.88
0	215 ± 152.7	575.75 ± 162.05	234.6 ± 204.98^{b}	0.94
20	181.77 ± 16.9	245 ± 17.9	228.8 ± 22.72^{b}	0.11
26	222.22 ± 37.23	277.5 ± 39.49	248.4 ± 49.95^{b}	0.67
32	241.44 ± 21.9	192 ± 23.26	239 ± 29.43^{b}	0.94
38	175.22 ± 15.4^{2}	272 ± 16.38^{1}	211.4 ± 20.72^{2b}	0.033
45	195.44 ± 15.9	252.5 ± 16.95	248.8 ± 21.44^{b}	0.061
52	192 ± 37.31	344.87 ± 39.58	319 ± 50.06^{a}	0.056
54	236.7 ± 17.38^{2}	361.75 ± 18.43^{1}	210.2 ± 23.31^{2b}	0.00

^{123} Mean values within rows having different superscripts differ significantly (*P*< 0.05)
^{abc} Mean values within columns having different superscripts differ significantly (*P*< 0.05)

Creatinine concentration

Plasma creatinine levels in Group 1, 2 and 3 buffaloes ranged from 1.56 ± 0.12 to 2.21 ± 0.14 mg dl^{-1}, 1.70 ± 0.08 to 2.58 ± 0.23 mg dl^{-1} and 1.84 ± 0.17 to 2.22 ± 0.29 mg dl^{-1}, respectively. Creatinine was significantly higher (*P*< 0.05) inGroup 3as compared to Group 1 and 2 across day26 postpartum. Creatinine was significantly higher (*P*< 0.05) in Group 3 as compared to Group 1across days20, 52 and 54 postpartum. Overall creatinine concentrationdid not differ significantly (*P*> 0.05) between the groups. In a study, the high cationic diets fed during prepartum period increased the creatinine concentration within 14 days in milk (Diehl *et al.*, 2018). High anionic diet feeding resulting in high protein degradation. Although creatinine concentration is generally associated with renal function but diet may slightly alter its level. Creatinine concentration is not altered by different levels of DCAD feeding (Farooq *et al.*, 2014) in goats. Creatinine levels obtained for three groups are given in Table 6.

Table 6: Plasma creatinine levels (mg dl^{-1}, Mean ± SEM) in buffaloes

Day of calving	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=5)	*P* value (*P*< 0.05)
-7	2.21 ± 0.14^{a}	1.93 ± 0.15^{a}	2.040 ± 0.19	0.49
0	1.66 ± 0.21^{b}	2.58 ± 0.23^{b}	2.220 ± 0.29	0.14
20	1.56 ± 0.12^{2b}	1.76 ± 0.13^{12a}	2.040 ± 0.16^{1}	0.037
26	1.64 ± 0.086^{2b}	1.71 ± 0.09^{2a}	2.120 ± 0.11^{1}	0.004
32	1.75 ± 0.16^{b}	1.72 ± 0.17^{a}	1.980 ± 0.21	0.42
38	1.62 ± 0.09^{a}	1.70 ± 0.1^{a}	1.900 ± 0.13	0.10
45	1.80 ± 0.12^{a}	1.70 ± 0.13^{a}	1.840 ± 0.17	0.853
52	1.58 ± 0.07^{2a}	1.70 ± 0.08^{12a}	1.962 ± 0.1^{1}	0.008
54	1.62 ± 0.08^{2b}	1.72 ± 0.08^{12a}	1.980 ± 0.1^{1}	0.017

^{123}Mean values within rows having different superscripts differ significantly (*P*< 0.05)
^{abc}Mean values within columns having different superscripts differ significantly (*P*< 0.05)

Glucose concentration

Plasma glucose in Group 1, 2 and 3 buffaloes ranged from 67.55 ± 2.84 to 99.44 ± 8.00 mg dl^{-1}, 67.00 ± 2.97 to 110.87 ± 8.49 mg dl^{-1} and 68.00 ± 5.34 to 93.4 ± 10.74 mg dl^{-1}, respectively. Buffaloes in Group 3 had significantly high (*P*< 0.05) glucose levels as compared to Group 1 and 2across38, 52 and 54 days postpartum.The fact attributes to low insulin secretion in response to metabolic acidosis induced by low cationic diets. Overall glucose concentration did not differ significantly (*P*> 0.05) between the groups. Glucose concentration differed significantly (*P*< 0.05) from day 7 prepartum to the day of calving. At parturition, glucagon and glucocorticoid secretion rises that increased the glucose levels (Janovick*et al.*, 2011). Glucose concentration did not differ with different levels of DCAD feeding (Ramos-Nieves*et al.*, 2009, Mohanrao *et al.*, 2016). Glucose levels obtained for three groups are given in Table 7.

Table 7: Plasma glucose levels (mg dl^{-1}, Mean ± SEM) in buffaloes

Day of calving	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=5)	*P* value (*P*< 0.05)
-7	74.33 ± 2.63^{b}	68.25 ± 2.79^{b}	72.40 ± 3.53^{b}	0.66
0	99.44 ± 8.00^{a}	110.87 ± 8.49^{a}	93.40 ± 10.74^{a}	0.65
20	72.77 ± 2.92^{b}	70.37 ± 3.10^{b}	77.00 ± 3.93^{b}	0.40
26	73.66 ± 2.80^{b}	67.00 ± 2.97^{b}	75.40 ± 3.76^{b}	0.71
32	78.55 ± 4.42^{b}	67.12 ± 4.69^{b}	79.00 ± 5.93^{b}	0.95
38	67.55 ± 2.84^{2b}	68.37 ± 3.01^{2b}	81.40 ± 3.81^{1b}	0.009
45	76.44 ± 3.98^{b}	74.00 ± 4.22^{b}	68.00 ± 5.34^{b}	0.22
52	71.33 ± 1.87^{2b}	70.12 ± 1.99^{2b}	79.60 ± 2.51^{1b}	0.016
54	71.77 ± 2.47^{2b}	71.62 ± 2.62^{2b}	80.60 ± 3.32^{1b}	0.047

[123]Mean values within rows having different superscripts differ significantly (*P*< 0.05)
[abc]Mean values within columns having different superscripts differ significantly (*P*< 0.05)

Total protein concentration

Total protein concentration in Group 1, 2 and 3 buffaloes ranged from 6.65 ± 0.36 to 9.61 ± 0.33 g dl^{-1}, 6.46 ± 0.32 to 10.06 ± 0.56 g dl^{-1} and 9.02 ± 0.41 to 10.06 ± 0.46 g dl^{-1}, respectively. Total protein levels obtained for three groups are given in Table 8.

Table 8: Plasma total protein levels (g dl^{-1}, Mean ± SEM) in buffaloes

Day of calving	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=5)	*P* value (*P*< 0.05)
-7	8.40 ± 0.32^{1a}	6.46 ± 0.32^{2b}	9.02 ± 0.41^{1}	0.00
0	7.81 ± 0.56^{2a}	10.06 ± 0.56^{1a}	9.92 ± 0.71^{1}	0.032
20	6.65 ± 0.36^{2b}	9.22 ± 0.36^{1b}	10.06 ± 0.46^{1}	0.00
26	7.71 ± 0.44^{2a}	9.12 ± 0.44^{1b}	9.36 ± 0.56^{1}	0.033
32	8.18 ± 0.52^{a}	8.77 ± 0.52^{b}	9.74 ± 0.66	0.082
38	8.47 ± 0.37^{a}	9.17 ± 0.37^{b}	9.46 ± 0.47	0.12
45	9.61 ± 0.33^{a}	8.70 ± 0.33^{b}	9.72 ± 0.42	0.84
52	8.72 ± 0.32^{a}	8.71 ± 0.32^{b}	9.74 ± 0.40	0.066
54	8.45 ± 0.31^{2a}	8.83 ± 0.31^{2b}	10.00 ± 0.40^{1}	0.007

(Table continued)

(Table continued)

[123] Mean values within rows having different superscripts differ significantly (*P*< 0.05)
[abc] Mean values within columns having different superscripts differ significantly (*P*< 0.05)

Group 2 total protein was significantly low (*P*< 0.05) as compared to Group 1 and 3acrossday 7prepartum. Group 1 total protein was significantly high (*P*< 0.05) as compared to Group 2 and 3acrossday of calving, day 20 and 26 postpartum. In Group 3buffaloestotal protein was significantly high (*P*< 0.05) as compared to Group 1 and 2 across54 day postpartum. Total protein was significantly low (*P*< 0.05) in Group 1 as compared to Group 3 over the period of sampling. Total protein was significantly lower in high anionic diet fed cows compared to control (Grünberg *et al.,* 2011). Increased duration of negative DCAD feeding is associated with decrease in serum total protein but remains within the normal range.

CONCLUSIONS

Concluding the investigation, it was observed that DCAD diet influenced the various biochemical parameters. Plasma urea nitrogen decreased with the feeding of high cationic diets. Creatine Kinase activity increased in high cationic diet fed buffaloes compared to low cationic diet fed buffaloes. Creatinine and glucose levels were not affected with DCAD feeding. Total protein concentration was higher in low cationic diet.

ACKNOWLEDGMENTS

The work has been funded by the All India Coordinated Research Project (AICRP) on Nutritional and Physiological Interventions for Enhancing Reproductive Performance in Animals, Indian Council of Agricultural Research (ICAR), New Delhi functional in Guru Angad Dev Veterinary and animal Sciences University, Ludhiana, Punjab, India.

CONFLICT OF INTEREST

None.

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