

**AMELIORATION OF CLIMATIC STRESS BY FEEDING
DIETARY CATION ANION DIFFERENCE BASED DIETS IN
GROWING CALVES**



THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF

**DOCTOR OF PHILOSOPHY
IN
ANIMAL NUTRITION**

BY

MADHU SUMAN
M.V.Sc. (Animal Nutrition)

**DIVISION OF DAIRY CATTLE NUTRITION
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
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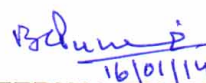
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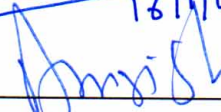
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EXTERNAL EXAMINER

(
16/1/14)
(Dr. HARJIT KAUR)
MAJOR ADVISOR & CHAIRMAN
(GUIDE)

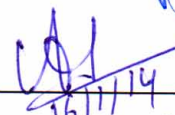
Members, Advisory Committee

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Principal Scientist, DCN Division

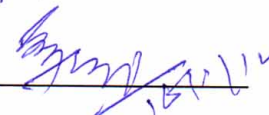
(
16/1/14)


2. **Dr. A.K. TYAGI**
Principal Scientist, DCN Division

3. **Dr. ANJALI AGGARWAL**
Principal Scientist, DCP Division

(
16/1/14)

4. **Dr. S.K. ATREJA**
Principal Scientist, ABC Division

(
16/1/14)

5. **Dr. R.C.UPADHYAY**
Principal Scientist, DCP Division

(
16/1/14)



DAIRY CATTLE NUTRITION DIVISION
NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL -132001 (HARYANA)

Dr. HARJIT KAUR
Principal Scientist

CERTIFICATE

This is to certify that the thesis entitled " **AMELIORATION OF CLIMATIC STRESS BY FEEDING DIETARY CATION ANION DIFFERENCE BASED DIETS IN GROWING CALVES** " submitted by MADHU SUMAN in partial fulfillment of the requirement for award of the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION of the National Dairy Research Institute (Deemed University), Karnal (Haryana) is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Date: Sept, 7 , 2013


(Dr. HARJIT KAUR)
MAJOR ADVISOR & CHAIRMAN
(GUIDE)



Dedicated to...
My Family
&
Respected Guide



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Date: 7, 9, 2013


(Madhu Suman)

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STUDENT

Madhu Suman

MAJOR ADVISOR

Dr. Harjit Kaur

ABSTRACT

Macro minerals (Ca, P, Mg, Na, K, Cl, and S) content of commonly used feeds and fodders was estimated. An experiment each in summer and winter months was conducted for 120 days to examine the influence of varying DCAD levels on growth, physiological variables, certain biochemical, immunological and endocrine parameters in KF crossbred calves. Eighteen female crossbred calves in summer and winter months each were blocked into three equal groups on the basis of average body weight. The calves either received a basal diet (control) or a +150 / +250mEq/Kg DM DCAD diet (W_1 , W_2) during winter months or +250 / +350 mEq/Kg DM DCAD (S_1 , S_2) diet during summer months. Blood samples were collected fortnightly during both seasons. After 3 months of dietary treatments, a metabolic trial of 7 days was conducted in both seasons. There was no significant effect of varying DCAD diet on physiological variables (RR, PR, RT) during winter and summer months, though variations were observed due to changes in environmental temperature and THI within a season and between seasons as well as during morning and afternoon values. The overall DMI was significantly more ($P<0.05$) in treatment groups, during winter (W_2) and summer (S_2) months. The growth rate per day averaged 410.42, 440.19 and 484.67 g in control, W_1 and W_2 groups, whereas it was 300.11, 332.01, 383.13 g in control, S_1 and S_2 groups, respectively which was significantly more ($P<0.05$) in high DCAD diets in both the seasons. Plasma macro minerals (K, Ca, Mg, Cl and S), micro minerals (Zn, Cu and Fe) and plasma glucose level were found to be similar in all the three groups during both the seasons. However, plasma Na concentration in S_2 group was significantly higher ($P<0.05$) as compared to the control in summer months whereas no effect was observed on Na level in winter months. The blood pH was ($p<0.05$) maximum (7.38) in W_2 group while it was minimum (7.33) in control group. In summer months, maximum blood pH was 7.40 in S_2 group. There was no effect of varying DCAD diet on FRAP value, SOD and catalase activity in both seasons. Immunity parameters were not affected in winter months, but significant increase ($P<0.05$) in plasma total Ig (mg/ml), IgG (ng/ml) and IgM (ng/ml) was observed in +350 mEq/kg DM DCAD fed group in summer months. Plasma T_3 and T_4 was significantly higher ($P<0.05$) in W_2 and S_2 groups. Plasma cortisol concentration averaged 3.67, 3.62 and 3.53 ng/ml, respectively in control, W_1 and W_2 groups and was not affected by treatments. In summer months, plasma cortisol concentration was 4.24, 3.85 and 3.79 ng/ml, in control, S_1 and S_2 groups, respectively showing significantly ($P<0.05$) low values in treatment groups. N intake, balance and urine pH increased significantly ($P<0.05$) with increasing level of DCAD in both the seasons. Na and K intakes were significantly more ($P<0.01$) in treatment groups as compared to control group in both the seasons, however Na and K balance ($p<0.01$) were significantly more in S_2 group whereas, only K balance was more ($p<0.01$) in W_2 as compared to control. There was no effect of seasons and dietary treatments on digestibility of nutrients. Intake and balance of Cl, S, Ca and P were not affected by positive DCAD diet in summer and winter months. It is inferred that positive DCAD diet of +250 and +350 mEq/Kg DM improved the nutrient intake and growth of crossbred calves by ameliorating climatic stress.

बढ़ती बछड़ों में आहार केटायन आयनों के फर्क पर आधारित आहार खिलाने से जलवायु तनाव का सुधार

छात्रा
मधु सुमन

मुख्य मार्गदर्शक
डॉ. हरजीत कौर

सारांश

वर्तमान अध्ययन में, विभिन्न प्रकार के पशु-खाद्यों और चारे में मैक्रो-मिनरल की मात्रा का आंकलन किया गया। तदपश्चात् अलग-अलग डी.सी.ए.डी. मात्रा का संकर बछड़ों के वृद्धि-प्रदर्शन, कार्यिकी-परिवर्तन, और कुछ जैव-रासायनिक, रोग-प्रतिरक्षात्मक एवं अन्तः-स्त्रावी घटकों पर सर्दियों और गर्मियों में 120 दिन तक प्रभाव देखा गया। प्रत्येक गर्मी और सर्दी महीने में 18 मादा संकर बछड़ों को 3 वर्गों में विभाजित किया गया, प्रत्येक समूह में उनके शारीरिक वजन के अनुसार 6 पशु रखे गए। सर्दी महीने के दौरान संकर करन-फ्राइज बछड़ों को या तो आधारीय खुराक या +150/+250 मिली. तुल्यांक प्रति किग्रा. शुष्क-पदार्थ डी.सी.ए.डी. खुराक (W_1 , W_2) और +250/+350 मिली. तुल्यांक प्रति किग्रा. शुष्क-पदार्थ डी.सी.ए.डी. खुराक गर्मी के महीनों में दी गयी। दोनों ऋतुओं के दौरान प्रत्येक पखवाड़े के अन्तराल पर रक्त-संग्रह किया गया। 3 माह के खाध्य-उपचार के उपरांत 7 दिनों का उपापचय परीक्षण किया गया। अलग-अलग डी.सी.ए.डी. खुराक का सर्दी व गर्मी महीनों में कार्यिकी परिवर्तनों (श्वसन दर, नाडी दर, गुदा तापमान) पर कोई सार्थक प्रभाव नहीं था। सर्दी में W_2 व गर्मी में S_2 समूह में समस्त शुष्क-पदार्थ ग्रहण व पोषक ग्रहण सार्थक रूप ($P < 0.05$) से अधिक पाए गए। सर्दी महीनों में नियंत्रित समूह (कंट्रोल) व उपचारित समूह W_1 एवं W_2 की औसत वृद्धि दर क्रमशः 410.42, 440.19 और 484.67 ग्राम थी वहीं गर्मी महीनों में नियंत्रित समूह व उपचारित समूह S_1 एवं S_2 की औसत वृद्धि दर क्रमशः 300.11, 332.01, 383.13 ग्राम थी। सर्दी व गर्मी महीनों के दौरान संकर मादा बछड़ों के शारीरिक वजन प्राप्ति पर +250/ +350 मिली. तुल्यांक प्रति किग्रा. शुष्क-पदार्थ डी. सी. ए. डी. खुराक का धनात्मक सार्थक प्रभाव देखा गया। दोनों ऋतुओं में प्लाज्मा मैक्रो-मिनरल्स (K, Ca, Mg, Cl, S), माइक्रो-मिनरल्स (Zn, Cu, Fe) व प्लाज्मा ग्लूकोस स्तर सभी समूहों में सामान्य पाए गए। यद्यपि गर्मी ऋतु में S_2 समूह की कुल प्लाज्मा सोडियम सांद्रता नियंत्रित समूह की तुलना में सार्थक रूप ($P < 0.05$) से उच्च पायी गयी वहीं सर्दी ऋतु में सोडियम स्तर पर कोई प्रभाव नहीं देखा गया। W_2 समूह की रक्त-pH अधिकतम (7.38) थी और नियंत्रित समूह (कंट्रोल) की न्यूनतम (7.33) थी लेकिन गर्मी ऋतु में अधिकतम रक्त- pH S_2 समूह की थी। दोनों ऋतुओं में अलग-अलग डी.सी.ए.डी. खुराक का फ्रैप-विश्लेषण, एस.ओ.डी. और केटालेज-सक्रियता पर कोई प्रभाव नहीं हुआ। सर्दी महीनों में रोग-प्रतिरक्षात्मक घटकों पर कोई प्रभाव नहीं हुआ परन्तु कुल प्लाज्मा इम्युनोग्लोबिंस (मिली.ग्राम/मिली.ली.) में सार्थक ($P < 0.05$) वृद्धि देखी गयी। गर्मी महीने में 350 मिली.तुल्यांक प्रति किग्रा. शुष्क-पदार्थ डी.सी.ए.डी. पोषित समूह में IgG (नेनो.ग्राम/मिली.ली.) और IgM (नेनो.ग्राम / मिली.ली.) स्तर में वृद्धि देखी गयी। सर्दी और गर्मी ऋतु में W_2 एवं S_2 समूह का प्लाज्मा T_3 व T_4 स्तर सार्थक रूप ($P < 0.05$) से उच्च था। प्रयोग की समाप्ति पर, सर्दी ऋतु में प्लाज्मा कोर्टिसोल सांद्रता नियंत्रित समूह व उपचारित समूह W_1 एवं W_2 में क्रमशः 3.67, 3.62 तथा 3.53 नेनो.ग्राम /मिली.ली. थी, और नियंत्रित समूह (कंट्रोल) द्वारा अप्रभावित थी वहीं गर्मी ऋतु में नियंत्रित समूह (कंट्रोल) व उपचारित समूह S_1 एवं S_2 की क्रमशः 4.24, 3.85 तथा 3.79 नेनो.ग्राम /मिली.ली. थी जो कि नियंत्रित समूह (कंट्रोल) की तुलना में सार्थक रूप ($P < 0.05$) से निम्न थी। दोनों ऋतुओं में नाइट्रोजन ग्रहण व संतुलन और मूत्र pH में सार्थक रूप ($P < 0.05$) से बढ़ती हुई डी.सी.ए.डी. खुराक के साथ वृद्धि देखी गयी। ग्रीष्म ऋतु प्रयोग के दौरान, सोडियम व पोटेशियम अंतर्ग्रहण सार्थक रूप ($P < 0.05$) से नियंत्रित समूह (कंट्रोल) की तुलना में उपचारित समूहों में अधिक था, यद्यपि सोडियम व पोटेशियम संतुलन S_2 समूह में अधिक था परन्तु सम्बंधित ऋतु में पोटेशियम संतुलन W_2 समूह में नियंत्रित समूह (कंट्रोल) की तुलना में अधिक था। सर्दी व गर्मी महीनों में धनात्मक डी.सी.ए.डी. खुराक का पोषक पाचकता, Cl, S, Ca तथा P अंतर्ग्रहण व संतुलन पर कोई प्रभाव नहीं हुआ। यह निष्कर्ष निकल गया कि बछड़ों में वातावरण तनाव से राहत दिलाने हेतु धनात्मक डी.सी.ए.डी. एक महत्वपूर्ण कौशल है जो कि पोषक अंतर्ग्रहण, जैव-रासायनिक, रोग-प्रतिरक्षात्मक एवं अन्तः-स्त्रावी घटकों में सुधार करता है।

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LIST OF ABBREVIATIONS

μmole	é .	Micro Mole
μg	é .	Micro Gram
μl	é .	Micro Liter
°C	é .	Degree Centigrade
AAS	é .	Atomic Absorption Spectrophotometer
ADF	é .	Acid Detergent Fibre
ADG	é .	Average Daily Gain
AOAC	é .	Association of Official Analytical Chemists
Ca	é .	Calcium
cm	é .	Centimeter
CoA	é .	Coenzyme A
CP	é .	Crude Protein
Cu	é .	Copper
DCAB	é .	Dietary Cation Anion Balance
DCAD	é .	Dietary Cation Anion Difference
DM	é .	Dry Matter
DMI	é .	Dry Matter Intake
EDTA	é .	Ethylene Diamine Tetra Acetate
EE	é .	Ether Extract
FeCl ₃	é .	Ferric Chloride
FeSO ₄	é .	Ferrous Sulfate
FRAP	é .	Ferric Reducing Antioxidant Power Assay
g	é .	Gram
H ₂ SO ₄	é .	Sulphuric Acid
Hb	é .	Haemoglobin

HCl	é .	Hydrochloric Acid
HClO ₄	é .	Perchloric acid
Ig	é .	Immunoglobulin
IU	é .	International Units
K	é .	Potassium
K ₂ CO ₃	é .	Di-Potassium carbonate
KF	é .	Karan-fries
L	é .	Litre
LCT	é .	Lower Critical Temperature
M	é .	Molar
ME	é .	Metabolizable Energy
mEq	é .	Milliequivalents
mg	é .	Milli Gram
ml	é .	Milli Liter
mM	é .	Milli Mole
N	é .	Normal
Na	é .	Sodium
NaHCO ₃	é .	Sodium Bicarbonate
NDF	é .	Neutral Detergent Fibre
nm	é .	Nanometer
NRC	é .	National Research Council
OD	é .	Optical Density
OM	é .	Organic Matter
P	é .	Phosphorus
ppm	é .	Parts Per Million
ROS	é .	Reactive Oxygen Species

rpm	é .	Revolutions Per Minute
S	é .	Sulphur
SOD	é .	Superoxide Dismutase
TDN	é .	Total Digestible Nutrients
THI	é .	Temperature Humidity Index
TPTZ	é .	Tripyridyl Triazine
U/l	é .	Units/ liter
UCT	é .	Upper Critical Temperature
UV	é .	Ultra Violet
v/v	é .	Volume/ Volume
VFA	é .	Volatile Fatty Acids
w/v	é .	Weight/ Volume
Zn	é .	Zinc

CHAPTER – 1

Introduction

1. INTRODUCTION

Climatic stress is a phenomenon that 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 in order to maintain homeostasis. These reactions include increased/ decreased feed intake, redistributed blood supply and increased evaporative heat loss via increased respiration and sweating. As a result, digestive function, nutrient absorption and metabolism, physiological functions such as acid-base regulation and animal growth get altered. Thermal stress in results in increased demand for net energy for maintenance resulting in subsequent reduction in energy for tissue growth and production (Nisa *et al.*, 1999). Heat stress has negative effect on feed consumption and metabolic activities, whereas in cold stress, animal's maintenance requirement is increased and more energy is converted to heat resulting in less efficient digestion of feed (NRC, 1981). The availability of metabolizable energy for productive processes would be limited unless additional energy is provided to compensate for increased energy demands in cold environment. However, changes in thermal environment also induce alteration in activity and function of the digestive system such as, reduction in rumination and nutrient absorption that are independent of changes in feed intake (Collier *et al.*, 2006). 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.*, 2006). These changes are necessary for adaptability and survivability of the animal. 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, 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. They play a key role in the transfer of fluids from cell to cell,

tissue to tissue and organ to organ which ultimately play a vital role to fulfill the diverse needs of all productive processes of the animals. It has been observed that metabolic or systemic acidosis is aggravated in the hot summer because rapid respiration by animals to dissipate heat reduces carbonic acid concentration in the blood leading to respiratory alkalosis and excessive loss of HCO_3^- in the urine reduces the buffering capacity of the blood. There is loss of K through excess saliva, increased urination and increased sweat which increases the requirement of this mineral in the diet of the animals. Minerals which have a major impact on acid base metabolism are sodium (Na), potassium (K) and chloride (Cl).

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 (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. The calculation of DCAD requires converting the various anions and cations in the diet into milliequivalents (mEq) because acid-base balance is affected by electrical charge rather than mass. The DCAD equation involves subtracting the mEq of anions from the mEq of cations and the result can be positive or negative DCAD. A positive linear relationship exists between blood acid base status and DCAD level.

Optimal DCAD is dependent on the stage of lactation, production levels and weather conditions. The thermal stress imposes some unique metabolic conditions that require elevated DCAD and dietary Na and K specifically. During climatic stress, the best DCAD may be at the upper end of the optimal DCAD range. Serum DCAD increased linearly with increasing DCAD level in the diets and it has a positive association with blood pH thus improving blood buffering capacity (Hu and Murphy, 2004). Blood pH is determined by the number of cation and anion charges absorbed into the blood. It is known that an alteration in blood pH effect the production and effectiveness of insulin (Robertson, 1987), growth hormone (Challa *et al.*, 1993) and parathyroid hormone (Goff and Horst, 1992). Hence, DCAD diet can also influence the altered endocrine system during climatic stress. Supplementation of Na and K in the form of bicarbonate/

carbonate helps in better regulation of acid-base balance in the blood. Positive DCAD based diets can be a useful strategy during thermal stress to increase dry matter intake (DMI) and resultant positive influence on the growth. It is assumed that increased DMI in calves fed high DCAD diet might be due to increased buffering capacity due to alkalogenic nature of high DCAD diet (Sarwar *et al.*, 2007a). Decreased growth rate in calves fed lower or negative DCAD diet might be due to metabolic acidosis induced by negative or low DCAD. High DCAD diets have not only proved to increase DMI, water intake, growth and production, but are also useful in mitigating the effects of summer and winter stress (Sarwar *et al.*, 2007a; Shahzad *et al.*, 2008b). It has been reported that energy and nitrogen balance are also enhanced by the positive DCAD diet (Shahzad *et al.*, 2007; Sarwar *et al.*, 2011).

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. In tropical countries like India, growing calves are well known victims of high and low temperature and humidity and these variations in temperature are likely to affect the DMI, physiological and blood biochemical parameters which ultimately affect the profitability of dairy enterprise. Scientific information regarding alteration of DCAD on performance of crossbred calves in our country is limited. No work has been done on feeding DCAD diets to growing calves for combating climatic stress. NRC (2001) has highlighted the importance of DCAD diet in regard to climatic stress, but there is no clear cut recommendation of its supplementation level. Therefore, the present study has been designed to establish the DCAD level for crossbred calves under winter and summer seasons with the following objectives:

1. Formulation of DCAD based diets for growing animals in summer and winter months
2. To study the effect of different levels of DCAD based diets on nutrient intake, utilization and growth performance of crossbred calves in summer and winter months
3. To observe changes in blood acid-base, minerals and immunity status of crossbred calves fed DCAD based diets

CHAPTER – 2

Review of Literature

2. REVIEW OF LITERATURE

The ability of livestock to breed, grow and lactate to their maximum genetic potential and capacity to maintain health is strongly affected by the thermal environment. Animals are homoeothermic so they have to maintain their temperature within normal range to maintain homeostasis. Climatic stress is one of the major concerns for growing animals, especially in the tropical regions of the world. Hot and humid environmental conditions stress the animals and reduce intake of the nutrients necessary to support growth and maintenance. However despite species and breed, dissipation of heat is obtained by non-evaporative or sensible heat loss (conduction, convection and radiation) and evaporative water loss (cutaneous and respiratory; Johnson, 1987). The immediate response of the animals to climatic stress is reduction of feed intake. If the exposure is prolonged, the reduced feed intake is followed by hormonal changes and decline of metabolic rate and enzymatic activities (Webster, 1983). The entire physiological and metabolic events activated due to the climatic stress lead to alteration in growth, health, production and reproduction. Dietary minerals are integral part of all biological functions in the animal body. They play a key role in the transfer of nutrients from cell to cell, tissue to tissue and organ to organ, thus playing an important role in fulfilling the diverse needs of production, growth and reproduction of animals. In animal body, transfer of different nutrients is achieved through the electrical potential, generated due to the difference in positively and negatively charged particles. The minerals may be cations (positively charged) and anions (negatively charged) and the difference between these cations (Sodium, Potassium) and anions (Chloride, Sulphur) is referred as dietary cation anion difference (DCAD) or dietary cation anion balance (DCAB; Tucker *et al.*, 1992). The DCAD also affects the acid base status which plays a key role in body physiology (Sanchez, 2003). Feeding high DCAD diet to growing calves might be an important nutritional strategy which acts by improving acid base status, digestibility of nutrients and decreasing the climatic stress. The literature on the effect of DCAD in ameliorating climatic stress has been reviewed under the following headings:

- 2.1 Dietary cation anion difference (DCAD)**
- 2.2 Effect of positive DCAD diet on blood acid- base physiology**
- 2.3 Animal responses to heat stress**
- 2.4 Effect of cold stress on animals**
- 2.5 Assessment of climatic stress**
- 2.6 Ameliorative effect of positive DCAD diet on feed intake and utilization during thermal stress**
- 2.7 DCAD based diet effects on various blood parameters**
- 2.8 Effect of DCAD on urinary pH and excretion of minerals**
- 2.9 Effect of positive DCAD diet on growth during thermal stress**
- 2.10 Positive DCAD diet effects on endocrine regulation**

2.1 Dietary cation anion difference (DCAD)

Dietary cation-anion difference (DCAD) is a way to balance the electrical charge of the cations and anions in the diet. These electrical charges affect blood buffering capacity and acid-base status. Blood pH is ultimately determined by the number of cation and anion charges absorbed into the blood. The term dietary cation anion difference is referred to the difference between certain dietary cations and anions (Tucker *et al.*, 1992). Shohl and Sato (1923) were the first to propose that mineral interrelationships were related to acid-base status. Shohl (1939) proposed that maintenance of normal acid-base equilibrium required excretion of excess dietary cations and anions. Minerals interrelationships were found to affect numerous metabolic processes. Leach (1979) and Mongin (1980) concluded that mineral interrelationships have profound influences to maintain acid-base homeostasis. It was found that net acid intake was related to the difference between dietary cations and anions. The monovalent macromineral ions, Sodium (Na), Potassium (K) and Chloride (Cl) were found to be the most influential elements to effect acid base status in poultry (Mongin, 1980), whereas Na, K, Cl and S (Sulphur) were found to be the most influential elements in the case of dairy cattle (Dishington, 1975). Several other minerals have been tested in various equations to verify that acid-base status is the major physiological event controlled by these minerals, or more specifically, acid-base status is

regulated by hydrogen ion (H^+) concentration in blood, which is affected by the buffering capacity of blood or tissue.

Mongin (1981) proposed a three-way interrelationship among dietary Na, K and Cl. He proposed that the sum of Na + K $\bar{}$ Cl [mEq per 100 g dry matter (DM)] could be used to predict net acid intake. This sum commonly has been referred to as the dietary cation-anion balance (Tucker *et al.*, 1988) or dietary electrolyte balance (West *et al.*, 1991). However, Sanchez and Beede (1991) coined the term dietary cation-anion difference (DCAD). Numerous equations have been cited in the literature for calculating DCAD value of the diet by subtracting the amount of various anions from the cations as listed below:

1. DCAD (mEq) = (Na + K) $\bar{}$ (Cl+S) (Ender *et al.*, 1971)
2. DCAD (mEq) = (Na + K) $\bar{}$ Cl (Mongin, 1981)
3. DCAD (mEq) = (Na + K + 0.15 Ca + 0.15 Mg) $\bar{}$ (Cl + 0.20S + 0.30P)
(Horst and Goff, 1997)
4. DCAD (mEq) = (Na + K + 0.38 Ca + 0.30 Mg) $\bar{}$ (Cl + 0.60S + 0.50P)
(Horst and Goff, 1997)
5. DCAD (mEq) = (Na + K) $\bar{}$ (Cl + 0.6S) (Goff *et al.*, 2004)

The calculation of DCAD requires converting the various anions and cations in the diet into milliequivalents (mEq). This is necessary because acid-base balance is affected by electrical charge rather than mass. DCAD is reported in mEq/kg of diet. The DCAD equation involves subtracting the mEq of anions from the mEq of cations and the result can be positive or negative DCAD. If more cations than anions enter the blood from the digestive tract, blood pH will increase. Milliequivalents for a particular element are calculated by dividing its equivalent weight by 1000. Each element is converted to its ionic state by dividing its concentration (%) in the feed by its mEq weight. The factors for conversion of various minerals used to calculate DCAD value of feed to its mEq/kg DM are given in Table 2.1.

By manipulating DCAD, one can promote optimal DMI to keep animals productive and healthy. Optimal DCAD is dependent on the stage of lactation, production levels and weather conditions. The latest equation i.e. (Na + K) $\bar{}$ (Cl + 0.6 S) proposed by Goff *et al.* (2004) discounts the acidifying effects of S by 40%

compared to the original four-mineral equation $(Na + K) \bar{=} (Cl + S)$. The equation, $[(Na + K) \bar{=} (Cl + 0.6S)]$ appears to be the most accurate in predicting blood pH and standard base excess from dietary mineral composition (Goff *et al.*, 2004). Charbonneau *et al.* (2006) conducted a meta-analysis using different DCAD equations and observed that this equation $[(Na + K) \bar{=} (Cl + 0.6S)]$ is most highly associated with clinical milk fever ($R^2=0.44$) and urinary pH ($R^2=0.85$) compared to other equations.

Table 2.1 Calculation of mEq weights of different minerals

Mineral	Atomic weight	Charge	Eq.wt.	mEq wt./1000	Conversion factor (1/ mEq wt.)
Na	23	1	23	0.023	435
K	39	1	39	0.039	256
Cl	35	-1	35	0.035	282
S	32	-2	16	0.016	624

2.2 Effect of positive DCAD diet on blood acid-base physiology

A positive linear relationship exists between blood acid base status and DCAD level. During heat stress, the expiration of carbon dioxide (CO_2) exceeds the rate of its formation in the body. The partial pressure of CO_2 in blood declines, creating a deficit of blood carbonic acid and resulting in respiratory alkalosis. This leads to elevated blood and urine pH and reduced net acid excretion rate. The animals compensate for high blood pH by excreting HCO_3^- ions into the urine. Alkalemia depresses the rate of renal secretion of hydrogen ions, but increases the excretion of filtered HCO_3^- , which subsequently leads to reduction in blood HCO_3^- concentration. During the cooler part of the day, blood and urine pH of cows in heat stress treatments fall below those of their thermoneutral counterparts, and blood HCO_3^- lowers. The lower concentration and pool size of HCO_3^- represent compensated metabolic acidosis because of the loss of the blood base HCO_3^- and a decline in blood pH.

Minerals are important in maintaining acid-base equilibrium. Na and Cl are the major ions found in extracellular fluids. Plasma Na concentration is normally maintained within narrow limits. It is the primary determinant of alterations in

plasma tonicity and is important in the maintenance of acid-base balance in body fluids. The retention of K largely within cells and of Na in extracellular fluids is accomplished primarily by the action of specific ATP-consuming pumps ($\text{Na}^+\text{-K}^+\text{-ATPase}$), located within the cell membrane, that pump K into cells in exchange for Na ions. Whereas Na is actively moved across the intestinal wall by an energy requiring process, Cl and K diffuse from ingesta within the gastrointestinal tract across the intestinal wall, the rate being determined by their relative concentration in ingesta vs. the mucosal cells of the intestinal wall. Thus, when absorption is passive as is the case for Cl and K, the concentration of these ions in the feed and the quantity of feed in the intestine can significantly affect the amount absorbed by the gut. During heat stress and the associated perturbations of acid-base physiology, the demand for cations by the kidney is increased. Because of the alkalemia resulting from respiratory alkalosis, the rate of renal secretion of hydrogen ion is depressed.

It has been found by many researchers that positive DCAD diet i.e. cationic diet affects the blood pH as well as acid-base status (Jackson *et al.*, 1992; Roche *et al.*, 2005). The main reason for increased blood pH and HCO_3^- at high DCAD diet is due to its alkaline properties. Moreover, when the body faces an acid load, it is compensated through respiratory rate that reduced partial pressure of carbon dioxide (pCO_2) and carbonic acid (H_2CO_3) (Hill, 1990). A number of other researchers (Chan *et al.*, 2006; Sarwar *et al.*, 2007a; Shahzad *et al.*, 2007 and Li *et al.*, 2008) have also reported similar findings. Similarly, high DCAD diets decreased blood H^+ and thus resulted in increased blood HCO_3^- (Block, 1994) as blood HCO_3^- responses were inversely related to H^+ changes that reflected the metabolic nature of the acid challenge at low DCAD diet. Hence positive DCAD diet can be a useful strategy to compensate for metabolic acidosis which occurs during climatic stress.

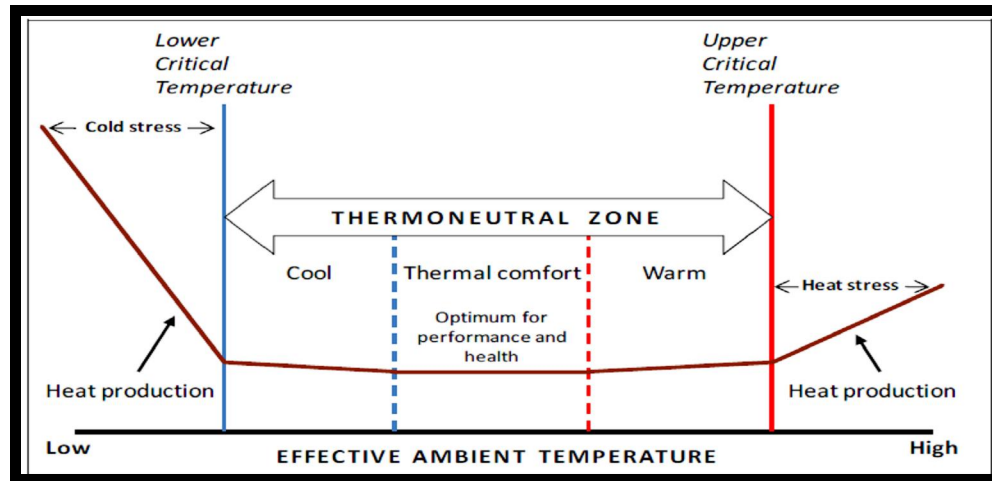
It has also been found that cattle in heat-stress conditions lose K more quickly through increased perspiration, panting and urination, resulting in less blood-buffering capacity by the animal's body. Hot weather lowers DMI, rumination and salivary production, all of which adversely influence production and rumen health. Positive DCAD provides the ration with additional K to replace levels lost through normal daily functions and act as a blood buffer to alleviate the effects of heat stress. K is a critical regulator of sweat glands during heat stress,

helping the animals to keep cool and maintain rumen integrity during heat stress conditions. During heat stress, the best DCAD may be at the upper end of the optimal DCAD range. For example, a ration with 1.5% K, 0.5% Na, 0.3% Cl, and 0.25% S (DM basis) has a DCAD of +36 mEq/100 g of dietary DM. If the Cl and S concentrations exceed those listed above, the first formulation step should be to try to reduce Cl and S concentrations in the basal ration by replacement of high anion-containing ingredients with other ingredients. If this is not possible, inclusion of more Na and (or) K from sodium bicarbonate and (or) potassium bicarbonate can be used to increase ration DCAD.

2.3 Animal responses to heat stress

Climatic stress is a phenomenon that can impart physical and economic losses to livestock production in temperate, sub-tropical and tropical regions of the world. Temperature stressed animals undergo a series of metabolic and physiological changes. These changes are necessary for adaptability and survivability of the animal. Climatic stress has strong impact on growth, productive and reproductive processes of the animals. Stress occurs when an animal experiences changes in the environment that stimulate body responses aimed at re-establishing homeostatic conditions (Mumma *et al.*, 2006). Each homeothermic animal, such as dairy cattle and buffalo, has an optimal ambient temperature zone or the so called "thermoneutral zone" within which no additional energy above maintenance is expended to heat or cool the body (Yousef, 1985). When environmental temperature move out of the thermo-neutral zone (or comfort zone), animals begin to experience either heat stress or cold stress. The thermoneutral zone is bounded by the lower critical temperature (LCT) and the upper critical temperature (UCT; Figure 2.1). The ambient temperatures above the UCT activate the thermoregulatory evaporative heat loss processes (Yousef, 1985) in response to the signals of the hypothalamic thermoregulatory center. As a consequence, the blood flow from internal organs to peripheral tissues increases, sweating and panting increases, and feed intake and nutrient absorption decreases (Shearer and Beede, 1990). LCT is the environmental temperature at which animal need to increase metabolic heat production to maintain body temperature.

Fig. 2.1 The thermoneutral zone showing the lower critical temperature (LCT) and the upper critical temperature (UCT)



Hot and humid environmental conditions stress the animals and reduce intake of the nutrients necessary to support milk yield and body maintenance. These conditions evoke a series of drastic changes in biological functions which include depression in feed intake, efficiency of utilization, disturbances in metabolism of water as well as nutrients including minerals balances, enzymatic reactions, hormonal secretions and blood metabolites, resulting in impairment of growth, production and reproduction performances. Thermal stress is of major concern for growing animals because of the poor growth performance, immuno-suppression and high mortality rate (Nesamvuni *et al.*, 2012).

2.3.1 Feed intake

Voluntary intake of feed tends to decrease as ambient temperature increases and increases when ambient temperatures decreases. Lower feed intake reduced digestive tract motility, ruminal contraction rates, rate of passage of ingesta, increased gut fill and mean retention time (Schneider *et al.*, 1988). Heat stress even reduced the rate of blood flow to the portal-drained viscera (McGuire *et al.*, 1989). Typically, feed intake of cows at 40°C may be reduced by 20-40% compared with that of cows in thermo-neutral environments (Sanchez *et al.*, 1994 and Khan *et al.*, 2012). The reduction in feed intake, with the corresponding decline in heat generated by ruminal fermentation and body metabolism, aids in the maintenance of heat balance (Collier *et al.*, 2006).

Minerals are also more easily depleted during hot summer months. Electrolyte concentrations, particularly Na and K are reduced in ruminal fluid of heat-stressed cattle due to increased loss of Na in the urine and K in the sweat (Silanikove *et al.*, 1998). Due to the summer stress, there is open mouth breathing which results in loss of salivary bicarbonates along with reduced partial pressure of CO₂ resulting in more loss of renal bicarbonates. So overall, there is effect on the ruminal buffering capacity ultimately affecting the feed intake of the animals.

2.3.2 Rumen health

Heat stress also adversely affects rumen health. Cows dissipate heat via panting and this increases respiration rate resulting in enhanced CO₂ being exhaled. Due to the hyperventilation induced decrease in blood CO₂, the kidneys secrete HCO₃⁻ to maintain this ratio. This reduces the amount of HCO₃⁻ that can be used (via saliva) to buffer and maintain a healthy rumen pH (Hill, 1990). In addition, panting cow drool saliva and drooling reduces the quantity of saliva that would have normally been deposited in the rumen. Increased drooling directly increases the risks of rumen acidosis and indirectly enhances the risk of negative side effects of an unhealthy rumen i.e. laminitis, milk fat depression, etc. (Kadzere *et al.*, 2002). Furthermore, due to reduced feed intake, heat-stressed cows ruminate less and therefore generate less saliva. The reduction in the amount of saliva produced and salivary HCO₃⁻ content and the decreased amount of saliva entering the rumen make the heat stressed animals much more susceptible to sub-clinical and acute ruminal acidosis (Marai and Haezeb, 2010).

2.3.3 Sweating

Sweating and panting provide evaporative cooling, one of the most effective ways to lower the cow's body temperature. These cooling mechanisms change the animal's mineral requirements, and so one should change the mineral content of the diet to compensate for the effects of heat stress. Unlike humans, who lose Na mostly in sweat, cattle secrete primarily K when they sweat. Animal's large surface area actually allows for significant sweating during heat stress, and K losses via sweat can be substantial. When cattle are heat stressed,

mineral losses occur by sweating mainly as potassium carbonate and potassium bicarbonate (Gaughan and Mader, 2009).

2.3.4 Growth

There appears to be several factors contributing to slower growth and smaller body size, including greater maintenance requirements during hot weather, poor appetite and lower quality forages which are influenced by the same environmental conditions that slow down growth in animals (NRC, 2001). When growing animals suffer from heat stress, average daily gain is lowered and gross efficiency of converting nutrients to tissue is reduced (Ames *et al.*, 1980). Heat stress also affects body composition and biometric parameters of growing heifers (Lacetera *et al.*, 1994). Exposure of pregnant dairy cows to high ambient temperature influences growth and development of foetus (Collier *et al.*, 1982). Baccari *et al.* (1983) observed increase in body temperature, reduced feed intake, growth rate, plasma triiodothyronine and growth hormone in heat stressed growing dairy heifers. Young Holstein Friesian calves exposed to heat stress showed significant reduction in body capacity and impairment of growth of anatomical parts (Lacetera *et al.*, 1994). The decline in thyroid hormones in the heat stressed buffaloes may be responsible for the decline in daily body weight gain with elevated temperature (Habeeb *et al.*, 2007). Heat stress not only affects the growth of the calves but also deteriorates their health (Shahzad *et al.*, 2007 and Hill *et al.*, 2011).

2.3.5 Respiratory alkalosis

With increased respiration, the expiration of CO₂ exceeds the rate of its formation in the body. The partial pressure of CO₂ of blood declines creating a deficit of blood carbonic acid and resulting in respiratory alkalosis (Wildman *et al.*, 2007). The cow compensates for the high blood pH by excreting HCO₃⁻ ions in the urine (Hu and Murphy, 2004). Alkalemia depresses the rate of renal secretion of hydrogen ions, but increases the excretion of filtered HCO₃⁻, which subsequently leads to a reduction in blood HCO₃⁻ concentration. A more normal ratio of HCO₃⁻ to pCO₂, and H₂CO₃ to HCO₃⁻ required maintain normal blood pH, but in heat stress, acid base status of the animals get altered due to loss of

filtered HCO_3^- , a compensatory response to the respiratory alkalosis (Block, 1994 and Spanghero, 2004).

2.3.6 Renal compensation

During heat stress and the associated perturbations of acid-base physiology, the demand for cations by the kidney is increased. In order to correct the acid-base imbalance, excretion of the HCO_3^- anions in the urine increases dramatically (West, 2003). Because of the alkalemia resulting from respiratory alkalosis, the rate of renal secretion of hydrogen ion is depressed. The excretion of HCO_3^- must be accompanied by the excretion of a cation either Na or K; however, excretion of Na is more as compared to K (Kadzere *et al.*, 2002). During heat stress, urinary concentration of K declines (El-Nouty *et al.*, 1980). Plasma K concentration is decreased, which might reflect a need for K in other processes, such as sweating (Mallonee *et al.*, 1985).

Nisa *et al.* (1999) noted that, during prolonged heat stress, plasma aldosterone concentration of Holstein cows declined. This decline was associated with reduced concentrations of Na and K in blood serum and K in urine. However, urinary Na excretion increased, perhaps to aid in the conservation of K. Urinary excretion of Na increased 80% in a hot environment compared with excretion in a cooler environment, but urinary excretion of K increased only by 18% (West *et al.*, 1991). The increased demand of Na for renal excretion and of K for sweating is consistent with the idea that dietary requirements of these minerals increase during heat stress (Marai and Haebe, 2010).

2.3.7 Hormonal changes

Hormonal changes that occur in response to heat stress may play an integral role in the decline in growth and productivity. Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma glucocorticoid concentrations are perhaps two of the most important responses of the animals to climatic stressful conditions. Adrenal corticoids, mainly cortisol, elicit physiological adjustments that enable animals to tolerate stressful conditions. In other words, it is thermogenic and consequently reduces adrenocortical activity, under thermal stress (Colleir *et al.*, 1982). Therefore,

cortisol is involved in adaptation to short and long term heat stress (Norman and Litwack, 1987 and Beraidinell *et al.*, 1992). However, the effects of high temperatures on cortisol levels are inconsistent (Correa-Calderon *et al.*, 2004). Concentration of cortisol is altered by acute and chronic heat exposure (Christison and Johnson, 1972) and by changes in photoperiod (Leining *et al.*, 1980).

In Friesian calves, plasma cortisol concentration increased from 11 to 29 ng/ ml when exposed to direct solar radiation of the hot summer (Yousef *et al.*, 1997). Chronic thermal stress decreased cortisol level by 45% in heat stressed Friesian calves (Marai and Haebe 2010). Abily *et al.* (1975) found that cortisol level decreased during prolonged heat exposure after a temporary increase in the beginning of heat stress.

Magdub *et al.* (1982) reported that during heat stress, there was significant reduction in concentrations of triiodothyronine (T_3) and thyroxine (T_4) in plasma and in milk of lactating cows. In young calves (aged 6 months), acute heat exposure (33-43°C, 40-60% RH) induced decreases in plasma T_3 (35.25%) and T_4 (17.59%) concentrations (Nessim, 2004). However, a significant increase in T_3 but not in T_4 level was observed during heat stress in crossbred cattle (Singh *et al.*, 1984). The thyroid hormones, T_3 and T_4 decline in response to heat stress which is probably an attempt to reduce metabolic heat production in the animal. The decline in T_3 in heat stressed buffaloes may be responsible for the decline in growth performance during the hot season (Habeeb *et al.*, 2000 and Habeeb *et al.*, 2007).

Mader and Kreikemeier (2006) observed that the levels of T_3 and T_4 were changed with changing environmental conditions in cold stress, gastrointestinal tract motility increases in cattle due to an elevated metabolic rate, resulting from an increase in thyroid hormone activity and feed intake is often enhanced in cattles exposed to cold environment. However, in heat stress, reduced metabolic rate compromised productive capability (Marai and Haebe, 2010).

Corticotropin-releasing hormone stimulates somatostatin release from the hypothalamus, which can inhibit secretion of somatotropin (ST) and thyroid stimulating hormones from the pituitary and down-regulate the thermogenic effects of both ST and thyroid hormones. Plasma growth hormone concentration and growth hormone secretion rate declined with hot temperatures (35°C)

(Mitra *et al.*, 1972). Growth hormone content in the milk of low, medium, and high production groups declined when THI exceeded 70, possibly reflecting suppressed production of growth hormone so that metabolic heat production is reduced (Igono *et al.*, 1985). Plasma growth hormone reductions that occurred in heat-stressed cows did not occur in thermoneutral conditions (McGuire *et al.*, 1991). The change in plasma growth hormone level was probably, a response to the effects of high temperature rather to reduced nutrient intake.

El Nouty *et al.* (1980) documented the simultaneous relationship between plasma aldosterone level and urine electrolyte concentration in bovines during thermal stress. During prolonged heat exposure, plasma aldosterone level was reported to decline, concurrent with this, there was a significant fall in serum and urinary K^+ (Marai and Haezeb, 2010).

2.3.8 Metabolic changes

Cells generate small amounts of free radicals or reactive oxygen species (ROS) during their normal metabolism. Although low levels of ROS are essential in many biochemical processes, accumulation of ROS may damage biological macromolecules i.e. lipids, proteins, carbohydrates and DNA (Kumar *et al.*, 2011a). External factors such as heat, trauma, ultrasound, infections, radiations, toxins, etc. can lead to increased free radicals that may lead oxidative stress (Halliwell *et.al.*, 1992). Altan *et al.* (2003) demonstrated that heat stress increased lipid peroxidation which was associated with production of large number of free radicals capable of initiating peroxidation of polyunsaturated fatty acids. Heat stress may lead to increased production of transition metal ions (TMI), which can donate electron to oxygen forming superoxide or H_2O_2 which is further reduced to an extremely reactive OH radical causing oxidative stress (Agarwal and Prabhakaran, 2005).

2.3.9 Immunological changes

High environmental temperature not only affects performance parameters, but also various physiological (Koelkebeck and Odom, 1995; Maak *et al.*, 2003; Tao *et al.*, 2006 and Star *et al.*, 2008) and immunological parameters (Mashaly *et al.*, 2004 and Star *et al.*, 2009). Immune cells intentionally generate free radicals in an effort to control invading pathogens - a phenomenon which is known as a

respiratory burst. These reactive species are bundled within white blood cells, in an effort to subject pathogens to an unfavorable environment within the animal. Immune cells are particularly sensitive to oxidative damage because their cell membrane contains high concentration of polyunsaturated fatty acids (PUFA) which is very susceptible to peroxidation (Spears and Weiss, 2008).

Cook *et al.* (2002) reported that prolonged exposure to severe heat stress is responsible for a decline of immune cells' reactivity, which may contribute to the higher occurrence of infections during summer. In calves during hot weather, less serum IgG was observed in the summer compared to spring season (West, 2003). White blood cells (WBCs), red blood cells (RBCs) and globulin as indicators of body immunity are, in general, adversely affected by exposure to heat stress (Marai and Haezeb, 2010). Stressors have been associated with increased circulatory concentration of glucocorticoids. They have also been linked with decreased functioning of the cells of the immune system. Excessive production of ROS due to heat stress renders harmful effect on the cells of immune system. The steroids that suppressed pituitary hypophyseal axis (PHA) induced lymphocyte proliferation in a concentration-dependent manner. Rozell *et al.* (1992) concluded that antibodies against cortisol are capable of reducing bioactivity of steroids that strongly suppressed lymphocyte proliferation. Heat stress reduced serum IgG1 in calves has been associated with an increased cortisol concentration transfer (Olsen *et al.*, 1980).

Extreme cold stress also reduced colostral immunoglobulin transfer. Thus, environmental extremes can influence disease resistance in dairy calves (Kumar *et al.*, 2010) thereby leading to reduced growth.

2.4 Effect of cold stress on animals

The performance, health and well being of cattle are strongly affected by climate. Cold stress (below the thermoneutral zone) increased maintenance requirement of livestock (Todini, 2007). At ambient temperature below the lower limit of the thermoneutral zone, animals convert more energy to heat and digest the feed less efficiently (NRC, 1981). Unless additional energy is provided to compensate for increased energy demands in cold environment, the availability of metabolizable energy (ME) for productive processes would be limited (McBride and Christopherson, 1984). In addition, changes in the thermal environment

caused by variations in temperature, wind, humidity, precipitation and radiation induce a variety of physiological responses in animals (Mader, 2003). Hypothermia occurs when the body temperature drops well below normal. In general terms, with cattle, mild hypothermia occurs with a body temperature of 30-32°C (86-89°F), moderate hypothermia at 22-29°C (71-85°F) and severe hypothermia below 20°C (68°F) (Shafie *et al.*, 1994; Marai *et al.*, 1997 and Marai *et al.*, 2000). As rectal temperature drops below 28°C (82°F), cows are not able to return to normal temperature without assistance through warming and the administration of warm fluids. As hypothermia progresses, metabolic and physiological processes slow down and blood is diverted from the extremities to protect the vital organs. Teats, ears and testes are prone to frostbite. In extremes, respiration and heart rate drop, animals lose consciousness and die. Environmental changes evoke predictable responses in the nervous, circulatory, renal and endocrine systems that allow the animals to adjust to the altered environment (Abdel- Hafez, 2002).

2.4.1 Nutrient intake and digestibility

It has been found that when temperature is in the range of 5-15°C, the feed intake is stimulated 2-5%, whereas the feed intake is reduced by 3-10% in the temperature range from 25-35°C (Kadzere *et al.*, 2002). There is a marked depression in the feed intake when temperature is >35°C with high humidity (NRC, 2001). Feed intake increases when ambient temperature drops below the LCT of the animal. The increased DMI under prolonged cold exposure conditions has been attributed to increased ruminal rate of passage as a result of enhanced gastrointestinal tract motility (Bernier *et al.*, 2012). Johnson *et al.* (1987) mentioned that in cold weather, animals show a higher appetite but the utilisation of feeds was lowered by 10%, with possible effect of the rumination activity. Graham *et al.* (1982) reported that shorn sheep exposed to cold stress during grazing increased feed intake by 20-40% to compensate for heat loss, this response is directly related to the activity of the thyroid gland (Todini, 2007). The elevation of thyroid activity results in increased rumino-reticulum motility and higher rate of passage of digesta (Gonyou *et al.*, 1979 and Shahzad *et al.*, 2011). In contrast, cows consuming only range forage ate less at lower temperature when compared to those kept at normal temperature (Adams *et al.*, 1986).

Further, a 44% reduction in feed intake was observed between 2-5 days interval when temperature averaged about -15 and -27°C (Adams *et al.*, 1986). Kartchner, (1996) also found that intake of cows grazing on winter forage was below maintenance during harsh than during mild weather. Nicholason *et al.* (1980) reported reduced feed digestibility in steers exposed to cold temperature. Graham *et al.* (1982) indicated that cold temperature increased both faecal and urinary energy losses, resulting in decreased ME availability. It is generally accepted that cold exposure decreases digestibility of feed in confined ruminants (Kennedy *et al.*, 1982; Adam *et al.*, 1986 and Mader, 2003).

2.4.2 Passage rate

Feedlot cattle exposed to cold temperature showed increase in the rumination activity (Nisa *et al.*, 1999). Various authors associated this increase in the rumination to higher rate of passage of digesta through the gut and subsequent decrease in nutrient digestibility (Sarwar *et al.*, 1996 and Nisa *et al.*, 2004). Increased rumino-reticulum motility during cold exposure enhances the rate of passage of small particles from the rumen by promoting their mixing, sorting and fluid propulsion (NRC, 2001 and Shahzad *et al.*, 2011). Nicholson *et al.* (1980) reported that cold exposure reduces the digestibility of long, chopped and ground-pelleted forms of hay, suggesting the rate of particle size reduction for the long and chopped hay might have been enhanced by cold exposure. Cold exposure might alter chemoreceptor reflexes by influencing ruminal blood flow and hence absorption. There is evidence for enhanced blood flow to the ruminant's digestive tract during moderate cold stress, which may enhance volatile fatty acid (VFA) absorption from the rumen (Nisa *et al.*, 1999). This response is relative to the greater release of ruminal VFA in to the portal blood. Kennedy *et al.* (1982) reported that lowering the concentration of ruminal VFA would reduce one of the inhibitory inputs to the gastric centre which consequently results in increased motility.

2.5 Assessment of climatic stress

Both increased and decreased environmental temperatures adversely affect physiological variables like rectal temperature, pulse and respiration rate. Increased ambient temperature during heat stress increased respiration

frequency, pulse rate and rectal temperature which in turn impose higher energy demand upon the animal body. Increased respiration rate is the first reaction when animals are exposed to environmental temperature above the thermo-neutral zone (Moran, 1999). During summer, the respiration rate is higher than in winter, whereas high humidity interferes in evaporative heat loss and enhances stress level which results in alteration in physiological responses (Abi-Saab and Saleim, 1995). The rectal temperature is an indicator of thermal stress and may be used to assess the adversity of the thermal environment (Silanikove, 2000).

Deviation from the normal rectal temperature has been considered as an index of discomfort. Body temperature indicates the extent to which the thermoregulatory mechanism has been successful in maintaining a thermal balance. Soly (2001) reported an increase in rectal temperature as the ambient temperature increased in both group of animals maintained inside and outside the shelter and the observations recorded during morning and evening were found to be significantly ($P < 0.05$) different. The rectal temperature is often used as a representative measurement of animal core temperature, for practical purposes. Cattle are homoeothermic so they strive to maintain their body temperature within a fairly narrow range, even under adverse climatic conditions. An increase in the ambient air temperature from 18 to 35°C is accompanied by significant increases in rectal temperature (Shafie *et al.*, 1994; Marai *et al.*, 1997 and Marai *et al.*, 2000). Rectal temperatures of 42°C and above are considered as dangerous.

The physiological parameters were markedly lower during winter than during summer (Marai *et al.*, 1997). When temperature starts to decline in winter, particularly when it gets closer to 0°C (or 32°F), it has an adverse effect on cattle's productivity and efficiency. If exposed to more cold, the internal mechanisms are unable to replenish the heat that is being lost resulting in a drop in core temperature. The pulse rate reflects primarily the homeostasis of circulation along with the general metabolic status. The pulse and respiration rate increases on exposure to high environmental temperature and decrease during low environmental temperature.

2.5.1 Importance of temperature humidity index (THI)

The degree of heat stress on the cattle and buffalo can be estimated by the THI. THI is the sum of the effects of ambient air temperature and relative humidity (LPHSI, 1990; Marai *et al.*, 1997 and Marai *et al.*, 2000). THI also offers a method of combining two of the more important and easily measured weather factors into a possible measure to compare temperature and humidity data and animal response at different locations. There are different formulas to calculate THI, for example: $THI = 0.72 (T_{db} + T_{wb}) + 40.6$ (NRC, 1981), with T_{db} and T_{wb} in degrees Celsius. The temperature humidity index threshold for dairy cattle has been calculated to be 72 (Thom, 1959), however, for high producing dairy cows, this value has been reduced to 68 (Zimbelman *et al.*, 2009) because of the concurrent rise in metabolic heat production which is associated with the vast amount of milk synthesis (Hansen, 2007). LPHSI (1990) quantified heat stress, as, up to 72= no heat stress, 72-74= moderate heat stress, 74-78= severe heat stress and 78 and more= very severe heat stress. Practically, the increase of THI in a range of 72 to 81 reduces milk yield and total digestible nutrient (TDN) intake, in dairy cows. The effect was greatest when THI exceeded 76 (West, 1993).

2.6 Ameliorative effect of positive DCAD diet on feed intake and utilization during thermal stress

There are various strategies to minimise the effect of climatic stress like, physical modifications of environment, genetic modification of breeds and nutritional management (Beede and Collier, 1986). Various nutritional tools have been used to improve productive and reproductive performances. The difference between certain minerals called as DCAD plays a pivotal role in ameliorating thermal stress as some unique metabolic conditions caused due to thermal stress require elevated DCAD. Several nutritional studies have been suggested, with variable success, to maintain the buffering capacity in the rumen and blood of ruminants consuming high concentrate diets. Among these, the addition of buffers such as sodium bicarbonate ($NaHCO_3$) does not only prevent the reduction in ruminal pH (Owens *et al.*, 1998) but also improves blood buffering capacity (Sarwar *et al.*, 2007b and Shahzad *et al.*, 2007). The favourable effects of $NaHCO_3$ supplementation in intensive ruminant farming systems are well documented in the literature (Belibasakis and Triantosa, 1991; West *et al.*, 1991).

and Sanchez *et al.*, 1997). Keeping this in view, the use of NaHCO_3 as cationic salt in DCAD studies, has been realized by several researchers to divert the electrical composition of diet towards more alkaline so as to counteract the slight ruminal acidosis which animals may experience when fed on nutrient rich diet as well as during thermal stress.

2.6.1 Nutrient intake

Heat stress reduces voluntary feed intake. It has been observed that DCAD diet mainly cationic diet positively affects the feed intake as well as nutrients digestibility. Tucker *et al.* (1992) studied the DCAD effect in heifers and mature cows and observed increase in feed intake of cows with positive DCAD value. The plausible explanation for decrease in DMI in cows fed low DCAD diet was due to un-palatibility of anionic salts used to reduce the DCAD level. Oetzel and Barmore (1993) also observed an increasing trend in DMI as DCAD was increased from -109 to +313 mEq/ kg DM. The addition of anionic salts in low DCAD diets decreased palatability and caused slight metabolic acidosis; ultimately affecting the nutrient intake (Block, 1994; Oetzel and Barmore, 1993; Vagnoni and Oetzel, 1998). Increased DMI in high DCAD fed animals might be attributed to increased blood HCO_3^- , acid base balance (Sanchez and Beede, 1994) and rumen pH (Tucker *et al.*, 1991c).

West *et al.* (1992) observed increased feed intake when DCAD level was increased from 12 to 46 mEq/100g DM in heat stressed cows. Jackson *et al.* (1992) reported a quadratic response in feed consumption in dairy calves fed diets containing DCAD, 0, 21, 37 and 52 mEq/100 g DM. Minimum (3.79 kg/d) and maximum (4.41 kg/d) feed intake was observed in calves fed 0 and 37 mEq/100 g of DM, respectively, while a significant reduction (3.93 kg/d) was observed at 52 mEq/kg DM. A linear increase in DMI and DMI/100 kg with greater DCAD has been observed by many researchers (Delaquis and Block, 1995a; Spanghero, 2004; Hu *et al.*, 2007; Wildman *et al.*, 2007 and Apper-Bossard *et al.*, 2009). An increased DCAD from negative or null to highly positive values increased DMI and milk yield (Hu and Murphy, 2004 and Apper-Bossard *et al.*, 2009).

Shahzad *et al.* (2007) opined that increase in DMI with increase in DCAD might be due to increased rumen pH making the ruminal environment alkaline,

which is a pre-requisite for optimum ruminal microbial activity. Lambs fed diet with +330 DCAD value consumed 27.68% more feed than those fed -110 mEq/kg DCAD diet and a constant increase in CP and NDF intakes was also observed with increasing DCAD level of the diet (Sarwar *et al.*, 2007b). Buffaloes fed +33 DCAD concentration consumed 27.1% more DM than those fed -11 DCAD concentration, however, DMI difference in buffaloes fed +22 and +33 DCAD was non-significant (Shahzad *et al.*, 2008b). Hersom *et al.* (2010) found that DMI (% of BW) was 11% greater for high-DCAD (19.73 mEq/d) compared with low-DCAD (-5.6 mEq/d) fed cows.

Sarwar *et al.* (2011) reported that nutrient intake increased ($P < 0.05$) with increasing levels of dietary NaHCO_3 , minimum (6.23 kg/d) and maximum (7.45 kg/d) DM intakes were recorded in calves fed no NaHCO_3 and 1.6% NaHCO_3 diets, respectively. The crude protein (CP) consumption was highest (1176 g/d) for calves fed the 1.6% NaHCO_3 diet and lowest (984 g/d) in calves fed the no NaHCO_3 diet. A constant increase in CP and neutral detergent fibre (NDF) intake was observed with increased DCAD level of diet, calves fed +330 and +110 mEq/kg DCAD diets consumed maximum (836.13 g/d) and minimum (681.8 g/d) CP, respectively (Shahzad *et al.*, 2007). Calves fed +110 and +220 mEq/kg had 681.8 and 807.7 g/d CP intake, respectively (Shahzad *et al.*, 2007). Shahzad *et al.* (2011) reported that the DMI in buffaloes fed on high cationic (+220 mEq), low cationic (+110 mEq), low anionic (-110 mEq) and high anionic (-220 mEq) diets were 13.95, 13.20, 12.51 and 12.09 kg/day, respectively. Maximum and minimum DMI was recorded in buffaloes fed on high cationic and high anionic diets. Luebke *et al.* (2011) reported that DMI increased linearly as DCAD increased and it was greatest for steers consuming the basal diet (+8 mEq/100 g DM), least for -32 mEq/100 g DM, and intermediate for -2 and -12 mEq/100 g DM. Biological studies executed under different environment to know response of DCAD on ruminant animal performance reflected that positive DCAD can enhance nutrients intake due to its favourable influence on rumen dynamics and blood chemistry. However, the extent of nutrients intake varies depending on the level of DCAD, diet composition, animal productive potential and environment.

2.6.2 Nutrient digestion

Rogers *et al.* (1982) reported that DM digestibility increased in cows when fed 2.0% NaHCO₃ in a mixed ration of 75% concentrate and 25% corn silage diet. Snyder *et al.* (1983) also pointed out improved DM, OM, ADF and NDF digestibility in dairy animals when NaHCO₃ was supplemented in their diets. Stokes and Bull (1986) observed that supplementation of NaHCO₃ in corn silage based diets enhanced the DM, OM, ADF, gross energy and cellulose digestibility in early lactating cows.

Sarwar *et al.* (2007b) also found increased digestibility of DM, NDF and CP in lambs fed -110 compared to those fed +110, +220 and +330 mEq/kg DCAD diets. The digestibility of DM, ADF, NDF and CP was found to increase slightly in buffaloes fed -11 DCAD compared to those fed +11, +22 and +33 mEq/100 g DCAD concentrations (Shahzad *et al.*, 2007). Sarwar *et al.* (2011) reported that DM, CP, NDF and ADF digestibility decreased linearly with increasing levels of NaHCO₃ in the diet and highest DM and CP digestibility were recorded for calves fed 0% NaHCO₃ and lowest for 1.6% NaHCO₃ fed group. Increased digestibility of nutrients in negative DCAD diet might be attributed to increased rumen retention time of nutrients due to reduced DMI, because a positive correlation between nutrient digestibility and rumen retention time has been well documented (Sarwar *et al.*, 1996; Nisa *et al.*, 2004 and Nisa *et al.*, 2006).

However, Tucker *et al.* (1991c) and Delaquis and Block (1995a) did not find any significant effect of varying DCAD (481 and 327 mEq/kg DM) on NDF and ADF digestibility in dry cows. Shahzad *et al.* (2008b) also reported that the DCAD concentrations did not alter the digestibility of DM, NDF, ADF and N in cows which might be attributed to the speculation that DCAD had no significant effect on ruminal fermentation pattern in lactating cows.

Alkaline nature of high DCAD diet or sodium bicarbonate feeding may be used as a nutritional tool to enhance rumen ecology aimed to utilize nutrients more efficiently, however, its response in terms of nutrients utilization depends on the nature of diet, rumen dynamics (which may differ with age) and productive potential etc.

2.6.3 Energy balance

Vicini *et al.* (1988) observed that sodium bicarbonate supplementation in cows fed control rations (50% corn silage and 50% concentrate) improved energy balance than those cows which were fed control rations only. The improved energy balance (21.5 vs. 15.5 Mcal ME/d) in sodium bicarbonate supplemented cows was due to increased DMI. Moore *et al.* (2000) investigated the effect of various DCAD levels in heifers and reported that energy balance was higher (3.75 Mcal/d) in heifers fed +15 mEq/100 g DM DCAD diet than those (0.09 Mcal/d) fed -15 mEq/100 g DM DCAD diet. Moore *et al.* (2000) also observed that prepartum cows fed high DCAD level (+15 mEq/100 g DM) showed better (8.42 Mcal/d) energy status when compared (6.01 Mcal/d) with cows fed low DCAD level (-15 mEq/100 g DM). The decreased energy balance at low DCAD diet was due to reduced DMI. High DCAD diet increased rumen pH (Tucker *et al.*, 1988) that eventually increased DMI leading to improved energy balance. Apper-Bossard *et al.* (2009) also reported that energy balance was increased by increasing DCAD due to increased DMI.

2.6.4 Nitrogen balance

The DCAD had no effect on nitrogen intake and excretion in early lactating cows (Delaquis and Block, 1995b) on two DCAD levels (258.1, 55.5 mEq /Kg DM) resulting in equal absorption of N. During mid lactation, intake, absorption, retention and balance of N increased in animals fed high DCAD diets that might be associated with increased feed intake. No effect of DCAD on N balance was observed during late lactation, which revealed that acid base perturbation was not sufficient to affect the protein metabolism (Phromphetcharat *et al.*, 1981; Welbourne *et al.*, 1988; Delaquis and Block, 1995 a,b).

DCAD had no significant effect on N intake, digestibility and retention in dry cows (Delaquis and Block, 1995 a,b), which might be due to small difference in DCAD level used in their study. Delaquis and Block (1995c) observed that urine N was not affected from DCAD levels in cows fed alfalfa hay and corn silage based diets, though slight increase was observed in cows fed alfalfa hay based diets. The probable reason for this might be attributed to higher N content

of alfalfa hay than corn silage. Shahzad *et al.* (2007) noticed increase in nitrogen intake and balance with increasing DCAD level of the diet. Shahzad *et al.* (2011) reported that the faecal and urinary N excretion increased with increasing DCAD levels. Sarwar *et al.* (2011) also reported that N intake increased with increasing NaHCO_3 level, consequently, N intake was the highest (188.1 g/d) in the calves on the 1.6% NaHCO_3 diet and lowest (157.5 g/d) on the 0% NaHCO_3 diet, calves fed the 1.6% NaHCO_3 diet retained more N than those on the other diets, resulting in the highest N balance in calves.

2.7 DCAD based diet effects on various blood parameters

2.7.1 Blood acid-base status

Metabolic or systemic acidosis is aggravated in the hot summer because rapid respiration by calves to dissipate heat reduces carbonic acid concentration in the blood leading to respiratory alkalosis and excessive loss of HCO_3^- in the urine reduces the buffering capacity of the blood. A positive linear relationship exists between blood acid base status and DCAD level. Jackson *et al.* (1992) observed that blood pH and HCO_3^- were lower in calves fed 0 DCAD diet than those fed 21, 37 and 52 mEq/100 g of DM DCAD diets. Increased blood bicarbonate concentration has been reported in response to increasing DCAD in the diet of growing beef steers (Ross *et al.*, 1994a). High DCAD diets decreased blood H^+ and thus resulted in increased blood HCO_3^- (Block, 1994). Cows fed low DCAD diets (-40, - 51 and -63 mEq/kg of DM) had reduced blood pH than those fed high DCAD diet (+203 mEq/kg of DM), though the difference was not significant (Vagnoni and Oetzel, 1998).

Moore *et al.* (2000) reported that blood pH and HCO_3^- were lower in cows and heifers fed -15 DCAD diet than those fed 0 DCAD diet. Similarly, Roche *et al.* (2005) also reported that by increasing DCAD from 23 to 88 mEq/100 g of DM, blood pH and HCO_3^- concentration increased in early lactating cows. Similar findings have been reported by various other researchers (Chan *et al.*, 2006; Sarwar *et al.*, 2007a; Shahzad *et al.*, 2007 and Li *et al.*, 2008). Hersom *et al.* (2010) observed that a treatment x day interaction existed for blood pH and high DCAD (19.73 mEq/d) fed cows, and blood pH was 0.05 and 0.04 units greater than low-DCAD fed cows (-5.6 mEq/d) on 21 and 42 d, respectively. Similarly Sarwar *et al.* (2011) also reported that blood pH was the lowest (7.26) in calves

fed the 0% NaHCO_3 diet and highest (7.57) in those fed the 1.6% NaHCO_3 diet, an increase in blood HCO_3^- concentration in calves was also recorded with increase in NaHCO_3 concentration in the diet. Sahazad *et al.* (2011) studied that blood pH increased linearly with increase in DCAD level; the values being 7.43 and 7.34 in buffaloes fed on high cationic and high anionic diets, respectively, similarly, blood HCO_3^- concentration in buffaloes also increased from 23.12 to 27.52 mmol/l with increase in DCAD.

The main reason for increased blood pH and HCO_3^- at high DCAD diet is due to its alkaline properties and a gradual reduction of dietary anions particularly chloride. Chloride is absorbed from the posterior part of the intestine in exchange of HCO_3^- when excess of Na^+ , to maintain electrical neutrality, thereby resulting in reduced blood HCO_3^- and increased H^+ . Blood HCO_3^- responses were inversely related to H^+ changes that reflected the metabolic nature of the acid challenge at low DCAD diet. Eventually, the greater DCAD concentration increased the blood HCO_3^- and reduced H^+ , the reverse was true for negative DCAD concentrations (Block, 1994).

2.7.2 Plasma Calcium

Plasma Ca concentration was higher in prepartum cows fed low DCAD diet compared to those fed high DCAD diet (Tucker *et al.*, 1992). Cows fed anionic diets prepartum showed higher levels of Ca in postpartum period (Joyce *et al.*, 1997). Kumar *et al.* (2004) observed that there is significantly higher level of plasma Ca from the day of parturition to 30 days after parturition, in the anionic group as compared to cationic group. Feeding anionic diets before and after parturition improved total Ca in cows (Kumar *et al.*, 2004; Li *et al.*, 2008 and Wu *et al.*, 2008). Improved plasma Ca concentration is due to increased Ca absorption, Ca mobilization from bones and renal reabsorption of Ca due to slight metabolic acidosis induced by low DCAD diet (Shahzad *et al.*, 2008a). Sarwar *et al.* (2011) reported that serum Ca increased with decreasing NaHCO_3 levels in the diet. Serum Ca concentration increased linearly as the DCAD level decreased, it was 10.98 mg/dl in buffaloes fed on high anionic diet and 9.43 mg/dl in buffaloes fed on high cationic diet (Shahzad *et al.*, 2011). Feeding a low DCAD diet increased the flow of Ca through the readily exchangeable Ca pool (Takagi and Block, 1988) which increased ionized Ca concentration in the blood.

The improved Ca status of the animal helps in reducing the occurrence of milk fever at the time of calving.

2.7.3 Plasma Sodium and Potassium

Fredeen *et al.* (1988b) noticed that plasma Na concentration increased as DCAD was increased in the diet. Serum Na concentration was elevated in cows receiving high DCAD, probably because the high DCAD diets contained higher Na concentrations (Wildman *et al.*, 2007). Shahzad *et al.* (2011) observed that serum Na concentration increased linearly with increase in the DCAD levels, it was maximum (144.84 mEq/l) in buffaloes fed on high cationic (+22 mEq/100 g DM) diet and minimum (138.57 mEq/l) with high anionic diet (-22 mEq/100 g DM). Similarly Sarwar *et al.* (2011) reported that serum Na concentration increased ($P < 0.05$) with increasing levels of NaHCO_3 in the diet while K, Cl, S and Mg concentrations remained unaltered. The higher serum Na^+ content was primarily due to the higher dietary concentration of this mineral, which significantly altered the serum cation-anion balance. Serum DCAD ($\text{Na}^+ + \text{K}^+$) - ($\text{Cl}^- + \text{S}^-$) increased linearly with increasing DCAD level in the diets, it has a positive association with blood pH thus improving blood buffering capacity (Tucker *et al.*, 1988; Hu and Murphy, 2004; Sarwar *et al.*, 2007a and Shahzad *et al.*, 2008b). These results are inconsistent with West *et al.* (1991) who observed that serum Na^+ remained unaffected with varying levels of DCAD.

Tucker *et al.* (1988) observed non-significant effect on serum Na or K concentration but Cl concentration decreased with increasing DCAD. Jackson *et al.* (1992) did not observe any change in plasma Na and K content of calves fed with a diet containing DCAD value of 0, 21, 37 and 52 mEq/100g of DM. Similar results were found by West *et al.* (1992) who reported that serum Na and K were not altered when DCAD level was increased from 120.4 to 464.1 mEq/kg DM. Sarwar *et al.* (2007b) reported that serum Na and K remained unaltered by DCAD level. The non-significant effect of high DCAD diet on Na and K might be due to their excessive excretion through kidney.

2.7.4 Plasma Chloride

Low DCAD diets resulted in increased Cl concentration in pregnant and lactating goats (Fredeen *et al.*, 1988a) which reflects the Cl content of the diet.

Tucker *et al.* (1988) reported a linear increase in plasma Cl^- concentration with decrease in the DCAD levels (+20 to -10 mEq/kg of DM) which might be due to supplementation of CaCl_2 in the diet. Tucker *et al.* (1991b) also observed similar effects of DCAD on plasma Cl^- concentration in growing cattle. A linear decline in serum chloride concentration with increasing DCAD concentration was also supported by Roche (2000). Actually Cl^- is absorbed in exchange of HCO_3^- to keep the body electrically neutral that results in decreased HCO_3^- and blood pH (Block, 1994).

Wildman *et al.* (2007) observed no effect of varying positive DCAD diet on the serum Cl^- content. A linear increase in serum Cl^- concentration was observed with decrease in the DCAD levels, it was maximum (103.4 mEq/l) in buffaloes fed on high anionic diet and minimum (98.3 mEq/l) in buffaloes fed on high cationic diet (Shahzad *et al.*, 2011). Sarwar *et al.* (2011) reported that serum Cl^- remained unaltered with increasing levels of NaHCO_3 in the diet. Increased serum Cl^- concentration with decreased DCAD concentration might be attributed to inverse relation between blood Cl^- and HCO_3^- concentrations (Hu and Murphy, 2004).

2.7.5 Plasma Magnesium

Oetzel *et al.* (1988) reported that plasma Mg content was significantly higher in the anionic group (-7.5 mEq/ 100g DM) of cows as compared to the cationic group (18.9 mEq/100g DM). West *et al.* (1992) reported that the DCAD based diet had non-significant effect on serum Mg concentration. However, Jackson *et al.* (1992), recorded higher level of plasma Mg in calves fed with diet containing DCAD value of 0 as compared with the calves fed DCAD diets of 21, 37, 52 mEq/ 100g DM. Roche *et al.* (2003a) observed a linear increase in serum Mg concentration as DCAD level was decreased in the diet. It might be due to metabolic acidosis induced by low or negative DCAD diet. A decrease in serum Mg concentration was observed with high DCAD (Wildman *et al.*, 2007). Shahzad *et al.* (2008a) observed decreased serum Mg concentration in buffaloes fed -11 DCAD compared to those fed +33 DCAD diet and explained that decreased serum Mg concentration in buffaloes fed -11 DCAD concentration might be due to high dietary Ca content, which might have reduced Mg absorption. Sarwar *et al.* (2011) and Shahzad *et al.* (2011) observed no effect of different DCAD diets on plasma Mg concentration.

2.7.6 Plasma Phosphorus

DCAD had non-significant effect on serum P concentration (Tucker *et al.*, 1988 and West *et al.*, 1992). Contrary to this, Roche *et al.* (2005) reported increased plasma P concentration as DCAD was increased in the diet. Shahzad *et al.* (2008a) reported that serum phosphorus concentration remained unaltered across all diets (-11, +11, +22 and +33 mEq/100g DM). Shahzad *et al.* (2011) observed that serum P concentration remained unaltered at all levels of DCAD, which revealed the lack of marked effect of DCAD on P concentration in the blood.

2.7.7 Plasma Sulphur

Delaquis and Block (1995c) observed increased plasma sulfide (S^{2-}) concentration with decreasing DCAD in the diet due to renal regulation of its absorption. Shahzad *et al.* (2008a) reported that serum S concentration showed a decreasing trend with increasing DCAD. However Sarwar *et al.* (2011) and Shahzad *et al.* (2011) did not find any change in serum S concentration with increasing levels of DCAD or $NaHCO_3$ in the diet

2.8 Effect of DCAD on urinary pH and excretion of minerals

2.8.1 Urine pH

Any change in urine pH is due to alteration in blood pH which reflects the ability of kidney to withstand changes in metabolic challenge. However, there is a minimum (4.5) threshold limit for reduction in urine pH in the mammalian body (Roche *et al.*, 2000). A dramatic reduction in urine pH has been reported with increased anion (Cl or S) or decreased cation (Na or K) in the diet (Waterman *et al.*, 1991). It is well documented that increased dietary cations increase the urine pH (Jackson *et al.*, 1992; Jackson and Hemken, 1994; Pehrson *et al.*, 1999 and Shahzad *et al.*, 2008 a,b). Urinary pH is generally used as an indicator of metabolic acid or alkali load (Wu *et al.*, 2008).

Hu *et al.* (2007) reported increased urine pH with increasing DCAD level in the diet. Decrease in urine pH in low or negative DCAD diets is associated with excess Cl and S inclusion in the diet. Increased urine H^+ at low DCAD diet was due to acidogenic properties of $CaCl_2$ (Tucker *et al.*, 1991b). Jackson *et al.* (2001) found that urine pH was higher for calves fed +200 DCAD diet than for

those fed +0 DCAD diet (8.09 vs. 6.80). Shahzad *et al.* (2007) observed a linear increase in urine pH with an increased DCAD level. Sarwar *et al.* (2007b) reported that lambs fed +110 and +220 DCAD diet had a urine pH of 7.43 and 7.68, respectively. Urine pH increased in high-DCAD (19.73 mEq/d) cows and decreased in low-DCAD cows (-5.6 mEq), the decrease of urine pH by 1 unit in low-DCAD cows demonstrates the capacity of the body to disseminate an acid load from the blood into other body tissues and fluids (Hersom *et al.*, 2010). Luebke *et al.* (2011) also reported that urinary pH increased as DCAD increased. A linear increase in urine pH ($P < 0.05$) was observed with increasing levels of NaHCO_3 in the diet, the lowest (7.33) and highest (8.02) urine pH readings ($P < 0.05$) were recorded in calves fed the 0% NaHCO_3 and 1.6% NaHCO_3 treatment diets, respectively (Sarwar *et al.*, 2011). The findings reflect that increase or decrease in urine pH in DCAD fed animals was because of the alkaline or acidic nature of the diet, respectively, which is the function of salts used for the respective mineral composition.

2.8.2 Urinary minerals excretion

Urinary Na and K concentrations increased while Cl concentration decreased as DCAD increased from -10 to +20 mEq/100 g DM. Decreased urinary Cl concentration in cows fed high DCAD (Tucker *et al.*, 1992) might be attributed to increased Cl content of the diet (Tucker *et al.*, 1988 and 1992). West *et al.* (1992) also pointed out that urinary Na and K concentration increased in cows fed high DCAD diet while its reverse was true for Cl concentration. Wildman *et al.* (2007) reported that fractional excretion of Na was elevated in cows receiving high DCAD, probably because the high DCAD diets contained higher Na concentration.

Buffaloes fed on high cationic diet had higher intake and excretion of Na^+ and K^+ while these were lower in buffaloes fed on high anionic diet (Shahzad *et al.*, 2008b and 2011). The Cl content of the anionic diet induced acid load (Budde and Crenshaw, 2003) and it is also retained in excess quantity in the body. An increased Cl retention with increasing Cl intake was also observed by Golz and Crenshaw (1991) in young animals. Due to physiological regulation of osmotic pressure, the excess dietary Cl must either be excreted with a counter balance of

cations or stored in a body compartment (bone) with a replacement of another anion.

Tucker *et al.* (1988) pointed out that increasing DCAD from -10 to +20 mEq/100 g DM increased urinary cation anion difference. Similarly, West *et al.* (1991) observed a linear increase in urinary cation anion difference with increasing DCAD. Urinary cation anion difference also tended to increase as DCAD level was increased from 120.4 to 464.1 mEq/kg DM (West *et al.*, 1992). This increased urinary cation anion difference at high DCAD diet might be attributed to decreased Cl content of the diet. Tucker *et al.* (1991c) reported that dietary supplementation with 2% Na bicarbonate had no effect on urinary Cl concentration. Jackson *et al.* (2001) reported that calves fed the 0-DCAD diet had higher Cl concentration in the urine than those fed the 200-DCAD diet (0.61 vs. 0.51 mg of Cl /mg of creatinine).

Tucker *et al.* (1988) reported lower urinary Ca concentration in calves fed 2% dietary Na bicarbonate. Joyce *et al.* (1997) reported that urinary excretion of Ca was higher in cows fed -7 mEq/100 g DM DCAD diet compared to those fed +30 and +35 mEq/100 g DM DCAD diets. Borucki Castro *et al.* (2004) and Li *et al.* (2008) also observed increased urinary excretion of Ca at low DCAD diet while it tended to decrease at high DCAD diet. The increase in urinary Ca at low DCAD was due to high plasma Ca concentration. This excess amount of plasma Ca is filtered through kidneys resulting in increased urinary excretion of Ca (Takagi and Block, 1988) as its concentration increased with increasing plasma Ca concentration, blood H⁺ and urine H⁺. Moreover, higher plasma Ca concentration indicates greater availability of Ca for metabolic functions and is helpful to assess Ca status of the animal. Feeding low DCAD diet can increase urinary Ca excretion due to slight metabolic acidosis. Jackson *et al.* (2001) observed that calves fed 0-DCAD dietary treatment had higher Ca concentration in the urine than those fed 200-DCAD dietary treatment. Urinary excretion of P is positively associated with increased H⁺ both in blood and urine (Oetzel *et al.*, 1988 and Tucker *et al.*, 1992). Dietary DCAD content had no effect on urinary P concentration; however, urinary concentration of P decreased linearly with increasing dietary Ca content (Jackson *et al.*, 2001). Excretion of P tended to increase as DCAD level was decreased in the diet (Shahzad *et al.*, 2008b).

Tucker *et al.* (1992) pointed out that urinary excretion of Mg increased during first week postpartum in cows fed low DCAD diet. The increase in urinary excretion of Mg is due to its positive association with plasma Mg concentration. When low DCAD diet is fed to the animals, it induces slight metabolic acidosis which results in increased plasma Mg concentration (Roche *et al.*, 2003a), excreting more urinary Mg. Wildman *et al.* (2007) reported decrease in Mg fractional excretion was observed with high DCAD. Urinary excretion of Mg increased with decreasing DCAD level in the diet, it was maximum (3.98 g/day) and minimum (3.31 g/day) in buffaloes fed on high cationic and low cationic diets respectively (Shahzad *et al.*, 2011). It can be concluded that mineral composition of diet may have close association with urinary mineral composition.

2.9 Effect of positive DCAD diet on growth during thermal stress

Positive DCAD based diet can be a useful strategy in case of thermal stress to increase DMI and resultant positive influence on growth. Wheeler and Hruska (1981) reported an increased weight gain in steers fed 100 mEq/kg of DM DCAD diet due to increased feed consumption. Fettman *et al.* (1984) recorded increased weight gain in calves fed 0.10% Cl diet compared to those fed 0.45% Cl diet and the increase in weight gain in calves fed low Cl (0.10%) diet was attributed to increased feed consumption. Xin *et al.* (1991) studied the effect of 100 and 200 mEq [(Na + K) - (Cl + S)]/ kg of DM on feed intake and observed that calves fed positive DCAD diet had higher growth rate compared to calves fed negative DCAD diet. In another study, growing dairy calves were fed diets varying in cation-anion difference of 0, 210, 370, and 520 mEq/kg dietary DM and it was found that feed intake and average daily gain responded quadratically being greatest at 370 mEq and lowest with 0 mEq (Jackson *et al.*, 1992). Jackson and Hemken (1994) also reported that calves fed +130 mEq DCAD diet had greater growth rates than those fed diets with -180 mEq.

Sarwar *et al.* (2007b) reported that lambs fed +330 and -110 DCAD diet had 159 and 126 g/d weight gain, respectively. Shahzad *et al.* (2007) observed increased weight gain with an increased DCAD level of diet in growing buffalo calves; calves fed +330 and +110 DCAD diets had 698 and 625 g/d weight gain, respectively. Sarwar *et al.* (2011) observed that calves fed the 0% NaHCO₃ showed the lowest (625 g/d) weight gain and those fed the 1.6% NaHCO₃ diet

showed the highest weight gain (735 g/d). Decreased growth rate in calves fed low DCAD diet was due to metabolic acidosis induced by low DCAD diet. Moreover, when the acid balance of the diet is deviated toward acidosis apart from the homeostatic welfare, most metabolic pathways cannot work optimally and thus they are more involved in homeostatic regulation than growth (Shahzad *et al.* 2007). Hence it is evident indicate that there should be an optimum dietary cation-anion difference for growing dairy calves and a low or negative cation-anion difference may be deleterious to bone strength and growth.

2.10 Positive DCAD effects on endocrine regulation

Climatic stress leads to alteration in body's physiological response resulting in alteration in the body acid-base balance. Supplementation of Na and K in the form of bicarbonate/ carbonate also help in better regulation of acid-base balance in the blood (Sanchez *et al.*, 1994). It is known that an alteration in blood pH affects the production and effectiveness of insulin (Schade *et al.*, 1981 and Robertson, 1987), growth hormone (Challa *et al.*, 1993) and parathyroid hormone (Gaynor *et al.*, 1989; Goff *et al.*, 1991 and Goff and Horst, 1992). Positive DCAD based diets can be useful strategy in case of thermal stress to increase DMI and resultant positive influence on the growth. Alteration in DCAD also affects the DMI and growth in calves (Fettman *et al.*, 1984; Jackson *et al.*, 1992; Jackson and Hemken, 1994). It is assumed that increased DMI in calves fed high DCAD diet might be due to increased buffering capacity due to alkalogenic nature of high DCAD diet (Block, 1994).

Sarwar *et al.* (2007b) found that lambs fed +330 and -110 DCAD diet had 159 g/d and 126 g/d weight gain, respectively. Any change in DCAD induces certain changes in blood chemistry for example if DCAD decreases; it causes an increase in blood H^+ and decrease in blood HCO_3^- , blood pH and urine pH (Block, 1994 and Spanghero, 2004). Hassan *et al.* (2011) observed that sodium bicarbonate and potassium chloride alone or their combinations into diets of hens in hot climate significantly decreased aspartate transaminase (AST) and alanine transaminase (ALT) enzymes compared to the control group. Challa *et al.* (1993) studied the effect of chronic metabolic acidosis and observed that acidotic conditions led to growth impairment, reduced food efficiency, and amplitude-specific inhibition of pulsatile secretion of GH axis. Helena *et al.* (2009) observed

that reduction in potassium intake causes hypokalemia, which leads to growth retardation, It also down regulated IGF-I mRNA expression despite simultaneous upregulation of the IGF-I receptor, due to a compensatory mechanism. Espino *et al.* (2005) found that feeding of anionic diet (-88.9mEq/ Kg DM) to sheep induced a non-respiratory systemic acidosis in association with a mild increase in plasma cortisol concentration without changes in plasma ACTH levels. Higher serum cortisol was noticed in buffaloes fed -110 than those fed +330 mEq/kg diet (Shahzad *et al.*, 2007). High concentration of cortisol can influence the immunoglobulin production and thereby can influence the productive performance of animals. Positive DCAD diet is likely to influence the acid-base status of the animal and can be a useful strategy to ameliorate the climatic stress.

CHAPTER – 3

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in Dairy Cattle Nutrition Division of National Dairy Research Institute, Karnal. The entire study comprised of two phases. Phase 1 involved formulation of DCAD diets and Phase 2 comprised of two experiments. Experiment-I was conducted during winter season (December 10, 2011 - April 8, 2012) whereas Experiment-II was conducted during summer season (April 15, 2012 - August, 12, 2012). A brief description of experimental techniques followed during the course of study is presented in this chapter.

3.1 Phase 1: Formulation of DCAD based diets for growing animals in summer and winter months

3.1.1 Estimation of various minerals in feeds and fodder samples

As per the first objective, commonly used feed and fodder samples (16) were collected from different locations. One gram of representative feed sample was digested in 100 ml kjeldahl flask containing glass beads to avoid bumping at very low heat initially by adding adequate amount of tri-acid mixture ($\text{HNO}_3:\text{HClO}_4:\text{H}_2\text{SO}_4$ 3:1:1) and then at higher temperature till the contents became clear and perchloric acid (HClO_4) fumes ceased to come out. The final volume was made to 25 ml and filtered to remove silica and other insoluble residues from digested samples. A blank was processed simultaneously by using the tri-acid mixture. Then, a series of working standards were run in Atomic Absorption Spectrophotometer for the preparation of the standard curve. Finally, the samples were run and the readings (OD) were noted. Concentration of Na, K, Ca, Mg and trace minerals (Zn, Cu, Fe) was calculated with the help of a standard curve. Chloride content of the samples was determined by the method of Chapman and Pratt (1961). Sulphur content in the samples was estimated by turbidimetric method (Massoumi and Cornfield, 1963) and phosphorus by Photometric method (AOAC, 2005).

3.1.2 Formulation of DCAD (mEq/ kg DM) diet

- A. The mEq weight ($\text{mg} \times \text{valency} / \text{atomic weight}$) of the minerals (Na, K, Cl and S) as well as the dietary cation anion difference (DCAD) value of feeds & fodders were calculated using formula:

DCAD (mEq) = (Na + K) i (Cl + 0.6S) (Goff *et al.*, 2004).

The DCAD values of feeds and fodders used for feeding to the calves are presented in Table 3.1.

B. Adjusting DCAD for winter and summer seasons

- (i) Formulation of basal diet as per nutrient requirements (NRC, 2001).
- (ii) Added appropriate cationic salt (NaHCO_3 and K_2CO_3) to achieve desired DCAD (mEq/ kg DM) for winter experiment i.e. +150 and +250 in groups W_1 and W_2 in winter (Table 3.2) and +250 and +350 mEq/ kg DM in groups S_1 and S_2 during summer experiment (Table 3.3).
- (iii) Quantity of salts changed according to the dry DMI (vary according to body weight) to adjust DCAD value of the diet. However, Tables 3.2 and 3.3 show the initial fortnight DCAD mEq/ kg DM of the diet.

3.2 Phase 2: Effect of positive DCAD diets on growing animals in summer and winter months

3.2.1 Experiment 1: To study the effect of +150 and +250 mEq/ kg DM DCAD diet on crossbred calves during winter months

a) Location of the Study

The study was conducted at the Cattle Yard of National Dairy Research Institute (NDRI), Karnal, Haryana. Karnal is situated on an altitude of 250 meter above mean sea level, latitude and longitude positions are $29^{\circ} 42''$ N and $79^{\circ} 54''$ E, respectively.

b) Selection and distribution of experimental animals

In the winter season experiment, 18 female Karan Fries calves (5 to 9 months) were selected from the herd of National Dairy Research Institute (NDRI), Karnal. They were divided into 3 groups having 6 crossbred calves in each group, on body weight and age basis as control, W_1 and W_2 . The details of experimental animals are given in Table 3.4. The experiment was continued for 120 days from December 10, 2011 - April 8, 2012.

c. Feeding and management of animals

The crossbred calves of the three different groups were offered the same basal diet, but the animals in W_1 and W_2 groups were offered +150 and +250

Table 3.1 Calculation of DCAD value (mEq/ kg DM) of feeds and fodders

Ingredients	mEq Weight				DCAD
	Na	K	Cl	S	(mEq/kg DM)
Concentrate mixture	200.1	299.52	375.91	187.2	11.4
Maize fodder	17.4	407.04	468.12	87.36	-96.1
Berseem fodder	108.75	419.84	112.8	424.32	161.2
Oats fodder	87	409.6	338.4	68.64	117.0
Wheat straw	160.95	552.96	282.00	87.36	379.5

Table 3.2 Formulation of DCAD diet for crossbred calves in winter months

Ingredients			DCAD (mEq/ kg DM)		
	Qty. (kg)	DM (kg)	Control	W ₁	W ₂
Concentrate mixture	2.0	1.8	20.5	20.5	20.5
Maize fodder	20.0	2.4	-230.6	-230.6	-230.6
Wheat straw	1.0	0.9	341.5	341.5	341.5
NaHCO ₃ (Control)	0		0		
NaHCO ₃ (W ₁)	0.0241			286.8	
NaHCO ₃ (W ₂)	0.0434				516.5
K ₂ CO ₃ (Control)	0		0		
K ₂ CO ₃ (W ₁)	0.0241			349.1	
K ₂ CO ₃ (W ₂)	0.0434				628.6
Total		5.1	131.4	767.3	1276.6
DCAD (mEq/kg DM)			25.8	150.5	250.3

Table 3.3 Formulation of DCAD diet for crossbred calves in summer months

Ingredients			DCAD (mEq/ kg DM)		
	Qty (kg)	DM (kg)	Control	S ₁	S ₂
Concentrate mixture	2.0	1.8	20.5	20.5	20.5
Oats fodder	20.0	4.0	468.1	468.1	468.1
Wheat straw	0.0	0	0.0	0.0	0.0
NaHCO ₃ (Control)	0		0		
NaHCO ₃ (S ₁)	0.0365			434.4	
NaHCO ₃ (S ₂)	0.0585				696.2
K ₂ CO ₃ (Control)	0		0		
K ₂ CO ₃ (S ₁)	0.0365			528.7	
K ₂ CO ₃ (S ₂)	0.0585				847.3
Total		5.80	488.6	1451.7	2032.2
DCAD (mEq/ kg DM)			84.2	250.3	350.4

Table 3.4 Details of crossbred calves during winter experiment

Treatment	Animal No.	Date of Birth	Age (Days)	Body weight
Control	KF 7403	28.01.2011	261	169.0
	KF 7404	30.01.2011	263	245.0
	KF 7432	31.03.2011	250	178.8
	KF 7447	18.04.2011	232	128.9
	KF 7467	14.07.2011	146	89.4
	KF 7482	24.07.2011	136	81.5
Mean ± SE			214.7±23.8	148.8±25.2
W₁	KF 7405	01.02.2011	265	194.0
	KF 7407	04.02.2011	262	138.0
	KF 7411	09.03.2011	257	123.0
	KF 7414	11.03.2011	255	139.5
	KF 7415	14.03.2011	252	173.0
	KF 7438	07.04.2011	243	122.0
Mean ± SE			255.7±3	148.3±11.9
W₂	KF 7419	19.03.2011	247	146.0
	KF 7422	25.03.2011	255	181.0
	KF 7423	26.03.2011	254	177.5
	KF 7435	03.04.2011	247	149.0
	KF 7436	04.04.2011	246	131.2
	KF 7437	06.04.2011	244	109.0
Mean ± SE			248.8±1.9	149.6±11.2

mEq/ kg DM DCAD diet by the 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 the 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. Fortnightly body weight of experimental animals was recorded to observe change in growth rate due to dietary treatments.

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 from the skin. Fresh drinking water was provided twice a day.

3.2.2 Experiment 2: To study the effect of +250 and +350 mEq/ kg DM DCAD diet in crossbred calves during summer months

Eighteen female Karan Fries calves (9 to 12 months) were selected from the herd of National Dairy Research Institute (NDRI), Karnal. All the calves were apparently free from anatomical, physiological and infectious disorders. They were divided into three groups of six animals each on the basis of their body weight as control, S_1 and S_2 (Table 3.5).

a) Housing and management of animals

Animal experimentation was performed in compliance with regulations set by the cattle yard, NDRI and approved by the Institutional Animal 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 from the skin. Fresh drinking water was provided twice a day.

b) Feeding of animals

All the growing crossbred calves (Karan Fries) were fed isocaloric and iso-nitrogenous diet as per NRC (2001) feeding standard, whereas diets of S_1 , S_2 groups were formulated to have +250, +350 mEq/kg DM DCAD during summer season. The energy and protein requirements of all the calves were met by feeding concentrate mixture and available green fodder (maize/ Jowar) in the ratio of 40:60 and the feeding trial was conducted for 120 days during summer season.

The concentrate mixture was offered in the morning, whereas, chaffed green fodder and wheat straw was offered at 11:00 am. Concentrate mixture (CP 19.81% and TDN 70%) was prepared by mixing maize 33%, groundnut cake 21%, mustard oil cake 12%, wheat bran 20%, deoiled rice bran 11%, and mineral mixture 2% and common salt 1%. The roughages and concentrate mixture were analyzed for proximate principles (AOAC, 2005) and fibre fractions (Goering and Van Soest, 1970).

c) Body weight and DM intake

The animals were weighed before feeding and watering in the morning on two consecutive days at the start of experimental feeding and thereafter at fortnightly intervals during the experimental period of 120 days. DM intake was recorded daily by subtracting the residual DM from the quantity of DM of feeds offered.

3.3 Sampling and observations during summer and winter experiments

3.3.1 Blood sampling

Blood samples (8 ml) were collected in sterile heparinised vacutainer (BD VacutainerTM, UK) 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 summer as well as winter seasons. 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 3000 rpm for 15 minutes and plasma was stored at -20°C in different aliquots for the analysis of various biochemical constituents. Plasma was used for the analysis for energy metabolites (glucose), metabolic

Table 3.5 Details of crossbred calves during summer experiment

Treatment	Animal No.	Date of Birth	Age (Days)	Body weight
Control	KF 7437	06.04.2011	374	156.4
	KF 7438	08.04.2011	372	169.4
	KF 7481	23.07.2011	262	149.2
	KF 7482	24.07.2011	263	138.9
	KF 7488	29.07.2011	268	139.3
	KF 7492	03.08.2011	273	135.9
Mean ± SE			302.0±22.5	148.2±5.3
S₁	KF 7411	09.03.2011	401	167.3
	KF 7467	14.07.2011	271	168.9
	KF 7477	19.07.2011	266	137.4
	KF 7483	25.07.2011	260	130.1
	KF 7485	26.07.2011	259	150.8
	KF 7491	30.07.2011	255	150.9
Mean ± SE			285.3±23.1	150.9±6.34
S₂	KF 7407	02.02.2011	380	201.4
	KF 7474	17.07.2011	264	148.9
	KF 7478	20.07.2011	267	139.3
	KF 7484	25.07.2011	260	157.9
	KF 7490	29.07.2011	254	141.8
	KF 7495	05.07.2011	275	110.5
Mean ± SE			283.3±19.0	149.9±12.2

hormones (Thyroxine, tri-iodothyronine and Cortisol), immunological parameters (Total immunoglobulin, IgG and IgM), antioxidant status (FRAP assay, SOD and catalase activity) and minerals.

3.3.2 Recording of climatic variables and Temperature Humidity Index

Microclimatic data viz., dry bulb temperature, wet bulb temperature, minimum and maximum temperature and relative humidity was recorded at 7.30 A.M and 2.30 P.M using Zeal (UK) thermometer every day during experimental periods. Temperature humidity index (THI) was calculated using the formula: $THI = 0.72 (T_{db} + T_{wb}) + 40.6$ (NRC, 1981)

Where,

T_{db} = dry bulb temperature ($^{\circ}\text{C}$)

T_{wb} = wet bulb temperature ($^{\circ}\text{C}$)

3.3.3 Measurement of physiological responses

a) Respiration rate (RR)

Respiration rate of the animals was recorded from visual observation of inward and outward abdominal movements. One outward movement was counted as one respiration and respiration rate was expressed as breaths per minute.

b) Pulse rate (PR)

Pulse rate of the animals were counted by observing the pulsation of middle coccygeal artery at the base of the tail and the results were expressed as pulse rate per minute.

c) Rectal temperature

Rectal temperature was recorded using digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 minutes.

3.3.4 Metabolism trial

A metabolic trial for a period of 7 days was conducted in the mid of the experiment to estimate nutrients utilization and mineral balances. Proper record of water intake, feeds offered and residue left, feces and urine voided were maintained during the trial period.

3.3.5 Collection of feed, residue, faeces and urine samples

During metabolic trial, weighed amount of concentrate mixture and green fodder was offered daily to the calves and the weight of the residues was recorded every day to find out the actual intake. The amount of faeces and urine voided by the animals in 24 h was recorded daily. The total faeces collected daily from each animal, were mixed thoroughly in a plastic trough and representative sample was taken to laboratory for aliquoting and further analysis. Similarly, urine voided by the individual animal during 24 h was collected in a container, having 25 ml 25% sulphuric acid, and mixed thoroughly before taking the sample into clean dried plastic bottles for further analysis.

3.3.6 Aliquoting of faeces and urine

To determine N content of the fresh dung, 1/500th aliquot of faeces was taken into pre-weighed plastic bottle for individual animal. Aliquot of wet faeces was preserved with 25% H₂SO₄ daily. At the end of the collection period, contents in each bottle were mixed thoroughly and weighed. At the end of trial, representative sample (about 5 g) of preserved dung was taken for digestion and subsequent N estimation. For DM estimation in dung of individual animal, 1/100th aliquot of faeces was taken daily in to pre-weighed aluminum tray, which was kept in hot air oven at 100^oC for drying till it got a constant weight. After drying, the sample weight was recorded. The dried dung samples for all the 7 days were pooled, ground (1-2 mm) and stored in clean plastic jar for analysis of proximate principles and fiber fractions. Similarly, an aliquot (1/500th) of urine voided by an individual animal was collected daily in to glass bottles containing H₂SO₄. Pooled representative samples of urine were used for N estimation.

3.4 Research techniques

3.4.1 Preparation of feed, faeces, plasma and urine samples for mineral analysis

One gram each of feed and faeces and 1 ml each of plasma and urine samples were digested separately by adding adequate amount of tri-acid mixture (HNO₃:HClO₄:H₂SO₄ 3:2:1) in 100 ml kjeldahl flask containing glass beads to avoid bumping at very low temperature initially and then at high temperature till the contents were clear and perchloric acid (HClO₄) fumes ceased to come out.

The final volume was made to 25 ml and filtered to remove silica and other insoluble residues from digested samples.

a) Estimation of Ca, Mg, Na, K, Zn, Cu, and Fe

Minerals in feed, faeces, urine and plasma (Ca, Mg, Na, K, Zn, Cu, and Fe) were estimated by using atomic absorption spectrophotometer (Model Z-5000, Polarized Zeeman atomic absorption spectrophotometer, Hitachi High-Technologies Corporation, Tokyo, Japan) after digestion. Standard solutions of Na (0.06-1 ppm), K (0.06-2 ppm), Ca (2-20 ppm), Mg (0.6-6ppm) Cu, Zn and Fe (0.5-5 ppm each) were prepared. Estimations were carried out on atomic absorption spectrophotometer using acetylene as fuel and air as an oxidant. Specific hollow cathode lamps were used for the determination of various minerals. The instrument was set zero density with the blank. The procedure described in AAS manual for preparation of stock, standard solutions and choice of instrumental conditions were followed (Table 3.6). A series of working standards were run in atomic absorption spectrophotometer for the preparation of the standard curve (Fig. 3.1 to 3.8). Finally the samples were run and the readings (OD) were noted. Concentrations of minerals were estimated with the help of a standard curve.

b) Estimation of Phosphorous in feed, residue, plasma, faeces and urine samples

Phosphorus content in sample was estimated by Photometric method (AOAC, 2005).

Reagents:

- 1) Molybdo-vanadate reagent: Dissolved 40 g ammonium molybdate in 400 ml of hot distilled water and cooled. Dissolved 2 g ammonium metavanadate in 250 ml of hot distilled water and cooled and added 250 ml of 70% perchloric acid. Gradually added molybdate solution to vanadate solution with constant stirring and diluted to 2 L.
- 2) Phosphorus standard:
 - A) Stock solution: 2 mg P/ml. Dissolved 8.788 g pure dry KH_2PO_4 in distilled water and diluted to 1 L.
 - B) Working Solution: 0.1 mg P/ml - Diluted 50 ml stock solution to 1 L.

Procedure:

- Digested the sample by wet oxidation procedure using tri-acid.
- Took suitable aliquot (20 ml) containing 0.5-1.5 mg P in 100 ml volumetric flask.
- Added 20 ml molybdo-vandate reagent and diluted to 100 ml volume with water.
- Mixed the contents thoroughly and allowed the solution to stand for 10 minutes for proper development of colour due to formation of phosphomolybdate-vandate complex.
- For preparation of standard curve, took standard solutions containing 0.5, 1.0, 1.5 mg P into 100 ml volumetric flasks. Treated in the same way as mentioned for samples.
- Read percent transmission at 400 nm and plotted the standard curve.
- Calculated the concentration of P in the sample from the standard curve.

c) Estimation of Sulphur

(i) Feed, faeces and urine samples:

Sulphur in samples was estimated by turbidimetric method (Massoumi and Cornfield, 1963).

Reagents:

- Standard sulphur solutions: 0.444 g of sodium sulphate (anhydrous) was dissolved in distilled water and volume was made to 1 L so as to prepare a standard solution containing 100 ppm of S. Different dilutions were made to get 10, 20, 30, 40 and 50 ppm S solutions for preparation of standard curve.
- Nitric acid (25%, v/v)
- Perchloric acid (70%)
- Barium chloride
- Acetic-phosphoric acid: 900 ml of acetic acid was mixed with 300 ml of orthophosphoric acid.
- Gum acacia-acetic acid solution: 5 g of gum acacia was dissolved in 500 ml of hot distilled water. The hot solution was filtered through Whatman filter paper No. 42, the filtrate was cooled and then diluted to 1 L with acetic acid.

Table 3.6 Conditions for mineral estimation by atomic absorption spectrophotometer

Mineral	Wave length (nm)	Lamp current (mA)	Fuel flow (L/min)
Ca	422.7	9	2.4
Mg	285.2	9	2.2
Na	589.0	12	2.2
K	766.5	12	2.4
Cu	324.8	9	2.2
Zn	213.9	6.5	2.0
Fe	248.3	15	2.2

Fig. 3.1 Standard curve for sodium

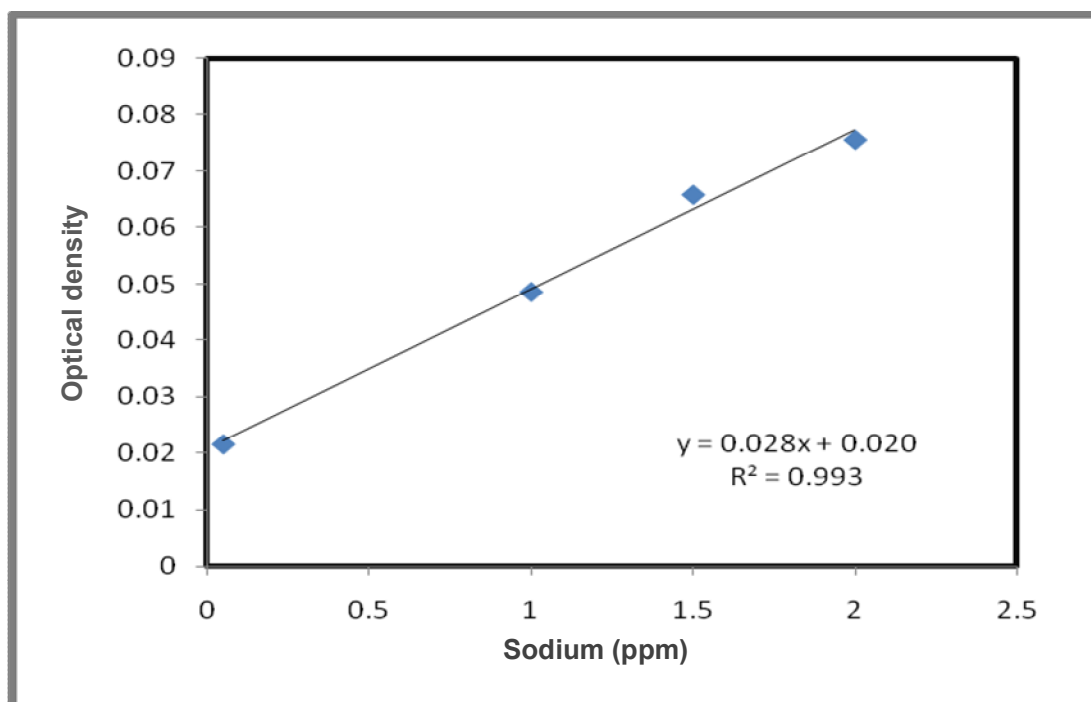


Fig. 3.2 Standard curve for potassium

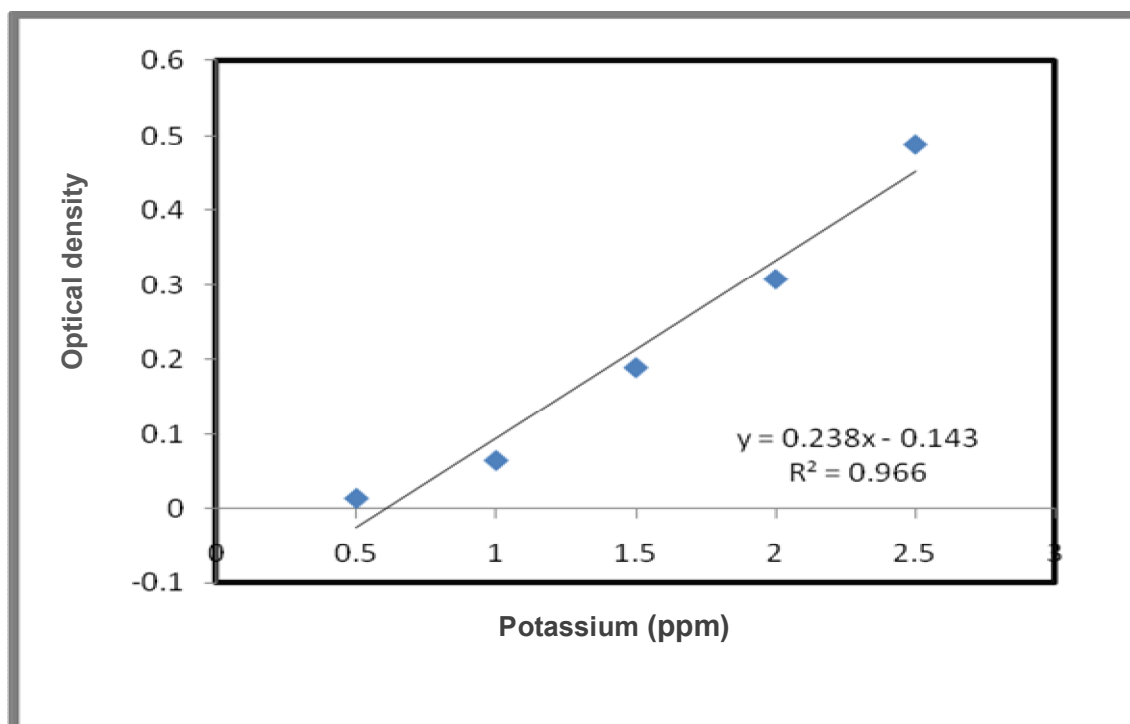


Fig. 3.3 Standard curve for calcium

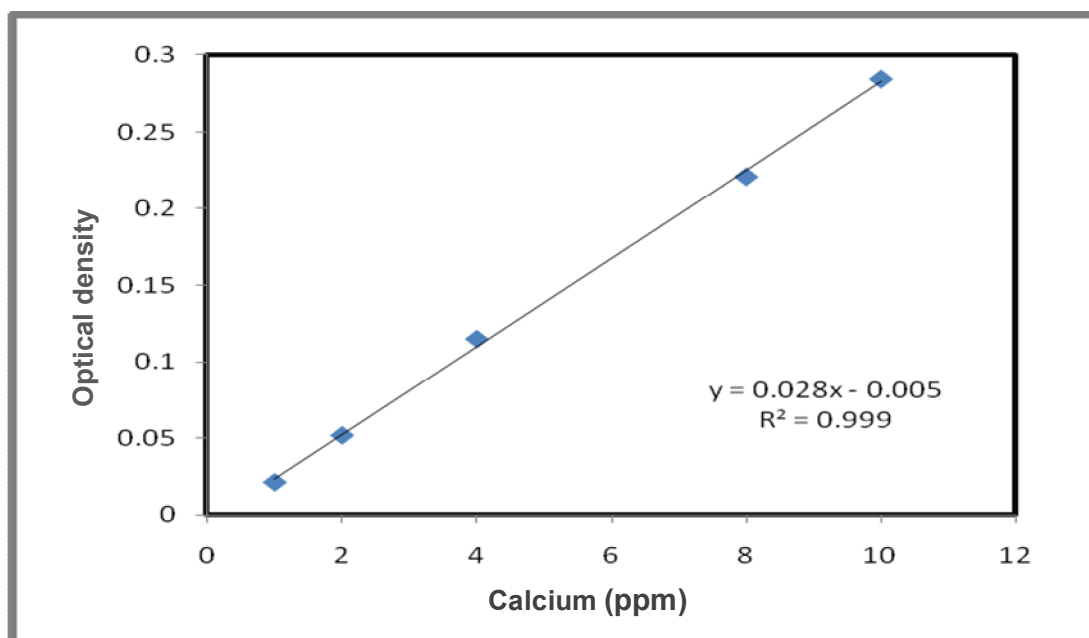


Fig. 3.4 Standard curve for phosphorus

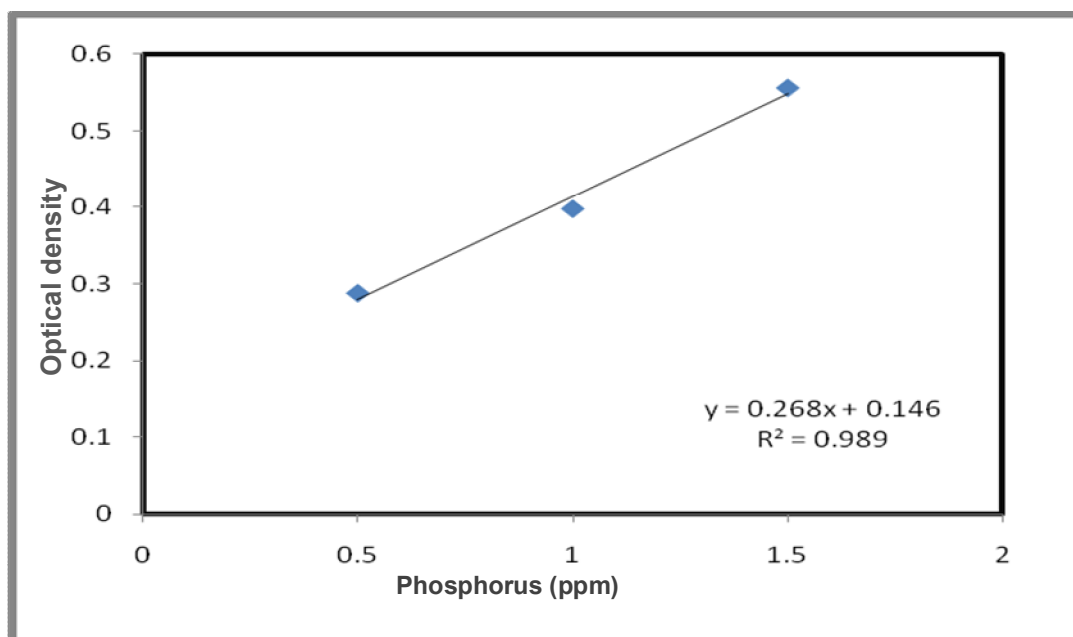


Fig. 3.5 Standard curve for magnesium

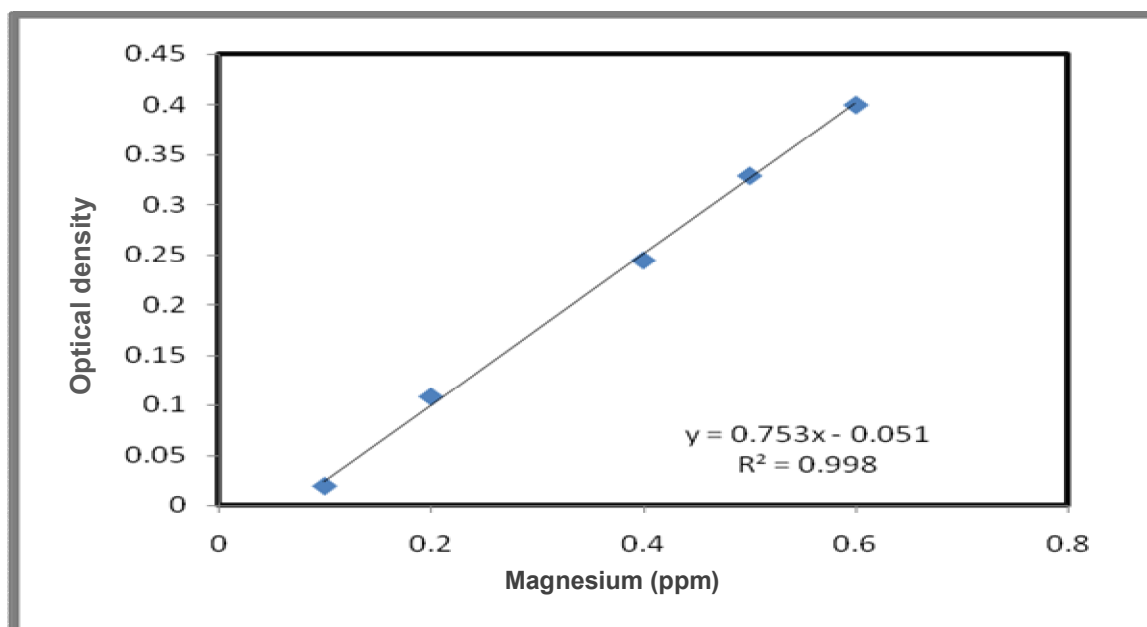


Fig. 3.6 Standard curve for zinc

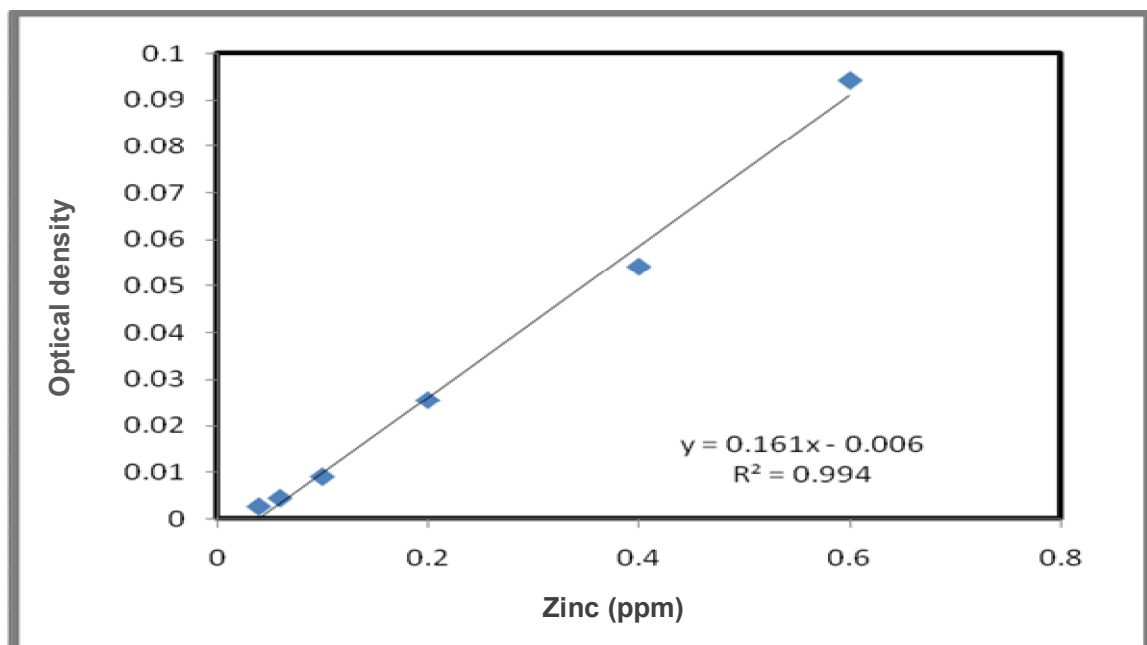


Fig. 3.7 Standard curve for copper

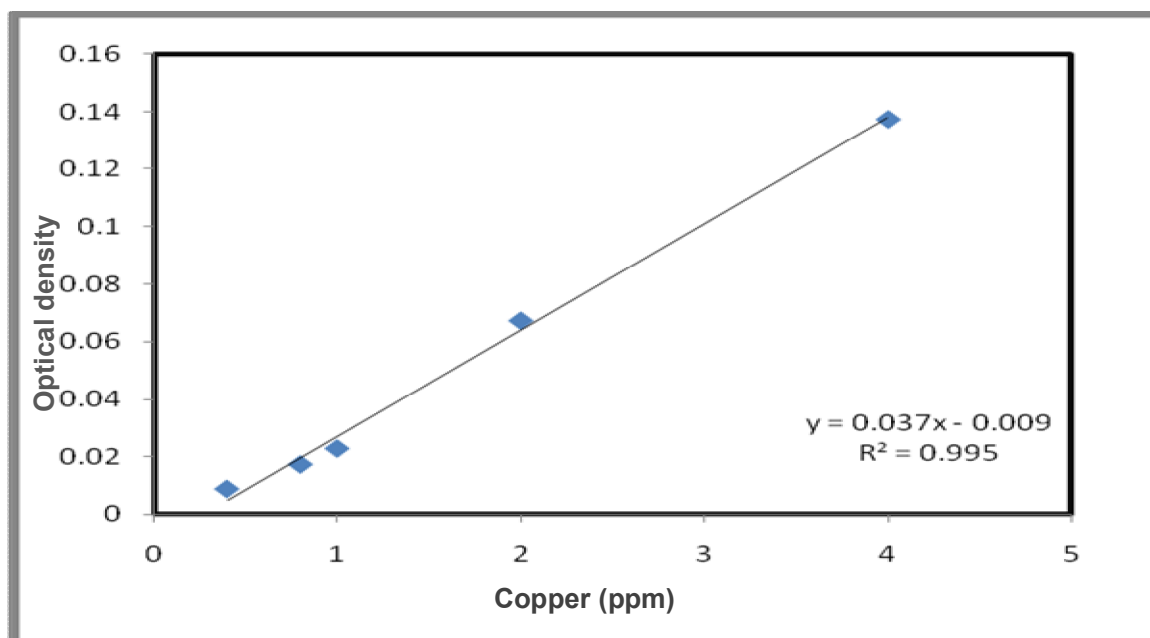
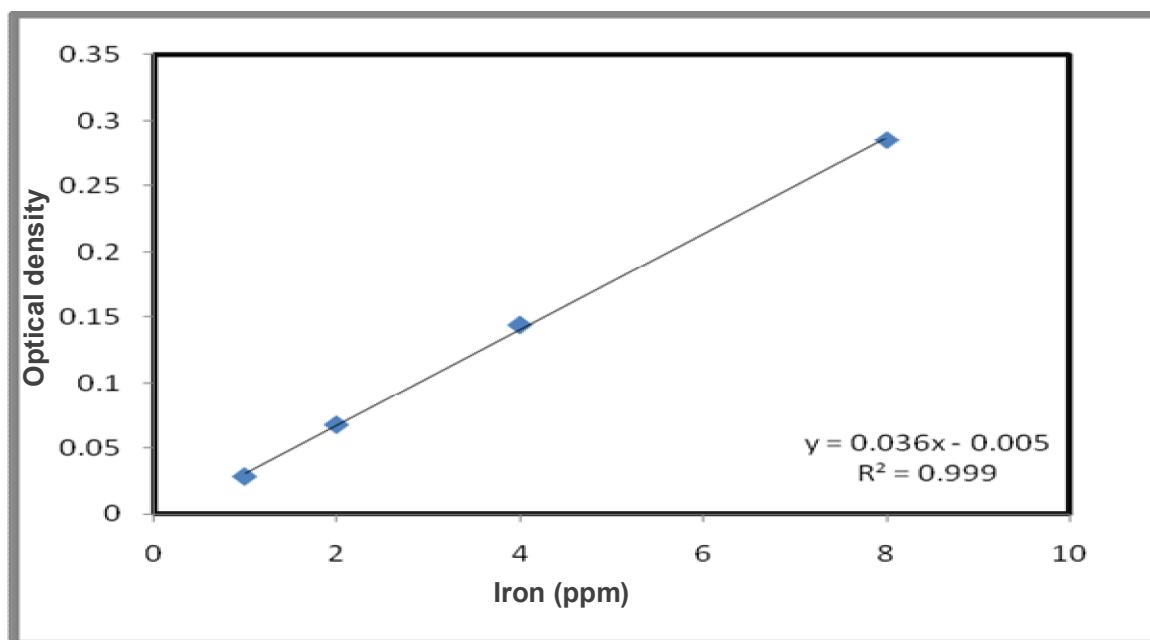


Fig. 3.8 Standard curve for iron



Procedure:

Approximately 1 g of feed and fodder sample was taken in a 100 ml volumetric flask and 10 ml of concentrated nitric acid was added. This was digested at slow heat to get a clear solution. Then 1 ml of perchloric acid was added and heated till the brown fumes ceased to come out and solution became clear. The flask was removed from the hot plate and cooled. Volume was made to 100 ml with distilled water and mixed well. From this, 15 ml was taken in a 50 ml volumetric flask and 5 ml of 25% (v/v) nitric acid was added followed by the addition of 4 ml of acetic-phosphoric acid solution and 1 g barium chloride while shaking the flask. Then 3.5 ml of gum-acacia acetic acid solution was added and the volume was made to 50 ml with distilled water. After proper mixing, the solution was allowed to stand for 15 minutes and the absorbance was measured in a spectrophotometer (Specord-200) at 440 nm. The measurements were done each time against a reagent blank. The absorbance of the sample solution was compared with that of S standard and the S content was expressed on percentage basis.

(ii) Plasma Sulphur:

The plasma inorganic sulphur level was determined by a turbidimetric method as described by Krigsheld *et al.* (1979).

Reagents:

- Trichloro-acetic acid (5%)
- Barium chloride
- Dextran powder
- BaCl₂ reagent was prepared by adding 20 g of BaCl₂ in 10 gm of dextran and the total volume was made to 1 L.

Procedure:

To 0.5 ml of plasma, 2 ml of trichloroacetic acid (5%) was added. The mixture was allowed to stand for 10 min at room temperature. After centrifugation, 1 ml of the clear supernatant was added to 0.25 ml of BaCl₂ reagent and the absorbance was read after 35 min in spectrophotometer

(Specord-200) at 350 nm. The absorbance of the sample solution was compared with that of S standard (Fig. 3.9).

d) Estimation of Chloride

(i) Feed, faeces and residue samples:

The chloride levels were determined the method as described by Chapman and Pratt (1961).

Principle:

The samples are digested by dry ashing procedure. The sample test solution containing Cl is titrated by standard AgNO_3 in the presence of CrO_4 in an alkaline medium (pH 8.2). As long as some Cl persists in the solution, formation of red Ag_2CrO_4 is momentary. When Cl in solution is exhausted through precipitation as AgCl , the red precipitate of Ag_2CrO_4 sharply signals the end point.

Reagents:

0.1N Sulphuric acid, 0.1N Sodium bicarbonate, 0.5% Phenolphthalein indicator, 0.05N Sodium chloride, 0.05N Silver nitrate solution, Potassium chromate indicator.

Procedure:

- 25 ml of test solution was taken in an Erlenmeyer flask
- The pH of the solution was adjusted to 8.2 just colourless to phenolphthalein end point by adding 0.1N Sodium bicarbonate.
- To this, 1 ml of K_2CrO_4 indicator was added and the solution was titrated with standard AgNO_3 solution till the appearance of first permanent red colouration due to the precipitation of Ag_2CrO_4 , and reading was taken. Blank was prepared in the similar manner.

Calculations:

$$\text{mEq/ 100 g sample} = (S-B) \times T \times 0.05 \times 100/ 25 \times \text{Weight of sample}$$

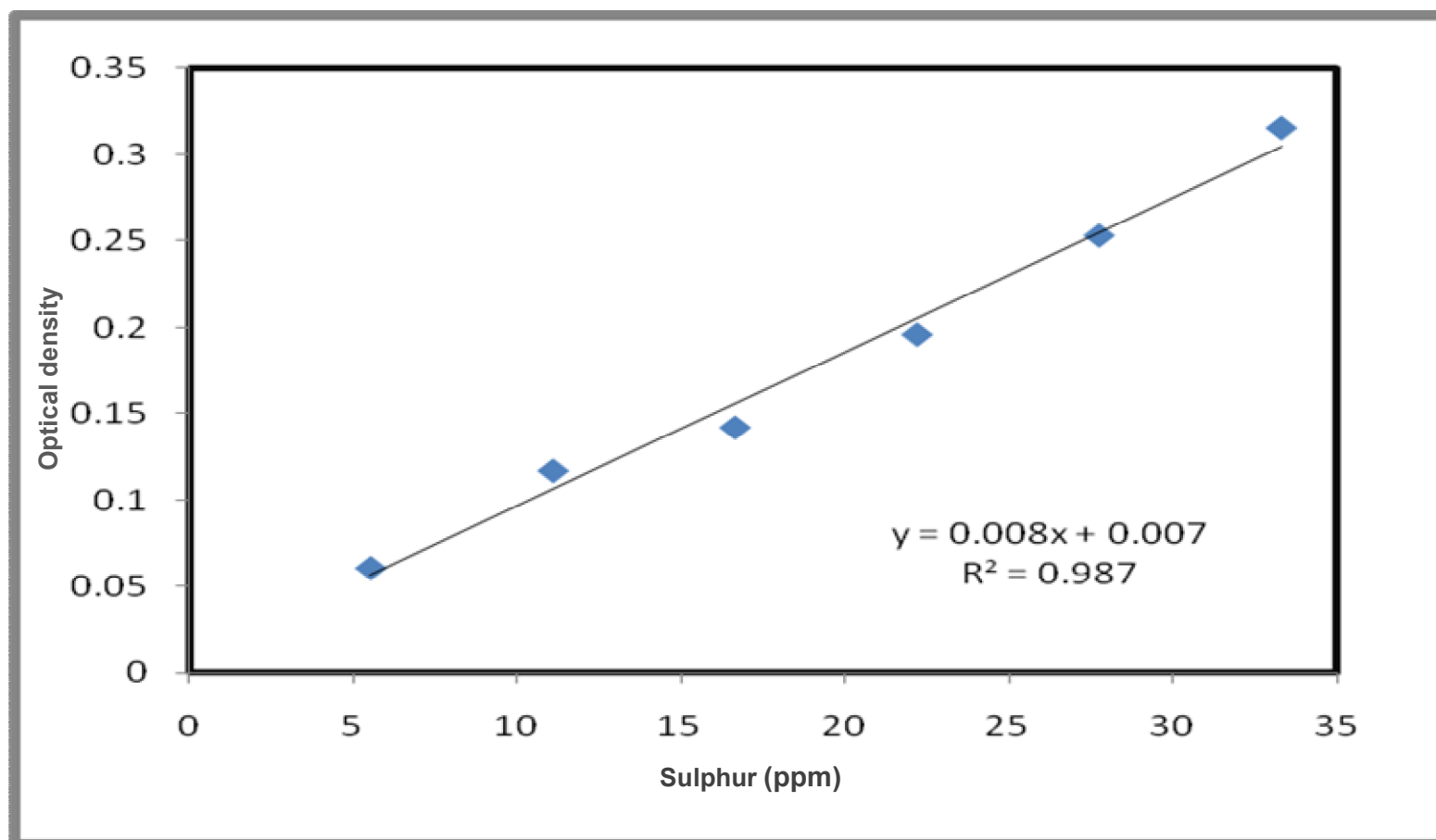
$$\% \text{ Cl in the sample} = \text{mEq/100 g sample} \times \text{mg equivalent weight of Cl}$$

S = ml of AgNO_3 used for precipitating Cl of sample

B = ml of AgNO_3 used for precipitating Cl of blank

T = Total volume of sample test solution prepared in ml

Fig. 3.9 Standard curve for sulphur



(ii) Plasma and urine samples:

Chloride was estimated in blood plasma and urine samples using kit supplied by Avecon Healthcare Pvt Ltd., Ambala. The principle of the assay was that the chloride ions react with mercuric thiocyanate to release thiocyanate ions which in turn react with ferric thiocyanate. The absorbance of the red colored complex at 505 nm is proportional to the chloride concentration.

Procedure:

Six tubes were taken and marked as blank, standard and test in duplicate. Added 1000 µl of chloride reagent to all the tubes marked as blank, standard and test. Then, added 10 µl of chloride standard and plasma/ urine to the tube marked as standard and test. The contents were mixed well and incubated at 37°C for 3 min and read the O.D. on photometer at 505 nm, within 15 minutes. The concentration of chloride was calculated as per formula and expressed in mmol/L.

$$\text{Chloride (mmol/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Concentration of standard = 100 mmol/L

3.4.2 Estimation of chemical composition of feed, faeces and residues

The samples of feeds offered to animals i.e. concentrate mixture, wheat straw and green fodder (Maize/ Jowar/ Berseem) and left over residue were analyzed for proximate principles viz. dry matter (DM), crude protein (CP), ether extract (EE) and total ash as per standard procedure of AOAC (2005). Cell wall components (NDF), ligno-cellulose component (ADF) and acid detergent lignin (ADL) were estimated by the method of Goering and Von Soest (1970).

3.4.3 Estimation of different blood biochemical parameters

a) Glucose

Glucose was determined in plasma using r̃Glucose 96 well assay kitò (Item No.10009582) from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbour, MI 48108, USA.

Principle:

Glucose is oxidized into delta-gluconolactone with concomitant reduction of the FAD dependant enzyme glucose oxidase. The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce H₂O₂. Finally, with horseradish peroxidase as a catalyst, H₂O₂ reacts with 3,5-dichloro-2-hydroxybenzenesulphonic acid and 4-amineantipyrine to generate a pink dye with an optimal absorption at 514 nm.

Assay procedure:

1. Added 5 μ l of sample or standard to appropriately labeled wells.
2. Added 500 μ l of the enzyme mixture forcefully down the side of each tube.
Tap tubes a couple of times to mix thoroughly.
3. Tubes are placed in an incubator at 37°C for 10 minutes.
4. From each tube, 150 μ l (in duplicate) was taken into the 96 well plates.
6. Read the absorbance at 500-520 nm using a plate reader.

Calculations:

$$\text{Plasma glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

b) Triiodothyronine (T₃)

The T₃ was estimated in plasma sample by $\bar{T}_3$ ELISA assay from Endocrine Technologies, Inc. USA.

Test principle:

In T₃ EISA test, T₃ specific antibody is coated on microtitre wells. A measured amount of plasma, a certain amount of goat anti - T₃ antibody, and a constant amount of T₃ conjugated with horseradish peroxidase is added to the microtitre well. During incubation, the T₃ and conjugated T₃ compete for the limited binding sites on the anti - T₃ antibody. A colour is developed after addition of colour developing reagent; the intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T₃ in the sample.

Reagents:

Antibody coated microtitre plate, T₃ HRP conjugate, TMB colour reagent, stop solution (2N HCl), wash buffer.

Standards: Reference standards in range; 0, 0.5, 1.0, 2.5, 5.0, 10 and 20 ng/ml

Assay procedure:

- 50 µl standards, specimens were dispensed in to appropriate wells.
- 100 µl enzyme conjugate reagent was added in to each well.
- The incubation of microtitre plate was carried out at 37°C for 2 hr.
- Incubation mixture was removed and plate was washed 5 times with wash buffer and all the residual water droplets were removed with the help of adsorbent paper.
- 100 µl TMB solution was dispensed in to each well and the plate was mixed gently for 10 sec.
- Then, the plate was incubated in the dark at room temperature for 20 min.
- Reaction was stopped after addition of 50 µl of stop solution. After gently mixing the plate, the colour changed from blue to yellow.
- Optical density was taken at 450 nm with ELISA reader.

Calculations:

A standard curve was plotted for standards against its concentration in ng/ml, and sample concentration was calculated from the standard curve (Fig. 3.10).

c) Thyroxine (T₄)

The T₄ was estimated in plasma sample by $\bar{T}_4$ ELISA assay from Endocrine Technologies, Inc. USA.

Test principle:

In T₄ EISA test, a certain amount of T₄ specific antibody is coated on microtitre wells. A measured amount of plasma and constant amount of T₄ conjugated with horseradish peroxidase is added to the microtitre well. During incubation, the T₄ and conjugated T₄ compete for the limited binding sites on the anti T₄ antibody. A colour is developed after addition of colour developing reagent; the intensity of the colour formed is proportional to the amount of

enzyme present and is inversely related to the amount of unlabeled T₄ in the sample.

Reagents:

Antibody coated microtitre plate, T₄ HRP - conjugate, TMB colour reagent, stop solution (2N HCl), wash buffer.

Standards: Reference standards in range; 0, 5, 10, 25, 50, 100 and 300 ng/ml

Assay procedure:

- 50 µl standards, specimens were dispensed in to appropriate wells.
- 100 µl T₄ HRP -conjugate was added in to each well.
- The incubation of microtitre plate was carried out at 37⁰C for 1 hr.
- Incubation mixture was removed and plate was washed 5 times with wash buffer and all the residual water droplets were removed with the help of adsorbent paper.
- 100 µl TMB colour reagent was dispensed in to each well and the plate was mixed gently for 10 sec.
- Plate was incubated in the dark at a room temperature for 20 min.
- Reaction was stopped after addition of 50 µl of stop solution. After gently mixing the plate, the colour changed from blue to yellow.
- Optical density was taken at 450 nm with ELISA reader.

Calculations:

A standard curve was plotted for standards against its concentration in ng/ml, and sample concentration was calculated from the standard curve (Fig. 3.11).

d) Plasma Cortisol

Cortisol was estimated in plasma by Cortisol EIA from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbor, MI 48108, USA.

Reagent preparation:

1. EIA Buffer- 10 ml EIA Buffer Concentrate + 90 ml ultrapure water

Fig. 3.10 Standard curve for tri- iodothyronine (T₃)

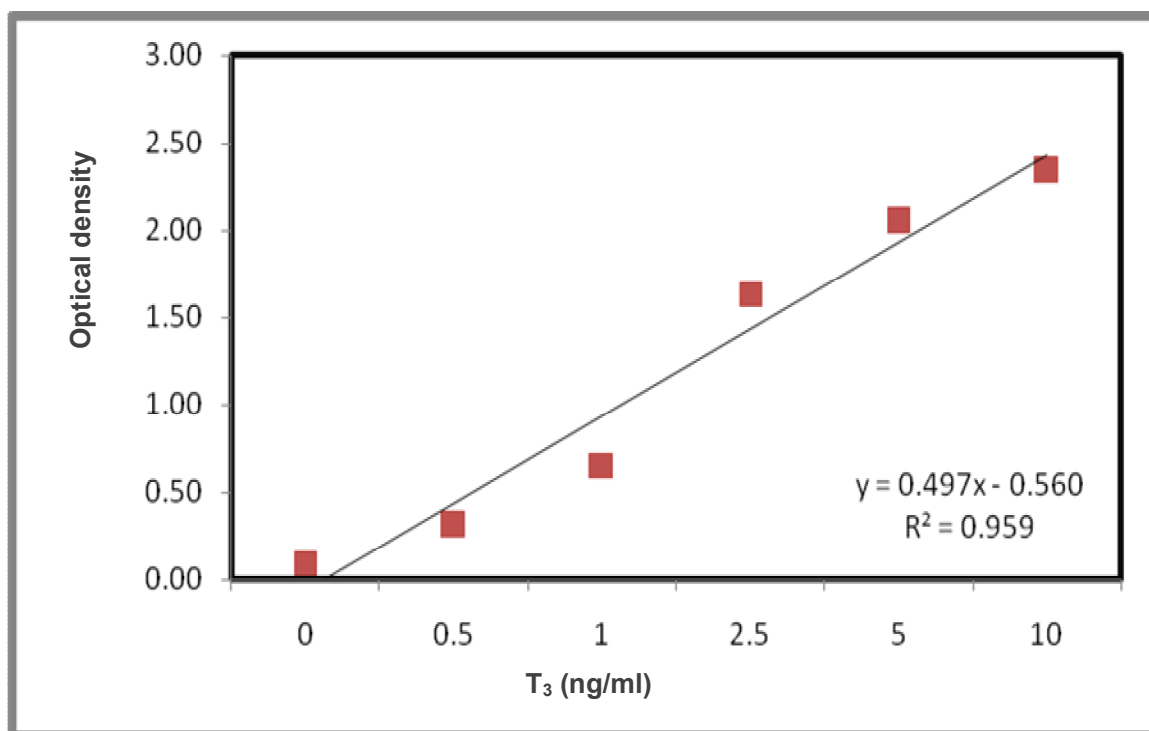
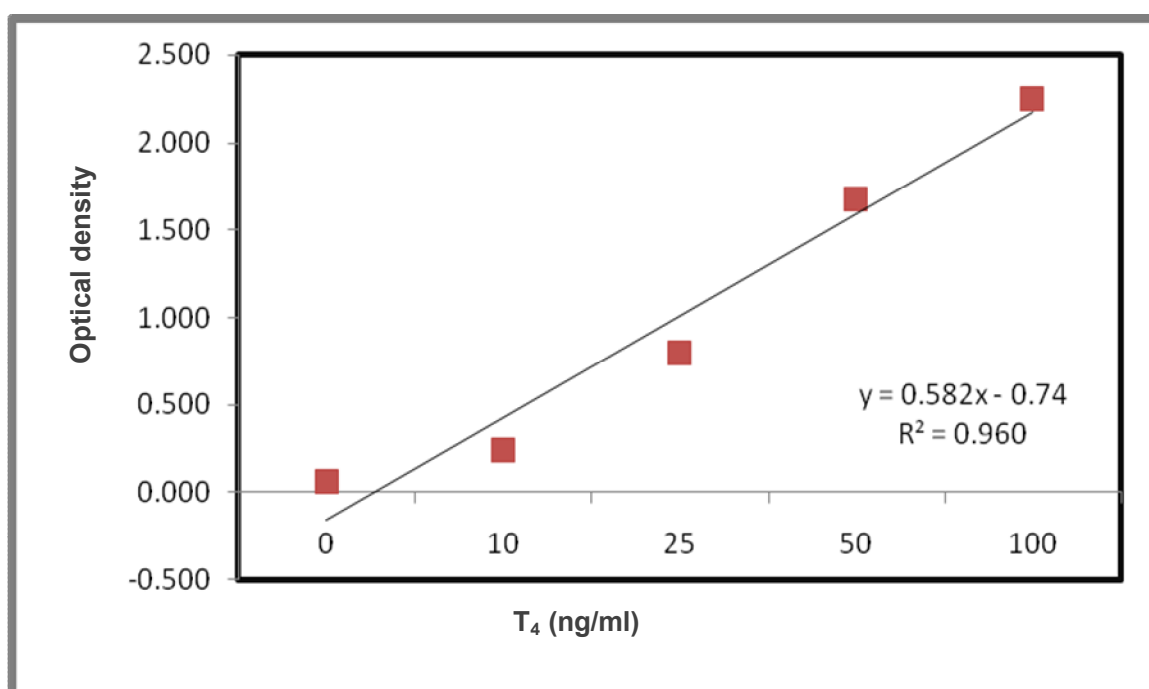


Fig. 3.11 Standard curve for thyroxine (T₄)



2. Wash Buffer- 5 ml Wash Buffer Concentrate + 2 L ultrapure water + 1 ml Polysorbate 20 (i.e. dilution of Wash Buffer Concentrate at 1:400 and adding polysorbate 20 @ 0.5ml/ L of wash buffer).
3. 100 dalton Cortisol AChE Tracer- Reconstituted with 6 ml EIA Buffer.
4. 100 dalton Cortisol EIA Monoclonal Antibody- Reconstitute with 6ml EIA buffer.
5. Cortisol EIA Antiserum- Reconstituted with 6 ml EIA Buffer.
6. Cortisol EIA Standard- Standards were prepared with concentration of 4000, 1600, 640, 256, 102.4, 41.0, 16.4 and 6.6 pg/ ml by serial dilution with EIA Buffer.

Assay Protocol:

Reagent (µl)	Blank	TA	NSB	B ₀	Standard
EIA Buffer	-	-	100	50	-
Standard/Sample	-	-	-	-	50
Tracer	-	-	50	50	50
Antibody	-	-	-	50	50

7. Ellman's Reagent- Prepared just before use by adding 20 ml of ultrapure water.

Assay procedure:

1. Assay plate was incubated at 4⁰C overnight covered with a plastic film.
2. All the wells were washed 5 times with wash buffer.
3. About 200 µl Ellman's reagent was added to all wells except TA well.
4. 5 µl of tracer was added only to the total activity wells.
5. Covered plate was incubated for 90-120 min. with gentle shaking.
6. OD was taken by ELISA Reader at wavelength of 420 nm.

Calculations:

1. Calculated average value of NSB, B₀, standard and sample.
2. NSB average was subtracted from the B₀ average which was called corrected B₀.
3. Calculated B/ B₀ for the remaining wells.
4. A standard curve was plotted of logit (B/B₀) versus concentration and concentration for sample was calculated (Fig. 3.12).

e) Plasma Total Antioxidant Activity

Total antioxidant activity was measured by Ferric Reducing Antioxidant Power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing easily reduced oxidant system present in stoichiometric excess.

Principle:

At low pH, reduction of Ferric Tripyridyl Triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593 nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe^{3+} to Fe^{2+}) ion formation. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

Reagents:

(1) FRAP Reagent:

(A) Acetate buffer 3.0 mM, pH 3.6: Weighed 3.1 gm sodium acetate trihydrate and added 16 ml of glacial acetic acid and the volume was made to 1.0 L with distilled water.

(B) FeCl_3 20 mM in 40 mM HCl

(C) TPTZ 10 mM

The working FRAP reagent was prepared by mixing A, B and C in the ratio of 10:1:1 at the time of use.

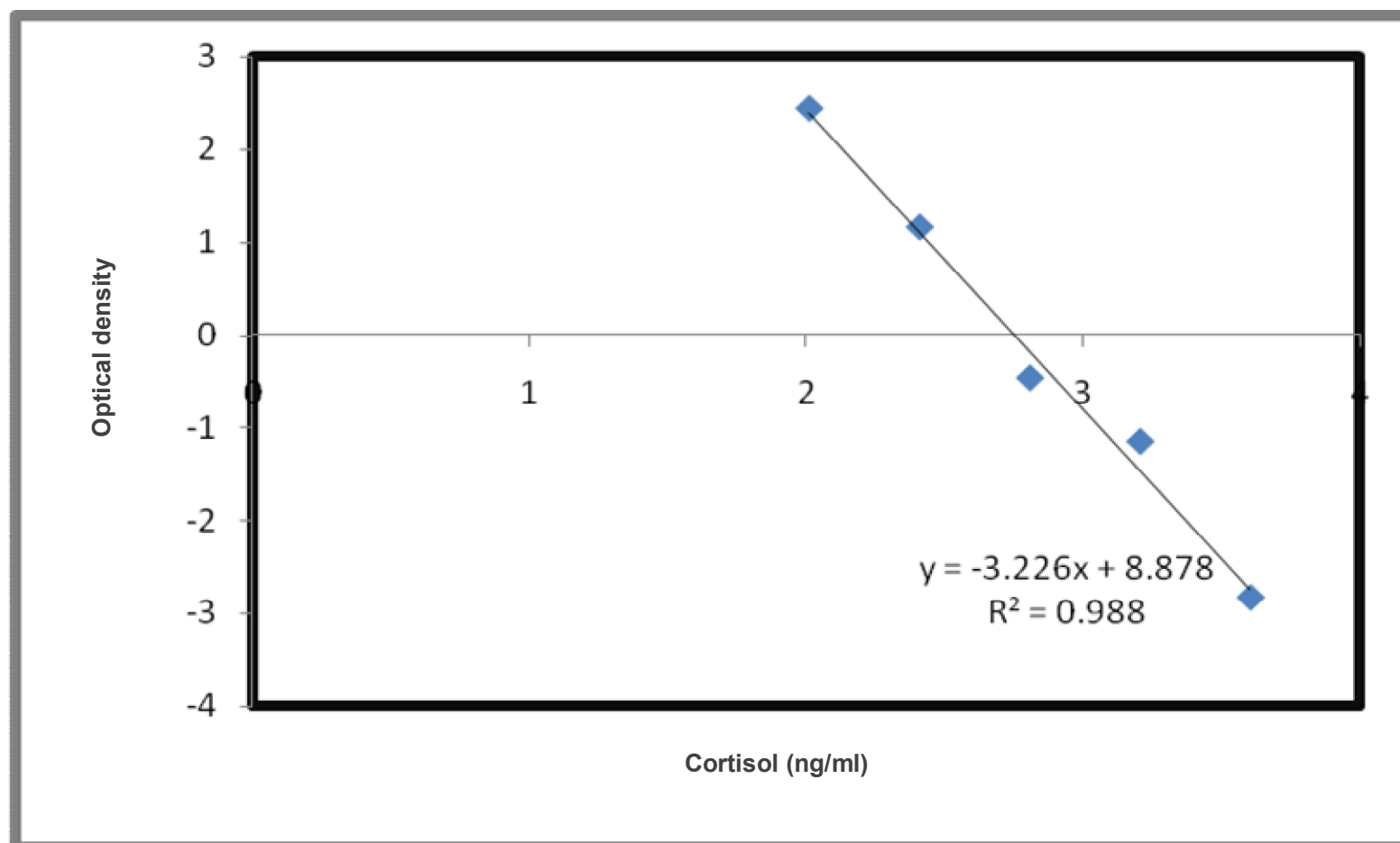
(2) FeSO_4 1mM

(3) Ascorbic acid 1000 μM

Procedure:

Plasma (100 μl) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorbance was measured after 4 minute. Ascorbic acid standards (100 μM -1000 μM) were processed in the same way.

Fig. 3.12 Standard curve for cortisol



Calculations: Results were calculated as follows.

$$\text{FRAP value of sample } (\mu\text{mol/L}) = \frac{A-B}{X-Y} \times 2 \times 1000$$

A = Reading of sample at 0 minute

B = Reading of sample at 4 minute

X = Reading of standard at 0 minute

Y = Reading of standard at 4 minute

2 = FRAP value of 1000 μM standard

f) Superoxide Dismutase (SOD)

Superoxide Dismutase was determined in plasma samples by $\text{\textcircled{S}}$ Superoxide Dismutase Assay kit from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbor, MI 48108, USA.

Reagent Preparation:

1. Assay Buffer- The content of vial (3 ml) was diluted with 27 ml of HPLC-grade water.
2. Sample Buffer- 2 ml of sample buffer was diluted with 18 ml of HPLC-grade water.
3. Radical Detector- 50 μl of radical detector solution + 19.95 ml of assay buffer.
4. SOD Standard- Standards were prepared with SOD activity of 0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 U/ml by serial dilution with sample buffer.
5. Xanthine Oxidase- 50 μl of enzyme + 1.95 ml of sample Buffer.

Assay Protocol:

1. SOD Standard Wells- 200 μl of diluted radical detector and 10 μl of standard per well with increasing activity of SOD.
2. Sample Wells- 200 μl of diluted radical detector and 10 μl of sample per well.
3. Initiated the reaction by adding 20 μl of diluted xanthine oxidase to each well and shook the plate carefully for a few seconds to mix.
4. Incubated the plate on shaker for 20 minutes at room temperature.
5. OD was taken, using ELISA reader at 460 nm.

Calculations:

1. Calculated the average absorbance (Abs) of each standard and sample.
2. Calculated linearized rate (LR) for all the standards. For this, absorbance of standard A was divided by absorbance of all the standards.
3. Calculated linearized rate (LR) for all the samples. For this, absorbance of standard A was divided by absorbance of all the samples.
4. Plotted the linearized SOD standard rate (LR) as a function of final SOD activity (U/ml) and SOD activity of the samples was calculated using linear regression equation of standard curve (Fig. 3.13).

$$\text{SOD (U/ml)} = [(\text{sample LR} - \text{intercept})/\text{slope}] \times 0.23/0.01 \times \text{sample dilution}$$

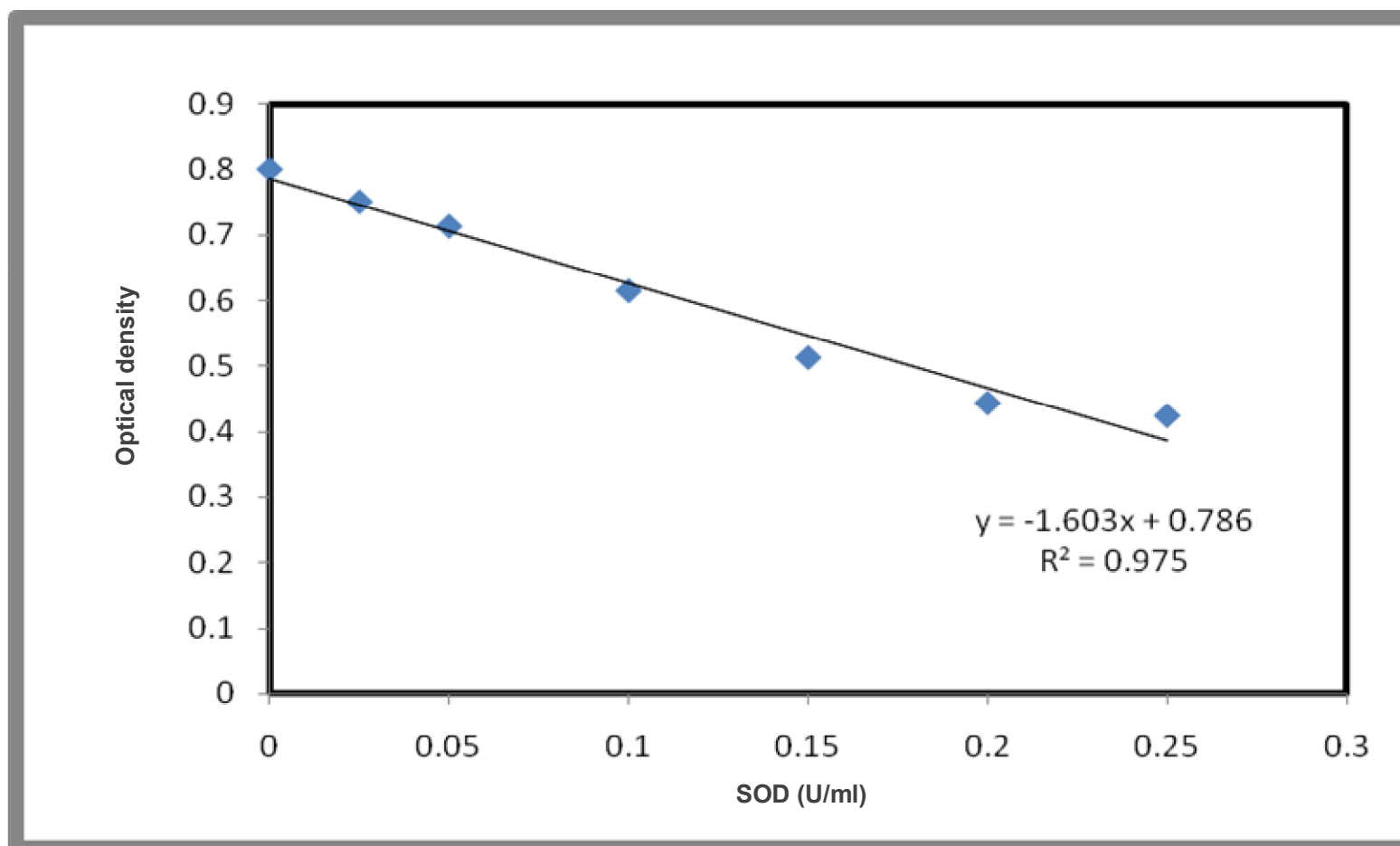
g) Catalase (CAT)

Catalase was determined in plasma samples by Catalase Assay kit from Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbor, MI 48108, USA.

Reagents:

1. Assay Buffer- 2 ml of assay buffer was diluted with 18 ml of HPLC-grade water.
2. Sample Buffer- 5 ml of sample buffer was diluted with 45 ml of HPLC-grade water.
3. Catalase (Control) - Reconstituted lyophilized bovine liver CAT by adding 2 ml sample buffer in the vial. From this reconstituted enzyme, 100 μ l was diluted with 1.9 ml of diluted sample buffer.
4. Potassium Hydroxide (10 M): Added 4 ml of cold HPLC-grade water in the vial, placed on ice and vortexed to yield a 10 M solution.
5. Hydrogen Peroxide (8.82 M): Diluted 40 μ l of hydrogen peroxide solution with 9.96 ml of HPLC-grade water.
6. Formaldehyde Standard- Standards were prepared with concentration of 0, 5, 15, 30, 45, 60 and 75 μ M by serial dilution with sample buffer.
7. Catalase Purpald (Chromagen) - It is 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole in 0.5 M HCl.
8. Catalase Potassium Periodate

Fig. 3.13 Standard curve for superoxide dismutase



Assay Protocol:

1. Formaldehyde Standard Wells- Added 100 µl of assay buffer + 30 µl of methanol + 20 µl of standard (A-G) with increasing concentration.
2. Positive Control Wells- Added 100 µl of assay buffer + 30 µl of methanol + 20 µl of CAT control.
3. Sample Wells- Add 100 µl of assay buffer + 30 µl of methanol + 20 µl of sample to each well.
4. Initiated the reaction by adding 20 µl of diluted hydrogen peroxide to all wells and mixed carefully for a few seconds.
5. Incubated the plate on shaker for 20 min at room temperature.
6. Added 30 µl of potassium hydroxide to each well to terminate the reaction.
7. Added 30 µl of purpald (chromagen) to each well and incubated the plate at room temperature on the shaker for 10 min at room temperature.
8. Added 10 µl of potassium periodate to each well and incubated the plate on shaker for 5 min at room temperature
9. Read the absorbance using ELISA reader at 540 nm.

Calculations:

1. Calculated the average absorbance of each standard and sample.
2. Subtracted the average absorbance of standard A from itself and all other standards and samples.
3. Plotted the corrected absorbance of standards as a function of final formaldehyde concentration (µM) and the formaldehyde concentration of the samples were calculated using linear regression equation of standard curve (Fig. 3.14).

$$\text{Formaldehyde (}\mu\text{M)} = [(\text{sample absorbance} - \text{intercept})/\text{slope}] \times 0.17/0.02$$

$$\text{Catalase activity (nmol/min/ml)} = (\mu\text{M of sample}/20 \text{ min}) \times \text{sample dilution}$$

h) Total Immunoglobulins

Immunoglobulins in the plasma sample were estimated by Zinc turbidity method (Mc Ewan and Fisher, 1970).

Reagents: ZnSO₄, Fetal calf serum, Rabbit gamma globulin.

Test reagent:

It was prepared by taking 4.1 ml of 5% ZnSO₄ and the volume was made to 1 L with freshly prepared distilled water.

Procedure:

About 0.1 ml of plasma sample was taken in a clean dry test tube. Then, 12 ml of test reagent was added and kept at room temperature for 1 hr. The standards (3.12-50.00 mg/ml) were prepared in fetal calf serum and were processed similar to samples. OD was taken at 460 nm. Then the OD of samples was plotted against the standard curve and the concentrations of total immunoglobulin in the samples were estimated and expressed as mg/ml of plasma.

i) Assay for plasma IgG

IgG was determined in plasma by Bovine IgG ELISA kit, pink-one from Koma Biotech Inc. Ltd., Seoul, Korea.

Materials provided:

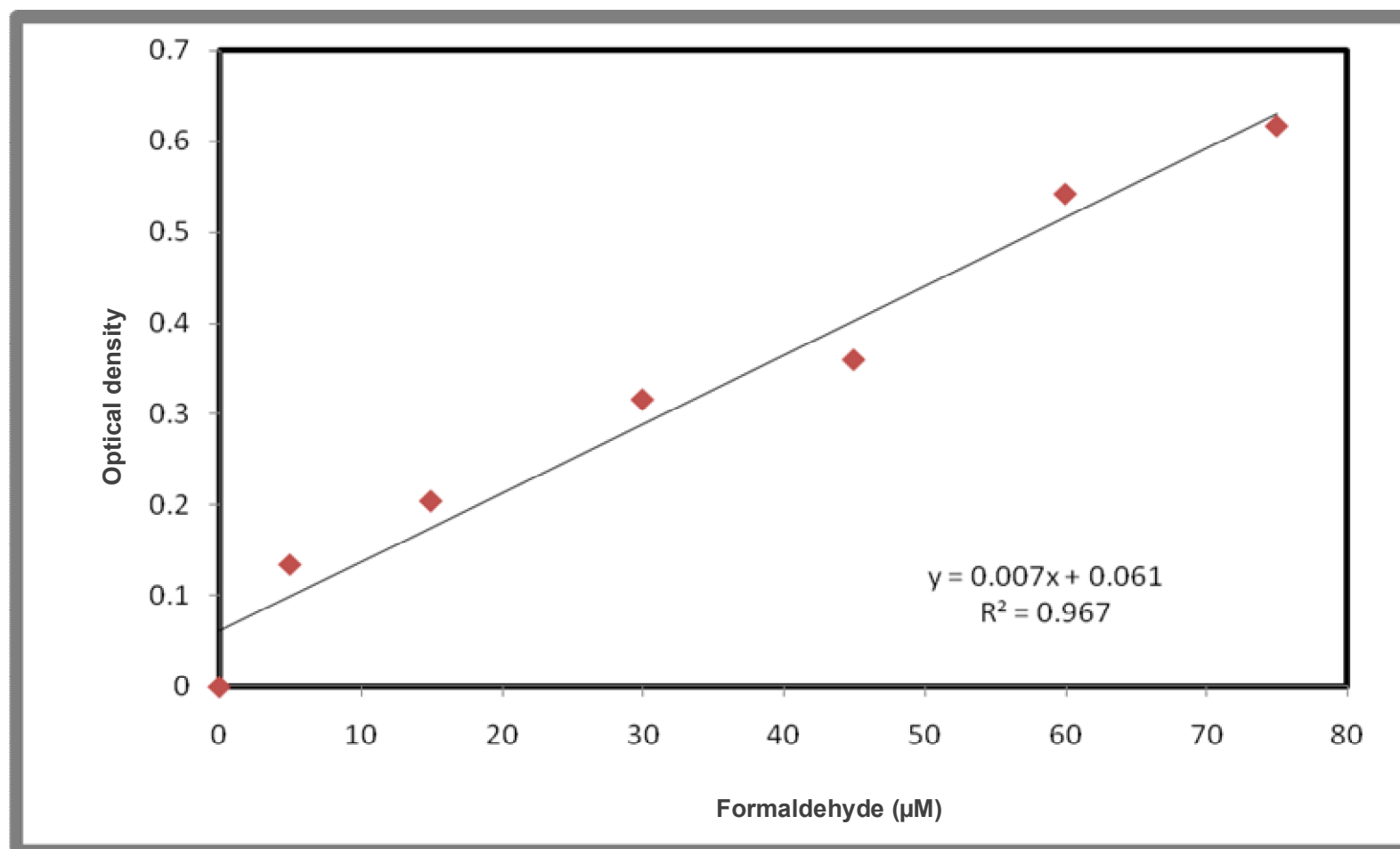
ELISA microplate, detection antibody, standard protein, assay diluents, PBS powder, tween-20 (50%), pink - ONE TMB solution and stop solution.

Reagent preparation:

1. Washing solution: Resolved the PBS powder (1 pouch) to sterile water and volume was made up to 1 L and then added 1 ml Tween-20 (50%) to the solution and mixed well.
2. Standard: Diluted the standard in the pink-ONE assay diluents at 1:2 serial dilutions as follows, and transferred 100 μ l of standard to assigned wells.

Step	Dilution Method	Standard conc.
A	1 μ l of standard + 1.2 ml of assay diluent	20,000 ng/ml
B	0.025 ml of step A + 0.975 ml of assay diluent	500 ng/ml
C	0.5 ml of step B + 0.5 ml of assay diluent	250 ng/ml
D	0.5 ml of step C + 0.5 ml of assay diluent	125 ng/ml
E	0.5 ml of step D + 0.5 ml of assay diluent	62.5 ng/ml
F	0.5 ml of step E + 0.5 ml of assay diluent	31.25 ng/ml
G	0.5 ml of step F + 0.5 ml of assay diluent	15.63 ng/ml
H	0.5 ml of step G + 0.5 ml of assay diluent	7.8 ng/ml

Fig. 3.14 Standard curve for catalase



3. Sample dilution: Sample was diluted to 1000 times with assay diluents.
4. Detection antibody: Detection antibody was diluted in assay diluents (1:20,000).

Procedure:

1. Added 200 μ l of washing solution to each well. Plate was washed three times. After wash, the plate was inverted to remove the residual solution and blotted on paper towel.
2. Added 100 μ l of standard or sample to each well in duplicate and plate was incubated at room temperature for at least 1 hr.
3. Aspirated the well to remove liquid and plate was again washed for 4 times as in step 1.
4. 100 μ l of diluted detection antibody was added per well and plate was incubated at room temperature for at least 1 hr.
5. Aspirated and plate was again washed for 4 times.
6. 100 μ l of pink One TMB colour development reagent was added to each well and plate was incubated for 10 min. at room temperature. Reaction was stopped after addition of 100 μ l of the stop solution.

Calculations:

Averaged the duplicate readings for each standard, control, and sample and these were subtracted with the average zero standard optical density. Standard curve was drawn by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and best fit curve was drawn through the points on the graph (Fig. 3.15).

j) Assay for plasma IgM

IgM was determined in plasma of cows by Bovine IgM ELISA kit, pink-one from Koma Biotech Inc. Ltd., Seoul, Korea.

Materials provided:

ELISA microplate, detection antibody, standard protein, assay diluents, PBS powder, tween-20 (50%), pink - ONE TMB solution and stop solution.

Reagent preparation:

1. Washing Solution: Resolved the PBS powder (1 pouch) to sterile water and made 1 L and then added 1 ml tween-20 (50%) to the solution and mixed well.
2. Standard: Diluted the standard in the pink-ONE assay diluents at 1:2 serial dilutions as follows, and transferred 100 μ l of standard to assigned wells.

Step	Dilution Method	Standard conc.
A	1 μ l of standard + 1.8 ml of assay diluent	1000 ng/ml
B	0.025 ml of step A + 0.975 ml of assay diluent	500 ng/ml
C	0.5 ml of step B + 0.5ml of assay diluent	250 ng/ml
D	0.5 ml of step C + 0.5ml of assay diluent	125 ng/ml
E	0.5 ml of step D + 0.5ml of assay diluent	62.5 ng/ml
F	0.5 ml of step E + 0.5ml of assay diluent	31.25 ng/ml
G	0.5 ml of step F + 0.5ml of assay diluent	15.63 ng/ml

3. Sample dilution: Sample was diluted to 1000 time with assay diluents.
4. Detection antibody: Detection antibody was diluted in assay diluents (1:20,000).

Procedure:

1. Added 200 μ l of washing solution to each well. Plate was washed three times. After wash, the plate was inverted to remove the residual solution and blotted on paper towel.
2. Added 100 μ l of standard or sample to each well in duplicate and plate was incubated at room temperature for at least 1 hr.
3. Aspirated the well to remove liquid and plate was again washed for 4 times as in step 1.
4. 100 μ l of diluted detection antibody was added per well and plate incubated at room temperature for at least 1 hr.
5. Plate was aspirated and washed 4 times.
6. 100 μ l of pink One TMB colour development reagent was added to each well and plate was incubated for 12 min. at room temperature. Reaction was stopped after addition of 100 μ l of the stop solution.
7. The plate was read at 450 nm using ELISA reader.

Fig. 3.15 Standard curve for IgG

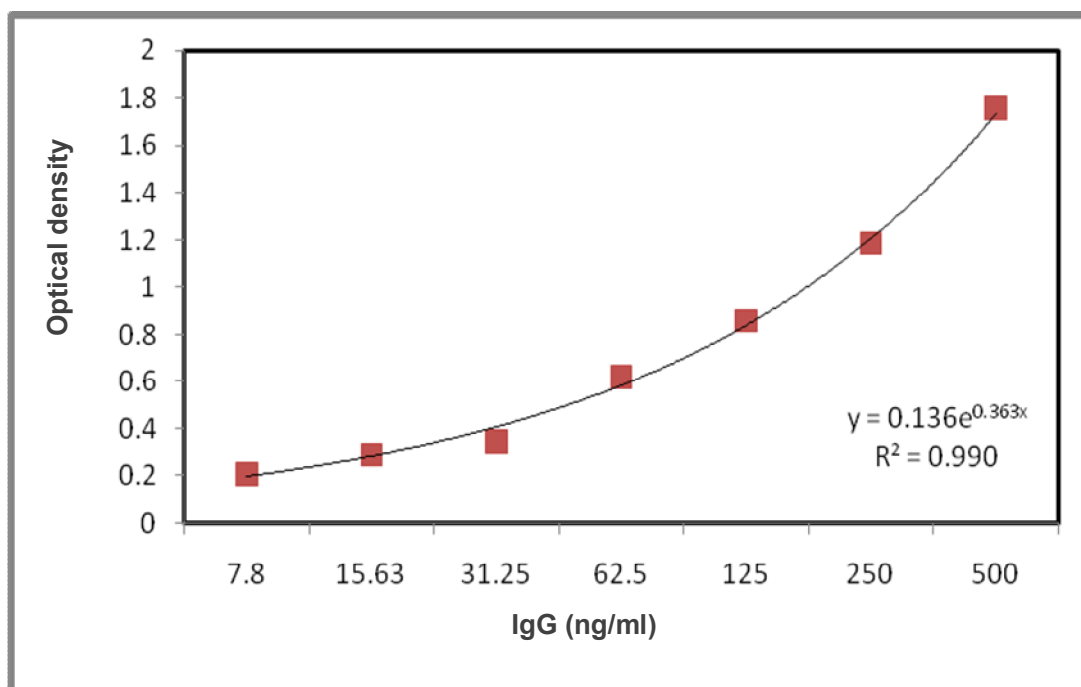
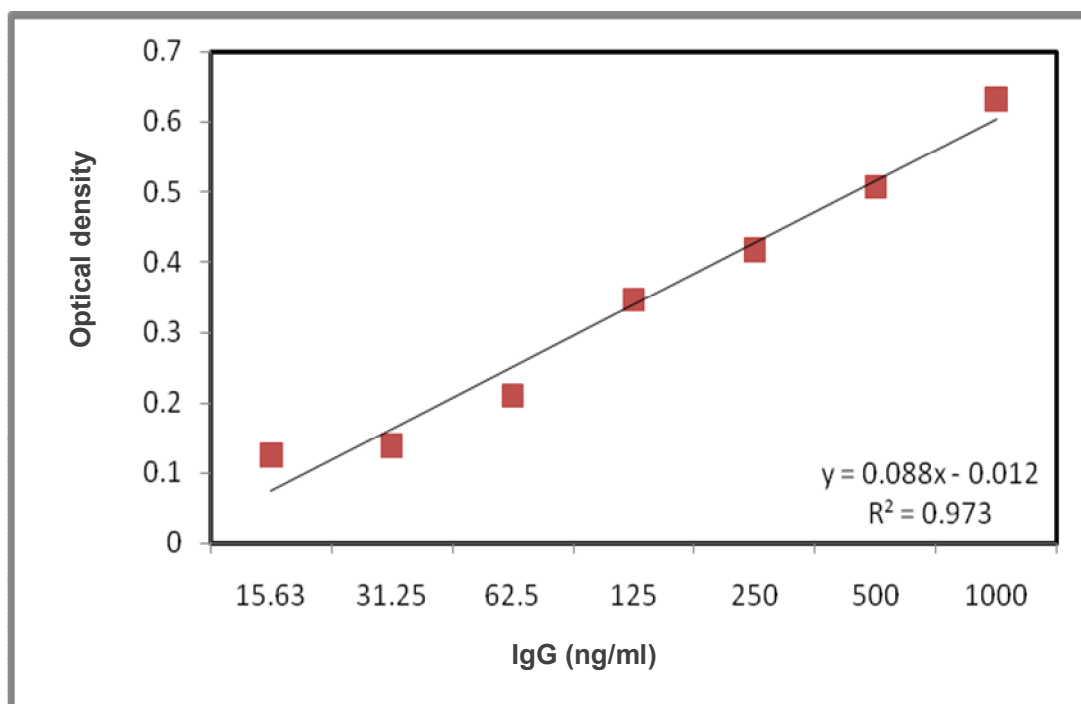


Fig. 3.16 Standard curve for IgM



Calculations:

Averaged the duplicate readings for each standard, control and sample and these were subtracted with the average zero standard optical density. Standard curve drawn by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and best fit curve was drawn through the points on the graph (Fig. 3.16).

3.5 Statistical Analysis

The data obtained from both the experiments was analyzed by two way ANOVA as described in Snedecor and Cochran (1994). The test of significance among the different treatments was also analyzed (SPSS, 1998).

CHAPTER – 4

Results and Discussion

4. RESULTS AND DISCUSSION

The results of experiments conducted on growing crossbred calves, to study the effect of feeding +150, +250 mEq/ kg DM DCAD in winter season (Experiment 1) and +250, +350 mEq/ kg DM DCAD (Experiment 2) in summer season, on various parameters (growth performance, plasma mineral profile of major and trace minerals, mineral balances, biochemical parameters, antioxidant status, immunity and hormonal changes) are presented in this section.

4.1 Minerals content of different feedstuffs

The major minerals (Ca, P, Mg, Na, K, Cl, and S) content of different feeds and fodders is presented in Table 4.1. The Ca content in wheat straw was 0.30%. Berseem (leguminous fodder) contained higher Ca, to the extent of 1.67%. Cereals and cereal by-products were deficient in Ca content. P content varied from 0.13 to 0.69% in roughages and from 0.40 to 1.05% in concentrate feed ingredients. The compound feed contained 1.16% Ca and 0.71% P. All feeds and fodders contained sufficient amount of Mg and P except wheat straw. Recommended concentrations for dietary Mg are reported to be within the range 0.2-0.4% of total DMI (Underwood and Suttle, 1999; Goff, 2000).

The Na and K contents in different concentrate feed ingredients varied from 0.01-0.10% and 0.48-2.40%, respectively. The variation in fodders tested was 0.01-1.08% for Na and 0.92-3.24% for K. The compound feed contained 0.99% Na and 1.30% K. The Na content was only 0.18% in wheat straw, whereas, berseem fodder contained highest amount of Na (1.08%), followed by oat fodder (0.67%). Na content was low in maize fodder (0.17%), cowpea (0.03%), jowar (0.02%) and barley fodder (0.01%). Similarly, there was wide variation with regard to K content among different green fodders. K content in dry and green fodders was higher (0.92-3.24%) than the concentrate feed ingredients (0.48-2.40%). Similar findings were also reported by Singh *et al.* (2002) and Garg *et al.* (2011) that K content in dry and green fodders was higher (3.41%) than the concentrate feed ingredients (1.02%). Higher K content

of feedstuffs may be due to its selective uptake from the soil and due to application of K based fertilizers to the soil.

All the concentrate ingredients tested contained low level of Cl in the range of 0.07-0.16% and S in the range of 0.07-0.28%. The Cl content in various roughage sources varied from 0.4-1.66%. Cl content in compound feed was 1.33% which might be due to the addition of sodium chloride in it. The S content varied from 0.16-0.45% in green fodders. The variation in S content of plants depends largely on the amount of S in plant proteins in the form of S-containing amino acids (McDowell, 1992).

4.2 Experiment 1: Effect of feeding +150, +250 mEq/ kg DM DCAD to crossbred calves during winter months

4.2.1 Chemical composition of the experimental diet

The feeds offered viz. concentrate mixture; wheat straw and fodder (Maize/ berseem) to the calves were analyzed for their major mineral contents (Table 4.1) and proximate composition and fibre fractions (Table 4.2). Chemical and mineral composition of feeds and fodders was similar to the earlier reports (Senthil Kumar, 2004; Halder *et al.*, 2009 and Meena, 2012).

4.2.2 Environmental conditions during the experiment

The maximum and minimum temperature ($^{\circ}\text{C}$) during morning time ranged from 16.5-33.5 and 4.4-15.6, respectively throughout the experimental period (December - April). During afternoon, the corresponding temperatures ranged from 19.5-34.1 and 5.8-18.1 $^{\circ}\text{C}$ (Table 4.3) which were higher than the morning temperatures. The lowest maximum morning temperature was observed in III fortnight (Jan., 9-Jan., 23), whereas minimum morning temperatures were observed in fortnight I (Dec., 10-Dec., 24). The highest maximum and minimum temperatures during morning and afternoon were observed in fortnight VIII (March, 24-April, 8). The relative humidity in morning and afternoon ranged from 81-98% and 30-60%, respectively. The THI throughout the experimental period varied from 49.1-67.3 and 61.1-81.8 during morning and afternoon, respectively. Except in the last fortnight (VII and VIII) afternoon value for

Table 4.1 Macro minerals content of different feeds and fodders (DM basis)

	Macro Minerals						
	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Cl (%)	S (%)
Critical level*	< 0.30	< 0.25	< 0.20	< 0.06	< 0.80	< 0.20	< 0.20
Concentrates							
Compound feed	1.16	0.71	0.71	0.99	1.30	1.33	0.47
Bajra grain	0.03	0.40	0.13	0.04	0.81	0.09	0.26
Maize grain	0.01	0.45	0.22	0.03	0.48	0.08	0.09
Cotton seed cake	0.14	0.52	0.80	0.03	1.37	0.07	0.15
Groundnut cake	0.78	0.41	0.46	0.02	1.86	0.10	0.07
Mustard oil cake	0.84	1.05	0.66	0.10	1.30	0.09	0.18
Guar meal	0.42	0.48	0.40	0.01	1.54	0.10	0.27
Soybean meal	0.51	0.92	0.33	0.04	2.40	0.10	0.13
Deoiled rice bran	0.07	0.66	0.32	0.02	1.93	0.09	0.28
Wheat bran	0.18	0.54	0.46	0.06	1.78	0.16	0.18
Roughages							
Barley fodder	0.58	0.30	0.58	0.01	1.56	0.50	0.32
Berseem	1.67	0.40	0.54	1.08	3.24	0.40	0.30
Cow pea fodder	1.08	0.43	1.10	0.03	0.92	0.60	0.25
Jowar fodder	0.70	0.57	0.50	0.02	2.41	0.80	0.16
Maize Fodder	0.97	0.38	0.53	0.17	2.00	1.66	0.45
Oats fodder	0.45	0.69	0.82	0.67	1.90	1.20	0.31
Wheat straw	0.30	0.13	0.08	0.18	2.05	1.00	0.35

*Critical level: Concentrations below which are low or considered deficient (McDowell *et al.*, 1992), based on requirements for cattle (NRC, 2001)

Table 4.2 Chemical composition of feed ingredients DM basis (%)

Feed/ fodder	CP	NDF	ADF	EE	Ash
Concentrate mixture	21.23	34.17	21.83	4.72	5.04
Maize fodder	8.93	54.38	23.47	1.62	7.85
Berseem fodder	17.21	55.62	21.84	1.46	7.18
Oats fodder	11.26	48.84	30.65	1.95	12.76
Wheat straw	3.17	67.85	40.20	0.76	12.04
Jowar fodder	10.6	61.36	41.11	1.61	11.80

Table 4.3 Environmental parameters recorded during the winter months

Fortnight	Max.Temp (°C)		Min.Temp (°C)		Relative Humidity (%)		THI	
	M	A	M	A	M	A	M	A
0 (Nov. 26,- Dec. 9,2011)	26.1	26.4	12.1	15.8	94.0	43.0	59.0	71.6
I (Dec. 10,- Dec. 24,2011)	19.4	22.8	4.4	5.8	97.0	48.0	49.1	64.1
II (Dec. 25,2011- Jan. 8,2012)	18.4	21.7	6.9	8.3	98.0	60.0	54.6	62.3
III (Jan. 9 ĩ Jan. 23,2012)	16.5	19.5	5.6	7.6	93.0	59.0	50.1	61.1
IV (Jan.24 - Feb. 7,2012)	19.1	21.6	6.0	7.4	88.0	49.0	51.7	64.1
V (Feb. 8 ĩ Feb. 22,2012)	20.7	21.8	6.6	6.9	83.0	43.0	51.4	65.8
VI (Feb. 23- March 8,2012)	24.3	26.9	8.6	10.1	85.0	40.0	56.7	68.9
VII (March 9- March 23,2012)	26.3	27.4	10.9	12.9	82.0	36.0	58.3	72.0
VIII (March 24- April 8,2012)	33.5	34.1	15.6	18.1	81.0	30.0	67.3	81.8

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm

THI (72 and 81.8), all other values during the whole experiment were below 72, indicating cold stress on the animals (LPHSI, 1990). Srikandakumar *et al.* (2003) and Gantner *et al.* (2011) stated that THI value below 72 is used to indicate cold stress on the animals. The experimental animals were under more cold stress during the months of December to February as the lowest THI values were observed in these months. The THI observed during winter trial was lower as compared to THI recorded during the summer trial (Table 4.25).

4.2.3 Physiological variables

The effect of +150, +250 mEq/ kg DM DCAD diet on respiration rate (RR), pulse rate (PR) and rectal temperature (RT) in crossbred calves is presented in Tables 4.4, 4.5 and 4.6, respectively. The mean values of respiration rate (breaths/ min) during the morning and afternoon were 20.7, 21.2, 21.5 and 24.7, 25.3, 25.3 at the start of experiment and increased to 30.3, 29.5, 29.5 and 31.0, 30.8, 30.8 breaths/ min at the end of the experimental period of eight fortnights in control, W₁ and W₂ groups, respectively. The respiration rate in all the three groups of calves was comparatively higher in the afternoon as compared to morning values due to higher environmental temperature and THI in the afternoon. There was numerical increase in the respiration rate during the VI and VII fortnights due to increase in the environmental temperature and THI, there was significant increase ($P<0.01$) in RR in the VIIIth fortnight when the average environmental temperature reached to 33.8⁰C and THI increased to 74.6 indicating heat stress on the animals. However, there was no significant difference in the respiration rate among three different groups in the eight fortnights.

The results obtained in the present study are in agreement with Itoh *et al.* (1997) and Brown-Brandl *et al.* (2003) who reported that respiration rate fell progressively in cold stress and increased with increase in the ambient temperature. Gaughan *et al.* (1999) found that cattle anticipate temperature changes and have a tendency to decrease respiration rate prior to a decrease in ambient temperature. Eigenberg *et al.* (2000) also reported that respiratory rate positively correlates with ambient

temperature. As the experiment started in December when the environmental temperature was low, so the RR was less as compared to the end of experimental period in the month of April when temperature started to increase (Table 4.3).

Throughout the experimental period of eight fortnights, the pulse rate varied from 60.2-66.0, 60.2-66.7 and 62.0-66.0 in the mornings showing no significant difference among groups as well as between fortnights (Table 4.5). But in the afternoon, the variation in pulse rate during different fortnights was more i.e. 64.3-76.0, 65.0-75.3 and 65.0-75.0 in the three respective groups. Significant variation in pulse rate in the afternoons was observed in the VIIth and VIIIth fortnight in control group, whereas it was observed only in the VIIIth fortnight in W₁ and W₂ groups which might be due to higher environmental temperature and THI value during this period. The overall mean values of pulse rate (beats/min) during the morning and afternoon observations in control, W₁, W₂ groups were 63.3, 63.4, 63.6 and 69.9, 69.5, 69.9 beats/min, respectively showing no significant difference ($P>0.05$) among the three experimental groups, Koga *et al.* (1999) also reported decrease in pulse rate with a decrease in ambient temperature and relative humidity. Koubkova *et al.* (2002) observed that the pulse rate decreased significantly during cold period as compared to the hot period. The mean values of pulse rate of growing and adult sahiwal cattle during morning increased by 4.00 and 2.83 beats/min whereas during afternoon the pulse rate increased by 4.83 and 8.00 beats/min respectively during summer as compared to spring season (Dandage, 2009;Chader bhan *et al.*, 2012). Chadra Bhan *et al.* (2012) reported that, the mean values of RR and PR during morning varied from 20.67 ± 0.49 to 26.16 ± 0.31 /min and 71.00 ± 0.49 to 69.50 ± 0.76 /min and in afternoon varied from 24.50 ± 0.48 to 29.83 ± 0.31 /min and 70.16 to 74.83 ± 0.31 /min, respectively in adult Sahiwal cattle during winter and summer months.

At the start and end of experiment the respective mean values of rectal temperature ($^{\circ}\text{C}$) during morning and afternoon were 38.1, 38.2, 38.2 and 38.3, 38.4, 38.4 which increased to 38.4, 38.5, 38.4 and 38.5, 38.6, 38.5 in the mornings and afternoons in the three respective groups

Table 4.4 Effect of positive DCAD diet on respiration rate (breaths/minute) in crossbred calves during winter months

Fortnight	Control		W ₁		W ₂	
	M	A	M	A	M	A
0	20.7 ^x ±1.1	24.7 ^x ±1.1	21.2 ^x ±1.1	25.3 ^x ±1.2	21.5 ^x ±0.6	25.3 ^x ±1.2
I	21.8 ^x ±0.8	24.7 ^x ±0.9	21.3 ^x ±0.8	23.7 ^x ±1.9	22.7 ^x ±1.0	25.2 ^x ±1.2
II	20.8 ^x ±1.0	23.7 ^x ±1.3	21.5 ^x ±1.0	24.3 ^x ±1.0	22.3 ^x ±1.3	24.7 ^x ±1.0
III	23.7 ^x ±0.8	25.3 ^x ±1.2	23.7 ^x ±0.8	25.0 ^x ±1.4	25.0 ^x ±1.2	24.8 ^x ±1.5
IV	23.3 ^x ±1.4	25.0 ^x ±1.3	23.7 ^x ±1.8	25.3 ^x ±1.2	24.0 ^{xy} ±1.5	25.7 ^x ±1.6
V	24.3 ^x ±1.9	25.7 ^x ±1.1	24.0 ^x ±1.5	26.0 ^{xy} ±1.6	24.5 ^{xy} ±1.5	26.2 ^x ±1.5
VI	25.2 ^{xy} ±1.7	26.5 ^{xy} ±1.0	25.7 ^{xy} ±1.3	26.7 ^{xy} ±1.3	25.8 ^{xy} ±1.4	26.8 ^x ±1.7
VII	26.0 ^{xy} ±1.5	28.5 ^{xy} ±1.5	26.2 ^{xy} ±1.5	28.3 ^{xy} ±0.9	26.3 ^{xy} ±1.5	28.8 ^{xy} ±2.0
VIII	30.3 ^y ±1.3	31.0 ^y ±1.1	29.5 ^y ±1.3	30.8 ^y ±1.1	29.5 ^y ±1.0	30.8 ^y ±2.0
Mean± SE	24.0±1.0	26.1±0.8	24.1±0.9	26.2±0.7	24.6±0.8	26.5±0.7

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;
Means having different superscript within a column differ significantly (P<0.05)

Table 4.5 Effect of positive DCAD diet on pulse rate (beats/minute) in crossbred calves during winter months

Fortnight	Control		W ₁		W ₂	
	M	A	M	A	M	A
0	62.7±1.8	65.5 ^x ±1.9	62.5±1.0	65.3 ^x ±2.2	62.7±1.0	65.0 ^x ±1.7
I	60.2±1.3	64.3 ^x ±1.9	60.2±1.1	65.0 ^x ±2.1	62.0±0.3	65.0 ^x ±2.2
II	61.0±0.9	67.5 ^{xy} ±0.7	62.2±1.3	68.4 ^{xy} ±1.3	62.4±0.7	68.6 ^{xy} ±1.1
III	62.4±1.4	68.0 ^{xy} ±2.1	63.7±1.3	68.7 ^{xy} ±2.1	62.6±2.3	68.9 ^{xy} ±2.3
IV	63.8±1.3	69.8 ^{xy} ±1.8	64.0±1.1	68.9 ^{xy} ±1.5	63.6±0.6	70.0 ^{xy} ±1.3
V	64.0±1.6	70.0 ^{xy} ±2.1	66.0±1.4	70.0 ^{xy} ±2.5	66.0±3.0	70.7 ^{xy} ±2.8
VI	65.3±1.5	72.7 ^{xy} ±3.5	65.3±2.1	71.0 ^{xy} ±1.3	65.2±3.0	72.5 ^{xy} ±2.1
VII	66.0±2.9	76.0 ^y ±1.0	66.7±3.5	72.8 ^{xy} ±1.4	66.0±2.0	73.3 ^{xy} ±1.5
VIII	64.7±3.6	75.3 ^y ±2.7	60.3±2.4	75.3 ^y ±2.4	62.0±3.5	75.0 ^y ±2.4
Mean± SE	63.3±0.7	69.9±1.4	63.4±0.8	69.5±1.1	63.6±0.6	69.9±1.2

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;

Means having different superscript within a column differ significantly (P<0.05)

Table 4.6 Effect of positive DCAD diet on rectal temperature (°C) in crossbred calves during winter months

Fortnight	Control		W ₁		W ₂	
	M	A	M	A	M	A
0	38.1 ^x ±0.1	38.3 ^x ±0.1	38.2 ^{xy} ±0.1	38.4 ^{xy} ±0.1	38.2 ^x ±0.1	38.4 ^x ±0.1
I	38.2 ^{xy} ±0.1	38.3 ^x ±0.1	38.2 ^{xy} ±0.1	38.3 ^x ±0.1	38.3 ^{xy} ±0.1	38.3 ^x ±0.1
II	38.2 ^x ±0.1	38.3 ^x ±0.1	38.2 ^x ±0.1	38.3 ^x ±0.1	38.2 ^x ±0.1	38.3 ^x ±0.3
III	38.2 ^{xy} ±0.1	38.3 ^x ±0.1	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.2	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.1
IV	38.1 ^x ±0.1	38.2 ^x ±0.1	38.2 ^x ±0.1	38.3 ^x ±0.1	38.2 ^x ±0.1	38.3 ^x ±0.1
V	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.2	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.1	38.4 ^x ±0.1	38.4 ^{xy} ±0.2
VI	38.2 ^{xy} ±0.1	38.3 ^{xy} ±0.2	38.2 ^{xy} ±0.1	38.4 ^{xy} ±0.1	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.1
VII	38.4 ^{xy} ±0.1	38.5 ^{xy} ±0.1	38.3 ^{xy} ±0.1	38.4 ^{xy} ±0.1	38.4 ^x ±0.1	38.5 ^y ±0.1
VIII	38.4 ^y ±0.2	38.5 ^y ±0.1	38.5 ^y ±0.1	38.6 ^y ±0.1	38.4 ^y ±0.1	38.5 ^y ±0.1
Mean± SE	38.2±0.1	38.3±0.1	38.3±0.1	38.4±0.1	38.3±0.1	38.4±0.1

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;
Means having different superscript within a column differ significantly (P<0.05)

(Table 4.6). The afternoon rectal temperature values were numerically higher than the morning rectal temperature values in all the three groups due to higher environmental temperature in the afternoons. The rectal temperature in all the three groups of calves started to increase from the Vth fortnight due to increase in environmental temperature and THI, but showed significant variation in the VIIIth fortnight than the start of the experiment when the mean environmental temperature and THI reached to 33.8°C and 74.6, respectively (Table 4.3). The overall mean values of rectal temperature (°C) in control, W₁, W₂ groups in morning and afternoon time were 38.2, 38.3, 38.3 and 38.3, 38.4, 38.4 showing no statistical (P>0.05) difference among groups. Cattle are homeothermic and strive to maintain the body temperature within a narrow range, but an increase in ambient temperature from 18-35°C is accompanied by significant increase in rectal temperature (Shafie *et al.*, 1994 and Marai *et al.*, 2000). Silanikove (2000) stated that rectal temperature is an indicator of thermal balance and may be used as an effective quantify tool to the harshness of the thermal environment. The results of the present study corroborate with the findings of Silanikove (2000) and Bernabucci *et al.* (2002) who reported that there is seasonal variation in rectal temperature. Valtorta and Gallardo (2004) reported that RT increased from 38.6°C at THI value of 68.4 to 40.8°C at THI of 77.9 in buffaloes. Chandra Bhan (2012) also observed less rectal temperature in Karan fries cows at lower THI as compared to higher THI.

In the present study, physiological parameters were affected by the environmental variables; however, there was no effect of positive DCAD diets on physiological parameters.

4.2.4 Effect of varying DCAD diet on dry matter intake

The fortnightly dry matter intake (kg/ d), DMI kg/ 100 kg BW and DMI g/ kg W^{0.75} of crossbred calves (KF) during an experimental period of 120 days in the control, W₁ (+150 mEq/ kg DM) and W₂ (+250 mEq/ kg DM) groups is presented in Table 4.7. In the beginning of the experiment, DMI averaged 3.87, 3.85 and 3.96 kg/ d which increased progressively to 5.35, 5.40 and 5.83 kg/ d at the end of 120 days of experimental feeding in

control, W_1 and W_2 groups, respectively due to increase in the body weight of the calves (Fig.4.1). An increase in the dry matter intake of the calves with increase in body weight is evident from various earlier studies (Lal, 1990; Saha, 1997; Ramachandran, 2003 and Aliarabi, 2005).

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. However, overall DMI kg/ 100 kg BW and DMI g/ kg $W^{0.75}$ averaged 2.65, 2.76, 2.79 and 95.49, 97.52, 101.29 in control, W_1 and W_2 groups, showing ($P > 0.05$) no significant difference due to dietary treatments. 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] increased from 0 to +45 mEq/100 g of DM during 84 d 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.

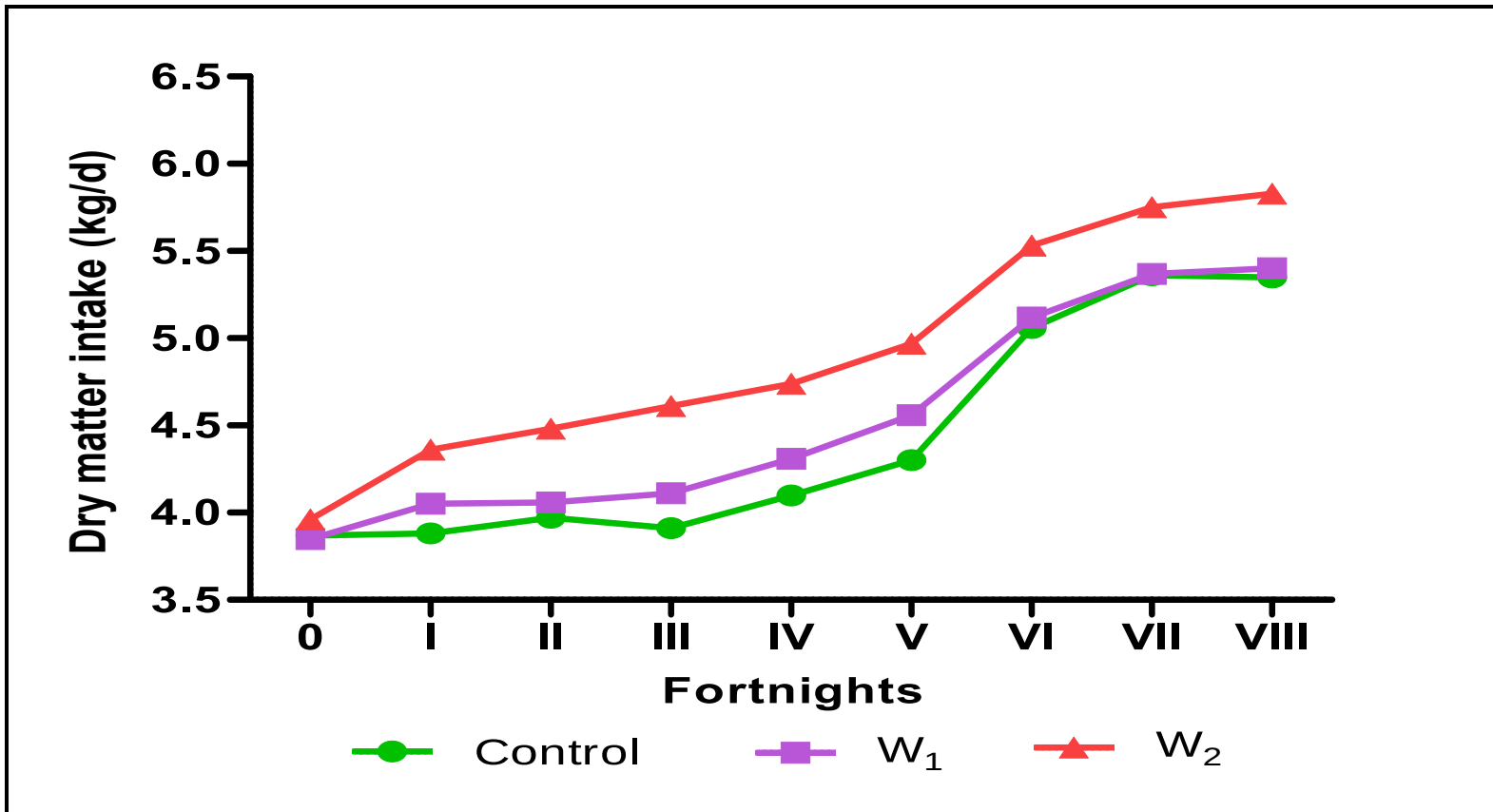
An optimum DCAD [(Na + K + 0.15 Ca + 0.15 Mg) - (Cl + 0.60S + 0.50P)] range of +15 to +20 mEq/100 g of DM (Roche *et al.*, 2000) has been indicated to positively affect DMI in dairy cows (Sanchez *et al.*, 1994; Roche *et al.*, 2005). An increase in nutrient intake with positive DCAD diet has also been reported by Jackson *et al.* (1992) and Shahzad *et al.* (2007, 2008a). Hu *et al.* (2007) in a review of 21 experiments, 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, which is a pre-requisite for optimum ruminal microbial activity. The influence of DCAD on DMI has a direct effect on the supply of nutrients for maintenance, growth, gestation, and lactation.

Table 4.7 Effect of positive DCAD diets on DM intake (kg/d), DMI (kg/100 kg BW) and DMI (g/kg W^{0.75}) in crossbred calves during winter months

Fortnight	DMI (kg/d)				DMI (kg/100 kg BW)				DMI(g/kg W ^{0.75})			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	3.87	3.85	3.96	0.08	3.01	2.67	2.71	0.18	100.09	92.36	94.02	4.55
I	3.88	4.05	4.36	0.08	2.82	2.66	2.78	0.15	95.38	93.22	98.20	3.88
II	3.97	4.06	4.48	0.08	2.72	2.55	2.75	0.13	93.66	90.49	98.01	3.50
III	3.91	4.11	4.61	0.12	2.54	2.51	2.74	0.11	88.52	89.48	98.59	3.05
IV	4.10	4.31	4.74	0.11	2.57	2.52	2.70	0.12	90.66	91.06	98.11	3.47
V	4.30	4.56	4.97	0.09	2.61	2.57	2.72	0.12	92.66	93.70	99.94	3.37
VI	5.06	5.12	5.53	0.11	2.88	2.78	2.92	0.11	104.20	102.33	108.07	2.91
VII	5.36	5.37	5.75	0.07	2.96	2.82	2.92	0.12	107.80	104.70	109.32	2.81
VIII	5.35	5.40	5.83	0.09	2.84	2.72	2.84	0.11	104.69	102.08	107.33	2.68
Mean	4.42^a	4.54^{ab}	4.92^b	0.05	2.65	2.76	2.79	0.04	95.49	97.52	101.29	1.19

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

Fig. 4.1 Effect of positive DCAD diets on DM intake (kg/d) in crossbred calves during winter months



4.2.5 Effect of positive DCAD diet on growth of crossbred calves

Average fortnightly body weights in crossbred calves of all the three groups are presented in Table 4.8, Fig. 4.2. The body weights at the start of the experiment averaged 148.77, 148.25 and 149.62 kg in control, W_1 and W_2 groups, respectively. The body weights at the end of 120 days experimental feeding averaged 198.02, 201.07 and 206.86 kg in the three respective groups. 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, W_1 and W_2 groups, respectively (Table 4.9, Fig. 4.3 and Fig. 4.4). Statistical analysis revealed that there was significantly more ($P<0.05$) gain (g/d) in W_2 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.

Wheeler and Hruska (1981) reported an increased weight gain in steers fed 100 mEq/kg DM DCAD diet due to increased feed consumption. In another study, growing dairy calves were fed diets varying in cation-anion difference of 0, 210, 370, and 520 mEq/kg dietary DM and it was found that feed intake and average daily gain responded quadratically being greatest at 370 mEq and lowest with 0 mEq (Jackson *et al.*, 1992). 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 (Table 4.7) 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.

4.2.6 Effect of positive DCAD diet on plasma minerals profile

4.2.6.1 Plasma Sodium and Potassium

The effect of feeding cationic diets on plasma Na and K levels is presented in Table 4.10. The overall average Na concentration in three respective groups was 127.85, 128.65 and 129.18 mEq/l. There was no significant effect ($P>0.05$) of treatments on the concentration of plasma Na. The K concentration (mEq/l) at 0 and 120 day of experimental feeding averaged 4.62, 4.57, 4.58 and 4.65, 4.70, 4.73 in the three respective groups. The concentration of K was also statistically similar ($P>0.05$) among three different groups, showing no effect of varying levels of DCAD on plasma K concentration. West *et al.* (1991) stated that varying DCAD levels (-116 to +312 mEq/Kg) did not affect the serum Na and K concentration. Jackson *et al.* (1992) also reported no change in plasma Na and K concentration of calves fed with a diet containing DCAD value of 0, 21, 37 and 52 mEq/100g DM. West *et al.* (1992) and Sarwar *et al.* (2007a) also reported that serum Na and K concentrations were not altered when DCAD level was increased. Since excess dietary Na and K may be excreted through kidneys (Hu and Murphy, 2004), so that may be the reason for no effect of dietary cations on the plasma Na and K concentrations.

4.2.6.2 Plasma Chloride and Sulphur

The fortnightly mean values of Cl and S are shown in Table 4.11. The overall plasma Cl (mEq/l) in control, W_1 and W_2 groups averaged, 97.23, 96.45 and 95.44 respectively, show no significant effect among different groups. The overall plasma S concentration (mEq/l) in control, W_1 and W_2 groups averaged, 2.41, 2.42 and 2.44 respectively, which was also statistically similar ($P>0.05$) among different groups. Both plasma Cl and S concentrations obtained in the present study were in the normal physiological range (Underwood and Suttle, 1999; NRC, 2001). Wildman *et al.* (2007) also observed no effect of varying positive DCAD (+41 or +58

Table 4.8 Fortnightly average body weights (kg) of crossbred calves during winter months

Fortnight	Control	W₁	W₂	SEM
0	148.77	148.25	149.62	9.36
I	155.93	156.39	156.61	9.26
II	161.15	162.58	165.29	9.08
III	165.82	167.34	171.32	9.14
IV	172.18	174.24	179.33	8.86
V	178.83	180.99	186.23	8.75
VI	184.86	187.08	192.53	8.51
VII	190.97	193.58	199.64	8.43
VIII	198.02	201.07	206.86	8.35
Mean	172.95	174.61	178.95	3.17

Table 4.9 Effect of positive DCAD diets on body weight gain (kg) and growth rate (g/d) in crossbred calves during winter months

Fortnight	Fortnightly body weight gain (kg)				Average growth rate(g/d)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
I	6.89	7.14	7.00	0.62	477.78	476.11	466.33	41.46
II	5.13	7.19	8.68	0.39	347.78	479.44	578.33	26.19
III	5.03	5.76	6.95	0.45	311.11	384.22	463.22	30.08
IV	6.99	6.90	8.01	0.57	424.44	460.00	533.89	38.21
V	6.42	6.75	6.90	0.70	442.78	450.00	460.00	46.95
VI	6.07	6.09	6.30	0.48	402.22	406.11	420.00	32.14
VII	6.21	6.49	7.11	0.36	407.22	432.78	473.89	24.09
VIII	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)

Table 4.10 Effect of positive DCAD diet on plasma sodium and potassium level (mEq/l) in growing crossbred calves during winter months

Fortnight	Sodium (mEq/l)				Potassium (mEq/l)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	125.09	125.18	125.42	1.17	4.62	4.57	4.58	0.05
I	124.37	124.54	125.85	1.39	4.60	4.61	4.62	0.05
II	124.10	127.21	127.78	1.34	4.60	4.61	4.64	0.09
III	125.07	126.73	127.82	1.59	4.50	4.63	4.65	0.08
IV	127.17	127.93	130.10	1.75	4.52	4.61	4.64	0.07
V	130.73	130.79	130.65	2.71	4.58	4.64	4.70	0.17
VI	130.97	130.24	131.25	1.57	4.65	4.66	4.72	0.04
VII	132.10	133.64	130.39	1.71	4.67	4.69	4.72	0.15
VIII	131.08	131.59	133.33	1.24	4.65	4.70	4.73	0.05
Mean	127.85	128.65	129.18	0.58	4.60	4.64	4.67	0.03

Table 4.11 Effect of positive DCAD diets on plasma chloride and sulphur (mEq/l) in growing KF calves during winter months

Fortnight	Chloride (mEq/l)				Sulphur (mEq/l)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	97.20	98.08	98.35	3.27	2.43	2.45	2.41	0.02
I	96.88	95.89	93.87	2.20	2.41	2.44	2.54	0.03
II	95.72	94.57	93.40	1.98	2.41	2.43	2.43	0.01
III	96.31	94.38	93.29	0.92	2.40	2.42	2.43	0.03
IV	98.89	97.68	96.21	1.21	2.38	2.40	2.43	0.02
V	98.15	97.57	97.08	1.67	2.42	2.42	2.43	0.02
VI	97.76	97.01	96.35	1.18	2.42	2.42	2.46	0.01
VII	97.71	96.92	95.37	1.51	2.40	2.41	2.44	0.02
VIII	96.44	95.98	95.01	0.92	2.38	2.39	2.40	0.02
Mean	97.23	96.45	95.44	0.59	2.41	2.42	2.44	0.01

Fig. 4.2 Effect of positive DCAD diet on fortnightly average body weights of crossbred calves during winter months

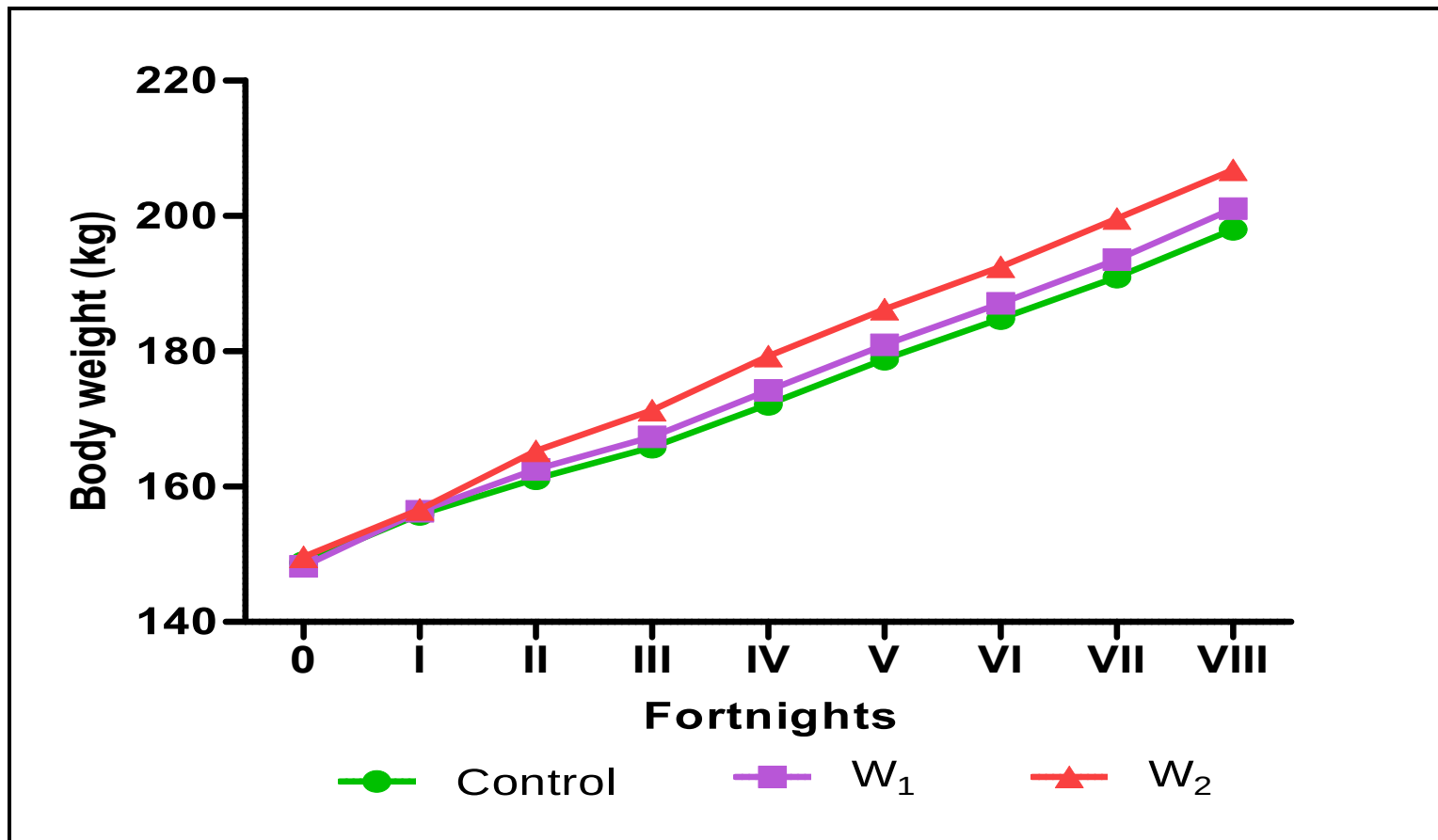


Fig. 4.3 Effect of positive DCAD diets on fortnightly body weight gain (Kg) in crossbred calves during winter month

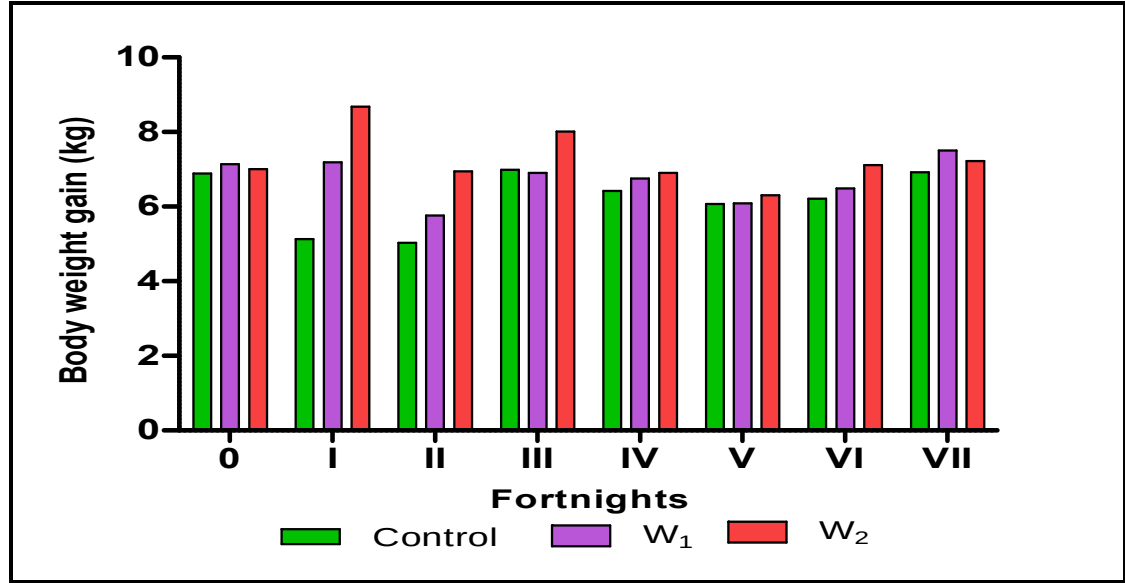
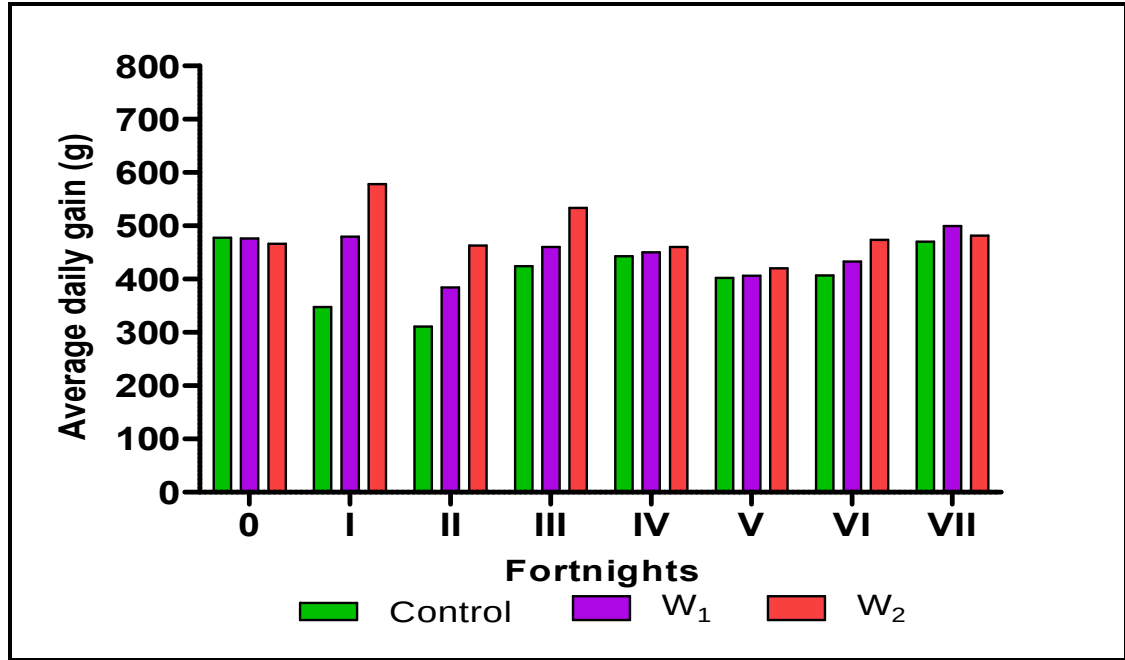


Fig. 4.4 Effect of positive DCAD diets on average daily gain (g) in crossbred calves during winter months



mEq/ 100g) diet on serum Cl and S contents. Sarwar *et al.* (2011) reported that serum Cl remained unaltered with increasing levels of NaHCO_3 in the diet. However, a linear increase in serum Cl^- concentration was observed with decrease in the DCAD levels, it was maximum (103.4 mEq/l) in buffaloes fed on high anionic diet and minimum (98.3 mEq/l) in buffaloes fed on high cationic diet (Shahzad *et al.*, 2011). Increased serum Cl concentration with decreased DCAD concentration might be attributed to inverse relation between blood Cl^- and HCO_3^- concentrations (Hu and Murphy, 2004). The similar Cl and S contents of diet in all experimental groups in the present study might have resulted in no change in plasma concentration of these minerals as it is reported that dietary concentration of minerals influence the plasma mineral concentration (Sarwar *et al.*, 2011).

4.2.6.3 Plasma Calcium, Phosphorus and Magnesium

The initial plasma Ca and P concentration (mg/dl) was 9.92, 9.85, 9.55 and 5.13, 5.72, 5.30 in the three respective groups. The overall plasma Ca and P averaged 9.37, 9.10, 8.80 and 5.12, 5.25, 5.23 mg/dl in control, W_1 and W_2 groups, respectively, showing no effect ($P>0.05$) of dietary treatments (Table 4.12). Similarly, the plasma Mg concentration (mg/dl) in control, W_1 and W_2 groups was 3.79, 3.51, 3.69 and 3.21, 3.30, 3.45, respectively at 0 and 120 days of experimental feeding, showing no effect ($P>0.05$) of treatments.

Similar to the present study Tucker *et al.* (1988) and West *et al.* (1992) reported that positive DCAD diet had non-significant effect on serum Ca, P and Mg concentrations. Though, there was no significant difference ($P>0.05$) in the plasma Ca level in different groups, but it was numerically less in the treatment groups which might be due to the fact that higher cationic diet reduces Ca absorption, Ca mobilization and renal reabsorption (Senthil Kumar, 2004; Li *et al.*, 2008; Wu *et al.*, 2008 and Shahzad *et al.*, 2008a). Shahzad *et al.* (2008a) also reported that serum Ca, P and Mg concentration remained unaltered across all diets (-11, +11, +22 and +33 mEq/100g DM). Shahzad *et al.* (2011) reported that serum P and Mg concentration remained unaffected at +11, +22 mEq/100g DM

DCAD, which revealed the lack of marked effect of positive DCAD on Ca, P and Mg concentration in the blood. Moreover it has been reported by several workers that plasma Ca, P and Mg levels are more altered by the anionic diet in comparison to cationic diet, as anionic diet cause acidosis which influences the functioning of parathyroid hormones and Ca and P homeostasis (Senthil Kumar, 2004; Shahzad *et al.*, 2008a and Shahzad *et al.*, 2011).

4.2.6.4 Plasma Zinc, Copper and Iron levels

Initially, plasma Zn concentrations (ppm) were 0.91, 1.06 and 0.91 in control, W₁ and W₂ groups, respectively (Table 4.13). At the end of the experimental period, the corresponding values in control, W₁ and W₂ groups averaged 1.11, 1.05 and 1.04 ppm showing no effect ($P>0.05$) of +150 and +250 mEq/kg DM DCAD diet. The average plasma concentration of Zn found in this study in the all group was within the normal range for growing cattle (Underwood and Suttle, 1999; NRC, 2001). The overall mean Cu level was 0.54, 0.58 and 0.57 ppm in control, W₁ and W₂ groups, respectively (Table 4.13), showing no significant ($P>0.05$) effect of positive DCAD diet on plasma Cu level. The mean values of plasma Fe as shown in Table 4.13 also did not vary due to dietary treatments. Both plasma Fe and Cu concentrations were in the normal physiological range (Underwood and Suttle, 1999; NRC, 2001). The present study is in agreement with the findings of Roche *et al.* (2005) who reported no effect of increasing DCAD (+23 to +88 mEq/ 100g of DM) on plasma trace mineral content.

4.2.7 Blood pH

The effect of different DCAD diets on the blood pH is presented in Table 4.14 and Fig. 4.5. The blood pH was significantly higher in IIIrd, Vth and VIIIth fortnights ($P<0.05$) in +250 mEq/ kg DCAD fed calves as compared to control group. The overall mean pH throughout the experimental period was ($P<0.05$) highest (7.38) in calves fed +250 mEq/ Kg DCAD and was minimum (7.33) in the control group. West *et al.* (1991) reported a decrease in blood pH (7.32 vs. 7.42) in cows fed low DCAD diet

Table 4.12 Effect of positive DCAD diets on plasma calcium, phosphorus and magnesium level (mg/dl) in crossbred calves during winter months

Fortnight	Calcium (mg/dl)				Phosphorus (mg/dl)				Magnesium (mg/dl)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	9.92	9.85	9.55	0.35	5.13	5.72	5.30	0.21	3.79	3.51	3.69	0.14
I	10.10	10.11	10.35	0.43	5.04	5.06	4.83	0.08	3.42	3.51	3.66	0.10
II	9.05	8.35	8.63	0.19	4.93	4.92	5.13	0.09	3.27	3.60	3.54	0.11
III	8.64	8.43	7.61	0.45	5.37	5.30	5.35	0.24	3.32	3.76	3.51	0.13
IV	9.85	9.50	9.17	0.27	4.63	4.56	4.58	0.17	3.37	3.93	3.46	0.18
V	8.17	8.06	7.29	0.45	5.59	5.84	5.96	0.12	3.41	3.58	3.66	0.10
VI	8.86	8.87	8.24	0.29	5.21	5.28	5.03	0.10	3.38	3.41	3.72	0.13
VII	9.87	8.95	9.27	0.40	5.00	5.22	5.08	0.12	3.35	3.48	3.48	0.10
VIII	9.90	9.77	9.07	0.42	5.22	5.40	5.84	0.22	3.21	3.30	3.45	0.08
Mean	9.37	9.10	8.80	0.13	5.12	5.25	5.23	0.06	3.39	3.56	3.57	0.04

Table 4.13 Effect of positive DCAD diets on plasma zinc, copper and iron concentration (ppm) in crossbred calves during winter months

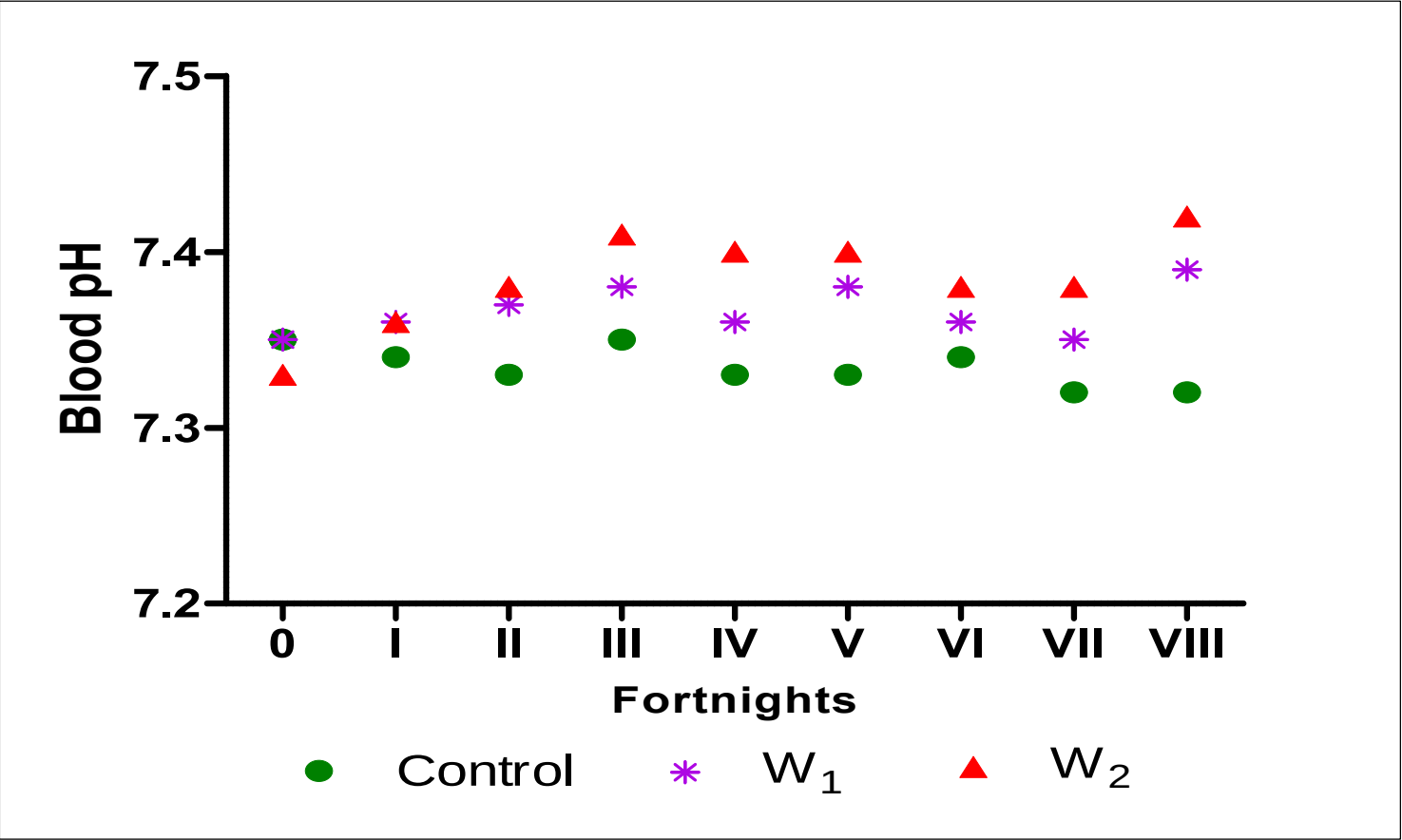
Fortnight	Zinc (ppm)				Copper (ppm)				Iron (ppm)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	0.91	1.06	0.91	0.91	0.61	0.54	0.55	0.03	2.37	2.02	2.74	0.21
I	0.83	0.90	1.05	0.83	0.52	0.60	0.53	0.03	2.34	2.35	2.46	0.13
II	0.85	0.87	1.02	0.85	0.61	0.56	0.60	0.04	2.54	2.69	2.67	0.21
III	0.86	0.95	0.97	0.86	0.47	0.64	0.65	0.04	2.44	2.41	2.43	0.20
IV	0.97	0.99	1.02	0.97	0.49	0.61	0.55	0.06	1.85	2.14	2.10	0.23
V	0.96	1.05	1.04	0.96	0.55	0.54	0.57	0.02	2.26	2.25	1.90	0.17
VI	0.89	1.00	1.04	0.89	0.49	0.50	0.57	0.02	2.67	2.36	2.49	0.22
VII	0.88	0.85	1.06	0.88	0.61	0.61	0.59	0.03	2.89	2.90	2.69	0.15
VIII	1.11	1.05	1.04	1.11	0.51	0.64	0.65	0.03	2.45	2.80	2.81	0.13
Mean	0.92	0.94	1.02	0.02	0.54	0.58	0.57	0.01	2.42	2.44	2.48	0.07

Table 4.14 Effect of positive DCAD diet on blood pH and glucose level (mg/dl) in growing KF calves during winter months

Fortnight	Blood pH				Glucose(mg/dl)			
	Control	W ₁	W ₂	SEM	Control	W ₁	W ₂	SEM
0	7.35	7.35	7.33	0.02	60.09	59.07	59.25	2.18
I	7.34	7.36	7.36	0.02	59.32	58.42	59.57	1.84
II	7.33	7.37	7.38	0.01	60.14	59.94	62.04	2.14
III	7.35 ^a	7.38 ^{ab}	7.41 ^b	0.01	61.36	63.37	60.94	1.95
IV	7.33	7.36	7.40	0.01	61.77	62.65	65.32	1.67
V	7.33 ^a	7.38 ^{ab}	7.40 ^b	0.01	64.40	66.75	66.75	1.76
VI	7.34	7.36	7.38	0.01	65.28	64.50	65.82	1.30
VII	7.32	7.35	7.38	0.01	59.30	64.87	63.20	1.18
VIII	7.32 ^a	7.39 ^{ab}	7.42 ^b	0.01	60.80	64.98	64.50	0.91
Mean	7.33^a	7.37^{ab}	7.38^b	0.02	61.38	62.73	63.04	0.68

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

Fig 4.5 Effect of positive DCAD diet on blood pH in growing crossbred calves during winter months



compared to those fed high DCAD diet. A high DCAD diet leads to increase in blood pH due to more HCO_3^- absorption and H^+ excretion (Tucker *et al.*, 1992 and Spanghero, 2004). Roche *et al.* (2005) also observed an increased blood HCO_3^- in cows fed increased DCAD level (+23 to +88 mEq/100 g of DM). So increase in the blood pH in present finding might be due to increase in blood bicarbonate due to supplementation of alkaline salt i.e. sodium as well as potassium bicarbonate (Block, 1994).

4.2.8 Plasma glucose

On an average, glucose level (mg/dl) was 61.38, 62.73 and 63.04 in control, W_1 and W_2 groups, respectively exhibiting no significant difference ($P>0.05$) of positive DCAD diet (Table 4.14). 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).

4.2.9 Effect of DCAD diet on antioxidant status and immunity

4.2.9.1 Plasma total antioxidant activity

Average plasma total antioxidant activity ($\mu\text{mol/l}$) in the blood samples drawn fortnightly in control, W_1 and W_2 groups has been presented in Table 4.15. Initial and final plasma total antioxidant activity i.e. mean FRAP value in control, W_1 and W_2 groups was 2219.46, 2134.58 and 2184.27 and 1910.51, 1974.06 and 2022.05 $\mu\text{mol/l}$, respectively. The overall mean of FRAP value averaged 1993.28, 2000.21 and 2058.71 $\mu\text{mol/l}$ in three respective groups showing no significant ($P>0.05$) effect of positive DCAD diet. The observed FRAP values are within the range reported by various workers (Ganie, 2012; Vaidya *et al.*, 2012).

4.2.9.2 Plasma superoxide dismutase and catalase activities

Effect of varying DCAD diets on plasma SOD (U/ml) and catalase activities (nmol/ min/ ml) in growing crossbred calves were estimated

fortnightly and the results obtained are presented in Table 4.15. Mean plasma SOD (U/ml) level in control, W₁ and W₂ groups varied from 2.19-3.08, 2.23-3.14 and 1.97-2.99 during 120 days of experimental feeding. Overall plasma SOD concentration (U/ml) averaged 2.60, 2.68 and 2.79, respectively showing no significant effect ($P>0.05$) of dietary treatments on this parameter.

The overall mean plasma catalase activity was 51.14, 51.49 and 52.22 (nmol/ min/ ml) in three respective groups. There was no significant effect ($P>0.05$) of varying dietary DCAD levels on plasma catalase activity. The values of SOD and catalase activity were in the normal range as reported by other workers (Kumar *et al.*, 2010, 2011a; Manish *et al.*, 2011 and Ganie, 2012). However, literature is not available on the effect of varying DCAD diets on antioxidative enzymes. Kumar *et al.* (2010) observed that the supplementation of electrolyte, ascorbic acid polyphosphate and zinc oxide decreased the activity of plasma SOD and catalase of heat stressed buffaloes due to the fact that ascorbate and zinc interfere with the actions of catalase and SOD.

4.2.9.3 Plasma total Immunoglobulin (Ig), plasma IgG and IgM levels

Total immunoglobulin concentration (0 d) in control, W₁ and W₂ groups was 30.96, 31.23 and 33.09 (mg/ml) and the concentration in corresponding groups at 120 d of experimental feeding was 30.22, 31.25 and 31.74 (mg/ml; Table 4.16). Statistical analysis of data revealed that the total immunoglobulin concentration was not affected significantly ($P>0.05$) by the treatment diet.

The initial total IgG concentration of calves in control, W₁, W₂ groups averaged 22.94, 23.51 and 24.37 mg/ml respectively (Table 4.16). At the end of 120 d experimental feeding, the total IgG concentration in control, W₁, W₂ groups was 23.39, 21.82 and 23.71 mg/ml and the variation among groups was non-significant ($P>0.05$). Similarly the overall average IgM concentration in control, W₁, W₂ groups was 7.72, 7.78 and 7.87 ng/ml, respectively (Table 4.16). The IgM concentration among different groups was statistically similar ($P>0.05$). Results of the experiment revealed that +150 and +250 mEq/ kg DM DCAD diet had no

Table 4.15 Effect of positive DCAD Diet on FRAP level ($\mu\text{Mol/l}$), SOD (U/ml) and catalase activity (nmol/min/ml) in crossbred calves during winter months

Fortnight	FRAP level ($\mu\text{Mol/l}$)				SOD (U/ml)				Catalase activity (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
I	2159.42	2037.27	1997.93	62.03	3.08	2.84	2.84	0.11	46.85	48.53	47.47	0.96
II	1919.25	2039.34	1983.44	37.71	2.99	2.75	2.80	0.10	42.71	43.43	43.65	0.77
III	2033.13	1971.01	2068.32	76.23	2.97	3.06	2.93	0.11	39.82	42.29	44.29	1.40
IV	1841.76	1884.57	1959.79	56.92	2.99	2.74	2.81	0.06	50.39	52.07	53.96	1.25
V	1867.70	1889.75	2101.17	56.28	2.80	2.52	2.50	0.11	51.76	53.20	53.20	0.10
VI	1961.09	2035.02	2116.73	49.63	2.62	2.38	2.22	0.09	56.23	54.91	54.99	0.88
VII	2027.24	2036.32	2094.68	54.57	2.19	2.23	1.97	0.19	55.14	53.69	54.18	0.94
VIII	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

Table 4.16 Effect of positive DCAD Diet on plasma total immunoglobulin (mg/ml), total IgG (ng/ml) and total IgM concentration (ng/ml) in crossbred calves during winter months

Fortnight	Total immunoglobulin (mg/ml),				Total IgG (ng/ml)				Total 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
I	34.68	32.97	32.30	0.67	22.11	19.54	21.99	1.60	8.19	7.75	7.81	0.15
II	30.19	32.13	31.99	0.90	20.62	26.22	23.46	1.96	7.49	7.86	8.00	0.15
III	32.31	32.75	31.81	0.64	23.03	22.46	24.48	1.08	7.15	7.52	7.76	0.13
IV	33.86	33.94	35.37	0.57	25.82	25.47	20.56	1.18	7.85	8.19	8.30	0.15
V	30.18	31.10	32.07	0.88	21.53	28.19	23.23	1.69	8.05	8.03	8.29	0.17
VI	31.11	31.41	32.94	0.61	21.72	21.12	24.73	1.77	7.95	7.37	7.64	0.16
VII	30.31	31.56	32.15	0.55	20.69	18.21	21.51	1.87	7.41	7.73	7.56	0.08
VIII	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	32.61	0.26	22.42	22.95	23.11	0.53	7.72	7.78	7.87	0.05

effect on plasma immunoglobulin in the winter season. The literature on the supplementation of positive DCAD diets on plasma immunoglobulin levels in crossbred calves during winter season is not available, so the results cannot be discussed due to lack of literature.

4.2.10 Endocrine parameters

4.2.10.1 Plasma T₃ and T₄

The mean values of plasma T₃ and T₄ in calves during winter season have been presented in Table 4.17. The initial T₃ and T₄ (ng/ml) levels in blood plasma of calves were 0.91, 0.97, 1.00 and 23.13, 23.54, 22.46 in control, W₁, W₂ groups, respectively. The overall mean value of plasma T₃ and T₄ (ng/ml) was 1.08, 1.12, 1.18 and 23.88, 24.19, 26.02 in the three respective groups. It is evident from the table that the mean value of plasma T₃ and T₄ was significantly higher ($P<0.05$) in W₂ group as compared to control group.

Riis and Madsen (1985) and Blum *et al.* (1980) stated that blood thyroid hormone levels were correlated with quantity and quality feed intake in ruminant species. Dauncey (1990) also found that the quantity and quality of food eaten is a major factor determining the plasma concentrations of thyroid hormones. The increased T₃ and T₄ in W₂ group in the present study, might be attributed to increase in nutrient intake due to feeding of alkalogenic salt, which enhances the rumen ecology thereby increasing the efficiency of nutrients utilization (Shahzad *et al.*, 2007 and Luebbe *et al.*, 2011). The T₃ and T₄ levels also differ between the breeds, seasons and production levels. Prakash and Rathore (1991) reported significantly ($P<0.05$) lower serum T₃ and T₄ levels during summer season compared to winter season in goats. Rasooli *et al.* (2004) reported significantly lower ($P<0.01$) levels of T₃ and T₄ in Holstein heifers during hot summer (0.87 ± 0.03 and 51.61 ± 1.47 nmol/l) compared to the winter season (1.48 ± 0.06 and 56.93 ± 1.22 nmol/l). Similarly, Todini (2007) reported that thyroid hormones levels were maximum in winter, decreased in spring and reached to the lowest value in summer in both buffaloes and Friesian cows.

4.2.10.2 Plasma cortisol

Effect of feeding different positive DCAD diet on plasma cortisol in crossbred calves during winter is presented in Table 4.17. Cortisol levels at the start of experiment averaged 3.01, 3.31 and 3.11 ng/ml in three respective groups. The plasma cortisol levels were highest on fortnight III (Table 4.17) in all the groups of calves when the environmental temperature was lowest (Table 4.3) indicating that the cold stress was responsible for increased secretion of cortisol. Similar to this, Sasaki and Weekes (1986) and Kim *et al.* (2009) reported that exposure of animals to cold increased plasma cortisol level. The overall average plasma cortisol (ng/ml) during the entire experimental period was 3.67, 3.62 and 3.53 in control, W₁ and W₂ groups, respectively showing no significant difference ($P>0.05$) among the groups. Plasma cortisol levels observed during the present study were within the normal physiological range (Block *et al.*, 2001 and Maurya *et al.*, 2011). Similarly, Muneedera Kumar (2013) reported that cortisol levels vary from 3.55-3.92 ng/ml, in the buffalo calves during winter months.

4.2.11 Effect of DCAD diet on nutrients intake and their digestibility in female crossbred calves during metabolic trial

4.2.11.1 Nutrients intake

The DM, OM, CP and TDN intakes observed during the metabolic trial are presented in Table 4.18. The DM and OM intakes (kg) of calves were 4.67, 4.99, 5.27 and 4.22, 4.53, 4.76 in control, W₁, W₂ groups, respectively. It is evident that DM and OM intakes were significantly higher ($P<0.05$) in W₂ group as compared to control. The CP and TDN (kg) intakes were 0.70, 0.73, 0.76 and 2.77, 2.96, 3.13 in control, W₁ and W₂ groups, respectively, and were statistically more ($P<0.05$) in W₂ group as compared to control. Results of present study corroborate with the findings of Apper-Bossard *et al.* (2006); Wildman *et al.* (2007); Shahzad *et al.* (2007) and Hersom *et al.* (2010) who reported increase in the nutrients intake with positive DCAD diets. Calves fed +330, +220 and +110 mEq/kg

Table 4.17 Effect of positive DCAD Diet on T₃,T₄ and cortisol levels (ng/ml) in growing crossbred calves during winter months

Fortnight	T ₃ (ng/ml)				T ₄ (ng/ml)				Cortisol (ng/ml)			
	Contro I	W ₁	W ₂	SEM	Contro I	W ₁	W ₂	SEM	Contro I	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
I	0.99	1.00	1.16	0.09	23.01	23.88	23.89	0.88	3.30	3.57	3.31	0.31
II	1.10	1.12	1.20	0.09	24.13	24.30	25.97	0.87	4.24	3.85	4.01	0.32
III	1.07	1.12	1.21	0.11	24.42	24.64	27.01	0.85	5.15	5.45	4.97	0.25
IV	1.14	1.17	1.25	0.07	24.48	24.76	27.38	0.82	4.24	3.85	4.01	0.32
V	1.12	1.21	1.24	0.04	24.71	24.94	28.38	0.79	4.20	3.64	3.78	0.42
VI	1.10	1.20	1.23	0.03	24.23	24.52	26.63	0.77	3.00	3.07	2.66	0.18
VII	1.09	1.15	1.16	0.02	24.07	24.29	26.46	0.64	2.87	2.88	2.91	0.11
VIII	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

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

Table 4.18 Effect of positive DCAD Diets on nutrient intake (kg/d) in growing crossbred calves during metabolic trials in winter months

Particulars	Control	W₁	W₂	SEM
DM	4.67 ^a	4.99 ^{ab}	5.27 ^b	0.10
OM	4.22 ^a	4.53 ^{ab}	4.76 ^b	0.09
CP	0.70 ^a	0.73 ^{ab}	0.76 ^b	0.01
TDN	2.77 ^a	2.96 ^{ab}	3.13 ^b	0.06

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

DCAD diets consumed 836.13, 807.7 and 681.8 g/d CP, respectively (Shahzad *et al.*, 2007, 2008a). Sarwar *et al.* (2011) reported that nutrient intake increased ($P < 0.05$) with increasing levels of dietary NaHCO_3 due to increased cationic value of the diet. They recorded DM intake of 6.23 kg/d in calves fed no NaHCO_3 which increased to 7.45 kg/d DM when the calves were supplemented with 1.6% NaHCO_3 in the diet. The CP consumption was also highest (1176 g/d) for calves fed 1.6% NaHCO_3 and lowest (984 g/d) in calves fed no NaHCO_3 diet. So the response of positive DCAD on animal performance reflected that DCAD can enhance nutrients intake due to its favourable influence on rumen dynamics and blood chemistry. However, the extent of nutrients intake varies depending on the level of DCAD, diet composition, animal productive potential and environment.

4.2.11.2 Nutrients digestibility

Nutrients digestibility in calves were not influenced by dietary treatments i.e. supplementations of cationic salt to obtain +150, +250 mEq/kg DM DCAD diet (W_1 and W_2 groups).

DM and OM digestibility values were 69.03, 69.72, 69.83 and 71.60, 72.11, 72.46 in control, W_1 and W_2 groups, respectively (Table 4.19) and were statistically similar ($P > 0.05$) in all the groups. The digestibility of CP (74.03, 74.29, and 76.35) and EE (77.76, 78.35, and 78.47) were also similar ($P > 0.05$) in all the three groups. The NDF and ADF digestibility also remained same in all three respective groups, irrespective of dietary treatments. Delaquis and Block (1995a) studied the effect of different DCAD levels (481, 327 mEq/kg of DM) on NDF and ADF digestibility in dry cows and reported that DCAD had non-significant effect on ADF and NDF digestibility. The digestibility of DM, ADF, NDF and CP were found to be increased slightly in buffaloes fed +11 DCAD diet as compared to those fed +11, +22 and +33 DCAD levels (Shahzad *et al.*, 2007) confirming that anionic diet had influence on digestibility of nutrients and not cationic diet. Further experiments conducted by Shahzad *et al.* (2011) also showed that digestibility of DM, NDF, ADF and CP increased significantly in buffaloes fed on high anionic diet (-220 mEq/Kg DM) as compared to those fed on

high cationic diets (+220 mEq/Kg DM). Increased digestibility of nutrients in negative DCAD diet might be attributed to increased rumen retention time of nutrients due to reduced DMI (Sarwar *et al.*, 1996 and Nisa *et al.*, 2004). No effect of feeding positive DCAD diets on nutrient digestibility observed in the present experiment might be attributed to the fact that such diets had no significant effect on ruminal fermentation pattern of animals (Tucker *et al.*, 1991a; Delaquis and Block, 1995a; Shahzad *et al.*, 2008b). The effect of alkaline nature of high DCAD diet or Na/K bicarbonate feeding to enhance rumen ecology depends on the nature of diet, rumen dynamics (which may differ with age) and productive potential.

4.2.12 Effect of positive DCAD diet on nitrogen balance in crossbred calves

The data on balance of nitrogen in growing calves during metabolic trial are presented in Table 4.20. Nitrogen intake (g/d) averaged 99.25, 106.10 and 110.30 in control, W₁ and W₂, groups, respectively. Nitrogen intake (g/d) was significantly more ($P < 0.05$) in W₂ group as compared to control. Excretion of N (g/d) through faeces and urine was not influenced by positive DCAD ($P > 0.05$) and it was 44.47, 46.19, 47.28 and 29.01, 31.75, 32.94 g/d in the three respective groups. All the animals were found to be in positive nitrogen balance and values obtained were 26.28, 28.16 and 30.08 g/d, in control, W₁ and W₂ groups, respectively. Nitrogen balance was significantly ($P < 0.05$) higher in W₂ group as compared to control. The apparent nitrogen retention was not affected significantly ($P > 0.05$) by the dietary treatments.

The higher N intake and balance in the treatment groups might be due to increased DMI of the calves (Table 4.18). In mid lactating cows as well, intake, absorption, retention and balance of N increased by feeding high DCAD diets (55.5 mEq/Kg DM) which was associated with increased feed intake (Delaquis and Block, 1995b). Shahzad *et al.* (2007) also reported more N intake and balance in +22 mEq/100g DM (350.42 and 111.75 g/d) as compared to +11 mEq/100 g DM (323.68 and 99.5 g/d) diet. Shahzad *et al.* (2011) noticed that by increasing the DCAD level from +11 to +33 mEq/100g DM of diet, both nitrogen intake and balance were

Table 4.19 Effect of positive DCAD diet on digestibility coefficient of nutrients in crossbred female calves during metabolic trial in winter months

Particulars	Control	W₁	W₂	SEM
DM	69.03	69.72	69.83	0.49
OM	71.60	72.11	72.46	0.51
CP	74.03	74.29	76.35	0.63
EE	77.76	78.35	78.47	0.58
NDF	63.13	64.57	64.90	0.63
ADF	56.20	56.95	57.15	0.65

Table 4.20 Effect of positive DCAD diet on nitrogen balance in growing crossbred calves in winter months

Parameter	Control	W₁	W₂	SEM
N intake (g/d)	99.25 ^a	106.10 ^{ab}	110.30 ^b	1.86
Faecal N outgo (g/d)	44.47	46.19	47.28	1.59
Urinary N outgo (g/d)	29.01	31.75	32.94	1.10
N balance (g/d)	26.28 ^a	28.16 ^{ab}	30.08 ^b	1.01
Apparent N retention (%)	25.21	26.28	26.76	1.38

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

increased. Sarwar *et al.* (2011) further reported that nitrogen intake increased with increasing NaHCO_3 level in the diet and N intake was highest (188.1 g/d) in the calves fed 1.6% NaHCO_3 diet and lowest (157.5 g/d) in the 0% NaHCO_3 fed diet. In buffaloes fed on high DCAD diet (+220 mEq/ Kg DM), there was increased N intake due to increased DMI (Shahzad *et al.*, 2011). Nitrogen metabolism of animals may be influenced by different DCAD levels in the diet. The higher level of alkaline diet might prevent systemic acidosis and a concomitant drop in ruminal pH, so more desirable pH for rumen microbes might have increased the post-ruminal supply of amino acids by accelerating rumen microbial multiplication and eventually enhancing N balance (Sarwar *et al.*, 2011).

4.2.13 Urine pH

The influence of positive DCAD on monthly urine pH has been presented in Table 4.21. The urine pH on 60 and 120 days of treatment was significantly higher ($P < 0.05$) in W_2 (7.70 and 7.78) group as compared to control (7.34 and 7.36). The overall mean of pH was also significantly higher ($P < 0.05$) in W_2 group as compared to control. It is well documented that increased dietary cations increase the urine pH (Jackson *et al.*, 1992; Jackson and Hemken, 1994 and Shahzad *et al.*, 2008a). Jackson *et al.* (2001) reported increased urine pH (8.09) in dairy calves fed a high DCAD level (200 mEq/kg) in the diet compared to those fed a low (0 mEq/ kg) DCAD level (6.80). Sarwar *et al.* (2007b) reported that lambs fed +110 and +220 mEq/ kg DCAD diet had a urine pH of 7.43 and 7.68, respectively.

Urine pH had been used as an indicator of metabolic acid or alkali load (Sanchez *et al.*, 2002). The alteration in urine pH in the positive DCAD fed groups reflects alteration in blood pH and kidneys minimize this change by making the urine pH alkaline, by excreting more HCO_3^- and conserving H^+ (Roche *et al.*, 2003b). Increased urinary pH with increased DCAD level might be attributed to higher blood HCO_3^- and lower urine net acid excretion, implying that the acid load of the animals decreased rapidly as DCAD increased (Hu and Murphy, 2004; Spanghero, 2004). The findings reflect that increase in urine pH in positive DCAD fed animals was because of the alkaline nature of the diet.

4.2.14 Effect of feeding different DCAD diet on mineral balances during winter season

4.2.14.1 Sodium and Potassium balance

The intake, excretion and balance (g/d) of Sodium and Potassium are presented in Table 4.22. Sodium intake (g/d) was 29.08, 33.94 and 38.60 in three respective groups, showing that Na intake was significantly higher ($P<0.01$) in treatment groups W_1 and W_2 as compared to control. Faecal and urinary excretion of Na (g/d) also increased with increase in the level of Na in the diet, which was 4.07, 5.71, 7.56 and 18.88, 20.71, 21.25 in control, W_1 and W_2 groups, respectively. Faecal excretion (g/d) of Na was significantly more ($P<0.01$) in W_2 group as compared to control, whereas the urinary excretion of Na did not vary statistically ($p>0.05$) among groups. Na balance (g/d) was statistically similar ($P>0.05$) among groups. Percent Sodium retention was 20.93, 21.69 and 25.20 in control, W_1 and W_2 , groups, respectively, however there was no variation ($P>0.05$) observed among different groups.

Potassium intake (g/d) averaged 121.59, 136.44 and 144.23 in control, W_1 and W_2 groups, respectively. The K intake was significantly higher ($P<0.01$) in W_1 and W_2 groups as compared to control. Faecal and urinary excretion of K was 30.02, 30.41, 30.73 and 31.89, 33.81, 35.55 g/d in control, W_1 and W_2 groups, respectively. Urinary excretion was significantly more ($P<0.01$) in W_2 group as compared to control. K balance was significantly higher ($P<0.05$) in W_1 and W_2 (72.22 and 77.95 g/d) groups as compared to control (56.68 g/d) group of calves. The overall potassium retention (%) was 48.90, 52.89 and 53.89 in three respective groups and did not vary significantly ($P>0.05$) among groups. These finding are in line with Tucker *et al.* (1988) who observed that urinary and faecal mineral composition was closely associated with dietary mineral composition. West *et al.* (1992) also pointed out that urinary Na and K concentrations were more in cows fed +312.4 mEq/ kg DM DCAD diet (3.04 and 6.74) as compared to -116.6 mEq/ kg DM DCAD (2.79 and 4.78) diet. Similarly Shahzad *et al.* (2011) reported that buffaloes fed on high cationic diet had higher intake, excretion and retention of Na^+ and K^+ while

Table 4.21 Effect of positive DCAD diet on urine pH in crossbred calves during winter months

Day	Control	W₁	W₂	SEM
0	7.35	7.33	7.35	0.05
30	7.35	7.49	7.56	0.04
60	7.34 ^a	7.45 ^{ab}	7.70 ^b	0.10
90	7.35	7.58	7.68	0.09
120	7.36 ^a	7.53 ^{ab}	7.78 ^b	0.09
Mean	7.35^a	7.46^{ab}	7.57^b	0.04

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

Table 4.22 Effect of positive DCAD diet on sodium and potassium balances in crossbred calves in winter months

	Parameter	Control	W₁	W₂	SEM	Sig.
Na	Na Intake (g/d)	29.08 ^A	33.94 ^B	38.60 ^B	0.10	P<0.01
	Faecal Na outgo (g/d)	4.07 ^A	5.71 ^{AB}	7.56 ^B	0.38	P<0.01
	Urinary Na outgo (g/d)	18.88	20.71	21.25	0.78	NS
	Na balance (g/d)	6.13	7.52	9.80	0.85	NS
	Na retention (%)	20.93	21.69	25.20	2.16	NS
K	K Intake (g/d)	121.59 ^A	136.44 ^B	144.23 ^B	2.68	P<0.01
	Faecal K outgo (g/d)	30.02	30.41	30.73	0.76	NS
	Urinary K outgo (g/d)	31.89 ^A	33.81 ^{AB}	35.55 ^B	0.56	P<0.01
	K balance (g/d)	59.68 ^A	72.22 ^B	77.95 ^B	2.24	P<0.01
	K retention (%)	48.90	52.89	53.89	0.82	NS

Means having different superscripts within a row differ significantly. NS: Non-significant

these were lower in buffaloes fed on high anionic diet, the minimum (9.5 vs. 134.11 g/d) and maximum (12.42 vs. 150.56 g/d) Na^+ and K^+ balances, were observed with high anionic (-220 mEq/ kg DM) and high cationic diet (+220 mEq/ kg DM), respectively. The intake as well as excretion of Na and K was increased in the treatment groups as the treatment diets contained higher Na and K concentrations (Wildman *et al.*, 2007) and indicated that dietary mineral content influences absorption, excretion as well as retention of minerals.

4.2.14.2 Chloride and Sulphur balances

The intake, excretion and balance (g/d) of Chloride and Sulphur has been presented in Table 4.23. The Cl intake in control, W_1 and W_2 , groups was 103.31, 103.59 and 104.03 g/d and it was statistically similar ($P>0.05$) among groups. Faecal and urinary excretion of Cl was also similar ($P>0.05$) in all the three groups. The faecal and urinary outgo was 51.04, 52.31, 52.86 and 22.68, 22.62, 22.79 g/d in three respective groups. The Cl balance (g/d) and retention (%) were 29.59, 28.66, 28.38 and 28.38, 27.46, 26.97 in the three respective groups and not affected ($P>0.05$) by the level of positive DCAD diet.

The S intake was statistically similar among groups. Excretion of S was not affected by the positive DCAD diet and it was statistically similar ($P>0.05$) among groups. The faecal and urinary excretion (g/d) of S was 8.33, 8.22, 8.20 and 3.45, 3.93, 4.22 in the control, W_1 , W_2 groups respectively. S balance (g/d) and retention (%) in control, W_1 , W_2 , groups were 13.95, 13.80, 13.64 and 54.01, 53.15, 52.05 respectively. Tucker *et al.* (1991c) reported the same that dietary supplementation with 2% Na bicarbonate had no effect on urinary Cl (mg Cl/ mg creatinine) concentration (0.019), however, it was increased (0.056) by dietary KCl. Increased urinary Cl concentration in cows fed low DCAD might be attributed to increased Cl content of the diet (Tucker *et al.*, 1988; 1992). Shahzad *et al.* (2011) also observed that at low DCAD diet, there was more intake of Cl (244.22 g/ d), so more excretion of it (92.15 g/d) as compared to high DCAD diet where lesser intake (75.33 g/d) and less excretion of Cl (25.123 g/ d) were observed. They also reported similar

observations for excretion and retention of Cl and S. Since the diets in the present study were of high DCAD value and contained similar amounts of Cl and S in all the three groups, so, there was no difference in the metabolism of these two minerals in different groups (West *et al.*, 1992).

4.2.14.3 Calcium and Phosphorus balances

The Ca and P balance has been presented in Table 4.24. The Ca intake (g/ d) was 44.61, 44.93 and 45.11 in the three respective groups. The excretion (faecal and urinary) of Ca was not affected by the treatment. The Ca balance (g/d) and retention (%) were statistically similar ($P>0.05$) among groups, which were 15.98, 15.14, 15.25 and 35.47, 33.62, 33.52 in the three respective groups.

The P intake (g/d) in control, W_1 and W_2 groups was 28.81, 28.86 and 28.89, respectively. The faecal and urinary excretion of P (g/d) was 24.90, 24.06, 23.73 and 0.29, 0.30, 0.23 in control, W_1 , W_2 groups, respectively, and was not affected by the dietary treatments. P balance (g/d) and retention (%) in control, W_1 , W_2 , groups was 3.60, 4.48, 4.92 and 12.48, 15.65, 16.98 which was statistically similar ($P>0.05$) showing no effect of treatments. Joyce *et al.* (1997) reported that urinary excretion of Ca was higher in cows fed -7 mEq/100 g DM DCAD diet compared to those fed +30 and +35 mEq/100 g DM DCAD diets. Shahzad *et al.* (2011) reported that +220 mEq/ kg DM DCAD diet has less Ca intake (97.65 vs 181.35 g/d) and faecal (56.51 vs 90.14 g/d) and urinary excretions (0.60 vs and 2.01 g/d) as compared to -220 mEq/ kg DM DCAD diet.

Shahzad *et al.* (2007) reported that Ca intake (32.31 and 33.21 g/ d) and apparent retentions (14.06 and 14.16 g/d) in growing buffalo calves were statistically similar among +22 and +33 mEq/ 100 g DM DCAD diet fed groups. The P balance in early lactating buffaloes was not influenced by the +22 mEq/ 100 g DM (35.49 g/d) and +33 mEq/ 100 g DM DCAD diet (36.12 g/d) as reported by Shahzad *et al.* (2008a). The absorption, retention and excretion of Ca and P is more affected by the negative DCAD diet because metabolic acidosis affects parathyroid hormones (Kumar *et al.*, 2004; Li *et al.*, 2008 and Wu *et al.*, 2008) which was not the case in the present study.

Table 4.23 Effect of positive DCAD diet on chloride and sulphur balances in crossbred calves in winter months

	Parameter	Control	W₁	W₂	SEM	Sig.
Cl	Cl Intake (g/d)	103.31	103.59	104.03	1.05	NS
	Faecal Cl outgo (g/d)	51.04	52.31	52.86	1.41	NS
	Urinary Cl outgo (g/d)	22.68	22.62	22.79	0.38	NS
	Cl balance (g/d)	29.59	28.66	28.38	1.82	NS
	Cl retention (%)	28.38	27.46	26.97	1.61	NS
S	S Intake (g/d)	25.73	25.95	26.07	0.28	NS
	Faecal S outgo (g/d)	8.33	8.22	8.20	0.20	NS
	Urinary S outgo (g/d)	3.45	3.93	4.22	0.09	NS
	S balance (g/d)	13.95	13.80	13.64	0.32	NS
	S retention (%)	54.01	53.15	52.05	0.92	NS

NS: Non-significant

Table 4.24 Effect of positive DCAD diet on calcium and phosphorus balances in crossbred calves in winter months

	Parameter	Control	W₁	W₂	SEM	Sig.
Ca	Ca Intake (g/d)	44.61	44.93	45.11	0.41	NS
	Faecal Ca outgo (g/d)	27.56	28.59	28.62	1.79	NS
	Urinary Ca outgo (g/d)	1.06	1.19	1.23	0.05	NS
	Ca balance (g/d)	15.98	15.14	15.25	1.88	NS
	Ca retention (%)	35.47	33.62	33.52	4.14	NS
P	P Intake (g/d)	28.81	28.86	28.89	0.06	NS
	Faecal P outgo (g/d)	24.90	24.06	23.73	0.71	NS
	Urinary P outgo (g/d)	0.29	0.30	0.23	0.01	NS
	P balance (g/d)	3.60	4.48	4.92	0.71	NS
	P retention (%)	12.48	15.65	16.98	2.47	NS

NS: Non-significant

4.3 EXPERIMENT 2: Effect of positive DCAD supplementation on growing Karan Fries calves during summer season

4.3.1 Chemical composition of feedstuffs

During summer experiment, concentrate mixture, fodder (Oat/ Jowar/ Maize) and wheat straw were offered to the animals and analyzed for their minerals composition, proximate composition and fibre fractions (Tables 4.1 and 4.2). It was observed that the mineral and chemical composition of feed and fodders were in the normal range (Sheikh, 2012; Tyagi, 2007 and Meena, 2012).

4.3.2 Environmental variables

The mean values of maximum, minimum temperature, relative humidity and temperature humidity index in the morning and afternoon during summer season are given in Table 4.25. The environmental temperature and relative humidity varied from 32.3-41.2⁰C and 48-90% in the morning and 32.6-42.1⁰C and 17-75% in the afternoon, during 120 days of experimental period. The relative humidity at the start of experiment in the morning was 77% which decreased up to the month of May (fortnight IV) and then again increased to 90% at the end of experimental period. Relative humidity during afternoon at the beginning of the experiment averaged 30% which decreased to 17% on fortnight III and increased to 75% on VIIIth fortnight. Fortnightly THI at the start of experiment (1-15thApril) 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 in June, July and August months (Fortnights IV to VIII). The THI values varied from 67.9-80.7 and 80.2-86.1 in the mornings and afternoons during the entire experimental period of eight fortnights indicating that the calves were under stress throughout the experiment. Experimental animals from 16-May to June 14, 2012 were in severe heat stress and from June15, 2012 to the end of experiment (August 14, 2012) were in very severe heat stress (LPHSI, 1990). The THI observed during summer months was more than THI observed in winter months (Table 4.3).

LPHSI (1990) quantified heat stress, as, up to 72= no heat stress, 72-74= moderate heat stress, 74-78= severe heat stress and 78 and more=very severe heat stress. Practically, the increase of THI in a range of 72-81 reduces milk yield and TDN intake in dairy cows. The effect on the animal performance was greatest when THI exceeded 76 (West, 1993). Fuquary (1981) and Srikandakumar *et al.* (2003) stated that degree of heat stress on the cattle and buffalo can be estimated by the THI value and THI above 72 is used to indicate heat stress on animals. The temperature humidity index (THI) was higher in the present study indicating a higher level of thermal stress on the animals during the experimental period.

4.3.3 Physiological variables

The effect of different DCAD levels on respiration rate (RR), pulse rate (PR) and rectal temperature (RT) in crossbred calves is presented in Tables 4.26, 4.27 and 4.28, respectively. At the start of experiment, the respiration rate averaged 25.3, 24.8, 25.2 and 47.3, 48.2, 42.8 in the three respective groups during morning and afternoon. RR ranged from 25.3-61.2, 24.8-58.7, 25.2-55.3 in the mornings and 47.3-84.5, 46.0-83.3, 42.8-82.5 in the afternoons in control, S₁ and S₂ groups throughout the experimental period of eight fortnights. The maximum RR obtained in the IIIrd and IVth fortnights might be due to higher maximum and minimum temperatures (°C) reported in these fortnights, which were 40.9, 42.1 and 41.2, 41.9, respectively (Table 4.25). The overall mean RR (breaths/ min) in three respective groups in the morning hours during summer season averaged 49.0, 48.4, 47.8, respectively, which was not significantly (P>0.05) different from each other. Similarly, the overall means of RR in the afternoon observations averaged 69.0, 67.6 and 66.3 in the three respective groups and showed no significant (P>0.05) difference due to dietary treatments. But, the mean values of RR in S₁ and S₂ groups were numerically lower than the means observed in control group both in the mornings and afternoons.

All the three groups of experimental animals expressed diurnal rhythm in the respiration rate, which in fact is due the thermal effect of the

Table 4.25 Environmental parameters recorded during the summer months

Fortnight	Max.Temp (°C)		Min.Temp (°C)		Relative Humidity (%)		THI	
	M	A	M	A	M	A	M	A
0 (April 1 - April 15, 2012)	32.4	34.1	16.2	17.3	77.0	30.0	67.9	80.2
I (April 16- April 30,2012)	34.8	36.8	18.3	19.6	69.0	24.0	68.9	80.4
II (May1- May 15,2012)	37.6	38.7	21.0	21.6	54.0	20.0	71.8	82.8
III (May 16- May 30,2012)	40.9	41.9	24.1	24.8	48.0	17.0	74.8	84.1
IV (May 31 - June14,2012)	41.2	42.1	25.7	26.4	59.0	31.0	77.7	86.1
V (June 15- June29,2012)	40.6	41.6	27.3	27.8	65.0	38.0	79.3	85.6
VI (June 30- July 14,2012)	35.4	36.2	27.3	27.8	80.0	61.0	80.7	84.7
VII (July 15 ĩ July 29,2012)	34.5	35.2	26.6	27.2	86.0	64.0	80.2	84.0
VIII (July 30- August 13,2012)	32.3	32.6	26.2	27.0	90.0	75.0	78.9	83.0

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm

Table 4.26 Effect of positive DCAD diet on respiration rate (breaths/minute) in crossbred calves during summer months

Fortnight	Control		S ₁		S ₂	
	M	A	M	A	M	A
0	25.3 ^x ±1.4	47.3 ^x ±1.5	24.8 ^x ±1.1	48.2 ^x ±2.6	25.2 ^x ±1.1	42.8 ^x ±1.3
I	28.7 ^x ±1.8	47.5 ^x ±3.4	30.7 ^x ±2.5	46.0 ^x ±1.4	30.3 ^x ±1.3	45.5 ^x ±2.9
II	48.0 ^{xy} ±2.8	67.2 ^y ±3.0	46.3 ^y ±1.0	60.3 ^y ±3.0	43.7 ^y ±1.7	63.3 ^{xy} ±3.1
III	59.8 ^y ±3.0	84.5 ^z ±2.1	58.7 ^z ±2.8	83.3 ^z ±3.0	53.5 ^{yz} ±2.2	82.5 ^z ±2.0
IV	61.2 ^y ±3.6	83.8 ^z ±2.6	58.0 ^z ±4.1	82.7 ^z ±1.8	53.5 ^{yz} ±2.6	79.7 ^z ±1.9
V	57.0 ^y ±3.3	78.0 ^{yz} ±2.0	54.7 ^{yz} ±3.6	76.0 ^{yz} ±1.5	51.3 ^{yz} ±3.2	75.8 ^{yz} ±2.1
VI	55.2 ^y ±3.9	68.0 ^y ±3.1	50.5 ^{yz} ±1.5	67.7 ^y ±2.3	46.2 ^{yz} ±1.8	63.8 ^{xy} ±2.8
VII	56.2 ^y ±4.5	74.3 ^{yz} ±3.3	47.3 ^y ±2.3	70.5 ^y ±3.6	55.3 ^z ±3.1	65.3 ^y ±3.8
VIII	49.3 ^{xy} ±4.2	70.2 ^y ±2.5	46.7 ^y ±2.0	65.0 ^{xy} ±2.5	44.0 ^{yz} ±1.5	60.0 ^{xy} ±1.5
Mean	49.0±4.4	69.0±4.6	48.4±3.9	67.6±4.5	47.8±3.5	66.3±4.6

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;
Means having different superscript within a column differ significantly (P<0.05)

Table 4.27 Effect of positive DCAD diet on pulse rate (beats/minute) in crossbred calves during summer months

Fortnight	Control		S ₁		S ₂	
	M	A	M	A	M	A
0	63.6 ^x ±1.2	71.7 ^x ±1.6	66.7 ^x ±1.5	74.7 ^x ±1.2	64.5 ^x ±1.3	72.5 ^x ±1.3
I	66.7 ^x ±1.4	74.7 ^x ±1.1	69.3 ^{xy} ±2.5	76.3 ^y ±2.0	65.3 ^x ±1.6	74.7 ^{xy} ±1.6
II	68.3 ^{xy} ±1.4	77.0 ^{xy} ±2.1	65.3 ^x ±2.2	75.0 ^x ±2.1	67.3 ^{xy} ±1.3	75.7 ^y ±1.4
III	73.7 ^y ±1.4	84.3 ^z ±0.6	73.0 ^{yz} ±2.8	80.7 ^z ±1.2	72.0 ^{yz} ±1.9	78.7 ^z ±2.1
IV	75.0 ^z ±1.1	81.2 ^z ±1.4	74.2 ^z ±2.9	80.0 ^z ±0.5	72.5 ^{yz} ±3.6	79.7 ^z ±1.1
V	76.3 ^z ±1.0	78.7 ^y ±0.7	76.3 ^z ±1.7	80.0 ^z ±1.1	78.3 ^z ±1.6	77.8 ^{yz} ±0.5
VI	71.0 ^{xy} ±0.9	79.8 ^{yz} ±1.5	69.7 ^{xy} ±1.9	79.0 ^z ±1.7	68.3 ^y ±2.4	77.5 ^{yz} ±2.9
VII	71.7 ^{xy} ±2.0	75.3 ^{xy} ±0.7	72.0 ^{yz} ±1.8	75.3 ^{xy} ±1.0	72.7 ^{yz} ±2.3	74.3 ^{xy} ±1.0
VIII	74.0 ^{yz} ±1.8	76.3 ^{xy} ±0.6	71.3 ^{yz} ±2.8	76.0 ^y ±1.5	72.3 ^{yz} ±2.1	75.0 ^y ±1.1
Mean	71.2±1.4	77.7±1.3	70.7±1.2	77.6± 0.9	70.4±1.5	76.2±0.8

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;
Means having different superscript within a column differ significantly (P<0.05)

Table 4.28 Effect of positive DCAD diet on rectal temperature (°C) in crossbred calves during summer months

Fortnight	Control		S ₁		S ₂	
	M	A	M	A	M	A
0	37.8 ^x ±0.1	38.7 ^{xy} ±0.1	37.9 ^x ±0.2	38.8 ^{xy} ±0.1	37.9 ^x ±0.1	38.8 ^y ±0.2
I	38.8 ^y ±0.2	38.5 ^x ±0.1	38.8 ^y ±0.2	38.6 ^x ±0.1	38.7 ^z ±0.1	38.6 ^x ±0.1
II	38.5 ^y ±0.1	39.1 ^z ±0.1	38.7 ^y ±0.2	39.0 ^z ±0.1	38.6 ^z ±0.1	39.0 ^y ±0.1
III	38.6 ^y ±0.1	39.0 ^z ±0.1	38.4 ^{xy} ±0.1	39.0 ^z ±0.1	38.4 ^y ±0.1	39.0 ^y ±0.2
IV	38.7 ^y ±0.1	39.0 ^z ±0.1	38.6 ^y ±0.2	38.9 ^y ±0.1	38.8 ^z ±0.1	38.9 ^y ±0.1
V	38.6 ^y ±0.1	39.0 ^z ±0.1	38.7 ^y ±0.1	38.9 ^y ±0.1	38.5 ^{yz} ±0.1	38.8 ^{xy} ±0.1
VI	38.7 ^y ±0.1	38.9 ^{yz} ±0.1	38.5 ^y ±0.1	38.8 ^{xy} ±0.1	38.6 ^z ±0.1	38.8 ^{xy} ±0.1
VII	38.5 ^y ±0.1	38.8 ^y ±0.1	38.5 ^y ±0.1	38.7 ^x ±0.1	38.5 ^{yz} ±0.1	38.7 ^{xy} ±0.1
VIII	38.4 ^y ±0.2	38.7 ^{xy} ±0.1	38.4 ^{xy} ±0.2	38.7 ^x ±0.1	38.1 ^{xy} ±0.1	38.6 ^x ±0.1
Mean	38.5±0.1	38.9±0.1	38.4±0.1	38.8±0.1	38.3±0.1	38.8±0.1

M= observations recorded at 7:30 am, A= observations recorded at 2:30 pm;
Means having different superscript within a column differ significantly (P<0.05)

ambient temperature and THI which increased ($P < 0.05$) significantly in the afternoon as compared to the morning. Campbell and Lasley (1985) observed that respiration rate accelerated as ambient temperature increased in order to achieve heat dissipation. Increase in the respiration frequency may be used as an index of discomfort in large animals. Measuring respiration rate appears to be the most accessible and easiest approach for evaluating the degree of heat stress in farm animals (low: 40-60 breaths per min, medium high: 60-80, high: 80-120 per minute in cattle) as reported by Hahn *et al.* (1997). Similarly, Aggarwal and Upadhyay (1997); Kadzere *et al.* (2002) and Kumar (2005) reported that the physiological variable increased with increase in ambient temperature and THI. Bouraoui *et al.* (2002) also reported that THI was positively correlated to respiration rate ($r = 0.89$) and RR increased by 5 respirations per minute as THI increased from 68 to 78. Beatty *et al.* (2006) reported a linear increase in RR of *Bos taurus* from 75/ min at 26°C T_{wb} to 127/ min at T_{wb} 32°C .

Throughout the experimental period of eight fortnights, the pulse rate varied from 63.6-76.3, 65.3-76.3 and 64.5-78.3 in the mornings showing no significant difference among groups as well as between fortnights. But in the afternoon, the variation in pulse rate during different fortnights was more i.e. 71.7-84.3, 74.7-80.7 and 72.5-79.7 in the three respective groups. Significant variation in pulse rate in the afternoons was observed in the IIIrd, IVth and Vth fortnight in the experimental groups, which might be due to higher environmental temperature and THI value during this period. The overall mean values of pulse rate (beats/ min) during the morning and afternoon observations in control, S₁, S₂ groups were 71.2, 70.7, 70.4 and 77.7, 77.6, 76.2 beats/ min, respectively showing no significant difference ($P > 0.05$) among the three experimental groups. Mishra *et al.* (1995) reported a positive correlation between pulse rate and THI. Koubkova *et al.* (2002) reported significant increase in rectal temperature from 37.3 to 39.3°C, respiration rate from 28 to 81 per minute and pulse rate from 64 to 81 per minute when high yielding Holstein cows were exposed to high temperature conditions. Kumar (2005) observed that pulse rate in Karan-Fries cows increased from 58 (THI -68.6) to 70 (THI-

98.4) with increase in THI. Patir and Upadhyay (2007) reported that pulse rate of buffalo heifers increased from 39 to 70 beats/ min. after 4 h of exposure to 45°C indicating that the exposure was stressful. So it can be concluded from the present study that the calves were under thermal stress during this period which was responsible for increased pulse rate as compared to winter months (Table 4.5).

The mean values of rectal temperature (°C) in morning and afternoon time were 37.8, 37.9, 37.9 and 38.7, 38.8, 38.8 at the start of the experiment. It is evident from the table that morning rectal temperatures were lower than afternoon values in all the groups throughout the experiment exhibiting a diurnal pattern. The rectal temperature increased upto 39°C in all the groups in afternoons during II to V fortnights when the environmental temperature and THI were also highest (Table 4.25). Rectal temperature in the winter experiment was about 0.2-0.3°C less (Table 4.6) than the values observed in the summer experiment both during mornings and afternoons.

Rectal temperature obtained in the present study very much supports the view that heat stress increases the average body temperature from 38.6 to 39°C in cattle (Hahn, 1999; Valtorta and Gallardo, 2004). Significant increase in rectal temperature from 37.3 to 39.3°C was observed when HF cows were exposed to high ambient temperature (Koubkova *et al.*, 2002).

The afternoon rectal temperature values were higher than the morning rectal temperature values in all the three groups due to higher environmental temperature in the afternoon. Similarly, Igono and Johnson (1990) reported diurnal variation in the rectal temperature of HF cows. Joshi and Tripathy (1991) noticed an increase in rectal temperature from 102.0°F to 103.8°F when buffalo calves were exposed to 40.5°C for 8 hours daily for three months. Sethi *et al.* (1994) recorded 2.6°C rise in rectal temperature in buffaloes when exposed to direct sun rays in the months of June and July. Das *et al.* (1999) recorded rise in rectal temperature of buffalo calves from 38.7 to 40°C during the 24 h diurnal change during solar exposure in summer when the minimum and maximum ambient temperature was 27.1 and 44.1°C, respectively. Kumar

and Upadhyay (2007) observed significant increase in mean rectal temperature from 38.59⁰C in the morning at THI=76 to 39.51⁰C in the afternoon, when the THI was significantly high (THI=85).

In the present study it has been observed that the values for physiological parameters were higher in the summer as compared to winter months. These observations indicate that hot climate caused severe thermal stress to the animals which forced them to resort to compensatory mechanism to lose heat, but these extra mechanisms utilize energy, reduce feed intake that ultimately leads to decline in growth and production (Hahn, 1999 and Beatty *et al.*, 2006). However, there did not seem to be any significant effect of positive DCAD diet on physiological variables. Moreover, there is no literature available regarding the effect of positive DCAD diet on physiological variable in thermal stress, so these parameters cannot be discussed due to unavailability of sufficient literature.

4.3.4 Effect of DCAD diet on dry matter intake (DMI)

The daily DMI (kg/ d, kg/ 100 kg BW and g/ kg W^{0.75} BW) during summer season experiment has been presented in Table 4.29. The DMI (kg/ d) intake at the start of experiment averaged 3.27, 3.31 and 3.38 which increased to 5.29, 6.09 and 6.09 at 120 d of dietary treatments in the control, S₁ and S₂ groups respectively (Fig.4.6). The DMI was more in the treatment groups however, it was only significantly higher (P<0.05) in the S₂ group in IIIrd and VIIth, fortnight, as compared to control. The overall average DMI throughout the experimental period of eight fortnights was 4.36, 4.75 and 4.93 kg/ d in control, S₁ and S₂ groups, respectively which was significantly more (P<0.05) in the S₂ group as compared to control. The DMI increased with increase in the age of calves due to the increase in their body weight (Ramachandran, 2003 and Aliarabi, 2005).

The overall averaged daily DMI (kg/ 100 kg BW) and g/ kg W^{0.75} was 2.65, 2.78 and 2.86 and 94.85, 100.38 and 103.28 in control, S₁ and S₂ groups, respectively which was significantly more (P<0.05) in S₂ group as compared to control group. Jackson *et al.* (1992) reported a quadratic response in feed consumption in dairy calves fed diets containing DCAD

0, 21, 37 and 52 mEq/ 100 g DM, minimum (3.79 kg/ d) and maximum (4.41 kg/ d) feed intake was observed in calves fed 0 and 37 mEq/ 100 g DM, respectively, while a significant reduction (3.93 kg/ d) was observed at 52 mEq/kg DM. Oetzel and Barmore (1993) observed an increasing trend in DMI as DCAD increased from -109 to +313 mEq/ kg DM. Hu and Murphy (2004) conducted a meta-analysis and observed a linear increase in DMI with increasing DCAD levels in the diet. They reported a positive correlation between DCAD and DMI. Hu *et al.* (2007) also supported these findings that by feeding 22 or 47 mEq (Na + K - Cl - S)/100 g DM increased both DMI ($P < 0.01$) and DMI per unit of metabolic body size ($P < 0.01$) in cows.

Goff *et al.* (1991) reported that feeding negative DCAD diets reduced feed consumption due to poor palatability of salts. Spanghero (2004) reported a decrease in DMI by animals fed low DCAD diets. The plausible explanation for this decrease in DMI might be due to unpalatability of anionic salts (Goff *et al.*, 2004 and Shahzad *et al.*, 2008a, b) used to reduce the DCAD level. Another plausible explanation might be that low DCAD induces slight metabolic acidosis, which reduces DMI (Block, 1994). Increased DMI at high DCAD diets might be due to increased rumen pH (Tucker *et al.*, 1988; Sharif *et al.*, 2009 and Sharif *et al.*, 2010) that makes the ruminal environment alkaline, which is prerequisite for optimum ruminal microbial activity. Biological studies executed under different environment to know response of DCAD on ruminant animal performance reflect that the positive DCAD diet can enhance nutrients intake due to its favourable influence on rumen dynamics and blood chemistry which might be the reason for significantly more DMI intake in +350 mEq/ kg DM DCAD fed group. However, +250 mEq/ kg DM DCAD fed group showed only numerical increase in DMI.

4.3.5 Effect of positive DCAD diet on growth of crossbred calves

Body weight (kg) at the start of summer experiment in control, S_1 and S_2 groups averaged 148.18, 150.89 and 149.94 kg which increased to 181.92, 190.73 and 195.25 kg, respectively at 120 days of positive DCAD supplementation (Table 4.30, Fig. 4.7). The overall fortnight body weight

Table 4.29 Effect of positive DCAD diets on DM intake (kg/d), DMI (kg/100 kg BW) and DMI (g/kg W^{0.75}) in crossbred calves during summer months

Fortnight	DMI (kg/d)				DMI (kg/100 kg BW)				DMI(g/kg W ^{0.75})			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	3.27	3.31	3.38	0.04	2.23	2.21	2.31	0.09	77.43	77.15	80.16	2.31
I	3.39	3.58	3.85	0.10	2.25	2.31	2.50	0.08	78.90	81.54	87.82	2.41
II	3.76	3.96	4.12	0.08	2.42	2.47	2.61	0.09	85.39	87.91	92.40	1.42
III	4.13 ^a	4.56 ^{ab}	4.80 ^b	0.10	2.58	2.77	2.89	0.08	91.63	99.09	103.69	2.39
IV	4.48	4.74	5.08	0.12	2.73	2.80	2.96	0.09	97.56	100.99	107.10	2.10
V	4.80	5.18	5.26	0.16	2.85	2.90	2.98	0.10	102.64	108.08	107.93	3.37
VI	4.99	5.48	5.65	0.16	2.91	3.07	3.12	0.12	105.26	112.21	114.20	3.89
VII	5.16 ^a	5.86 ^{ab}	6.07 ^b	0.17	2.94	3.17	3.22	0.11	106.93	116.88	119.21	3.62
VIII	5.29	6.09	6.09	0.18	2.95	3.20	3.24	0.10	107.91	119.55	117.05	3.41
Mean	4.36^a	4.75^{ab}	4.93^b	0.07	2.65^a	2.78^{ab}	2.86^b	0.03	94.85^a	100.38^{ab}	103.28^b	1.24

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

Table 4.30 Effect of positive DCAD diet on fortnightly average body weights (kg) of crossbred calves during summer months

Fortnight	Control	S₁	S₂	SEM
0	148.18	150.89	149.94	4.61
I	153.71	156.06	155.49	4.42
II	158.34	160.92	160.27	4.34
III	163.24	166.08	167.59	4.24
IV	167.20	170.18	172.76	4.31
V	171.30	175.45	178.93	4.52
VI	174.42	180.04	183.87	4.48
VII	178.21	186.53	190.13	4.55
VIII	181.92	190.73	195.25	4.75
Mean	166.28^a	170.76^{ab}	172.77^b	1.76

Means having different superscripts within a row differ significantly

Fig. 4.6 Effect of positive DCAD diet on fortnightly average DM intakes (kg/d) of growing crossbred female calves during summer months

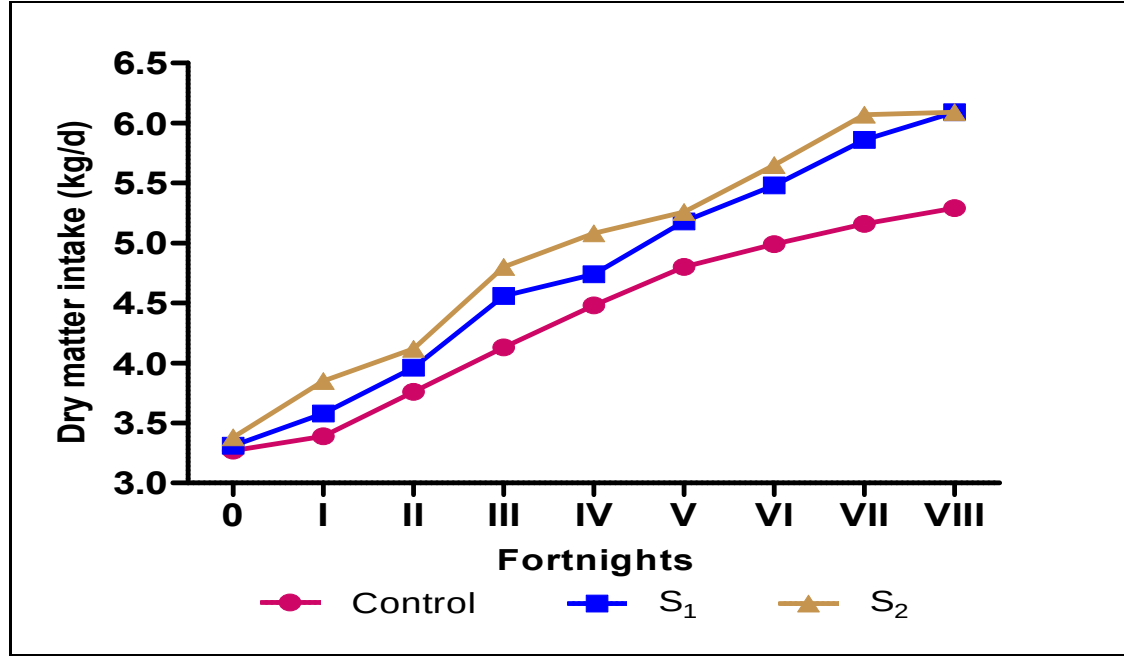
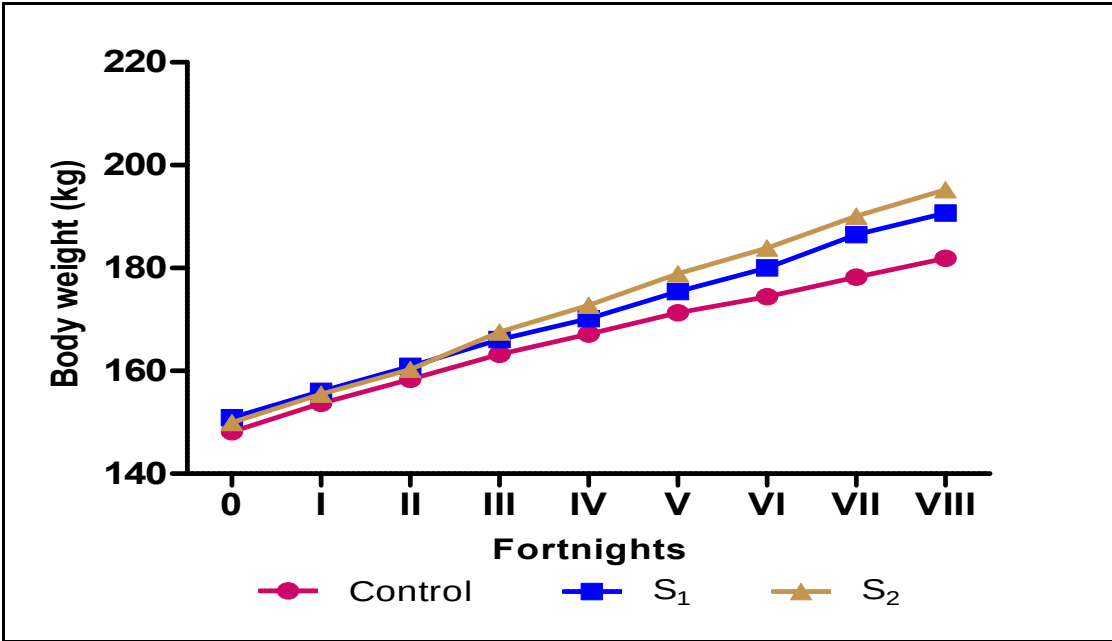


Fig. 4.7 Effect of positive DCAD diet on fortnightly average body weights (kg) of growing crossbred calves during summer months



(kg) was 166.28, 170.76, 172.77 in three respective groups, which was significantly higher ($P < 0.05$) in S_2 group as compared to control. The overall average fortnightly body weight gain (kg) and average daily gain (g) was 4.50, 4.98, 5.75 and 300.11, 332.01, 383.13 in control, S_1 and S_2 groups respectively (Table 4.31, Fig. 4.8 and Fig. 4.9). Statistical analysis of the data revealed that weight gain in S_2 group was significantly higher ($P < 0.05$) as compared to control showing the effect of dietary treatments on body weight gain over a period of four months in summer.

Similarly, Xin *et al.* (1991) studied the effect of 100 and 200 mEq [(Na +K) - (Cl + S)]/ kg DM and observed that calves fed positive DCAD diet had higher growth rate compared to calves fed negative DCAD diet. In another study, growing dairy calves were fed diets varying in cation-anion difference of 0, 210, 370, and 520 mEq/ kg dietary DM and it was found that feed intake and average daily gain responded quadratically being greatest at 370 mEq / kg DM and lowest with 0 mEq / kg DM (Jackson *et al.*, 1992). Similarly, Jackson and Hemken, (1994) studied that calves fed the +130 mEq had higher growth rates than those fed -180 mEq/ kg DM. Sarwar *et al.* (2007b) found that lambs fed +330 mEq/ kg DM had higher gain as compared to -110 mEq / kg DM DCAD diet. Shahzad *et al.* (2007) also observed increased weight gain with an increased DCAD level in growing buffalo calves; calves fed +330 and +110 mEq/ kg DM DCAD diets had maximum (698 g/ d) and minimum (625 g/ d) weight gains, respectively. Sarwar *et al.* (2011) observed that calves fed the 0% NaHCO_3 showed the lowest (625 g/d) weight gain and those fed the 1.6% NaHCO_3 diet showed the highest weight gain (735 g/d).

The plausible explanation of the higher weight gain in female crossbred calves fed positive DCAD diets is the higher feed consumption in these groups. High DCAD diet attributed its favourable effects on ruminal pH, rumen microbial activity, fermentation and passage rate from the rumen, resulting in higher nutrient consumption (Sarwar *et al.*, 1996; Nisa *et al.*, 2004 and Nisa *et al.*, 2006). 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 (Robert *et al.*, 2004) as CO_2 acts as an acid (carbonic acid) after

combining with water (Guyton and Hall, 2000). 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 DCAD 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 CO₂.

4.3.6 Effect of positive DCAD diet on plasma minerals

4.3.6.1 Plasma Sodium

Plasma Na concentration (mEq/ l) during summer season at the start of experiment averaged 124.46, 123.18, 123.39 in control, S₁, S₂ groups, respectively and the corresponding values at the end of the experiment were 123.89, 126.65, 127.99 mEq/ l (Table 4.32). The overall average plasma Na concentration during summer experiment in control, S₁ and S₂ groups was 124.06, 125.12 and 125.72 mEq/ l, respectively. The higher serum Na content was primarily due to the higher dietary concentration of this mineral which significantly altered the serum cation:anion balance (Sarwar *et al.*, 2011). The results of the present study corroborate with the findings of Fredeen *et al.* (1988b) who also noticed that plasma Na concentration increased as DCAD was increased in the diet. Roche *et al.* (2005) reported that in cows blood Na concentration increased ($P < 0.05$) curvilinearly as DCAD increased from +23 to +88 mEq/100 g of DM. Shahzad *et al.* (2011) also reported that serum Na concentration increased linearly with increase in the DCAD levels, it was maximum (144.84) and minimum (138.57) in buffaloes fed on high cationic (+220 mEq/ 100 g) and high anionic (+220 mEq/100 g) diets. Sarwar *et al.* (2011) found that serum Na concentration in buffalo calves increased linearly with increase in the DCAD levels due to increased intake of Na in high DCAD diet.

4.3.6.2 Plasma Potassium

The fortnightly changes in plasma K concentration (mEq/ l) due to positive DCAD diets have been given in Table 4.32. The overall mean

Table 4.31 Effect of positive DCAD diets on body weight gain in crossbred calves during summer months

Fortnight	Fortnightly body weight gain (Kg)				Average daily gain (g)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
I	5.20	5.17	5.55	0.48	347.11	344.44	370.00	28.82
II	4.63	4.86	4.78	0.41	308.67	323.89	318.33	27.09
III	4.90	5.17	7.00	0.56	326.96	344.44	433.33	37.55
IV	4.29	4.09	5.17	0.47	285.93	272.78	344.44	31.11
V	4.77	5.27	6.18	0.50	318.00	351.67	411.67	33.16
VI	3.57	4.59	5.27	0.74	238.22	306.11	351.56	49.16
VII	4.39	5.49	5.93	0.57	292.89	366.11	395.11	38.05
VIII	4.25	4.30	5.78	0.65	283.11	286.67	385.56	43.32
Mean	4.50 ^a	4.98 ^{ab}	5.75 ^b	0.20	300.11 ^a	332.01 ^{ab}	383.13 ^b	11.68

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

Table 4.32 Effect of positive DCAD Diet on plasma sodium and potassium level (mEq/l) in growing crossbred calves during summer months

Fortnight	Sodium (mEq/l)				Potassium (mEq/l)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	124.46	123.18	123.39	0.57	4.19	4.16	4.18	0.08
I	124.44	123.53	123.74	0.57	4.17	4.17	4.19	0.08
II	124.14	124.04	125.44	0.53	4.19	4.20	4.25	0.08
III	124.10	123.98	125.38	0.53	4.17	4.18	4.23	0.08
IV	124.05	124.83	126.05	1.10	4.13	4.21	4.25	0.08
V	123.99	124.77	125.99	1.10	4.11	4.19	4.23	0.08
VI	123.74	124.03	126.76	0.60	4.11	4.24	4.26	0.09
VII	123.73	125.05	126.78	0.60	4.12	4.26	4.28	0.09
VIII	123.89	126.65	127.99	0.77	4.13	4.27	4.29	0.07
Mean	124.06^a	125.12^{ab}	125.72^b	0.25	4.15	4.21	4.24	0.03

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

Fig. 4.8 Effect of Positive DCAD diet on fortnightly body weight gain (Kg) in growing crossbred calves in summer months

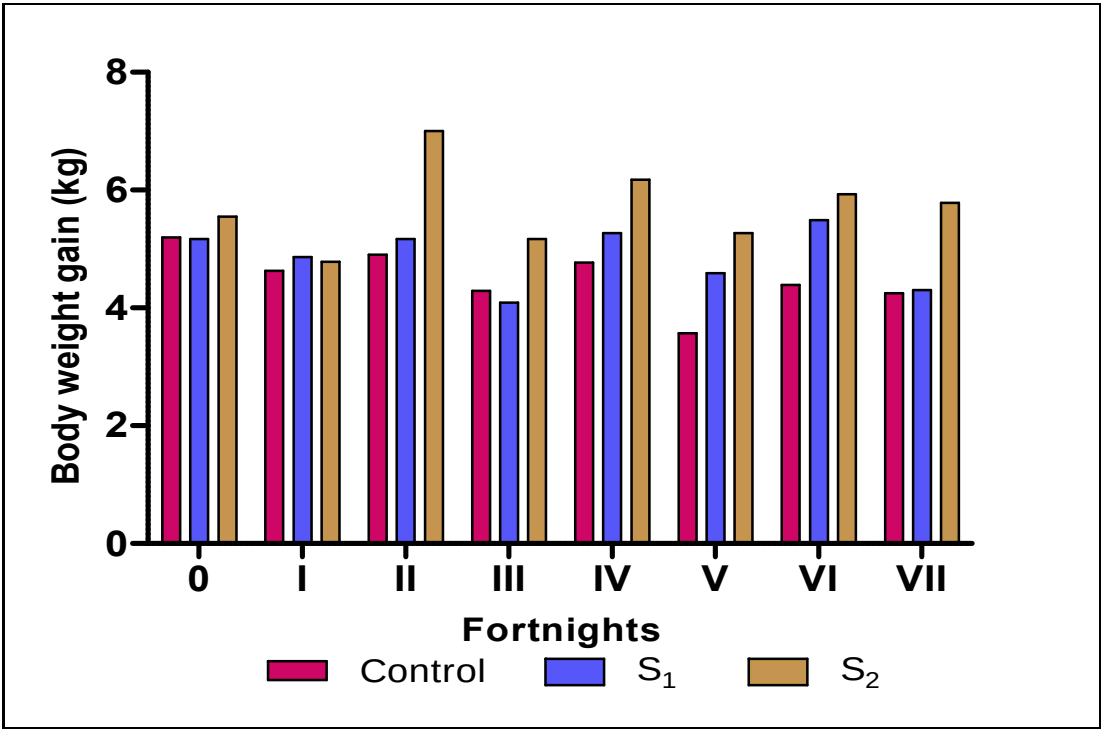
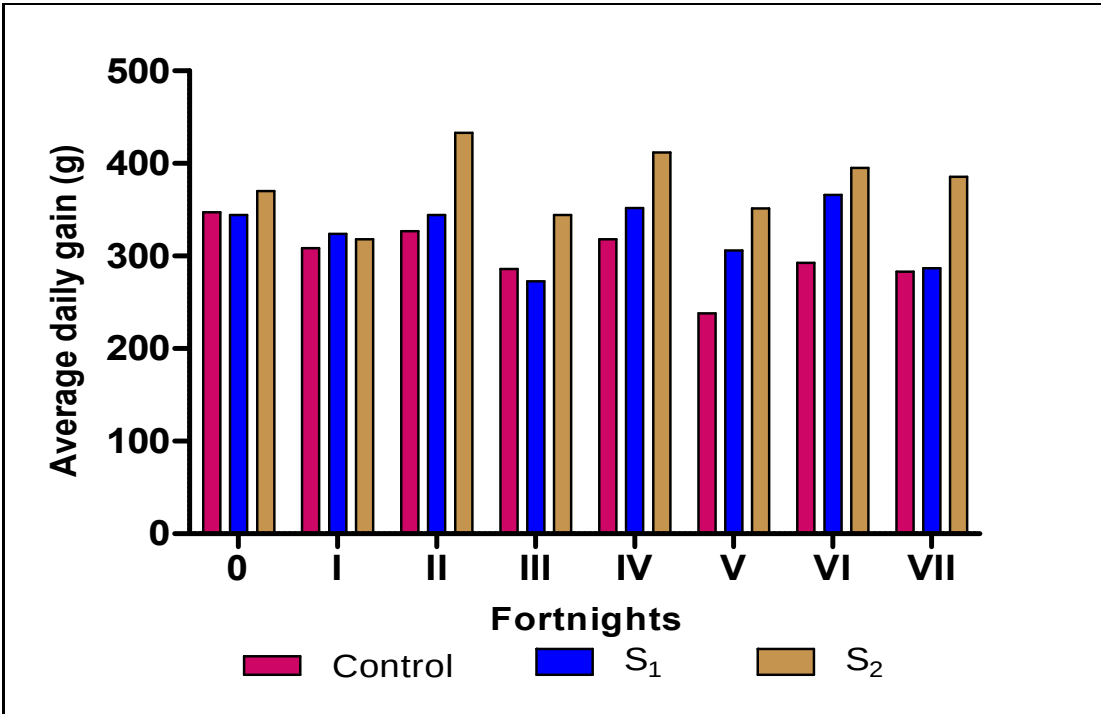


Fig. 4.9 Effect of Positive DCAD diet on average daily gain (g) in growing crossbred calves in summer months



plasma K concentration was 4.15, 4.21 and 4.24 mEq/ l in the three respective groups and it was statistically similar ($P>0.05$) in all the groups. However, plasma K levels increased numerically ($P>0.05$) in the treatment groups as compared to control. Tucker *et al.* (1988) observed non-significant effect on serum K concentration with increase in DCAD of diet (20 vs 10 mEq/ 100g DM) in cows. Jackson *et al.* (1992) also did not observe any change in plasma K content of calves fed diets containing DCAD value of 0, 21, 37 and 52 mEq/ 100g DM. Similar results were found by West *et al.* (1992) who reported that serum Na and K were not altered in heat stressed cows, when DCAD level was increased from 120.4 to 464.1 mEq/kg DM. Sarwar *et al.* (2007b) reported that in growing Thalli lambs, serum K remained unaltered by varying DCAD levels (-110, +110, +220, +330 mEq/ kg DM). The non-significant effect of high DCAD diet on plasma K might be due to their excessive excretion through kidneys to maintain homeostasis (Tucker *et al.*, 1988; Jackson *et al.*, 1992 and West *et al.*, 1992).

4.3.6.3 Plasma Chloride and Sulphur

The fortnightly average Cl and S levels in the plasma are presented in Table 4.33. The overall mean plasma Cl was 94.51, 93.64 and 92.76 (mEq/ l) in control, S₁ and S₂ groups, respectively. Statistical analysis of data did not show any significant ($P>0.05$) effect of different levels of DCAD on plasma Cl level of calves during summer experiment of 120 days. Tucker *et al.* (1991c) reported no difference in plasma Cl and S content of calves fed diets containing 0 or 2% Na bicarbonate. Wildman *et al.* (2007) also observed no effect of varying positive DCAD diet on serum Cl content of cows.

A linear increase in serum Cl⁻ concentration was observed with decrease in the DCAD levels, it was maximum (103.4 mEq/ l) in buffaloes fed on high anionic diet and minimum (98.3 mEq/ l) in buffaloes fed on high cationic diet (Shahzad *et al.*, 2011). Tucker *et al.* (1988) in dairy cows reported a linear increase in plasma Cl⁻ concentration with decrease in the DCAD levels (+20 to -10 mEq/ kg of DM) which might be due to the supplementation of CaCl₂ in the diet. It has been found that plasma Cl

level reflects the Cl content of the diet (Jackson *et al.*, 1992; Jackson and Hemken, 1994). In the present study, the Cl content of the diet was same in all groups, so that might be the reason for no effect on the plasma Cl level.

Plasma S (mEq/ l) levels on 0 and 120 d of experiment were 1.43, 1.48, 1.50 and 1.38, 1.39, 1.40 in control, S₁, S₂ groups respectively. The plasma S level was not statistically ($P>0.05$) different among groups. Sarwar *et al.* (2007b) reported no change in plasma S levels of growing Thalli lambs with varying DCAD levels (-110, +110, +220, +330 mEq/ kg DM). Sarwar *et al.* (2011) in buffalo calves (1.43-1.45 mEq/ l) and Shahzad *et al.* (2011) in buffaloes (1.53-1.55 mEq/ l) did not find any change in serum S concentration with increasing levels of DCAD or NaHCO₃ in the diet.

4.3.6.4 Plasma Calcium, Phosphorus and Magnesium

The mean values (mg/ dl) of plasma Ca, P and Mg in control, S₁ and S₂ groups are presented in Table 4.34. The initial plasma Ca concentration was 11.42, 11.24 and 11.03 mg/ dl in the three respective groups. The overall mean value was 11.11, 10.97 and 10.76 mg/ dl in control, S₁ and S₂ groups, respectively. There was no significant ($P>0.05$) difference on plasma Ca level among groups. The overall average P level was 6.84, 7.06 and 7.16 mg/ dl in control, S₁ and S₂ groups, respectively and it was also not affected by +250 and +350 mEq/ kg DM DCAD diets. The fortnightly concentration of plasma Mg in summer experiment was 3.10, 2.94 and 2.90 mg/ dl in control, S₁ and S₂ groups, respectively. The Mg level was statistically similar ($P<0.05$) among groups.

Similar to the present results, West *et al.* (1992) reported that positive DCAD diets of +180 and +312.4 mEq/ 100g DM in heat stressed cows had non-significant effect on serum Ca, Mg and P concentration. Jackson *et al.* (2001) reported that plasma Ca level in calves was not significantly altered by dietary DCAD varying in the range of 0 to 200 mEq/ Kg DM. Negative DCAD diets have been reported to influence the intake and excretions of Ca, P and Mg due to their action on the parathyroid hormones resulting in mobilization of these minerals from the bones

Table 4.33 Effect of positive DCAD diets on plasma chloride and sulphur levels (mEq/l) in growing crossbred calves during summer months

Fortnight	Chloride(mEq/l)				Sulphur(mEq/l)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	93.04	95.22	93.66	1.22	1.43	1.48	1.50	0.03
I	93.35	94.90	93.46	1.18	1.41	1.40	1.54	0.04
II	94.88	93.86	93.35	1.44	1.41	1.43	1.43	0.01
III	95.10	93.63	93.30	1.42	1.40	1.42	1.43	0.02
IV	94.97	93.11	92.70	1.36	1.38	1.40	1.43	0.02
V	94.76	92.59	92.19	1.24	1.42	1.42	1.43	0.02
VI	94.99	93.34	92.24	1.28	1.42	1.42	1.46	0.01
VII	94.66	92.99	91.89	1.23	1.40	1.41	1.44	0.02
VIII	94.86	93.14	92.05	1.08	1.38	1.39	1.40	0.02
Mean	94.51	93.64	92.76	0.42	1.45	1.42	1.41	0.01

Table 4.34 Effect of positive DCAD diets on plasma calcium,phosphorus and magnesium levels(mg/dl) in crossbred calves during summer months

Fortnight	Calcium (mg/dl)				Phosphorus (mg/dl)				Magnesium (mg/dl)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	11.42	11.24	11.03	0.57	7.46	7.68	7.21	0.30	2.33	2.59	2.63	0.13
I	11.19	11.15	10.66	0.35	7.36	7.79	7.11	0.28	2.46	2.46	2.52	0.07
II	10.44	10.16	10.03	0.36	6.31	6.18	7.22	0.23	2.58	2.51	2.51	0.08
III	10.39	9.85	9.65	0.36	6.41	6.28	7.44	0.22	3.06	2.68	2.61	0.09
IV	9.97	9.85	9.84	0.46	7.13	7.31	7.17	0.34	3.19	3.01	2.97	0.14
V	10.53	10.45	10.65	0.27	7.03	7.21	7.07	0.34	3.82	3.70	3.33	0.12
VI	12.42	12.77	12.41	0.15	6.38	6.61	7.08	0.33	4.06	3.49	3.69	0.18
VII	12.43	12.09	12.00	0.19	6.48	6.71	7.18	0.33	3.41	3.09	3.14	0.14
VIII	11.24	11.17	10.58	0.38	7.00	7.76	6.98	0.39	2.96	2.90	2.66	0.12
Mean	11.11	10.97	10.76	0.15	6.84	7.06	7.16	0.10	3.10	2.94	2.90	0.05

(Senthil Kumar, 2004; Li *et al.*, 2008; Wu *et al.*, 2008; Shahzad *et al.*, 2008a and Sarwar *et al.*, 2011). There was no difference in the Ca, P and Mg levels in calves during summer and winter seasons (Tables 4.12 and 4.34).

4.3.6.5 Plasma Zinc, Copper and Iron

The mean values for plasma Zn, Cu and Fe (ppm) for all the three groups are presented in Table 4.35. Overall mean Zn, Cu and Fe plasma level during the experimental period averaged 1.61, 1.59, 1.61; 0.81, 0.84, 0.84 and 2.08, 2.11, 2.06 ppm in control, S₁ (+250 mEq /kg DM), S₂ (+350mEq /kg DM) groups, respectively and these were similar across all groups (P>0.05). Results of the present study showed that positive DCAD diet did not affect the absorption and excretion of Zn, Cu and Fe contents thereby resulting in no change in their circulatory levels. The average plasma concentration of Zn, Cu and Fe found in this study in the all groups was within the normal range for growing cattle (Underwood and Suttle, 1999; NRC, 2001). The above results are in line with the findings of Roche *et al.* (2005) who observed that the concentrations of trace minerals were not affected with increasing DCAD levels (+23 to +88 mEq/ 100g DM).

4.3.7 Blood pH

The effect of varying positive DCAD diets on the blood pH has been given in Table 4.36 and Fig. 4.10. The overall pH in control, S₁ and S₂ groups was 7.34, 7.37 and 7.40, respectively. It has been found that blood pH was significantly more in (P<0.01) S₂ group as compared to the control group, but, the values were within the normal range (Houpt, 1984 and Robinson, 1992). Jackson *et al.* (1992) observed that a positive linear relationship exists between blood acid -base status and DCAD level. Blood pH and HCO₃⁻ were lower in calves fed 0 DCAD diet than those fed 21, 37 and 52 mEq/ 100 g of DM DCAD diets (Jackson *et al.*, 1992). Increased blood bicarbonate concentration has been reported in response to increasing DCAD in the diet of growing beef steers (Ross *et al.*, 1994b). High DCAD diets decreased blood H⁺ and thus resulted in increased blood HCO₃⁻ (Block, 1994). Cows fed low DCAD diets (-40, - 51 and -63 mEq/kg

DM) had reduced blood pH than those fed high DCAD diet (+203 mEq/kg DM), though the difference was not significant (Vagnoni and Oetzel, 1998). Similarly, Roche *et al.* (2005) also reported that altering DCAD from 23 to 88 mEq/100 g of DM increased blood pH and HCO_3^- concentration in early lactating cows. Hersom *et al.* (2010) observed that blood pH was 0.05 and 0.04 pH units greater in high DCAD (19.73 mEq/d) diet fed cows than low-DCAD fed cows (-5.6 mEq/d) on 21 and 42 d of treatment, respectively. Sarwar *et al.* (2011) also reported that blood pH was the lowest (7.26) in calves fed the 0% NaHCO_3 diet and highest (7.57) in those fed the 1.6% NaHCO_3 diet. Sahazad *et al.* (2011) opined that blood pH increased linearly with increase in DCAD level; the values being 7.43 and 7.34 in buffaloes fed on high cationic and high anionic diets, respectively.

The main reason for increased blood pH and HCO_3^- at high DCAD diet is due to its alkaline properties and a gradual reduction of dietary anions particularly Cl (Li *et al.*, 2008 and Sarwar *et al.*, 2007a). Chloride is absorbed from the posterior part of the intestine in exchange of HCO_3^- when excess of Na^+ is present in the diet in order to maintain electrical neutrality, thereby resulting in reduced blood HCO_3^- and increased H^+ . Blood HCO_3^- was inversely related to H^+ changes that reflected the metabolic nature of the acid challenge at low DCAD diet. Eventually, the greater DCAD concentration increased the blood HCO_3^- /blood pH and reduced H^+ , the reverse was true for negative DCAD concentrations (Block, 1994).

4.3.8 Plasma glucose

Mean plasma glucose level at start of experiment was 57.22, 55.16 and 58.93 mg/ dl, in control, S_1 and S_2 groups, respectively, whereas overall mean value was 55.25, 56.31 and 58.17 mg/ dl in the three respective groups (Table 4.36). There was no significant effect ($P>0.05$) of varying dietary DCAD levels on blood glucose concentration. Plasma glucose concentration in all the three groups was within the normal range (40.00 to 80.00 mg/dl) throughout the experimental period of 120 d (Upadhyay and Rao 1985; More *et al.* 2008 and Bhooshan *et al.* 2010).

Table 4.35 Effect of positive DCAD diets on plasma zinc, copper and iron concentration (ppm) in crossbred calves during summer months

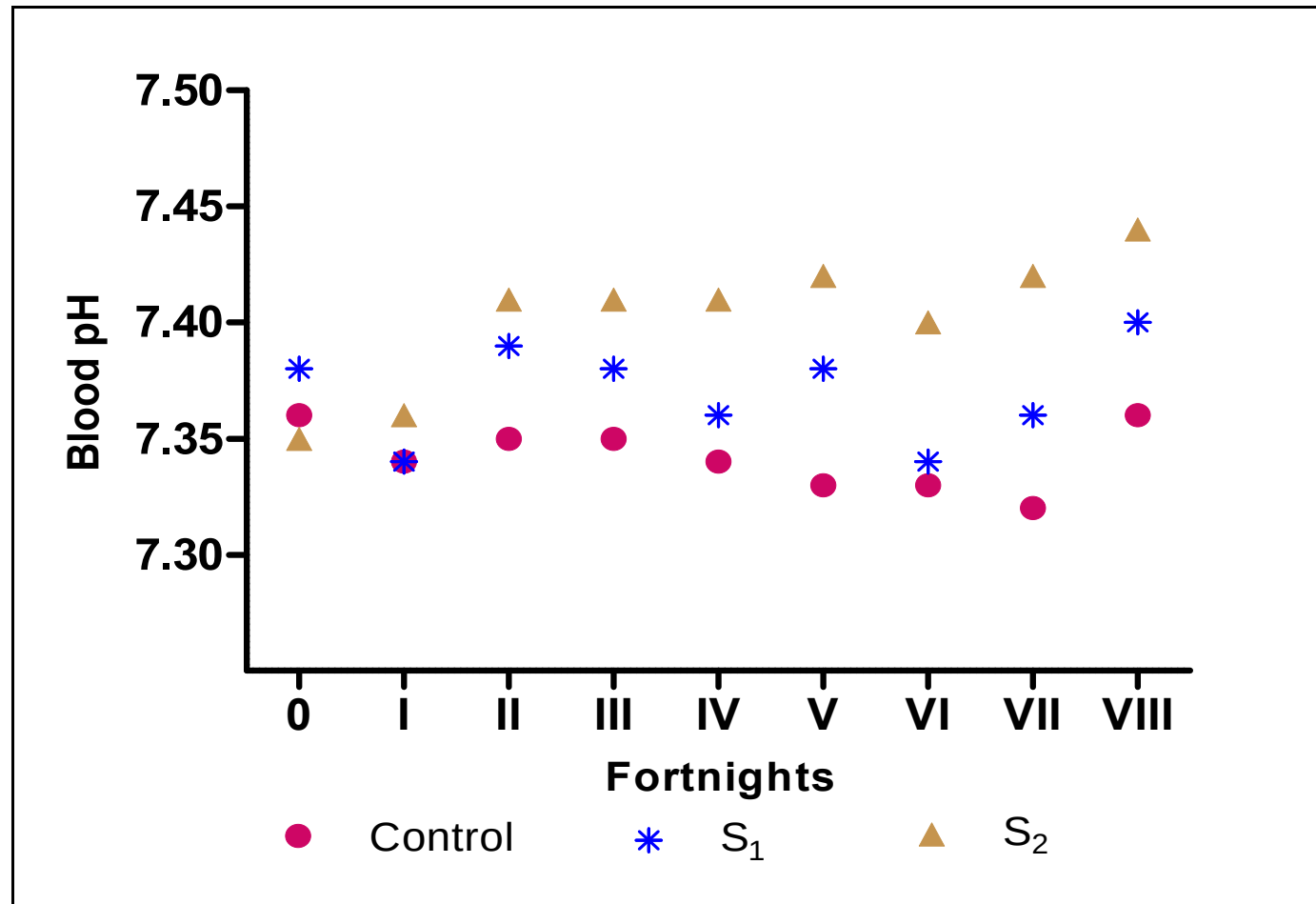
Fortnight	Zinc (ppm)				Copper(ppm)				Iron (ppm)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	1.71	1.85	1.72	0.06	0.79	0.78	0.78	0.27	2.54	2.49	2.30	0.18
I	1.44	1.41	1.41	0.06	0.76	0.74	0.76	0.20	2.38	2.37	2.35	0.12
II	1.17	1.26	1.26	0.06	0.75	0.90	0.92	0.37	2.58	2.58	2.63	0.72
III	1.33	1.28	1.25	0.05	0.84	0.86	0.87	0.25	2.16	2.28	2.18	0.52
IV	1.39	1.47	1.51	0.07	0.86	0.86	0.88	0.23	1.64	1.67	1.66	0.89
V	1.73	1.51	1.67	0.05	0.83	0.83	0.85	0.25	1.62	1.61	1.61	0.88
VI	1.97	1.90	2.00	0.08	0.81	0.83	0.82	0.25	1.85	1.86	1.80	0.13
VII	1.96	1.74	1.79	0.05	0.82	0.88	0.85	0.21	2.00	2.09	2.01	0.09
VIII	1.82	1.85	1.86	0.07	0.84	0.87	0.86	0.28	1.96	2.00	2.04	0.09
Mean	1.61	1.59	1.61	0.03	0.81	0.84	0.84	0.01	2.08	2.11	2.06	0.43

Table 4.36 Effect of positive DCAD Diet on blood pH and glucose level (mg/dl) in growing KF calves during summer months

Fortnight	Blood pH				Glucose(mg/dl)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	7.36	7.38	7.35	0.01	57.22	55.16	58.93	1.36
I	7.34	7.34	7.36	0.02	59.95	58.02	60.45	0.78
II	7.35	7.39	7.41	0.02	55.61	56.45	59.22	1.03
III	7.35	7.38	7.41	0.02	51.02	55.00	55.20	1.15
IV	7.34	7.36	7.41	0.02	51.03	53.22	53.55	1.41
V	7.33	7.38	7.42	0.02	53.50	55.00	56.46	1.05
VI	7.33	7.34	7.40	0.02	55.13	56.12	59.04	1.34
VII	7.32	7.36	7.42	0.02	56.29	58.80	59.54	0.99
VIII	7.36	7.40	7.44	0.02	57.52	59.00	61.20	0.79
Mean	7.34^A	7.37^{AB}	7.40^B	0.006	55.25	56.31	58.17	0.41

Means having different superscripts within a row differ significantly (P<0.01)

Fig. 4.10 Effect of positive DCAD diet on blood pH in growing crossbred calves during summer months



Muneedera Kumar (2013) also reported plasma glucose in the range of 54.96 to 55.95 mg/ dl in the heat stressed buffalo calves. Chan *et al.* (2005) in lactating dairy cows (+20, +35, or +50 mEq/kg DM) and Cho *et al.* (2006) in beef cows (-10 or +25 mEq/kg DM) did not observe any effect of DCAD diet on blood glucose in lactating cows.

4.3.9 Effect of DCAD diet on antioxidant status and immunity

4.3.9.1 Plasma total antioxidant activity (Ferric Reducing Antioxidant Power Assay, FRAP)

FRAP value at start of experiment was 2039.44, 2029.92 and 1981.78 $\mu\text{mol/l}$, respectively in control, S₁ and S₂ groups (Table 4.37). The overall mean FRAP value during the whole experimental period of 120 days was 2158.62, 2227.63 and 2279.49 $\mu\text{mol/l}$ in the three respective groups showing no significant ($P>0.05$) effect of positive DCAD diets. The FRAP value in blood plasma was within normal range (Ganie, 2012 and Vaidya *et al.*, 2012). However, Muneedera Kumar (2013) reported FRAP value in the range of 1007.96 to 1113.83 $\mu\text{mol/l}$ in buffalo calves. The literature is not available on the effect of varying DCAD diets on FRAP value. There was no variation in the FRAP value between summer and winter experiment due to dietary treatments.

4.3.9.2 Plasma Superoxide dismutase and Catalase activity

Effect of positive DCAD diet on SOD (U/ ml) and catalase activity (nmol/ min/ ml) in growing crossbred calves were estimated fortnightly and the results obtained are presented in Table 4.37. The overall plasma SOD activity (U/ ml) averaged 2.89, 2.85 and 2.78, respectively showing no significant difference ($P>0.05$) among groups. The catalase activity (nmol/ min/ ml) at the end of 120 days dietary treatments averaged 58.07, 56.60 and 56.13 in three respective groups showing no significant difference ($P>0.05$) among dietary treatments. The values of SOD and catalase activity were in the normal physiological range as reported by earlier authors (Kumar *et al*, 2010 and 2011a; Manish *et al*, 2011 and Ganie, 2012). In the present study, the SOD and catalase activity obtained in the summer months were slightly more than the winter months (Table 4.15).

Lallawmkimi (2009) studied the effect of winter and summer seasons on antioxidant status of growing heifers and lactating Murrah buffaloes and reported significantly higher SOD and catalase levels during summer compared to winter. Manish *et al.* (2011) also reported significantly higher concentration of SOD in goats during summer (THI >79) compared to pre-summer months (THI=69.5). Vitamin C supplementation significantly ($P<0.05$) decreased SOD and catalase activity in buffalo serum under hot-humid conditions compared to control group (Kumar *et al.*, 2010). Kumar *et al.* (2011b) observed effect of vitamin C supplementation in thermally exposed buffaloes and reported lower SOD activity (1942.0 ± 28.93 K/ g Hb) in treated group compared to the non-treated (2120.0 ± 60.93 K/ g Hb) buffaloes. Similarly, decreased activity of serum catalase (530.0 ± 8.22 U/ g Hb) in vitamin C supplemented group compared to non-supplemented group (584.0 ± 10.79 U/ g Hb) was observed in buffaloes exposed to thermal stress. The effect of positive DCAD diet on these anti-oxidative enzymes has not been studied earlier.

4.3.9.3 Plasma total Immunoglobulin (Ig), plasma IgG and IgM levels

The effect of positive DCAD diets on total plasma immunoglobulin concentration (mg/ ml) has been presented in Table 4.38. Plasma total immunoglobulin concentration on day 0 in the three respective groups averaged 28.96, 27.19 and 27.89 mg/ ml. The overall plasma total immunoglobulin concentrations during 120 d of experimental period was 29.24, 31.65 and 31.26 mg/ ml in three respective groups showing significantly ($P<0.05$) higher values in +250 and +350 mEq/ kg DM DCAD fed groups.

Fortnightly plasma IgG and IgM levels in the female crossbred calves are presented in Table 4.38. The plasma IgG at the start of experiment was 20.25, 20.48 and 22.80 ng/ ml which increased to 21.95, 22.32 and 22.36 ng/ ml in control, S_1 and S_2 groups, respectively at the end of summer season experiment. Overall mean plasma IgG level in S_2 group was significantly higher ($P<0.05$) than control group. The overall mean value of plasma IgM was 6.65, 7.10 and 7.13 ng/ ml in three

Table 4.37 Effect of positive DCAD Diet on FRAP level ($\mu\text{Mol/l}$), SOD (U/ml) and catalase activity (nmol/min/ml) in crossbred calves during summer months

Fortnight	FRAP level ($\mu\text{Mol/l}$)				SOD (U/ml)				Catalase (nmol/min/ml)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	2039.44	2029.92	1981.78	94.14	3.03	3.09	3.05	0.16	54.62	54.95	53.65	1.12
I	2180.02	2103.52	2124.22	96.59	2.83	2.89	2.85	0.16	51.12	52.62	51.98	1.13
II	2078.16	1962.73	2043.48	99.77	3.23	3.30	3.13	0.06	56.09	55.83	55.76	0.65
III	2229.61	2410.14	2485.11	54.99	3.03	3.10	2.93	0.06	52.59	53.50	54.42	0.74
IV	1937.16	2118.01	2190.68	89.88	2.81	2.56	2.61	0.14	57.76	56.97	56.56	0.44
V	2291.93	2109.73	2294.31	93.03	2.61	2.36	2.41	0.14	54.26	54.47	55.22	0.46
VI	2385.09	2585.92	2480.33	91.36	2.82	2.80	2.70	0.05	56.94	55.85	54.97	0.42
VII	2194.62	2523.81	2689.44	112.11	2.62	2.60	2.50	0.05	53.94	53.35	53.80	0.38
VIII	2091.51	2204.87	2226.09	87.31	3.06	2.96	2.85	0.06	58.07	56.60	56.13	0.45
Mean	2158.62	2227.63	2279.49	32.83	2.89	2.85	2.78	0.04	55.04	54.90	54.72	0.26

Table 4.38 Effect of positive DCAD Diet on plasma total immunoglobulin (mg/ml), total IgG (ng/ml) and total IgM concentration (ng/ml) in crossbred calves during summer months

Fortnight	Total immunoglobulin (mg/l)				Total IgG (ng/ml)				Total IgM (ng/ml)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	28.96	27.19	27.89	1.14	20.25	20.48	22.80	0.60	7.60	7.60	7.78	0.07
I	26.44	28.10	27.68	1.12	20.15	23.98	22.40	0.64	7.45	7.93	7.49	0.10
II	27.53	34.00	31.01	1.37	21.65	24.10	24.65	1.70	5.60	5.50	5.61	0.24
III	27.47	31.19	30.90	1.17	21.82	23.61	24.24	1.11	4.00	4.98	5.04	0.21
IV	27.90	31.91	30.28	0.96	19.52	19.18	21.84	0.64	6.33	7.98	8.28	0.55
V	26.29	30.05	30.66	1.10	21.14	21.11	21.54	0.86	7.21	7.45	7.42	0.08
VI	30.41	32.38	32.05	1.13	22.76	23.32	23.87	0.95	6.35	6.46	6.54	0.12
VII	31.38	32.09	32.72	1.45	19.69	21.58	21.79	1.07	7.37	7.75	7.80	0.17
VIII	36.81	37.94	38.19	0.82	21.95	22.32	22.36	0.92	7.91	8.21	8.25	0.07
Mean	29.24^a	31.65^b	31.26^b	0.44	20.99^a	22.19^{ab}	22.83^b	0.34	6.65^a	7.10^{ab}	7.13^b	0.11

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

respective groups which was significantly higher in S₂ group (P<0.05) as compared to control group.

It has been reported that high environmental temperature affects the immunological parameters (Mashaly *et al.*, 2004 and Star *et al.*, 2009). The red blood cell (erythrocytes) count was found to decrease by 12-20% in cattle under heat stress conditions (38°C) (Salem, 1980 and Habeeb *et al.*, 2000). It is well established that increase in thermal stress lowers the plasma IgG and IgM concentration (Tizard, 1992). Kelly *et al.* (1982) reported that HF cows exposed to constant temperature of 30°C for 24 h period resulted in 27% decrease in serum IgG. The exposure of animals to high environmental temperature also results in increased plasma glucocorticoid concentration (Wise *et al.*, 1988 and Silanikove, 2000). Studies in humans and laboratory animals under a large variety of experimental conditions have demonstrated that intense adrenocortical activity depressed immune cell activity (MacMurray *et al.*, 1983; Kok *et al.*, 1995; Elenkov and Chrousos, 1999 and Moynihan, 2003). In calves, heat stress reduced serum IgG1 associated with increased cortisol concentration (Stott *et al.*, 1976). So, increase in cortisol level is also one of the reasons for immunosuppression.

4.3.10 Endocrine parameters

4.3.10.1 Plasma thyroid hormones

The mean values for plasma triiodothyronine (T₃) and thyroxine (T₄) (ng/ dl) in all the groups are presented in Table 4.39. Overall mean of T₃ and T₄ hormones was 0.96, 0.98, 1.01 and 19.92, 21.24, 21.68 ng/ dl in control, S₁ and S₂ groups, respectively. The T₃ and T₄ hormones in S₂ group was significantly more (P<0.05) than in control. In the present study the thyroid hormone levels in the summer months were less as compared to winter months. The plasma T₃ and T₄ levels decreased under heat stress as compared to thermo neutral conditions (Magdub *et al.*, 1982; Yousef *et al.*, 1997 and Habeeb *et al.*, 2001) and their blood levels have been negatively correlated with RH, THI, rectal temperature and respiration rate (Perez *et al.*, 1997). In young (6 months) and old age buffalo calves, acute heat exposure (33-43°C, 40-60% RH) induced

decrease in plasma T₃ (35.25%) and T₄ (17.59%) (Nessim, 2004). Decreased thyroid hormone levels during heat stress are an adaptive response and also might be an attempt to reduce metabolic rate and heat production (West, 1999). Further, the decrease in thyroid activity may be due to the production of H₂O₂ radicals.

Thyroid hormones also decreased in the present study with increase in thermal stress, but decrease in the treatment groups is less than control. It has been reported that these hormones are the primary determinants of basal metabolic rate and have a positive correlation to weight gain/ tissue production, feed intake and energy and nitrogen balances (Magdub *et al.*, 1982; McCann *et al.*, 1992 and Caldeira *et al.*, 2007a,b), so these can be the reasons for more circulatory thyroid hormones in the treatment groups as compared to the control; as the more feed intake (Table 4.29), body weight gain (Table 4.31) and positive nitrogen balance (Table 4.42) was observed in the treatment groups. Higher T₃ and T₄ levels in treatment groups are indicative of less thermal stress in these animals due to feeding of high DCAD diets.

4.3.10.2 Plasma cortisol

The mean values of cortisol for all groups are presented in Table 4.39. Cortisol levels at the start of experiment averaged 3.33, 3.26 and 3.32 ng/ ml, whereas, overall cortisol concentration was 4.24, 3.85 and 3.79 ng/ ml, in control, S₁ and S₂ groups, respectively. Overall mean value of cortisol was significantly (P<0.05) lower in treatment groups than control group. In the present experiment, reduced cortisol value in S₁ and S₂ groups indicated that these animals had less stress compared to control group of calves. Plasma cortisol increased with increase in THI in all the three groups and maximum cortisol levels were obtained at IV and V fortnights of experiment when THI was reported maximum. It has been reported that cortisol level is affected by the thermal stress. Yousef *et al.* (1997) observed that plasma cortisol concentration increased from 11 to 29 ng/ ml due to direct solar exposure during heat stress in Friesian calves. Habeeb *et al.* (2001) also observed that plasma cortisol concentration increased from 3.8 to 6.5 ng/ ml when ambient temperature

Table 4.39 Effect of positive DCAD Diet on T₃, T₄ and cortisol levels (ng/ml) in growing crossbred calves during summer months

Fortnight	T ₃ (ng/ml)				T ₄ (ng/ml)				Cortisol (ng/ml)			
	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM	Control	S ₁	S ₂	SEM
0	1.00	0.98	0.98	0.02	19.76	21.37	20.39	0.89	3.33	3.26	3.32	0.12
I	0.94	0.98	0.99	0.08	20.22	21.21	21.27	0.50	3.83	3.65	3.64	0.15
II	0.93	0.98	0.98	0.02	20.88	22.05	22.65	0.76	4.13	3.78	3.72	0.31
III	0.91	0.95	1.00	0.01	20.79	21.96	22.56	0.27	4.49	4.14	4.09	0.26
IV	0.95	0.96	1.01	0.02	19.01	20.70	21.48	0.75	4.92	4.42	4.28	0.21
V	0.95	0.99	1.02	0.01	18.95	20.65	21.42	0.75	5.45	5.15	4.97	0.25
VI	0.99	0.99	1.04	0.01	20.35	21.91	22.68	0.73	4.56	3.73	3.58	0.23
VII	0.98	0.99	1.04	0.03	20.29	21.85	22.62	0.73	3.83	3.34	3.32	0.22
VIII	1.00	0.98	0.98	0.01	19.00	19.44	20.06	0.76	3.59	3.19	3.17	0.23
Mean	0.96 ^a	0.98 ^{ab}	1.01 ^b	0.01	19.92^a	21.24^{ab}	21.68^b	0.24	4.24^a	3.85^b	3.79^b	0.15

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

increased from 24 to 38°C in climatic chamber. Marai and Habeeb (2010) too reported significant rise in blood plasma cortisol levels due to increase in ambient temperature from 17.5°C to 37.1°C. However, the information regarding the effect of supplementation of DCAD diet on cortisol level in crossbred calves during summer season is not available, hence, the results cannot be discussed. It may be inferred from the results that positive DCAD diets are beneficial in reducing climatic stress.

4.3.11 Effect of positive DCAD diets on nutrients digestibility and intake in female crossbred calves during metabolic trial

4.3.11.1 Digestibility of nutrients

The mean values for nutrient digestibility in crossbred KF calves are presented in Table 4.40. The DM and OM digestibilities were 67.10, 67.32, 67.83 and 69.04, 70.23, 70.26 in control, S₁, S₂ groups, respectively. The OM and DM digestibilities were similar across all groups (P>0.05). CP and EE digestibilities were 72.32, 73.26, 73.43 and 76.05, 76.85, 78.66 in three respective groups and were also similar (P>0.05) in all the three groups. Similarly, the NDF and ADF digestibilities were not affected by the treatments (P>0.05). Similar findings were reported by Tucker *et al.* (1991a, b) and Delaquis and Block (1995a). Sarwar *et al.* (2007b) in lambs and Shahzad *et al.* (2007, 2008a, b) in buffaloes reported that the digestibility of DM, NDF and CP increased in anionic diet as compared to cationic diet. Sarwar *et al.* (2011) reported that DM, CP, NDF and ADF digestibilities decreased linearly with increasing levels of NaHCO₃ in the diet and highest DM and CP digestibilities were recorded for calves fed 0% NaHCO₃ and lowest for 1.6% NaHCO₃ fed group. Increased digestibilities of nutrients in negative DCAD diet might be attributed to increased rumen retention time of nutrients due to reduced DMI because a positive correlation exists between nutrients digestibility and rumen retention time (Sarwar *et al.*, 1996; Nisa *et al.*, 2004 and Nisa *et al.*, 2006). In present study, only cationic diets were used which might be the reason for no effect on the digestibility of nutrients (Hu and Murphy, 2004 and Apper-Bossard *et al.*, 2009).

4.3.11.2 Nutrient intake

The DM, OM, CP and TDN intakes observed during the metabolic trial are presented in Table 4.41. The DMI (kg/ d) of calves averaged 5.18, 5.67 and 5.74 in control, S_1 and S_2 groups, respectively. It is observed that DM intake was significantly higher ($P<0.05$) in the treatment groups as compared to control. CP and TDN (kg/ d) intakes were 0.66, 0.68, 0.71 and 2.97, 3.24, 3.28 in control, S_1 and S_2 groups, respectively and were statistically more ($P<0.05$) in treatment groups. The DCAD diets (+250 and +350 mEq/ kg DM) improved the nutrient intake in KF calves that might be due to the fact that the cationic diet attributed a higher rumen pH, blood HCO_3^- and acid-base balance (Sanchez *et al.*, 1994 and Shahzad *et al.*, 2008 a,b). It has been reported that cationic diet increased rumen buffering capacity in addition to an increased rumen fluid dilution rate (Rogers *et al.*, 1979). Rogers *et al.* (1982) also reported increased DMI in dairy cows when high level of NaHCO_3 was supplemented. They further stated that in addition to a buffering effect, NaHCO_3 also increased ruminal osmotic pressure and liquid dilution rate. In the rumen, NaHCO_3 is disassociated into Na^+ and HCO_3^- with non-buffering and buffering effects, respectively (Schneider *et al.*, 1986). Erdman (1988) hypothesized that rumen buffering reduces the extent of acidity produced by volatile fatty acid and lactic acid production in the rumen and therefore, improves the systemic acid-base status. A constant increase in crude protein (CP) and neutral detergent fiber (NDF) intake was observed with increased DCAD level of diets. Calves fed +330 and +110 mEq/ kg DCAD diets consumed maximum (836.13 g/ d) and minimum (681.8 g/ d) CP, respectively (Shahzad *et al.*, 2007). Sarwar *et al.* (2011) reported that nutrients intake increased ($P<0.05$) with increasing levels of dietary NaHCO_3 , minimum (6.23 kg/ d) and maximum (7.45 kg/ d) DM intakes were recorded in calves fed no NaHCO_3 and 1.6% NaHCO_3 diets, respectively. The CP consumption was highest (1176 g/ d) for calves fed the 1.6% NaHCO_3 diet and lowest (984 g/ d) in calves fed no NaHCO_3 diet. Shahzad *et al.* (2011) reported that the DMI in buffaloes fed on high cationic (+220 mEq), low cationic (+110 mEq), low anionic (-110 mEq) and high anionic (-220 mEq)

Table 4.40 Effect of positive DCAD diet on digestibility coefficient of nutrients in crossbred female calves during metabolic trial in summer months

Particulars	Control	S₁	S₂	SEM
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

Table 4.41 Effect of positive DCAD Diets on nutrient intake (Kg/d) in growing crossbred calves during metabolic trials in summer months

Particulars	Control	S₁	S₂	SEM
DM	5.18 ^a	5.67 ^b	5.74 ^b	0.10
OM	4.64	5.00	5.03	0.08
CP	0.66 ^a	0.68 ^{ab}	0.71 ^b	0.10
TDN	2.97 ^a	3.24 ^b	3.28 ^b	0.06

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

diets were 13.95, 13.20, 12.51 and 12.09 kg/ d, respectively. Maximum and minimum DMI was recorded in buffaloes fed on high cationic and high anionic diets. Luebbe *et al.* (2011) reported that DMI increased linearly as DCAD increased and it was greatest for steers consuming the basal diet (+8 mEq/ 100 g of DM), least for -32 mEq/ 100 g of DM, and intermediate for -2 and -12 mEq/ 100 g of DM. So it can be concluded that increased nutrient intake in the present study, might be due to increased rumen buffering capacity influencing rumen fermentation pattern by cationic diets.

4.3.12 Effect of positive DCAD diet on nitrogen balance

The results of nitrogen intake, outgo and balance during summer season are presented in Table 4.42. Nitrogen intake (g/ d) was 105.29, 108.65 and 113.90 in control, S₁ and S₂ groups, respectively. Nitrogen intake (g/ d) was significantly ($P<0.05$) higher in S₂ group as compared to control. Excretion of nitrogen (g/ d) through faeces and urine was influenced numerically by positive DCAD diet and it was 41.21, 47.67, 49.29 and 32.91, 33.93, 37.85 g/ d in three respective groups. All the animals were found to be in positive nitrogen balance and values obtained were 23.71, 25.43 and 27.56 g/ d in three respective groups, however S₂ group had significantly higher ($P<0.05$) nitrogen balance in comparison to control. The apparent N retention (%) was found to be similar ($P>0.05$) in all the dietary groups. The higher N intake and balance obtained in the treatment groups might be due to increased DMI of the calves. Higher level of cationic salt might also have contributed towards enhanced nitrogen balance by preventing systemic acidosis leading to more desirable pH for rumen microbes (Tucker *et al.*, 1991c) which might have increased the post-ruminal supply of amino acids by accelerating rumen microbial multiplication (Shahzad *et al.*, 2007). Similar, to the present study, Shahzad *et al.* (2007) reported more N intake and balance in +22 mEq/100 g DM diet (350.42 and 111.75 g/ d) as compared to the +11 mEq/100 g DM diet (323.68 and 99.5 g/ d). Sarwar *et al.* (2011) also found that N intake was highest (188.1 g/ d) in the calves fed 1.6% NaHCO₃ diet and lowest (157.5 g/ d) in the 0% NaHCO₃ fed diet.

4.3.13 Urine pH

The influence of positive DCAD on monthly urine pH has been presented in Table 4.43. The IVth fortnight urine pH was significantly higher in the S₂ group (8.01, $P < 0.05$) as compared to control (7.32). The overall urine pH was 7.30, 7.53 and 7.73 in control, S₁ and S₂ groups, respectively and also significantly higher in the S₂ group ($P < 0.01$) as compared to control. The findings of the present studies are in agreement with the findings of Jackson *et al.* (1992); Jackson and Hemken (1994) and Pehrson *et al.* (1999) that higher intake of dietary cations increased the urine pH. Shahzad *et al.* (2007) observed a linear increase in urine pH with increased DCAD levels. Sarwar *et al.* (2007b) reported that lambs fed +110 and +220 DCAD diet had a urine pH of 7.43 and 7.68, respectively. Urine pH increased in high-DCAD (19.73 mEq) cows and decreased in low-DCAD cows (-5.6 mEq). The decrease of urine pH by 1 unit in low-DCAD cows demonstrates the capacity of the body to disseminate an acid load from the blood into other body tissues and fluids (Hersom *et al.*, 2010). Luebke *et al.* (2011) also reported that urinary pH increased as DCAD increased. The findings reflect that increase or decrease in urine pH in DCAD fed animals was because of the alkaline or acidic nature of the diet, respectively, which is the function of salts used for the respective mineral composition. So, it can be inferred from the present study that cationic diet being alkaline in nature increases the urine pH.

4.3.14 Effect of different levels of DCAD diet on mineral balances

4.3.14.1 Sodium and Potassium balances

Na intake (g/ d) was 23.25, 32.54 and 48.88 in the three respective groups which increased ($P < 0.01$) with the level of DCAD diet (Table 4.44). Faecal and urinary excretion of Na (g/ d) also numerically increased with increase in the level of cationic diet, which was 3.98, 5.33, 5.41 and 12.25, 17.15, 19.30 g/d in control, S₁, S₂ groups, respectively. Na balance was significantly more ($P < 0.01$) in S₂ group (24.17 g/ d) in comparison to control (7.02 g/ d). Percent Na retention was significantly higher ($P < 0.01$) in S₂ group than in control group.

Table 4.42 Effect of positive DCAD diet on nitrogen balance in growing crossbred calves in summer months

Parameter	Control	S₁	S₂	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

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

Table 4.43 Effect of positive DCAD diet on urine pH in crossbred calves during summer months

Fortnight	Control	S₁	S₂	SEM
0	7.30	7.29	7.28	0.12
I	7.28	7.48	7.76	0.11
II	7.30	7.65	7.81	0.15
III	7.29	7.50	7.78	0.15
IV	7.32 ^a	7.70 ^{ab}	8.01 ^b	0.14
Mean	7.30^a	7.53^{ab}	7.73^b	0.07

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

Table 4.44 Effect of positive DCAD diet on sodium and potassium balances in crossbred calves in summer months

	Parameter	Control	S₁	S₂	SEM	Sig.
Na	Na Intake (g/d)	23.25 ^A	32.54 ^B	48.88 ^C	2.57	P<0.01
	Faecal Na outgo (g/d)	3.98	5.33	5.41	0.31	NS
	Urinary Na outgo (g/d)	12.25	17.15	19.30	1.91	NS
	Na balance (g/d)	7.02 ^A	10.06 ^{AB}	24.17 ^B	2.61	P<0.01
	Na retention (%)	29.95 ^A	31.05 ^{AB}	49.35 ^B	6.16	P<0.01
K	K Intake (g/d)	125.49 ^A	138.57 ^B	149.52 ^B	2.81	P<0.01
	Faecal K outgo (g/d)	27.06	30.83	31.41	1.23	NS
	Urinary K outgo (g/d)	18.70	19.15	20.69	2.01	NS
	K balance (g/d)	79.73 ^A	88.59 ^{AB}	97.43 ^B	2.55	P<0.01
	K retention (%)	63.12	63.57	64.63	1.71	NS

Means having different superscripts within a row differ significantly; NS: Non-significant

Potassium intake was significantly ($P < 0.01$) more in the treatment groups S_1 and S_2 i.e. 138.57 and 149.52 g/ d as compared to control (125.49 g/ d) (Table 4.44). Faecal and urinary excretion of K (g/ d) increased numerically with increase in the level of cationic diet which was 27.06, 30.83, 31.41 and 18.70, 19.15, 20.69 g/d in control, S_1 and S_2 groups, respectively. K balance was significantly more ($P < 0.01$) in the S_2 group (97.43 g/ d) as compared to control (79.73 g/ d). The K retention (%) was not ($P > 0.05$) affected by the treatments.

Findings of the present experiment indicated that Na and K intake as well as excretion was higher in the positive DCAD fed group. These results are in accordance with the findings of Tucker *et al.* (1991c) and West *et al.* (1992). Roch *et al.* (2005) also observed that Na excretion increased linearly with increasing DCAD. Similarly, Wildman *et al.* (2007) reported that fractional excretion of Na was elevated in cows receiving high DCAD, probably because the high DCAD diets contained higher Na concentrations. Shahzad *et al.* (2011) reported that buffaloes fed on high cationic diet had higher intake, excretion and retention of Na^+ and K^+ while these were lower in buffaloes fed on high anionic diet; the minimum (9.5 vs. 134.11 g/ d) and maximum (12.42 vs. 150.56 g/ d) Na^+ and K^+ balances were observed with high anionic (-220 mEq/ kg DM) and high cationic diet (+220 mEq/ kg DM), respectively. Hence, is evident that cationic diet influences the intake, excretion as well as balance of Na and K because it contains higher Na and K salts as compared to control group.

4.3.14.2 Chloride and Sulphur balance

Cl and S intake, excretion and balance (g/ d) are shown in Table 4.45. Cl and S intake (g/ d) was 78.05, 79.57, 80.13 and 16.06, 16.37, 16.48 in control, S_1 and S_2 groups, respectively. Cl and S intakes (g/ d) were similar ($P > 0.05$) in all the groups, as similar amounts were fed to all the groups. Faecal and urinary excretion of Cl and S (g/ d) was also similar ($P > 0.05$) in all the three groups. The Cl and S balance (g/ d) was 27.41, 27.34, 30.52 and 8.85, 9.51, 9.81 showing no statistical difference ($P > 0.05$) in the three groups. Similarly, percent retention of Cl and S were not affected by the dietary treatments. Tucker *et al.* (1988) and West *et al.*

(1992) reported that there was decreased Cl^- excretion at high DACD level and increased at low DCAD level. Shahzad *et al.* (2011) also reported that, intake, excretion as well as retention of Cl was higher in the anionic group as compared to cationic group, this may be due to the fact that CaCl_2 was used to reduce the DCAD of the diet; so intake of Cl^- was increased, which was manifested in urine and faeces. In the present study, anionic diets were not used which contain chloride salts, so, Cl metabolism was not affected. Delaquis and Block (1995a) recorded increased (8.7 vs. 36.4 mg/ d) urinary S in early lactating cows with decreased (258 vs. 55 mEq/ kg) DCAD levels. The plausible explanation of this might be that they increased S salt (0.20 vs. 0.49%) as compared to Cl salt (0.3 vs. 0.35%) to acidify the diet. However Shahzad *et al.* (2007) observed that different DCAD levels in the diet had no effect on S balance. In the present study we have not got any effect on the intake as well on retention of Cl and S due to the reason that there is a no anionic salt containing Cl and S elements in the treatments diets.

4.3.14.3 Calcium and Phosphorus balances

The Ca and P intake in control, S_1 , S_2 , groups was 40.07, 39.31, 38.47 and 26.34, 27.63, 28.17 g/ d, respectively. It has been found that Ca and P intake was statistically similar ($P>0.05$) among groups (Table 4.46). Similarly, there was no variation ($P>0.05$) observed for the Ca and P excretions. The Ca and P balance (g/ d) in control, S_1 , S_2 groups was 22.31, 21.89, 22.00 and 5.39, 8.98, 10.25 showing no significant difference ($P>0.05$) among groups. The Ca and P retention was also similar ($P>0.05$) among groups. Shahzad *et al.* (2007) also reported that Ca intake (32.31 and 33.21 g/d) and apparent retentions (14.06 and 14.16 g/ d) in growing buffalo calves were statistically similar among +22 and +33 mEq/ 100 g DM DCAD diet fed groups. Shahzad *et al.* (2008b) reported that the P balance in early lactating buffaloes was not influenced by the +22 mEq/ 100 g DM (35.49 g/ d) and +33 mEq/ 100 g DM DCAD diet (36.12 g/ d). It is reported that anionic diets influence the Ca and P metabolism (Schonewille *et al.*, 1994 and Roche *et al.*, 2003a). Sarwar *et al.* (2007b) observed that Ca intake, absorption, excretion and retention

Table 4.45 Effect of positive DCAD diet on chloride and sulphur balances in crossbred calves in summer months

	Parameters	Control	S₁	S₂	SEM	Sig.
Cl	Cl Intake (g/d)	78.05	79.57	80.13	0.70	NS
	Faecal Cl outgo (g/d)	37.13	36.06	33.47	0.69	NS
	Urinary Cl outgo (g/d)	14.59	15.11	16.14	1.89	NS
	Cl balance (g/d)	27.41	27.34	30.52	1.79	NS
	Cl retention (%)	34.55	34.07	37.31	2.53	NS
S	S Intake (g/d)	16.06	16.37	16.48	1.10	NS
	Faecal S outgo (g/d)	4.89	4.55	4.40	0.12	NS
	Urinary S outgo (g/d)	2.33	2.30	2.26	0.18	NS
	S balance (g/d)	8.85	9.51	9.81	0.23	NS
	S retention (%)	54.91	57.86	59.08	2.80	NS

NS: Non-significant

Table 4.46 Effect of positive DCAD diet on calcium and phosphorus balances in crossbred calves in summer months

	Parameter	Control	S₁	S₂	SEM	Sig.
Ca	Ca Intake (g/d)	40.07	39.31	38.47	0.40	NS
	Faecal Ca outgo (g/d)	15.85	17.17	17.88	0.65	NS
	Urinary Ca outgo (g/d)	0.32	0.25	0.20	0.03	NS
	Ca balance (g/d)	22.31	21.89	22.00	0.49	NS
	Ca retention (%)	57.45	55.07	54.31	1.36	NS
P	P Intake (g/d)	26.34	27.63	28.17	0.27	NS
	Faecal P outgo (g/d)	20.62	18.51	17.75	1.01	NS
	Urinary P outgo (g/d)	0.14	0.14	0.17	0.02	NS
	P balance (g/d)	5.39	8.98	10.25	0.82	NS
	P retention (%)	25.67	32.07	36.07	2.92	NS

NS: Non-significant

significantly increased in lambs fed -110 DCAD diet as compared to those fed +110, +220 and +330 DCAD diets. Increased urinary Ca excretion in lambs fed -110 diets might be due to slight metabolic acidosis, induced by a negative DCAD diet, but they did not get any significant difference in the Ca intake, absorption, excretion and retention in positive DCAD fed group similar to the present findings. Shahzad *et al.* (2011) also observed that Ca and P intake and excretions were more in the anionic group compared to cationic group. The plausible explanation for the effect of anionic diet on Ca and P metabolism is that metabolic acidosis affects parathyroid hormones which are linked with Ca and P metabolism. In the present study, only positive DCAD diets were used which being alkaline in nature is unable to exert any effect on the absorption and excretion of Ca and P.

CHAPTER – 5

Summary and Conclusion

5. SUMMARY AND CONCLUSION

In the present study, commonly used feed and fodder samples were collected and analyzed for macro minerals. The Ca content in wheat straw was 0.30%. Berseem (leguminous fodder) contained higher Ca, to the extent of 1.67%. Cereals and cereal by-products were deficient in Ca content. P in roughages and concentrate feeds varied from 0.13-0.69% and 0.41-1.05%, respectively. The compound feed contained 0.71% Ca and 1.16% P. The Na and K in different concentrate feed ingredients varied from 0.01-0.10% and 0.48-2.40%, respectively. The variation in fodders tested was 0.01-1.08% for Na and 0.92-3.24% for K. All the tested concentrate ingredients contained low level of Cl in the range of 0.07-0.16% and S in the range of 0.07-0.28%. The Cl content in various roughage sources varied from 0.40-1.66%. The S content varied from 0.16-0.45% in green fodders.

EXPERIMENT 1: Effect of feeding +150, +250 mEq/ kg DM DCAD to crossbred calves during winter months

An experiment was carried out with the objective of evaluating the efficacy of +150 and +250 mEq/ Kg DM DCAD diet in crossbred calves to mitigate the adverse effect of environmental stress on animal performance, nutrient utilization and immune status during winter months.

For this experiment, eighteen female crossbred KF calves of around 5-9 months of age were selected from the herd of NDRI, Karnal. They were divided into three groups of six animals in each group viz. control, W_1 and W_2 on the basis of body weight (Kg). The body weight at the start of experiment averaged 148.77, 148.25 and 149.62 kg in three respective groups. Calves of all the groups were fed basal diet consisting of concentrate mixture, maize or berseem fodder, whereas diet of W_1 and W_2 group calves was supplemented with NaHCO_3 and K_2CO_3 salt to achieve +150 and +250 mEq/ Kg DM DCAD value, respectively. Due to increase in body weight of the calves, the DMI increased, therefore, the amount of NaHCO_3 and K_2CO_3 salt was adjusted every fortnight to maintain +150

and +250 mEq/ Kg DM DCAD diet. Desired NaHCO_3 and K_2CO_3 salts were added in the concentrate mixture. Nutrient requirements of the animals were met as per NRC (2001). The duration of the experiment was 120 days (8 fortnights).

The maximum temperature, minimum temperature, relative humidity and dry-wet bulb temperature were recorded daily in the morning (at 7:30 am) and afternoon (at 2:30 pm) and THI was calculated. DM intake and physiological variables (RR, PR and RT) were recorded daily. The body weight of the animals was taken fortnightly in the morning before offering feed and water. Blood samples were collected at the start of the experiment (day 0) and then at fortnightly intervals. Total Ig, IgG, IgM levels, total antioxidant activity, superoxide dismutase activity, catalase activity, glucose, cortisol, thyroid hormones (T_3 and T_4), macro minerals (Na, K, Ca, Mg, P, Cl and S) and trace minerals (Zn, Cu and Fe) were estimated in blood plasma at fortnightly intervals.

THI, an indicator of environmental stress varied along with ambient temperature and relative humidity. The THI throughout the experimental period varied from 49.1-67.3 and 61.1-81.8 in the mornings and afternoons, respectively. The THI during the mornings and afternoons up to VIIth fortnight was below 72, indicating stress on the animals. The overall means of PR, RR and RT in all the three groups showed no significant difference. The overall DMI (Kg) in group W_2 (4.92) was significantly more ($P < 0.05$) as compared to control group (4.42). DMI Kg/ 100 kg BW and DMI g/ kg BW^{0.75} were 2.65, 2.76, 2.79 and 95.49, 97.52, 101.29 in control, W_1 and W_2 groups, respectively showing ($P > 0.05$) no significant difference between dietary treatments. The overall average body weight gain/fortnight was 6.21, 6.60 and 7.27 kg and growth rate was 410.42, 440.19 and 484.67 g/d in control, W_1 and W_2 groups, respectively. Statistical analysis revealed that there was significantly more ($P < 0.05$) weight gain in W_2 group as compared to control showing the positive effect of feeding +250 mEq/kg DM DCAD diet in female crossbred calves.

Plasma macro minerals concentration was not affected by the +150 and +250 mEq/ Kg DM DCAD diets in the winter season. The overall average Na concentration in three respective groups was 127.85, 128.65

and 129.18 mEq/ l. The K concentration (mEq/ l) at 0 and 120 d was 4.62, 4.57, 4.58 and 4.65, 4.70, 4.73 in three respective groups. The overall mean plasma Cl, S, Ca, P and Mg (mg/ dl) concentrations during 120 d experimental period averaged 97.23, 96.45, 95.44; 2.41, 2.42, 2.44; 9.37, 9.10, 8.80; 5.12, 5.25, 5.23 and 3.39, 3.56, 3.57 in control, W₁, W₂ groups, respectively. Plasma Zn, Cu and Fe were also not affected by the dietary treatments (P<0.05).

The overall blood pH was (p<0.05) maximum (7.38) in calves fed +250 mEq/Kg DCAD while it was minimum (7.33) in the control group. The mean levels of plasma glucose were unaffected by alkaline diets fed to the calves. There was no significant effect of +150 and +250mEq/ Kg DM DCAD diets on plasma total antioxidant activity (μ mol/ L), plasma SOD (U/ ml) and catalase activity (mmol/ min/ ml) during winter months. Total immunoglobulin concentration (0 d) in control, W₁, W₂ groups was 30.96, 31.23 and 33.09 (mg/ ml) and the concentration in corresponding groups at 120th d of experimental feeding was 30.22, 31.25 and 31.74 (mg/ ml). Statistical analysis of data revealed that the total immunoglobulin concentration was not affected significantly (P>0.05) by the treatment diets. Similarly, plasma IgG and IgM concentrations showed no effect of +150 and +250 mEq/ Kg DM DCAD diet. The average plasma T₃ and T₄ (ng/ ml) in winter months was 1.08, 1.12, 1.18 and 23.88, 24.19, 26.02 in three respective groups. It was found that the mean value of plasma T₃ and T₄ was significantly higher (P<0.05) in W₂ group as compared to control. The overall mean plasma cortisol (ng/ ml) during the 120 days experimental period was 3.67, 3.62 and 3.53 ng/ ml in control, W₁ and W₂ groups, respectively, and no variation (P>0.05) was observed between the groups.

In the present experiment, the OM, DM, NDF and ADF digestibility were affected by +150 and +250 mEq/ kg DM DCAD diets. It was evident that DM, OM, CP and TDN intakes were significantly improved in the +250 mEq/ kg DM DCAD group in winter months during metabolic trial. Nitrogen intake (g/d) was significantly more (P<0.05) in W₂ group as compared to control. Excretion of N (g/ d) through faeces and urine was not influenced

by positive DCAD diet and it (g/ d) was 44.47, 46.19, 47.28 and 29.01, 31.75, 32.94 in three respective groups. All the animals were found to be in positive nitrogen balance and values obtained were 26.28, 28.16 and 30.08 (g/ d), in control, W_1 and W_2 groups, respectively. N balance was significantly ($P<0.05$) higher in W_2 group, as compared to other two groups. The apparent nitrogen retention was not affected by the treatments. The overall urine pH was significantly higher ($P<0.05$) in the W_2 (7.57) group as compared to control (7.35).

Sodium intake (g/ d) was 29.08, 33.94 and 38.60 in three respective groups and was significantly higher ($P<0.01$) in W_2 group as compared to control group. Faecal and urinary excretion of Na (g/ d) also increased with increase in the level of Na in the diet which was 4.07, 5.71, 7.56 and 18.88, 20.71, 21.25 g/ d, respectively in control, W_1 and W_2 groups. Faecal excretion (g/ d) of Na was significantly more ($P<0.01$) in W_2 group as compared to control, whereas the urinary excretion of Na did not vary statistically ($P>0.05$) among groups. Na balance (g/ d) and Na retention (%) were statistically similar ($P>0.05$) among groups. Potassium intake (g/ d) was 121.59, 136.44 and 144.23 in control, W_1 and W_2 groups. The K intake was significantly higher ($P<0.01$) in W_1 and W_2 groups as compared to control. Urinary excretion of K was significantly more ($P<0.01$) in W_2 group compared to control group. However the K balance was significantly higher ($P<0.05$) in the W_1 and W_2 groups (72.22 and 77.95 g/ d) as compared to control (59.68 g/ d). The overall K retention (%) was 48.90, 52.89 and 53.89 in three respective groups and remained statistically ($p>0.05$) similar in all the three groups. The Cl and S intake in control, W_1 and W_2 groups was statistically similar ($P>0.05$) among groups. Faecal and urinary excretion was also similar ($P>0.05$) in all the three groups. The Cl and S balance (g/d) was 29.59, 28.66, 28.38 and 13.95, 13.80, 13.64, showing no variation ($p>0.05$) between groups. Cl and S retentions (%) were also not affected ($P>0.05$) by the level of positive DCAD diet supplementation. Similarly the Ca and P intakes, excretions, balance and retentions were not affected ($P>0.05$) by the DCAD diet. It was inferred that +250 mEq/ Kg DCAD diet significantly improved the growth rate of the calves without affecting the utilization of major and trace minerals.

EXPERIMENT 2: Effect of +250 and +350 mEq/ Kg DM DCAD diet on growing crossbred calves during summer season

Eighteen female crossbred KF calves of 8-12 months of age were selected from the herd of NDRI, Karnal. They were divided into three groups of 6 animals in each group viz. control, S_1 and S_2 on the basis of body weight (Kg). The body weight at the start of experiment averaged 148.18, 150.89 and 149.94 in three respective groups. Calves of all the groups were fed basal diet consisting of concentrate mixture, oat/ maize/ jowar fodder, whereas the diet of S_1 and S_2 calves were supplemented with NaHCO_3 and K_2CO_3 salt to achieve +250 and +350 mEq/ Kg DM DCAD value, respectively (NRC, 2001). The maximum temperature, minimum temperature, relative humidity and dry-wet bulb temperature were recorded daily in morning (at 7:30 am) and afternoon (at 2:30 pm) and THI was calculated. DM intake, environmental (THI) and physiological variables (RR, PR and RT) were recorded daily. The body weight of the animals was taken fortnightly in the morning before offering feed and water. Blood samples were collected at the start of the experiment (0 d) and then at fortnightly intervals.

Total Ig, IgG, IgM levels, total antioxidant activity, superoxide dismutase activity, catalase activity, glucose, cortisol, thyroid hormones (T_3 and T_4), macro minerals (Na, K, Ca, Mg, P, Cl and S) and trace minerals (Zn, Cu and Fe) were estimated in blood plasma at fortnightly intervals.

Fortnightly THI at the start of experiment (1-15th April) in the morning and afternoon was 67.9 and 80.2. The maximum THI in morning and afternoon observations was observed in V^{th} (79.3 and 85.6) and VI^{th} (80.7 and 84.7) fortnights. The THI values indicated that the calves were under thermal stress during most of the experimental period. The overall mean of RR (breaths/ min), PR (beats/ min) and RT ($^{\circ}\text{C}$) in three respective groups in the mornings during summer season averaged 49.0, 48.4, 47.8; 71.2, 70.7, 70.4 and 38.5, 38.4, 38.3, respectively which were not statically ($P>0.05$) different among groups. Similarly the overall means

of PR, RR and RT in the afternoon observations in all the three groups showed no significant ($P>0.05$) difference due to dietary treatments.

The DMI (kg/ d) was significantly higher ($P<0.05$) in the S_2 group as compared to control. The DMI (kg/ 100 kg BW) and g/ Kg^{0.75} were 2.65, 2.78, 2.86 and 94.85, 100.38, 103.28 in control, S_1 and S_2 groups, respectively, which was significantly more ($P<0.05$) in S_2 group as compared to control group. The overall fortnightly body weight (Kg) was 166.28, 170.76, 172.77 in three respective groups, which was significantly higher ($P<0.05$) in S_2 group as compared to control. The body weight gain (Kg)/ fortnight and average daily gain (g/ d) was 4.50, 4.98, 5.75 and 300.11, 332.01, 383.13 in control, S_1 , S_2 , respectively. Statistical analysis of the data revealed that body weight gain in S_2 group was significantly higher ($P<0.05$) as compared to control group showing the effect of dietary treatments on body weight gain over a period of four months in summer.

Plasma Na concentration during summer experiment, in control, S_1 and S_2 groups was 124.06, 125.12 and 125.72 respectively which was significantly higher ($P<0.05$) in S_2 group as compared to control group. The mean plasma K concentration (mEq/ l) was 4.15, 4.21 and 4.24 in the three respective group, showing no statistical ($P>0.05$) difference between groups. The average plasma Cl and S concentrations (mEq/ l) also did not show any significant ($P>0.05$) effect of different levels of DCAD on calves during summer months. Similarly plasma Ca, P and Mg (mg/ dl) levels did not vary with DCAD diet. Overall mean Zn, Cu and Fe plasma level during the experimental period averaged 1.61, 1.59, 1.61; 0.81, 0.84, 0.84 and 2.08, 2.11, 2.06 ppm in control, S_1 (+250 mEq/ Kg DM) and S_2 (+350 mEq/ Kg DM) groups, respectively and were similar across all groups ($P>0.05$).

It has been found that blood pH was significantly more in ($P<0.01$) S_2 (7.40) group as compared to the control (7.34) group. There was no significant effect ($P>0.05$) of varying dietary DCAD levels on blood glucose concentration. The average FRAP value was 2158.62, 2227.63 and 2279.49 $\mu\text{mol/l}$ in three respective groups showing no significant effect ($P>0.05$) of dietary treatments. The average plasma SOD (U/ ml) and catalase activity (mmol/ min/ ml) in control, S_1 , S_2 groups was 2.89, 2.85, 2.78 and 55.04, 54.90, 54.72, respectively showing no significant

difference ($P>0.05$) among dietary treatments. The total immunoglobulin concentrations was significantly ($P<0.05$) higher in +250 and +350 mEq/Kg DM DCAD fed groups. Plasma IgG and IgM levels in S_2 group were significantly higher ($P<0.05$) than control group.

Overall mean of T_3 and T_4 hormones was 0.96, 0.98, 1.01 and 19.92, 21.24, 21.68 ng/dl in control, S_1 and S_2 groups, respectively. The T_3 and T_4 hormones concentration in S_2 group was significantly more ($P<0.05$) than control. Plasma cortisol level was significantly ($P<0.05$) lower in treatment groups than control groups indicative of less stress in these calves.

The DM, OM, CP, EE, NDF and ADF digestibility were not affected by the treatments. During the metabolic trial, the DMI (Kg) of calves was 5.18, 5.67 and 5.74 in control, S_1 and S_2 groups, respectively. It has been observed that DM intake was significantly higher ($P<0.05$) in the treatment groups as compared to control. OM intake was not ($P>0.05$) affected by the treatments. CP and TDN (Kg) intakes were statistically more ($P<0.05$) in treatment groups as compared to control. N intake (g/d) was significantly ($P<0.01$) higher in S_2 group as compared to control. Excretion of nitrogen (g/d) through faeces and urine was influenced numerically ($P>0.05$) by positive DCAD diet and it (g/d) was 41.21, 47.67, 49.29 and 32.91, 33.93, 37.85 in three respective groups. All the animals were found to be in positive nitrogen balance and values obtained were 23.71, 25.43 and 27.56 (g/d) in three respective groups, however S_2 group had significantly higher ($P<0.05$) N balance in comparison to control. Apparent N retention (%) was found to be similar ($P>0.05$) in all the dietary groups.

Na intake (g/d) was 23.25, 32.54 and 48.88 in the three respective groups which increased ($P<0.01$) with the level of DCAD diet. Faecal and urinary excretion of Na (g/d) also numerically increased with increase in the level of cationic diet. Na balance was significantly more ($P<0.01$) in S_2 group (24.17 g/d) in comparison to control (7.02 g/d). Percent Na retention was significantly more ($P<0.01$) in S_2 group than control group. K intake was significantly more ($P<0.01$) in the treatment groups S_1 and S_2 i.e. 138.57 and 149.52 g/d as compared to control (125.49 g/d). Faecal and urinary excretion of K (g/d) also increased numerically with increase

in the level of cationic diet which was 27.06, 30.83, 31.41 and 18.70, 19.15, 20.69 g/ d, in control, S₁ and S₂ groups, respectively. K balance was significantly more ($P<0.01$) in the S₂ group (97.43 g/ d) as compared to control (79.73 g/ d). The K retention was not significantly ($P>0.05$) affected by the treatments. Cl and S balance and percent retention were not ($P>0.05$) affected by the dietary treatments. Similarly Ca and P balance, excretion and retentions were similar ($P>0.05$) across all the groups.

Conclusions

- Feeding positive DCAD diet (+250 mEq/ Kg DM) increased nutrient intake and body weight gain in crossbred calves during winter months. Plasma pH, thyroid hormones concentration and N and K balances were improved on feeding of +250 mEq/ Kg DM DCAD diet.
- There was no effect of feeding high DCAD diets (up to +250 mEq/ Kg DM) on plasma minerals, immunity and antioxidative parameters in growing calves in winter months.
- During summer months, positive DCAD diets (+250 and +350 mEq/ Kg DM) improved DMI, nutrient intake, nitrogen balance, Na and K balances and growth performance of growing crossbred calves.
- Immunity status and concentration of T₃ and T₄ hormones increased whereas cortisol level decreased in positive DCAD (+350 mEq/ Kg DM) diet fed calves during summer months.
- The THI values were higher in summer months as compared to winter months. The plasma macro minerals, glucose levels also not affected by seasons. Plasma thyroid hormone levels were higher in winter as compared to summer months.

CHAPTER – 6

Bibliography

6. BIBLIOGRAPHY

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