



Effect of dietary cation anion difference based diet on nutrient intake, acid base status and growth performance of crossbred calves in summer months

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ABSTRACT

The present study was undertaken used to determine the effect of positive dietary cation anion difference (DCAD) based diet on nutrient intake and growth of crossbred calves in summer. Female crossbred calves (18) were blocked into three equal groups on the basis of their average body weight. The calves received a basal diet (control) or one supplemented with either +250 (S₁) or +350 mEq/kg DM (S₂) of dietary cation anion difference based diet. The dry matter intake (DMI) was significantly higher in S₁ and S₂ groups as compared to control. There was no effect of dietary treatments on digestibility of nutrients. The overall average daily gain (ADG) was significantly higher in S₂ group as compared to control. Nitrogen intake, balance and urine pH increased significantly with increasing level of DCAD in diet. Sodium (Na) and potassium (K) intakes were significantly higher in treatment groups as compared to control group. However, Na and K balance were significantly higher in S₂ group as compared to control. Intake and balance of Cl (chloride), S (sulphur), Ca (calcium) and P (phosphorus) were not affected by positive DCAD diet. Positive DCAD diets of +250 and +350 mEq/kg DM improved the nutrient intake and growth of crossbred calves by ameliorating climatic stress.

Key words: Climatic stress, Crossbred calves, Dietary cation anion balance, Growth, Heat stress, summer months

Climatic stress can impart physical and economical losses to livestock production in temperate, subtropical and tropical regions of the world. Temperature stressed animals undergo a series of metabolic and physiological changes (Rojas-Downing *et al.* 2017). These changes ultimately affect the metabolism and physiological functions such as acid-base regulation. In thermal stress, there is an increased demand for net energy for maintenance which leads to reduction in energy for tissue growth and production (Nesamvuni *et al.* 2012). Nutritional balance is an important factor in combating thermal stress because such imbalance may be deleterious to the productive as well as reproductive performance of animals (Sharif *et al.* 2010). It has been observed that metabolic or systemic acidosis is aggravated in summer (Li *et al.* 2008, Carlos *et al.* 2018). Recent advances in minerals nutrition suggested that, the difference between certain cations (Na⁺, K⁺) and anions (Cl⁻, S⁻) may be of more significance for animal productivity than their individual effects (Rodney *et al.* 2018). Sanchez and Beede

(1991) coined the term DCAD which is a way to balance the electrical charge of the cations and anions in the diet. Positive DCAD based diets can be a useful strategy during thermal stress to increase DMI and resultant positive influence on the growth (Pawar *et al.* 2016). High DCAD diets not only proved to increase DMI, growth and production, but are also useful in mitigating the effects of summer stress (Sarwar *et al.* 2011). In tropical countries like India, growing calves are well known victims of high temperature and humidity and these variations in temperature are likely to affect the various physiological parameters which ultimately affect the profitability of dairy enterprise. Feeding high DCAD diet to growing calves might be an important nutritional strategy to ameliorate the adverse effects of climatic stress by improving acid base status and nutrients intake. Therefore, the present study was planned to determine the effect of different levels of DCAD based diet on nutrient intake, utilization and growth performance of crossbred calves in summer.

MATERIALS AND METHODS

Selection of animals and feeding management: Eighteen female Karan Fries (Tharparkar × Holstein Friesian) calves (5 to 9 months of age) were selected in the subtropical region (National Dairy Research Institute, Karnal) of India on the basis of average body weight. The experiment was conducted during summer months (15 April 2012 to 12

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

Feed ingredient	CP	NDF	ADF	EE	TA	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
Oats fodder	11.26	48.84	30.65	1.95	12.76	0.67	1.90	1.20	0.31	0.45	0.69
Wheat straw	3.17	67.85	40.20	0.76	12.04	0.18	2.05	1.00	0.35	0.30	0.13
Jowar fodder	10.6	61.36	41.11	1.61	11.80	0.02	2.41	0.80	0.16	0.70	0.57

ADF, Acid detergent fibre; Ca, calcium; Cl, chloride; CP, crude protein; EE, ether extract; K, potassium; Na, sodium; NDF, neutral detergent fibre; P, phosphorus; S, sulphur; TA, total ash.

August 2012) for 120 days. All the experimental procedure were in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India) for the care and use of animal for scientific purposes. The equation used for DCAD calculation was $DCAD = (Na + K) - (Cl + 0.6S)$ mEq/kg DM (Sanchez *et al.* 1991). Experimental animals were fed iso-nitrogenous and iso-caloric diet as per NRC (2001) standard and requirements of animal were fulfilled by feeding concentrate mixture, wheat straw and maize/berseem fodder. In concentrate mixture (19.81% CP and 2.85 Mcal ME/kg DM), appropriate cationic salts [sodium bicarbonate ($NaHCO_3$) and dipotassium carbonate (K_2CO_3)] were added to achieve desired DCAD (mEq/kg DM), i.e. +250 and +350 mEq/kg DM. Calves were housed on a concrete floor in separate pens and no mechanical means were used to control the temperature. Composition of roughage was estimated by drawing weekly samples. Body weight of the animals was recorded at fortnightly interval. A metabolic trial for seven days was conducted in the mid of the experiment. Microclimatic data, viz. dry bulb temperature, wet bulb temperature, minimum and maximum temperature and relative humidity were recorded at 7.30 and 14.30 h using thermometer (GH Zeal Ltd., London, United Kingdom) every day during experimental period. Temperature humidity index (THI) was calculated using the formula (NRC 1981): $THI = 0.72 (Tdb + Twb) + 40.6$; where Tdb, dry bulb temperature ($^{\circ}C$); Twb, wet bulb temperature ($^{\circ}C$).

Chemical and mineral analysis of feed samples: The roughage and concentrate were grounded individually, labeled and analyzed for proximate composition as per AOAC (2005) and cell wall constituents as per Goering and Van Soest (1970). Concentration of Na, K and Ca in feed, urine and faecal samples were analysed by atomic absorption spectrophotometer (Hitachi Z-5000, Hitachi Ltd., Japan). Cl content of the samples was determined by the method of 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: Statistical analysis of the data was by ANOVA as per Snedecor and Cochran (1994) with the help of software package (SPSS 1998). The effect of treatments was analysed by two-way ANOVA.

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \quad (i = 1 \dots I; j = 1 \dots J)$$

where μ , Overall mean; α_i , Row effect; β_j , Column effect and ε_{ij} , Random error for observation X_{ij}

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 d is presented in Table 1.

Environmental variables: The environmental temperature and relative humidity varied from 32.4 to 41.2 $^{\circ}C$ and 48 to 90% in the morning and 32.6 to 42.1 $^{\circ}C$ and 17 to 75% in the afternoon, respectively, during 120 d

Table 2. Environmental variables during the experimental period

Fortnight	Max. temp ($^{\circ}C$)		Min. temp ($^{\circ}C$)		Relative humidity (%)		THI	
	M	A	M	A	M	A	M	A
Initial	32.4	34.1	16.2	17.3	77.0	30.0	67.9	80.2
1	34.8	36.8	18.3	19.6	69.0	24.0	68.9	80.4
2	37.6	38.7	21.0	21.6	54.0	20.0	71.8	82.8
3	40.9	41.9	24.1	24.8	48.0	17.0	74.8	84.1
4	41.2	42.1	25.7	26.4	59.0	31.0	77.7	86.1
5	40.6	41.6	27.3	27.8	65.0	38.0	79.3	85.6
6	35.4	36.2	27.3	27.8	80.0	61.0	80.7	84.7
7	34.5	35.2	26.6	27.2	86.0	64.0	80.2	84.0
8	32.3	32.6	26.2	27.0	90.0	75.0	78.9	83.0

A, Observations recorded at 14:30 h; Initial, 01 to 15 April 2012; M, observations recorded at 7:30 h; THI, temperature humidity index; 1, 16 to 30 April 2012; 2, 01 to 15 May 2012; 3, 16 to 30 May 2012; 4, 31 to 14 June 2012; 5, 15 to 29 June 2012; 6, 30 to 14 July 2012; 7, 15 to 29 July 2012; 8, 30 July to 13 August 2012.

of experimental period (Table 2). Fortnightly THI at the start of experiment (01 to 15 April 2012) in the morning and afternoon was 67.9 and 80.2 which increased with the duration of the experiment. The highest fortnightly THI in morning and afternoon sessions was observed during June, July and August. Calves are homoeothermic animals so they have to maintain their temperature within normal range to maintain homeostasis. The THI was higher in present study indicating a higher level of thermal stress to the animals during the experimental period (LPHSI 1990).

Dry matter intake and digestibility of nutrients: A significant increase in DMI was observed with increase in DCAD concentration. The calves fed +350 mEq/kg DM consumed 12.55% higher DM ($P<0.05$) than control group. CP, ME (Mcal/d) and N intake increased ($P<0.05$) with increase in DCAD concentration (Tables 3 and 4). All the animals were in positive nitrogen balance, however S_2 group had significantly higher ($P<0.05$) nitrogen balance in comparison to control. Digestibility of nutrients (CP, EE, NDF and ADF) was similar ($P>0.05$) in all the three groups. The increased DMI might be due to effect of higher DCAD diet on ruminal pH, which is pre-requisite for optimum ruminal microbial activity and also on blood HCO_3^- and acid-base balance (Pacheco *et al.* 2018). In the rumen, NaHCO_3 is disassociated into Na^+ and HCO_3^- with non-

buffering and buffering effects, respectively; they also increased ruminal osmotic pressure and liquid dilution rate (Mao *et al.* 2017). Rumen buffering reduces the extent of acidity produced by volatile fatty acid and lactic acid and therefore, improves the systemic acid-base status (Gruenberg *et al.* 2011). So it can be concluded that positive DCAD can enhance nutrients intake due to its favourable influence on rumen dynamics and blood chemistry (Nisa *et al.* 2006). Nitrogen intake and balance also increased in cationic group which may be due to increased DMI, which increased the post-ruminal supply of amino acids by accelerating rumen microbial multiplication (Shahzad *et al.* 2007).

Urine pH: An increase in urine pH was recorded with increasing DCAD concentration during the experimental period (Table 5). The increase in urine pH with increase in high DCAD fed group is due to the alkaline nature of the diet (Shahzad *et al.* 2007, Luebke *et al.* 2011) as diet has direct effect on the urinary pH.

Growth performance: There was numerically more weight gain in the positive DCAD diet fed groups of growing Karan Fries calves in different fortnights (Table 6). The overall ADG was 25.60% more ($P<0.05$) in group fed on DCAD +350 mEq/kg DM compared to the control. High DCAD diet due to its favourable effects on

Table 3. Effect of positive DCAD diets on nutrient intake and their digestibility in growing crossbred calves

Particular	Control	S_1	S_2	SEM
<i>Nutrient intake</i>				
DM (kg/d)	5.10 ^a	5.67 ^b	5.74 ^b	0.10
OM (kg/d)	4.64	5.00	5.03	0.08
CP (kg/d)	0.66 ^a	0.68 ^{ab}	0.71 ^b	0.10
ME (Mcal/d)	12.11 ^a	13.14 ^b	13.28 ^b	0.16
<i>Nutrient digestibility (%)</i>				
DM	67.10	67.32	67.83	0.43
OM	69.04	70.23	70.26	0.68
CP	72.32	73.26	73.43	0.69
EE	76.05	76.85	78.66	1.17
NDF	58.02	58.38	58.97	0.57
ADF	53.74	54.24	54.44	0.68

^{a,b}Means having different superscripts within a row differ significantly ($P<0.05$). DM, Dry matter; OM, organic matter; CP, crude protein; ME, metabolizable energy, EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Table 4. Effect of positive DCAD diet on nitrogen balance in growing crossbred calves

Parameter	Control	S_1	S_2	SEM
N intake (g/d)	105.29 ^a	108.65 ^{ab}	113.90 ^b	2.35
Faecal N outgo (g/d)	41.21	47.67	49.29	1.32
Urinary N outgo (g/d)	32.91	33.93	37.85	3.97
N balance (g/d)	23.71 ^a	25.43 ^{ab}	27.56 ^b	1.68
Apparent N retention (%)	22.84	23.92	24.13	1.12

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

Table 5. Effect of positive DCAD diet on urine pH in crossbred calves

Months	N intake (g/d)			
	Control	S_1	S_2	SEM
Initial	7.30	7.29	7.28	0.12
1	7.28	7.48	7.76	0.11
2	7.30	7.65	7.81	0.15
3	7.29	7.50	7.78	0.15
4	7.32 ^a	7.70 ^{ab}	8.01 ^b	0.14

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

Table 6. Effect of positive DCAD diets on average body weight gain (g/d) in crossbred calves

Fortnight	Control	S_1	S_2	SEM
1	347.11	344.44	370.00	28.82
2	308.67	318.33	323.89	27.09
3	326.96	344.44	433.33	37.55
4	285.93	272.78	344.44	31.11
5	318.00	351.67	411.67	33.16
6	238.22	306.11	351.56	49.16
7	292.89	366.11	395.11	38.05
8	283.11	286.67	385.56	43.32
Mean	300.11 ^a	323.01 ^{ab}	376.95 ^b	11.68

1, 16 to 30 April 2012; 2, 01 to 15 May 2012; 3, 16 to 30 May 2012; 4, 31 to 14 June 2012; 5, 15 to 29 June 2012; 6, 30 to 14 July 2012; 7, 15 to 29 July 2012; 8, 30 July to 13 August 2012.

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

ruminal fermentation activity may result in higher nutrient consumption (Nisa *et al.* 2006, Meena 2012) which can be the reason for the higher weight gain ($P < 0.05$) in treatment groups as compared to control. It is also stated that metabolic activities in growing animals take place at a rapid rate, leading to higher production of CO_2 in the cells which makes the cellular environment acidic (Guyton *et al.* 2000, Iwaniuk *et al.* 2015). This slight acidic situation restricts the cells and its organelles to work optimally and consequently reduces cellular activities resulting in poor growth rate (Sarwar *et al.* 2011). The alkalogenic nature of the positive DCAD especially, +350 mEq/kg DM DCAD based diet might have allowed the cells to work to its optimal potential by sustaining the cellular environment slightly alkaline by counteracting the cellular acidity produced by CO_2 .

Na and K balances: Na and K intake was significantly higher ($P < 0.01$) in the high DCAD fed group (Table 7). The overall faecal and urinary outgo of Na and K was more in the high DCAD fed group, however it was not statistically significant. Na balance and retention was significantly higher ($P < 0.01$) in S_2 group as compared to control similar trend was observed for the K balance. It might be due to effect of positive DCAD based diet because the high DCAD based diets contained higher Na and K concentrations (Shahzad *et al.* 2011, Martin-Tereso *et al.* 2104).

Cl and S balances: Intakes as well as excretion of Cl and S (g/d) were similar ($P > 0.05$) in all the groups (Table 8). The Cl and S balance (g/d) and retention also showed no statistical difference ($P > 0.05$) in the groups. In present study, we did not observe any effect on the intake as well on retention of Cl and S showing no effect of cationic diet on sulphur metabolism (Shahzad *et al.* 2011). Moreover the diets in present study were of high DCAD value and contained similar concentration of Cl and S in all the three respective groups, which might be the reason for no difference in the metabolism of these two minerals in different groups.

Ca and P balances: The Ca and P intakes and excretion were statistically similar ($P > 0.05$) among groups (Table 9).

Table 7. Effect of positive DCAD diet on sodium and potassium balances in crossbred calves

Parameter	Control	S_1	S_2	SEM
<i>Sodium</i>				
Na intake (g/d)	23.25 ^a	32.54 ^b	48.88 ^c	2.57
Faecal Na outgo (g/d)	5.33	5.41	0.31	3.98
Urinary Na outgo (g/d)	12.25	17.15	19.30	1.91
Na balance (g/d)	7.02 ^a	10.06 ^{ab}	24.17 ^b	2.61
Na retention (%)	29.95 ^a	31.05 ^{ab}	49.35 ^b	6.16
<i>Potassium</i>				
K intake (g/d)	125.49 ^a	138.57 ^b	149.52 ^b	2.81
Faecal K outgo (g/d)	30.83	31.41	1.23	27.06
Urinary K outgo (g/d)	18.70	19.15	20.69	2.01
K balance (g/d)	79.73 ^a	88.59 ^{ab}	97.43 ^b	2.55
K retention (%)	63.12	63.57	64.63	1.71

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

Table 8. Effect of positive DCAD diet on chloride and sulphur balances in crossbred calves

Parameter	Control	S_1	S_2	SEM
<i>Chloride</i>				
Cl intake (g/d)	78.05	79.57	80.13	0.70
Faecal Cl outgo (g/d)	37.13	36.06	33.47	0.69
Urinary Cl outgo (g/d)	14.59	15.11	16.14	1.89
Cl balance (g/d)	27.41	27.34	30.52	1.79
Cl retention (%)	34.55	34.07	37.31	2.53
<i>Sulphur</i>				
S intake (g/d)	16.06	16.37	16.48	1.10
Faecal S outgo (g/d)	4.89	4.55	4.40	0.12
Urinary S outgo (g/d)	2.33	2.30	2.26	0.18
S balance (g/d)	8.85	9.51	9.81	0.23
S retention (%)	54.91	57.86	59.08	2.80

Table 9. Effect of positive DCAD diet on calcium and phosphorus balances in crossbred calves

Parameter	Control	S_1	S_2	SEM
<i>Calcium</i>				
Ca intake (g/d)	40.07	39.31	38.47	0.40
Faecal Ca outgo (g/d)	15.85	17.17	17.88	0.65
Urinary Ca outgo (g/d)	0.32	0.25	0.20	0.03
Ca balance (g/d)	22.31	21.89	22.00	0.49
Ca retention (%)	57.45	55.07	54.31	1.36
<i>Phosphorus</i>				
P intake (g/d)	26.34	27.63	28.17	0.27
Faecal P outgo (g/d)	20.62	18.51	17.75	1.01
Urinary P outgo (g/d)	0.14	0.14	0.17	0.02
P balance (g/d)	5.39	8.98	10.25	0.82
P retention (%)	25.67	32.07	36.07	2.92

The Ca and P retention was also similar ($P > 0.05$) among groups. It had been reported that anionic diets influence the Ca and P metabolism (Shahzad *et al.* 2011, Rodney *et al.* 2018) but in present study, only positive DCAD based diets were used which being alkaline in nature is unable to exert any effect on the absorption and excretion of Ca and P.

In conclusion, the findings revealed that the positive DCAD based diet improved DMI, nutrient intake, nitrogen balance, Na and K balances and growth performance of growing crossbred calves. However, more detailed work involving effect of positive DCAD based diet on the rumen ecosystem and at cellular level is required to establish the possible correlation among the mentioned activities of the DCAD based diet and deciding a particular level to the farming community.

REFERENCES

- AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, DC, USA.
- Carlos A P, Martin Francisco M, Noemi G, Jaime S C, Jose O, Aris Bionel C and Ricard Z. 2018. Influence of dietary cation-anion difference in finishing diets fed to Holstein steers during periods of high ambient temperature on feedlot performance and digestive function. *Journal of Applied Animal Research* 46: 729–33.

- Chapman H D and Pratt P F. 1961. *Method of Analysis for Soils, Plant and Water*. Division of Agriculture Sciences, University of California, Berkeley, USA.
- Goering H K and Van Soest P J. 1970. Forage fibre analysis. Agriculture Handbook No. 379, ARS USDA, Washington, DC.
- Gruenberg W, Donkin S S and Constable P D. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. *Journal of Dairy Science* **94**: 727–45.
- Guyton A C and Hall J E. 2000. *Textbook of Medical Physiology*. WB Saunders Company, USA.
- Iwaniuk M E and Erdman R A. 2015. Intake, milk production, ruminal and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. *Journal of Dairy Science* **98**: 8973–85.
- Li F C, Liu H F and Wang Z H. 2008. Effects of dietary cation-anion difference on calcium, nitrogen metabolism and relative blood traits of dry Holstein cows. *Animal Feed Science and Technology* **142**: 185–91.
- LPHSI. 1990. Livestock and poultry heat stress indices for cattle, sheep and goats. *Livestock and Poultry Heat Stress Indices*. Agriculture Engineering Technology Guide, Clemson University, Clemson, South Carolina, USA.
- Luebke M K, Erickson G E, Klopfenstein T J, Greenquist M A and Benton J R. 2011. Effect of dietary cation-anion difference on urinary pH, feedlot performance, nitrogen mass balance, and manure pH in open feedlot pens. *Journal of Animal Science* **89**: 489–500.
- Mao S, Huo W, Liu J, Zhang R and Zhu W. 2017. *In vitro* effects of sodium bicarbonate buffer on rumen fermentation, levels of lipopolysaccharide and biogenic amine, and composition of rumen microbiota. *Journal of the Science of Food and Agriculture* **97**: 1276–85.
- Martin-Tereso J, Wijlen H, Van Laar H and Verstegen W A. 2014. Periparturient calcium homeostasis of multiparous dairy cows fed rumen-protected rice bran or a lowered dietary cation/anion balance diet before calving. *Journal of Animal Physiology and Animal Nutrition* **98**: 775–84.
- Massoumi A and Cornfield A H. 1963. A rapid method for determining sulphate in water extract of soils. *Analyst* **88**: 321–22.
- Meena B. 2012. 'Evaluation of di-ammonium phosphate as a phosphorus source in crossbred female calves.' MVSc Thesis, National Dairy Research Institute (Deemed University), Karnal, India.
- National Research Council. 1981. *Effect of Environment on Nutrient Requirements of Domestic Animals*. National Academies Press, Washington, DC.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th edn. National Academies Press, Washington, DC.
- Nesamvuni E, Lekalakala R, Norris D and Ngambi J W. 2012. Effects of climate change on dairy cattle, South Africa. *African Journal of Agriculture Research* **7**: 3867–72.
- Nisa M, Khan M A, Sarwar M, Murtaza W S and Kim K S. 2006. Influence of re-growth interval on chemical composition, herbage yield, and digestibility and digestion kinetics *setaria sphacelata* and *cenchrus ciliaris* in buffaloes. *Asian Australasian Journal of Animal Science* **19**: 381–85.
- Pacheco C A, Montano-Gomez M, Torrentera N G, Ortiz J, Cano A B and Zinn R V. 2018. Influence of dietary cation-anion difference in finishing diets fed to Holstein steers during periods of high ambient temperature on feedlot performance and digestive function. *Journal of Applied Animal Research* **46**: 729–33.
- Pawar M, Srivastava A K, Chauhan H D, Kumar S and Damor V. 2016. Nutritional strategies to alleviate heat stress in dairy animals—a review. *International Journal of Livestock Research* **8**: 8–18.
- Rodney R M, Martinez N, Block E, Hernandez L L, Celi P, Nelson C D, Santos J E P and Lean I J. 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: vitamin D, mineral, and bone metabolism. *Journal of Dairy Science* **101**: 2519–43.
- Rojas-Downing M M, Nejadhashemi A P, Harrigan T and Woznicki S A. 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management* **16**: 145–63.
- Sanchez W K and Beede D K. 1991. Interrelationships of dietary Na, K, and Cl and cation-anion difference in lactation rations. Proceedings of Florida Ruminant Nutrition Conference, Gainesville, USA. pp 31.
- Sarwar M, Shahzad M A and Nisa M. 2011. Nutrient intake, acid base status and weight gain in water buffalo calves fed different dietary levels of sodium bicarbonate. *South African Journal of Animal Science* **41**: 94–103.
- Shahzad M A, Sarwar M and Nisa M. 2007. Nutrient intake, acid base status and growth performance of growing male buffalo calves fed varying level of dietary cation anion difference. *Livestock Science* **111**: 136–43.
- Shahzad M A, Sharif M, Nisa M, Sarwar M, Farooq Khalid M and Saddiq H A. 2011. Changing certain dietary cationic and anionic minerals: Impact on blood chemistry, milk fever and udder edema in buffaloes during winter. *African Journal of Biotechnology* **10**: 13651–663.
- Sharif M, Shahzad M A, Nisa M and Sarwar M. 2010. Influence of varying levels of dietary cation anion difference on nutrient intake, ruminal characteristics, nitrogen metabolism and *in situ* digestion kinetics in Nili Ravi buffalo bulls. *Animal Science Journal* **81**: 657–65.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. Iowa State University Press, Ames, Iowa, USA.
- Statistical Package for the Social Sciences. 1998. SPSS Base 8.0, SPSS Inc. Chicago, USA.