



Effect of positive dietary cation anion difference (DCAD) diet on blood biochemical and immunological parameters in crossbred calves in winter

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Received: 11 August 2013; Accepted: 19 December 2014

ABSTRACT

The present study was conducted for 120 days to examine the influence of varying dietary cation anion difference (DCAD) on certain biochemical and immunological parameters of crossbred calves in winter. Female crossbred Karan Fries (KF) calves (18) were grouped into 3 groups having 6 animals in each group on the basis of average body weight and fed either a basal diet or a +150 / +250mEq/kg dry matter (DM) DCAD diets (W₁, W₂) during winter. The overall dry matter intake (DMI) was significantly more in W₂ group as compared to control. The growth rate per day averaged 410.42, 440.19 and 484.67 g in control, W₁ and W₂ groups, respectively which was significantly more in high DCAD diets. Blood was collected at fortnightly interval. There was no effect of varying DCAD diet on FRAP (ferric reducing antioxidant power) assay, SOD and catalase activity in winter. Immunity parameters and plasma concentration of cortisol were not affected by treatments. However, the T₃ and T₄ concentrations were significantly higher in W₂ group compared to control. It was concluded that positive DCAD was not able to impose any effect on biochemical and immunological parameters but there is significant effect on the DMI and T₃ and T₄ concentrations.

Key words: Climatic stress, Crossbred calves, Dietary cation anion difference, Immunity

Climate stress is a phenomenon that can impart physical and economical losses to livestock production in temperate, subtropical and tropical regions of the world. The environmental changes also evoke predictable responses in the nervous, circulatory, renal and endocrine systems that allow animals to adjust to the altered environment (Collier *et al.* 2005). Cook *et al.* (2002) reported that prolonged exposure to severe climatic stress is responsible for a decline of immune cells reactivity, which may contribute to the higher occurrence of infections. In calves climatic stress leads to less serum IgG (West 2003), white blood cells (WBCs), red blood cells (RBCs) and globulin, which are indicators of body immunity in general, revealing adverse effect of exposure to climatic stress (Marai and Haebe 2010). Thermal stress is of major concern for growing animals because it may lead to poor growth performance, immuno-suppression and high mortality rate (Nesamvuni *et al.* 2012). Interactions between nutrition and stress result in nutrient deficiencies which affect the animal's ability to counter the stress (NRC 2001). So, nutritional balance is an important factor in combating thermal stress and imbalance in it, which otherwise may be deleterious to the productive as well as reproductive performance of animals (Sharif *et al.* 2010). Challenged by these reactions to

climatic stress, dietary requirements of macrominerals may differ from requirements of animals in thermoneutral environments. Dietary minerals are an integral part of all biological functions in the animal body. Recent advances suggested that difference between certain cations (Na⁺, K⁺) and anions (Cl⁻, S⁻) may be of more significance for animal productivity than their individual effects (Tucker *et al.* 1992). Sanchez and Beede (1991) coined the term dietary cation-anion difference (DCAD) which is a way to balance the electrical charge of the cations and anions in the diet. Therefore, the present experiment was designed to establish the DCAD level and to study the effect of positive DCAD diet on blood biochemical and immunity parameters in crossbred calves during winter.

MATERIALS AND METHODS

Location of the study: The study was conducted at the farm of the Institute, situated on an altitude of 250 m above mean sea level, latitude and longitude positions are 29° 42" N and 79° 54" E, respectively.

Selection and distribution of experimental animals: During winter, 18 female crossbred Karan Fries calves (5 to 9 months old) were selected and divided into 3 groups having 6 calves in each group, on body weight and age basis as control, W₁ and W₂.

Feeding and management of animals: The crossbred calves of the 3 different groups were offered the same basal diet, but the animals in W₁ and W₂ groups were offered

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+150 and +250 mEq/ kg DM DCAD diet by addition of cationic salts (NaHCO_3 and K_2CO_3) in the basal diet. Experimental animals were fed as per NRC (2001) standard and requirements of animal were fulfilled by feeding concentrate mixture, wheat straw and maize/ berseem fodder. The concentrate mixture was offered in morning whereas, the chaffed green fodder was offered at 11:00 AM. The dietary treatments were continued for 120 days. Concentrate mixture (CP 19.81% and TDN 70%) contained maize 33%, groundnut cake (oiled) 21%, mustard cake (oiled) 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%. Composition of roughage and concentrate mixture was estimated by drawing weekly samples. The feeds offered to the animals and residue left were recorded daily to find out the total DM intake of the animals.

Animal experimentation was performed in compliance with regulations set by the cattle yard, NDRI and approved by the Institutional Animals Ethics committee. The calves were housed in well ventilated stalls having facilities for individual feeding. The animals were washed and brushed daily to remove dust and faeces adhered to the skin. Fresh drinking water was provided twice a day.

Collection and processing of blood sample: Blood samples (8 ml) were collected in sterile heparinised vacutainer tubes from jugular vein puncture, posing minimum disturbance to the animal. Blood samples were taken on days 0, 15, 30, 45, 60, 75, 90, 105 and 120 with respect to day of treatments during winter. Day '0' represented the day of start of experiment. Immediately after collection, tubes were kept in ice and transported to the laboratory for further processing and centrifuged at 3,000 rpm for 15 min and plasma was stored at -20°C in different aliquots for the analysis of various biochemical constituents.

Estimation of blood biochemical parameters: Glucose was determined by assay kit. Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). Superoxide dismutase and catalase was determined in plasma samples by a kit. Immunoglobulins in the plasma sample were estimated by zinc turbidity method (Mc Ewan and Fisher 1970). IgG and IgM was determined by ELISA kit. The T_3 and T_4 by ELISA kit. Cortisol was determined in plasma samples by a kit.

Chemical and mineral analysis of feed sample: The roughage and concentrate were ground individually, labeled and analyzed for proximate composition as per AOAC (2005) and cell wall constituents as per Goering and Van Soest (1970). Concentrations of Na, K and Ca in feed, urine and faecal samples were analysed by atomic absorption spectrophotometer. Cl content of the samples was

determined as per Chapman and Pratt (1961). S content in the samples was estimated by turbidimetric method (Massoumi and Cornfield 1963) and P by photometric method (AOAC 2005).

Statistical analysis: The data obtained from both the experiments was analyzed by 2-way ANOVA (Snedecor and Cochran 1994). The test of significance among the different treatments was also analyzed (SPSS 1998).

All the experimental procedures described in this research were in compliance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals, India) for the care and use of animal for scientific purposes.

RESULTS AND DISCUSSION

Chemical and minerals composition of different feedstuffs: Chemical and mineral composition of feedstuffs offered to the calves during the experimental period of 120 days is presented in Table 1.

Effect of varying DCAD diet on dry matter intake: These increased progressively in control, W_1 and W_2 groups, respectively (Table 2). There was only numerical increase in DMI at DCAD diet of +150 mEq/ kg DM than the control group. The overall mean DMI in W_2 was significantly higher ($P < 0.05$) as compared to W_1 and control groups. Tucker *et al.* (1988) reported that a DCAD [(Na + K) – Cl] of 0, +10, and +20 mEq/100 g of DM increased DMI of lactating dairy cows compared with cows consuming rations with DCAD values of –10 mEq/100 g of DM. Growing beef steers (Ross *et al.* 1994b) and finishing beef steers (Ross *et al.* 1994a) also showed increased DMI as DCAD [(Na + K) – Cl] in the growing and finishing stages. Likewise, Vagnoni and Oetzel (1998) reported a 6.9% decrease in DMI by dry cows fed corn silage-alfalfa silage diets that had DCAD [(Na + K) – (Cl + S)] values of –5.1 compared with +20.3 mEq/100 g DM.

An optimum DCAD [(Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.60S + 0.50P)] range of +15 to +20 mEq/100 g of DM (Roche *et al.* 2000) was found to positively affect DMI in dairy cows (Sanchez *et al.* 1994, Roche *et al.* 2005). An increase in nutrient intake with positive DCAD diet was also reported by Jackson *et al.* (1992) and Shahzad *et al.* (2007 2008). Hu *et al.* (2007) reported peak DMI at DCAD values of 40 mEq/100 g of DM and an overall quadratic increase in DMI of dairy cow diets with increasing DCAD. The increase in DM intake in calves of W_2 group fed high DCAD diets might be due to increased rumen pH (Sharif *et al.* 2009, 2010) that makes the ruminal environment alkaline, a pre-requisite for optimum ruminal microbial activity. The influence of DCAD on DMI has a direct effect

Table 1. Chemical and minerals composition of feed ingredients DM basis (%)

Feed/ fodder	CP	NDF	ADF	EE	Ash	Na	K	Cl	S	Ca	P
Concentrate mixture	21.23	34.17	21.83	4.72	5.04	0.99	1.30	1.33	0.47	1.16	0.71
Maize fodder	8.93	54.38	23.47	1.62	7.85	0.17	2.00	1.66	0.45	0.97	0.38
Berseem fodder	17.21	55.62	21.84	1.46	7.18	1.08	3.24	0.40	0.30	1.67	0.40

Table 2. Effect of positive DCAD diets on DM intake (kg/d) in crossbred calves

Day	DMI (kg/d)			
	Control	W ₁	W ₂	SEM
0	3.87	3.85	3.96	0.08
15	3.88	4.05	4.36	0.08
30	3.97	4.06	4.48	0.08
45	3.91	4.11	4.61	0.12
60	4.10	4.31	4.74	0.11
75	4.30	4.56	4.97	0.09
90	5.06	5.12	5.53	0.11
105	5.36	5.37	5.75	0.07
120	5.35	5.40	5.83	0.09
Mean	4.42 ^a	4.54 ^{ab}	4.92 ^b	0.05

Table 3. Fortnightly average body weights (kg) of crossbred calves

Day	Control	W ₁	W ₂	SEM
0	148.77	148.25	149.62	9.36
15	155.93	156.39	156.61	9.26
30	161.15	162.58	165.29	9.08
45	165.82	167.34	171.32	9.14
60	172.18	174.24	179.33	8.86
75	178.83	180.99	186.23	8.75
90	184.86	187.08	192.53	8.51
105	190.97	193.58	199.64	8.43
120	198.02	201.07	206.86	8.35
Mean	172.95	174.61	178.95	3.17

Table 4. Effect of positive DCAD diets on body weight gain (kg) and growth rate (g/d) in crossbred calves during winter

Fortnight	Fortnightly body weight gain (kg)				Average growth rate (g/d)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
1	6.89	7.14	7.00	0.62	477.78	476.11	466.33	41.46
2	5.13	7.19	8.68	0.39	347.78	479.44	578.33	26.19
3	5.03	5.76	6.95	0.45	311.11	384.22	463.22	30.08
4	6.99	6.90	8.01	0.57	424.44	460.00	533.89	38.21
5	6.42	6.75	6.90	0.70	442.78	450.00	460.00	46.95
6	6.07	6.09	6.30	0.48	402.22	406.11	420.00	32.14
7	6.21	6.49	7.11	0.36	407.22	432.78	473.89	24.09
8	6.92	7.50	7.22	0.50	470.00	499.89	481.56	33.23
Mean	6.21 ^a	6.60 ^{ab}	7.27 ^b	0.20	410.42 ^a	440.19 ^{ab}	484.67 ^b	13.80

Means having different superscripts within a row differ significantly (P<0.05).

on the supply of nutrients for maintenance, growth, gestation and lactation.

Effect of positive DCAD diet on growth of crossbred calves: Average fortnightly body weights in crossbred calves of all the 3 groups are presented in Table 3. Statistical analysis of data did not show any significant (P>0.05) effect of different levels of DCAD diet on body weights of crossbred calves during winter trial of 120 days. The overall average weight gain/ fortnight was 6.21, 6.60 and 7.27 kg and average growth rate was 410.42, 440.19 and 484.67 g in control, W₁ and W₂ groups, respectively (Table 4.). Statistical analysis revealed that there was significantly more (P<0.05) gain (g/d) in W₂ group as compared to control showing the positive effect of feeding of +250 mEq/kg DM DCAD level on the body weight gain of female crossbred calves.

Jackson and Hemken (1994) also observed that calves fed DCAD diet of 13 mEq/ 100g DM diet gained 0.14 kg/d more weight than those fed diets containing -18 mEq/ 100g DM. Decreased growth rate in calves fed low DCAD diet was due to metabolic acidosis induced by low DCAD diet. Similarly Shahzad *et al.* (2007) reported an increase in the body weight gain with positive DCAD diet. In the present study, there was 7.3% increase in growth rate in +150 mEq/kg DCAD diet, which was not significantly (P>0.05) different then control group. The significant gain in the

growth rate was observed only at +250 mEq/ kg, DCAD diet which might be attributed to increased DMI due to the favourable effect of alkaline diet on the rumen dynamics and blood chemistry. The extent of nutrients intake varies depending on the level of DCAD, diet composition, animal productive potential and environment.

Plasma glucose: Glucose level (mg/dl) in control, W₁ and W₂ groups showed no significant difference (P>0.05) of positive DCAD diet (Table 5). Similar to our findings, Chan *et al.* (2005) and Cho *et al.* (2006) also observed no effect of DCAD diet on blood glucose in cows. The blood glucose levels observed in crossbred calves in the present study are in the normal physiological range (40.00 to 80.00 mg/dl) as reported by other workers (Upadhyay and Rao 1985, More *et al.* 2008 and Bhooshan *et al.* 2010).

Total antioxidant activity: Average plasma total antioxidant activity (mmol/ L) in the blood samples drawn fortnightly in control, W₁ and W₂ are presented in Table 6. Initial and final plasma total antioxidant activity i.e. mean FRAP value in control, W₁ and W₂ group was 2219.46, 2134.58 and 2184.27 and 1910.51, 1974.06 and 2022.05 mmol/ L respectively. The overall mean of FRAP value in 3 groups showed nonsignificant (P>0.05) effect of positive DCAD diet. The present study reflects no effect of DCAD on total antioxidant activity of young growing calves. Several authors also reported the similar range of FRAP in

Table.5 Effect of positive DCAD diet on glucose level (mg/dl) in growing KF calves

Day	Glucose (mg/dl)			
	Control	W ₁	W ₂	SEM
0	60.09	59.07	59.25	2.18
15	59.32	58.42	59.57	1.84
30	60.14	59.94	62.04	2.14
45	61.36	63.37	60.94	1.95
60	61.77	62.65	65.32	1.67
75	64.40	66.75	66.75	1.76
90	65.28	64.50	65.82	1.30
105	59.30	64.87	63.20	1.18
120	60.80	64.98	64.50	0.91
Mean	61.38	62.73	63.04	0.68

samples drawn at fortnightly intervals from calves fed on control diet as well as +150, +250 mEq/kg DM DCAD diet (control, W₁ and W₂ group) are presented in Table 7. Statistical analysis of data revealed that the total immunoglobulin concentration was not affected significantly ($P>0.05$) by the treatment diets and was similar among groups.

At the end of experimental feeding the total IgG concentration in control, W₁, W₂ group (Table 7) varied nonsignificantly among groups ($P>0.05$). Similarly the overall average of IgM concentration in control, W₁, W₂ group was statistically similar ($P>0.05$). Results of the experiments revealed that +150 and +250 mEq/kg DM DCAD diet did not influence the plasma immunoglobulin in winter. This might be due to the fact that such levels

Table 6. Effect of positive DCAD diet on FRAP ($\mu\text{Mol/l}$), SOD (U/ml) and catalase activity (nmol/min/ml) in crossbred calves during winter

Day	FRAPP ($\mu\text{Mol/l}$)				SOD (U/ml)				Catalase (nmol/min/ml)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	2219.46	2134.58	2184.27	49.48	2.74	3.14	2.99	0.13	60.46	58.26	56.81	1.16
15	2159.42	2037.27	1997.93	62.03	3.08	2.84	2.84	0.11	46.85	48.53	47.47	0.96
30	1919.25	2039.34	1983.44	37.71	2.99	2.75	2.80	0.10	42.71	43.43	43.65	0.77
45	2033.13	1971.01	2068.32	76.23	2.97	3.06	2.93	0.11	39.82	42.29	44.29	1.40
60	1841.76	1884.57	1959.79	56.92	2.99	2.74	2.81	0.06	50.39	52.07	53.96	1.25
75	1867.70	1889.75	2101.17	56.28	2.80	2.52	2.50	0.11	51.76	53.20	53.20	0.10
90	1961.09	2035.02	2116.73	49.63	2.62	2.38	2.22	0.09	56.23	54.91	54.99	0.88
105	2027.24	2036.32	2094.68	54.57	2.19	2.23	1.97	0.19	55.14	53.69	54.18	0.94
120	1910.51	1974.06	2022.05	53.65	2.78	2.50	2.40	0.09	56.93	57.06	61.39	1.82
Mean	1993.28	2000.21	2058.71	19.08	2.60	2.68	2.79	0.04	51.14	51.49	52.22	0.59

blood plasma (Ganie 2012, Vaidya *et al.* 2012). No previous literature has been reported regarding the effect of DCAD on total plasma antioxidant activity, therefore this result is not discussed in the light of previous literature.

Superoxide dismutase and catalase activity: Effect of DCAD supplementation on plasma SOD (U/ml) and catalase activity (mmol/min/ml) in growing calves was estimated fortnightly and the results obtained are presented in Table 6. No significant effect ($P>0.05$) of dietary treatments was observed on the plasma SOD levels.

No significant effect ($P>0.05$) of varying dietary DCAD levels on plasma catalase activity was observed. The values of SOD and catalase were in normal range as reported earlier (Manish *et al.* 2011, Ganie 2012, Kumar *et al.* 2010, 2011). In the present study there was no effect of cationic diet on the plasma antioxidative enzyme activity. Contrary to our findings, Kumar *et al.* (2010) observed that the supplementation of electrolyte decreased the activity of plasma SOD and catalase of heat stressed buffalo; results may be due to the fact that they have used ascorbic acid polyphosphate and zinc oxide in addition to salts, and ascorbate and zinc interferes with the actions of catalase and SOD.

Total immunoglobulin, IgG, IgM: Average total immunoglobulin concentration (mg/ml) in the blood

were not sufficient to evoke any changes in the immunological parameters.

T₃ and T₄: The mean values of plasma T₃ and T₄ in calves during winter are presented in Table 8. During winter the mean value plasma T₃ and T₄ was significantly higher ($P<0.05$) in W₂ group as compared to control and W₁ group. The values of T₃ and T₄ differ significantly between the breeds, seasons and production levels. Present results are in accordance with Rasooli *et al.* (2004) and Todini (2007), who reported that thyroid hormones were maximum during winter, decreased in spring and continued to decline reaching the lowest value in summer in both buffaloes and Friesians. Prakash and Rathore (1991) also showed significant ($P<0.05$) lower serum T₃ and T₄ levels during summer compared to winter in goats. The quantity and quality of food eaten is a major factor determining plasma concentrations of TH (Dauncey 1990). The increased T₃ and T₄ in W₂ group in this study, may be attributed to increase in nutrient intake, especially in the treatment group where alkalogenic salt were important nutritional tool to enhance rumen ecology aimed to utilize nutrients more efficiently (Shahzad *et al.* 2007, Luebbe *et al.* 2011). Similarly, Riis and Madsen (1985), Blum *et al.* (1980) also stated that blood TH levels were correlated with quantity and quality feed intake in ruminant species.

Table 7. Effect of positive DCAD diet on plasma total immunoglobulin (mg/ml), IgG (ng/ml) and IgM concentration (ng/ml) in crossbred calves during winter

Day	Total immunoglobulin (mg/ml)				IgG (ng/ml)				IgM (ng/ml)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	30.96	31.23	33.09	0.88	22.94	23.51	24.37	1.25	7.87	7.97	7.62	0.14
15	34.68	32.97	32.30	0.67	22.11	19.54	21.99	1.60	8.19	7.75	7.81	0.15
30	30.19	32.13	31.99	0.90	20.62	26.22	23.46	1.96	7.49	7.86	8.00	0.15
45	32.31	32.75	31.81	0.64	23.03	22.46	24.48	1.08	7.15	7.52	7.76	0.13
60	33.86	33.94	35.37	0.57	25.82	25.47	20.56	1.18	7.85	8.19	8.30	0.15
75	30.18	31.10	32.07	0.88	21.53	28.19	23.23	1.69	8.05	8.03	8.29	0.17
90	31.11	31.41	32.94	0.61	21.72	21.12	24.73	1.77	7.95	7.37	7.64	0.16
105	30.31	31.56	32.15	0.55	20.69	18.21	21.51	1.87	7.41	7.73	7.56	0.08
120	30.22	31.25	31.74	0.44	23.29	21.82	23.71	1.42	7.55	7.56	7.81	0.07
Mean	31.54	32.04	31.61	0.26	22.42	22.95	23.11	0.53	7.72	7.78	7.87	0.05

Table 8. Effect of positive DCAD Diet on T₃, T₄ and cortisol levels (ng/ml) in growing crossbred calves during winter

Day	T ₃ (ng/ml)				T ₄ (ng/ml)				Cortisol (ng/ml)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	0.91	0.97	1.00	0.04	23.13	23.54	22.46	0.57	3.01	3.31	3.11	0.20
15	0.99	1.00	1.16	0.09	23.01	23.88	23.89	0.88	3.30	3.57	3.31	0.31
30	1.10	1.12	1.20	0.09	24.13	24.30	25.97	0.87	4.24	3.85	4.01	0.32
45	1.07	1.12	1.21	0.11	24.42	24.64	27.01	0.85	5.15	5.45	4.97	0.25
60	1.14	1.17	1.25	0.07	24.48	24.76	27.38	0.82	4.24	3.85	4.01	0.32
75	1.12	1.21	1.24	0.04	24.71	24.94	28.38	0.79	4.20	3.64	3.78	0.42
90	1.10	1.20	1.23	0.03	24.23	24.52	26.63	0.77	3.00	3.07	2.66	0.18
105	1.09	1.15	1.16	0.02	24.07	24.29	26.46	0.64	2.87	2.88	2.91	0.11
120	1.18	1.10	1.19	0.02	22.76	22.87	25.98	0.64	3.05	2.99	2.98	0.16
Mean	1.08 ^a	1.12 ^{ab}	1.18 ^b	0.02	23.88 ^a	24.19 ^{ab}	26.02 ^b	0.26	3.67	3.62	3.53	0.10

^{a,b} Means having different superscript within row for respective parameter differ significantly (P<0.05).

Plasma cortisol: Effect of feeding different positive DCAD diet on plasma cortisol in crossbred calves during winter is presented in Table 4. The cortisol numerically increased gradually up to 45th day due to increase in cold stress. Similar to this, Sasaki and Weekes (1986) and Kim *et al.* (2009) also reported that exposure of animals to cold, increased plasma cortisol level. There was no significant difference (P>0.05) found among the groups. Plasma cortisol levels observed during the present study were within the normal physiological range (Block *et al.* 2001, Maurya *et al.* 2011). Results of winter trial surmise that +150 and +250 mEq/DM of DCAD level is not able to affect plasma cortisol.

The results revealed that feeding positive DCAD diet (+250 mEq/kg DM) can be used as a effective tool to enhance DMI, body weight gain and metabolic hormones, which are ultimately going to affect the productive and reproductive performance of animals. However, the immunity and antioxidative parameters in growing calves did not show any effect by feeding of +150 /+250mEq/kg DM diet.

ACKNOWLEDGEMENT

Facilities provided by National Dairy Research Institute, Karnal, for undertaking this study, are gratefully acknowledged.

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