STUDIES ON MINERALS, BIOMARKERS OF OXIDATIVE STRESS AND ENERGY STATUS DURING TRANSITION PERIOD IN DAIRY COWS

दुधारू गायों में परिवर्तन काल के दौरान खनिज, आक्सीकृत तनाव और ऊर्जा स्तर के जैव सूचकों पर अध्ययन

Subhash Chandra Kachhawaha

THESIS

DOCTOR OF PHILOSOPHY

(Veterinary Clinical Medicine, Ethics and Jurisprudence)

2015

Department of Veterinary Clinical Medicine, Ethics and Jurisprudence

College of Veterinary and Animal Science
Bikaner-334001

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Bikaner-334001Rajasthan University of Veterinary and Animal Sciences
Bikaner-334001

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – I

Date……

This is to certify that **Mr. SUBHASH CHANDRA KACHHAWAHA** had successfully completed the **comprehensive examination** held on **7 March 2014** as required under the regulation for the degree of Doctor of Philosophy.

(Anil Ahuja)

Head

Department of Clinical Veterinary Medicine, Ethics and Jurisprudence,

College of Veterinary and Animal Science,

Bikaner **-**334001

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – II

Date……..…

This is to certify that the thesis entitled **"STUDIES ON MINERALS, BIOMARKERS OF OXIDATIVE STRESS AND ENERGY STATUS DURING TRANSITION PERIOD IN DAIRY COWS"** submitted for the degree of Doctor of Philosophy in the subject of **Veterinary Clinical Medicine** of the **Rajasthan University of Veterinary and Animal Sciences, Bikaner** embodies bonafide research work carried out by **Mr. SUBHASH CHANDRA KACHHAWAHA** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation has been fully acknowledged. The draft of the thesis was also approved by the Advisory Committee on 18 February, 2015.

(Anil Ahuja)

(A.P. Singh)

Head

Major Advisor

Department of Clinical Veterinary Medicine,

Ethics and Jurisprudence

College of Veterinary and Animal Science

Bikaner -334001

DEAN

(B. K. Beniwal)

College of Veterinary and Animal Science,

Bikaner -334001

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – III

Date……..

This is to certify that the thesis entitled **"STUDIES ON MINERALS, BIOMARKERS OF OXIDATIVE STRESS AND ENERGY STATUS DURING TRANSITION PERIOD IN DAIRY COWS"** submitted by **Mr. SUBHASH CHANDRA KACHHAWAHA** to the **Rajasthan University of Veterinary and Animal Sciences, Bikaner,** in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the subject of **Veterinary Clinical Medicine, Ethics and Jurisprudence** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory. We therefore recommend that the thesis be approved.

(A.P. Singh) **(Anju Chahar)** **(D.K. Bihani)**

Major Advisor Advisor Advisor

(S.B.S.Yadav) **(Dr)** **(Hemant Dhadhich)**

Dean PG Nominee External Examiner Advisor

Approved

(Anil Ahuja)

Professor and Head

Department of Clinical Veterinary Medicine

Dean

Post Graduate Studies

Rajasthan University of Veterinary and Animal

Ethics and Jurisprudence science, Bikaner

College of Veterinary and Animal Science

Bikaner -334001

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – IV

Date……..…

This is to certify that **Mr. SUBHASH CHANDRA KACHHAWAHA** of the Department of **Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Science, Bikaner** has made all corrections/modifications in the thesis entitled "**STUDIES ON MINERALS, BIOMARKERS OF OXIDATIVE STRESS AND ENERGY STATUS DURING TRANSITION PERIOD IN DAIRY COWS"** which were suggested by the external examiner and the advisory committee in the oral examination held on…….. .The final copies of the thesis duly bound and corrected were submitted on ……, are enclosed herewith for approval.

(Anil Ahuja)

(A.P. Singh)

Professor and Head

Major Advisor

Department of Clinical Veterinary Medicine,

Ethics and Jurisprudence

College of Veterinary and Animal Science

Bikaner -334001

DEAN

College of Veterinary and Animal Science,

Bikaner -334001

Approved

Dean

Post Graduate Studies, Rajasthan University of Veterinary and Animal science, Bikaner

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Place: Bikaner

Date:

(Subhash Kachhawaha)

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Abbreviations

A/G-Albumin globulin ratio

ADP- Adenosine diphosphate

AHPR- Animal husbandry progress report

AL- Albumin

ALT- Alanine amino transferase

AST- Aspartate amino transferase

ATP- Adenosine triphosphate

BCS- Body condition score

BHBA-Beta hydroxyl butyratic acid

Ca- Calcium

CK-Clinical Ketosis

CMPT- Compton metabolic profile test

Cr-Creatinine

Cu- Copper

DA- Displaced abomasum

DCAD- Dietary cation-anion difference

ECF- Extra cellular fluid

EDTA-Ethylene diamine tetra acetic acid

ELISA-Enzyme-linked immunosorbent assay

FAO-Food and Agriculture Organization

Fe- Iron

G/dl- Gram per 100 ml

G-Globulin

GGT-Gamma glutamyl transferase

GSH- Reduce glutathione

GSH-Px-Glutathione peroxide

Hb- Haemoglobin

HBCS- High body condition score

HDL- High density lipoprotein

HFL- High fatty liver

HHPM-Herd Health and Productivity Management

ICF- Intracellular fluid

K-Potassium

LDA- left displacement of abomasum

LDL-Low density lipoprotein

LFL- Low fatty liver

MDA- Malondialdehyde

MET-Metritis

mg %- milligram/ 100 ml

Mg- Magnesium

mg/dl- Milligram per 100 ml

mg-Milligram

ml/dl- Millilitre per 100 ml

M-Mean

Mmol/l-Millimol per litre

Na- Sodium

NDDB- National dairy development board

NEB. Negative energy balance

NEFA- Non esterified fatty acid

NRC-National Research Council

PG-Propylene glycol

Pi- Inorganic phosphorus

Post Partum - Post partum transition period

Pre Partum - Pre partum transition period

PTH- Parathyroid hormone

PUN-Plasma urea nitrogen

r=Correlation coefficient

ROS- Reactive oxygen species

RP-Retention of placenta

SCK- Subclinical ketosis

SE- Standard error

SOD- Superoxide dismutase

TAG- Triacylglycerols

TCA- Tricarboxylic acid cycle

TMR- Total mixed ration

TP- Total protein

Zn-Zinc ……..

STUDIES ON MINERALS, BIOMARKERS OF OXIDATIVE STRESS AND ENERGY STATUS DURING TRANSITION PERIOD IN DAIRY COWS

PhD Thesis

Department of Clinical Veterinary Medicine, Ethics and Jurisprudence

College of Veterinary and Animal Science

Bikaner-334001

Rajasthan University of Veterinary and Animal Sciences

Bikaner-334001

Submitted by: **Subhash Chandra Kachhawaha**

Major Advisor: **Dr. Awadesh Pratap Singh**

ABSTRACT

The present study was conducted on 123 crossbred dairy cows of Bikaner, Jodhpur and Pali-Marwar districts of Western Rajasthan to evaluate the status of minerals, oxidative stress and energy in transition period. The blood samples taken from each animal in pre and post-partum periods were analyzed. The subclinical ketosis was diagnosed on the basis of plasma BHBA (> 1.2 mmol/L) and NEFA (> 0.7 mmol/L) levels during post-partum period. The overall prevalence of subclinical ketosis was recorded 23.57 per cent. The prevalence was highest in Pali-Marwar district (26.08 percent). Overall deficiency of Ca, Pi, Cu and Zn was 34.15, 40.65, 33.33 and 2.43 per cent, respectively in cows. The overall mean values of oxidative stress parameters of GSH and MDA levels were recorded as 2.423±0.071 and 2.811±0.109 mg/dl per gm Hb (38.813±0.729 and 49.753±1.118 nmol/ml per gm Hb) in healthy and subclinical ketotic cows, respectively. The overall mean values of plasma glucose, BHBA, NEFA, total protein, BCS levels in healthy and subclinical ketosis were 56.293±0.611 and 42.768±0.937 mg/dl; 0.753±0.030 and 1.289±0.042 mmol/l; 0.262±0.010 and 0.683±0.016 mmol/l; 6.816±0.046 and 7.221±0.071 g/dl; 3.392±0.029 and 2.982±0.044, respectively. Highly significant difference ($P \leq 0.01$) was observed for glucose, BHBA, NEFA, cholesterol, triglyceride, total protein, AST, GGT, GSH, MDA, BCS, Ca, Cu, milk production and significant difference ($p \leq 0.05$) was observed for creatinine, globulin, potassium levels in healthy and SCK dairy cows. Significant correlations were observed among glucose, NEFA, BHBA, MDA, BCS, AST, GGT in subclinical ketotic cows. Therapeutic trial was conducted using Propylene glycol and choline in subclinical ketotic cows. The result revealed highly significant increase in the value of glucose, milk production and decrease NEFA and BHBA levels. It was concluded that dairy cows were in oxidative stress during transition period. Though negative energy balance (NEB) is a physiologically normal process yet excessive NEB reflects poor adaptation and results in adverse effects on health and production after calving. Estimation of NEFA and BHBA for prevalence of subclinical ketosis is expensive and practically challenging. It is useful with in herd level testing of energy status. It can be broadly stated that the transition cow adapted to provide minimal risk of macro and micro mineral metabolism including calcium (Ca), inorganic phosphorus (Pi), copper (Cu) and zinc (Zn). Changes of AST and GGT enzymes were mainly caused by SCK in postpartum period cows. The levels of these liver enzymes were influenced by stage, health and energy status.

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वर्तमान अध्ययन के लिए 123 संकर नस्ल की गायों मे परिवर्तन काल में खनिज, ऑक्सीकृत तनाव और ऊर्जा की स्थिति का मूल्यांकन किया गया। अध्ययन के लिए पश्चिमी राजस्थान के बीकानेर, जोधपुर और पाली–मारवाड़ जिलों का चयन किया गया। अध्ययन के लिए ब्याने के पूर्व और पश्चात् की अवधि में दो बार रक्त के नमूने प्रत्येक जानवर के लिए गए। नमूनों के अध्ययन के लिए विभिन्न मापदंडों का विश्लेषण किया गया। ब्याने के बाद प्लाज्मा BHBA> 1.2 मिली मोल/लीटर और NEFA> 0.7 मिली मोल/लीटर के आधार पर लक्षणहीन कीटोसिस का मूल्यांकन किया गया। लक्षणहीन कीटोसिस की सम्पूर्ण व्याधि दर 23.57 प्रतिशत तथा पाली–मारवाड़ जिले में 26.08 प्रतिशत जो कि सर्वाधिक पायी गई थी। कैल्सियम, अकार्बनिक फास्फोरस, तांबा और जिंक के लिए कुल मिलाकर कमी क्रमशः 34.15, 40.65, 33.33 और 2.43 प्रतिषत पाई गई। GSH तथा MDA का सम्पूर्ण स्तर क्रमशः 2.423± 0.071 और 2.811± 0.109 मिलीग्राम/100 मिली लीटर प्रति ग्राम हीमोग्लोबिन; 38.813± 0.729 और 49.753±1.118 नेनोमोल/मिलीलीटर प्रति ग्राम हीमोग्लोबिन स्वस्थ और लक्षणहीन कीटोसिस मे पाया गया । सम्पूर्ण ऊर्जा स्थिति के लिए प्लाज्मा ग्लूकोज, BHBA, NEFA, कुल प्रोटीन, BCS के स्तर क्रमश: 56.293±0.611 और 42.768±0.937 मिलीग्राम/100 मिली लीटर; 0.753±0.030 और 1.289±0.042 मिलीमोल/लीटर; 0.262±0.010 और 0.683±0.016 मिलीमोल/लीटर; 6.816±0.046 और 7.221±0.071 ग्राम/100 मिलीलीटर; 3.392±0.029 और 2.982±0.044 स्वस्थ और लक्षणहीन कीटोसिस मे पाया गया था । ग्लूकोज, BHBA, NEFA, कोलेस्ट्रॉल, ट्राइग्लिसराइड, कुल प्रोटीन, AST, GGT, GSH, MDA , BCS, कैल्सियम, तांबा, दुग्ध उत्पादन में अत्यन्त महत्वपूर्ण अंतर (P≤0.01) और क्रिएटिनिन, ग्लोब्युलिन, पोटेशियम का स्तर लक्षणहीन कीटोसिस मे महत्वपूर्ण अंतर (P≤0.05) पाया गया । उपचार हेतु लक्षणहीन कीटोसिस गायों में प्रोपाईलिन ग्लाइकोल का उपयोग किया गया था। जिसके परिणाम स्वरूप अत्यधिक महत्वपूर्ण वृद्धि ग्लूकोज में तथा NEFA और BHBA का स्तर कम पाया गया था। प्रोपाईलिन ग्लाइकोल का प्रभाव दुध उत्पादन पर भी महत्वपूर्ण पाया गया। दुधारू गायों मे परिवर्तन काल के दौरान ऑक्सीकृत तनाव देखने को मिला । नकारात्मक ऊर्जा संतुलन एक सामान्य शरीर प्रक्रिया है, वहीं अत्यधिक नकारात्मक ऊर्जा संतुलन ब्यांत के बाद स्वास्थ्य तथा उत्पादन के ऊपर प्रतिकूल प्रभाव डालती है । लक्षणहीन कीटोसिस

को रोकने के लिए NEFA और BHBA का आकलन महंगा है और व्यावहारिक रूप से चुनौतीपूर्ण है। यह कहा जा सकता है की कैल्शियम, अकार्बनिक फास्फोरस, तांबा और जिंक सहित वृहद और सूक्ष्म खनिज उपापचय की न्यूनतम जोखिम प्रदान करने के लिए अनुकूल है। AST और GGT एंजाइमों का परिवर्तन काल में मुख्य रूप से प्रसवोत्तर अवधि की गायों में लक्षणहीन कीटोसिस के कारण वृद्धि होती है। यह यकृत एंजाइम पशु की अवस्था, स्वास्थ्य और ऊर्जा की स्थिति से भी प्रभावित होते है।

Table 24: Analysis of Variance For Liver Enzymes and Oxidative Stress Parameters Investigated Under The Study.

S.No.	EFFECT	DF	MEAN SQUARE						
			AST	ALT	GGT	GSH	MDA	MILK PRODUCTION	BCS
1.	DIST	2	450.828*	13.392NS	354.953**	0.886**	350.664**	21.635NS	0.773**
2.	HLT	1	1445.894**	52.308NS	707.961**	4.553**	3751.735**	899.544**	5.27**
3.	PRT	1	6261.306**	560.784**	1759.731**	0.006NS	28.007NS	0.043NS	0.133NS
4.	STG	1	154.878NS	8.435NS	87.432NS	2.115NS	843.787**	0 NS	6.934**
5.	DIST X HLT	2	430.031*	366.027**	92.548NS	0.053NS	173.904NS	5.948NS	0.254NS
6.	DIST X PRT	2	23.901NS	89.633NS	232.413*	1.839*	53.576NS	20.157NS	0.014NS
7.	DIST X STG	2	623.524**	20.25NS	53.429NS	0.016NS	340.078**	0 NS	0.021NS
8.	HLT X PRT	1	7.028NS	71.588NS	2.715NS	1.791NS	78.814NS	21.221NS	0.002NS
9.	HLT X STG	1	270.709NS	25.316NS	0.281NS	0.615NS	281.964*	0 NS	0.001NS
10.	PRT X STG	1	493.837*	5.184NS	30.659NS	0.092NS	71.486NS	0 NS	0.007NS
11.	REMAINDER	231	124.446	63.724	72.222	0.567	58.689	8.637	0.092

12.	ERROR		11.924	8.874	8.692	0.753	7.660	2.938	0.304

F statistic of corresponding effects as **= highly significant (P≤ 0.01). *= significant (P≤0.05), NS = Non significant

(i)

Table 25: Analysis of Variance for Different Biochemical Parameters Investigated Under the Study

S.No.	EFFECT	DF	MEAN SQUARE										
			GLUCOSE	NEFA	BHBA	TOTAL PROTEIN	ALBUMIN	GLOBULIN	AL/GL RATIO	PUN	CR	CHOLESTEROL	TRIGLYCERIDE
1.	DIST	2	82.607NS	0.025NS	0.111**	4.195**	1.259NS	3.787**	0.478NS	12.574**	0.307*	627.906*	179.777**
2.	HLT	1	5734.412**	5.541**	5.901**	5.134**	0.008NS	4.729*	0.535NS	6.506NS	0.319*	14223.79**	950.779**
3.	PRT	1	1781.667**	1.775**	12.558**	21.166**	8.052**	2.543NS	0.662NS	256.883**	2.102**	1684.401**	333.664**
4.	STG	1	196.176*	0.013NS	0.021NS	0 NS	2.444*	3.107*	0.986NS	2.09NS	0.089NS	79.263NS	28.526 NS
5.	DIST X HLT	2	275.978**	0.105**	0.202**	0.376NS	0.89NS	0.119NS	0.13NS	14.37**	0.285*	524.558NS	106.349**
6.	DIST X PRT	2	11.545NS	0.036NS	0.033NS	0.241NS	1.126NS	1.746NS	0.519NS	1.842NS	0.075NS	388.79NS	36.805NS
7.	DIST X STG	2	62.226NS	0.024NS	0.002NS	0.133NS	1.415NS	2.418*	0.523NS	3.988NS	0.28*	573.552NS	18.999NS
8.	HLT X PRT	1	199.916*	0.002NS	0.005NS	0.062NS	0.126**	0.011NS	0.002NS	2.462NS	0.039NS	553.242NS	26.831NS
9.	HLT X STG	1	0.025NS	0.184**	0.861**	4.404**	0.388NS	2.176NS	0.119NS	3.551NS	0.195NS	3544.67**	8.232NS
10.	PRT X STG	1	0.669NS	0.002NS	0.003NS	1.114*	0.503NS	0.119NS	0 NS	0.321NS	0.158NS	221.559NS	30.965NS
11.	REMAINDER		41.215	0.012	0.02	0.237	0.594	0.736	0.319	2.61	0.073	190.224	21.233
12.	ERROR		6.41994	0.111	0.144	0.487	0.77092	0.858	0.565	1.615	0.272	53.293	4.607

F statistic of corresponding effects as **= highly significant (P≤ 0.01). *= significant (P≤0.05), NS = Non significant

(ii)

i

Table 26: Analysis of Variance for Macro and Micro Minerals Investigated Under the Study

S.No.	EFFECT	DF	MEAN SQUARE							
			Ca	Pi	Mg	Na	K	Cu	Fe	Zn
1.	DIST	2	3.017NS	0.393NS	0.26NS	29.028NS	0.548NS	0.524**	0.443NS	0.02NS
2.	HLT	1	46.627**	3.213NS	0.081NS	8.434NS	6.237*	0.456**	1.1NS	0 NS
3.	PRT	1	0.026NS	0.103NS	0.425NS	177.447NS	0.442NS	0.013NS	0.198NS	0.078*
4.	STG	1	123.342**	32.264**	3.673**	530.654**	5.334*	0.546**	4.755**	1.55**
5.	DIST X HLT	2	10.726**	1.706NS	0.316NS	201.273*	2.943NS	0.095*	0.421NS	0.037NS
6.	DIST X PRT	2	2.624NS	1.272NS	0.048NS	78.607NS	0.253NS	0.035NS	0.624NS	0.031NS
7.	DIST X STG	2	18.628**	5.651**	1.209NS	40.791NS	0.769NS	0.067NS	0.351NS	0.004NS
8.	HLT X PRT	1	1.055NS	0.015NS	0.133NS	83.044NS	6.916**	0.021NS	0.221NS	0.035NS
9.	HLT X STG	1	0.292NS	0.887NS	1.007NS	2.79NS	27.103**	0 NS	1.485*	0.002NS
10.	PRT X STG	1	0.016NS	0.014NS	0.012NS	66.03NS	0.075NS	0.034NS	0.788NS	0NS
11.	REMAINDER		1.008	0.977	0.518	60.698	0.987	0.022	0.289	0.013
12.	ERROR		1.004	0.988	0.719	7.790	0.993	0.149	0.538	0.115

F statistic of corresponding effects as **= highly significant (P≤ 0.01). *= significant (P≤0.05), NS = Non significant

History Sheet of Dairy Animal

Sample no._____ Date of sample:-_____

1. Name of the farmer:_____
2. Address:_____
3. Total no. of animal(adult cow):- _____
4. Date of insemination/natural service:-_____
5. Pregnancy status :-_____Pregnancy diagnosis: Yes/No
6. Temp.:-_____Resp.:-_____Pulse rate:-_____
7. Stage of lactation:-_____
8. Previous Milk yield:-_____
9. Previous disease condition:-_____
10. Parturition history:-_____
11. Feeding status during dry period:-_____

A. What type of concentrate: - _____B. Roughages:-_____

12. Aware about the production diseases:- Yes/No:-_____
13. If yes what are the preventive measure are taken:-_____
14. Period of this occupation:-_____
15. Any regular test done by farmer for production diseases:-_____
16. Managemental practice:-

A. Housing:-_____B. Ticks control:-_____C. Mineral:-_____

17. In case of production disease how much milk loss occur approx.:-_____

18. Common diseases occur in herd:-1._____2._____3._____4._____

19. Cost of treatment in case of any production disease:-_____

20. Season of production disease occurrence:-_____

21. Meteorological data (Temp., Humidity and wind velocity)_____

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1. Introduction

Animal keeping is traditionally an integral part of Indian rural economy. With 1,90904 thousands cattle and 1,08,702 thousands buffaloes (Livestock census, 2012), India ranks first in number of animals, and has earned distinction of being the highest milk producer. With the advancement of modern farming system the number of high yielding animals are increasing and over the past several decades, milk production by crossbred cows has increased markedly. Presently, in India, the main focus is towards cross breeding practices to produce high yielding crossbred cows in order to meet the demand of milk and milk products. India's annual milk production has increased to 127.9 million tonnes in 2011-2012 from 21 million tonnes in 1968 (AHPR, 2013). Similarly dairy animals in Rajasthan produce 13.5 million tonnes of milk which accounts for 13.56 per cent of total milk yield in India. Rajasthan is rank 2nd in India for milk production and average milk production is 7.5 kgs/day and 3.5 kgs/day in crossbred and indigenous cow respectively. Compositions of dairy cattle population have changed gradually from lower producers to higher producers. Proportions of crossbred cows had shown increasing trends over the year.

Dairy farming is a business that people engage in for financial gain. Unfortunately, the quest for optimal economic efficiency frequently places the dairy cow in a state of compromised welfare or poor health. In both intensive and extensive dairy production systems internationally and nationally, the goals of economic efficiency and optimal dairy cow health are often in conflict. The relatively recent developments in animal breeding have produced dairy cows with enormous losses of body nutrients to support milk production. Production diseases of the dairy cow are associated with a level of production inconsistent with nutrient intake, provision of an unsuitable diet, an inappropriate breeding policy or an unsuitable environment. Production diseases are important for dairy cows as they constitute a major proportion of the common health problems encountered on dairy farms and because they predispose cows to infectious diseases, infertility, production losses and lameness; they compromise the health and welfare of dairy cows and ultimately reduce the farmer profitability (Mulligan and Doherty, 2008).

In the emerging scenario due to free international market, it has now become important to increase the production efficiency and quality of milk. This calls for a system where use of drugs is bare minimum, the diseases are prevented and the nutritional and metabolic problems are

predicted and corrected before any overt sickness and setting up economic targets, such as, productivity, fertility and health. This new system is called 'Herd Health and Productivity Management (HHPM), which since 1980s has replaced the traditional curative services system in developed countries (Blood *et al.,* 1978). In order to implement such a system the core requirement is real time animal performance recording related to production, breeding and health. The data then is periodically analyzed to identify problem areas and herd performances assessed by calculating few indices (Lissemore, 1989). Once the farm database is generated, it also enables protocol-based health and productivity services by veterinarians. For example, the farm activities can be scheduled as predicted by the programme. The system is focused on identifying sub-clinical problems, finding out the associated factors based on which problem-solving strategy can be developed (Henderson, 1980).

Diseases in farm animals have a significant economic impact on livestock production and incur substantial costs for societies in both developed and developing countries (FAO, 1962). According to some estimates, India suffers a loss of Rs 50 billion annually as a result of neglect in disease prevention and control. Economic losses due to diseases occur in the form of both direct and indirect losses. Direct losses occur on account of reduction in milk yield, quality and quantity of meat, work capacity and growth. Indirect losses occur due to decrease in productivity, life span of animal, decrease in fertility and decreased feed conversion efficiency. Among problems that cause losses in the productivity of animals, mineral deficiency remains at the top across states in India. Though required in small quantities, minerals are essential for optimal body functions of animals (Sharma *et al.,* 2003). Poor animal performance and reproductive problems in livestock are associated with micro-mineral deficiencies (Underwood, 1977; Sharma *et al.,* 2003).

The transition period for dairy cows generally extends from 3 weeks prior to parturition through 3 weeks after parturition (Smith and Risco 2005). During this period dairy cattle are at a high risk for most of the metabolic diseases which is characterized by marked changes in their endocrine status that are much more dramatic than at any other time in the lactation–gestation cycle and a reduction in feed intake when nutrient demand for the developing conceptus and the impending lactogenesis are increasing (Grummer, 1995).

Transition period is especially critical for health and subsequent performance of dairy cows (Castillo *et al.,* 2005). Dairy cattle are more susceptible to a variety of metabolic and infectious diseases during the transition period compared with peak lactation (Sordillo *et al.,* 2007; Sharma *et al.,* 2011). Physiological changes during transition period associated with rapid differentiation of secretory parenchyma, intense mammary gland growth and the onset of copious milk synthesis and secretion are accompanied by a high-energy demand and an increased oxygen requirement (Gitto *et al.,* 2002). This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Excessive production of free radicals and concomitant damage at cellular and tissue levels are controlled by cellular antioxidant defence systems. When ROS are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Trevisan *et al.,* 2001). There are growing evidences that oxidative stress is a threat to transition period and an increase in its level may lead to delivery/calving-related complications in both man and animals (Orhan *et al*., 2003; Castillo *et al.,* 2005; Dimri *et al*., 2010).

Despite the fact that the trace minerals are present in body tissues in very low concentrations (Arinola *et al,* 2008), they are very critical for normal body function as they serve as components of metallo-enzymes and enzyme cofactors, or as components of hormones of the endocrine system (Speer, 1996). Complex inter-relationships exist between certain micro-minerals, immune functions and disease resistance in cattle. Several micro-minerals have been shown to influence immune responses. The relationship between deficiencies of some micronutrients and disease resistance is less clear. Immune cells, like all other types of cells, require an adequate supply of trace elements for the structure and function of mentally-proteins that participate in housekeeping processes such as energy production and protection against reactive oxygen species (Chew, 1995).

Trace minerals with an antioxidant function include selenium (Se), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). While some nutrients have a role in directly quenching free radicals, these trace minerals have an indirect role in which they are required components of a

variety of antioxidant enzymes (Waldron, 2010). For example, enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase are considered to be an important defense system against free radical accumulation; superoxide dismutase converts superoxide to hydrogen peroxide while GSH-Px and catalase convert hydrogen peroxide to water. Superoxide dismutase is Cu, Mn and Zn dependent, GSH-Px is Se dependent and catalase is Fe dependent (Bowman *et al*., 2008; McDowell *et al*., 2007; Weiss, 2005).

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy balance (NEB) during the first week of lactation due to energy expenditure associated with milk production and limited feed intake, resulting in NEB, a high mobilization of lipids from body fat reserves, and hypoglycaemia (Veenhuizen *et al.*, 1991; Drackley, 1999; Bobe *et al*., 2004; Djokovic *et al*., 2007, 2011). Nutrition, age, heredity, body condition score (BCS), management and energy imbalance as various risk factors are possible causes of NEB, periparturient fatty liver and ketosis (Drackley, 1999; Bobe *et al*., 2004).

Production diseases may be considered 'a man-made problem' resulting in 'a breakdown of the various metabolic systems of the body under the combined strain of high production and modern intensive husbandry' (Payne, 1972). More recent definitions of production disease have been educated by the manipulate of high production and management on ‘factors such as animal behaviour, immunity and gene expression’, thus, the definition has been expanded to include not only ‘metabolic and nutritional diseases’ but diseases of an ‘infectious and genetic nature’ (Herdt, 2006). It is important to state that both production and environmental factors are equally implicit in the onset of production diseases. Drackley (2006) stated that ‘triggers for production disease likely lie at the interface between environmental stressors and productivity’. The common theme of all these diseases is their association with management and selection of animals for ‘efficient’ agricultural production (Herdt, 2006).

The importance of a particular (individual) production disease of the transition cow should not be considered in isolation as ketosis, fatty liver, clinical hypocalcaemia, retained placenta, metritis and displacement of the abomasum are all aetiologically inter-related. For example, over-conditioned dry cows are more likely to suffer from ketosis and fatty liver, both of which may suppress immunity directly or through an excessive negative energy balance route (Ingvartsen *et al*., 2003). Immunosuppression is thought to be the main cause of retained placenta (Le Blanc, 2008). Over-conditioned dry cows are also more likely to suffer from hypocalcaemia, which exacerbates immunosuppression and may cause dystocia and retained placenta (Houe *et al*., 2001). Thus, ketosis and milk fever are both related to each other and to retained placenta via more than one aetiological pathway. Because of these inter-relationships, production diseases of the transition cow regularly result in cascade effects that increase the incidence of infectious disease or other production diseases, reduce fertility, reduce milk production and increase lameness. Therefore, prevention of production diseases has consequences for dairy cow welfare and producer profitability long after the transition period ends. Some examples of these relationships include the observations that over-conditioned dry cows are four times more likely to experience milk fever (Houe *et al.,* 2001). Dairy cows with milk fever are eight times more likely to suffer from mastitis in the following lactation (Curtis *et al.,* 1983). Dairy cows in negative energy balance (NEB) in the pre-calving period are more likely to develop displacement of the abomasum in the following lactation (Le Blanc *et al.*, 2005). Those cows that have excessive NEB after calving or milk fever have reduced fertility performance (Borsberry and Dobson, 1989; Buckley *et al.,* 2003) and those with ruminal acidosis are likely to suffer from immunosuppression, excessive NEB and laminitis (Enemark, 2008). However, this improvement comes at the cost of higher incidence and increasing the risk of metabolic diseases, reproductive problems and compromised immune status leading to various disease conditions.

High producing crossbred cows need to mobilize body reserves to sustain milk production and enter into a state of negative energy balance, loosing their condition markedly and immune response of the animal is also compromised (Goff and Horst, 1997).

Though clinical effects become evident in few animals, sub-clinical disturbances may develop in larger proportion of animals. In general, incidence of sub-clinical disease is more important than clinical disease because in subclinical disease clinical signs are not evident to recognize the disease and animal continue to produce at a markedly reduced rate resulting into significant economic losses. Commonly acetonemia, fat cow syndrome, sub-clinical hypocalcemia, periparturient haemoglobinuria and Hypophosphetemia are the major disease

states. Apart from the direct effect of these, there are also indirect effects which make an animal more prone to infections of udder and uterus, reduce fore rumen motility, decrease appetite and hence decreased milk production (Randhawa and Chand, 2011).

In order to monitor, detect and predict such diseases, Compton Metabolic Profile Test (CMPT) was developed by Payne *et al*, (1970). This CMPT is done by comparing the average concentration of blood constituents viz. haemoglobin (Hb), packed cell volume (PCV), glucose, blood urea nitrogen (BUN), total plasma proteins (TPP), albumin, calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), zinc (Zn) and iron (Fe) of a group of cows to the defined mean concentration values.

Animal response also can be useful for adjustment of specific mineral nutrition. In late gestation dairy cows, a low dietary cation-anion difference (DCAD) decreases the risk of milk fever in early lactation. Adjustment of DCAD by dietary calculation is difficult because Na, K, Cl and S contents of feedstuffs are usually unknown, but a urinary pH around 6.5 is indicative of effective anion addition (National Research Council, 2001).

Veterinary health delivery system in India primarily revolves around treatment of individual animals, whereas, sickness identification is largely based on owner's perception and presence of overt signs. The services largely restricts to treating sick animals, Artificial insemination, vaccination and worm treatment when infestation is evident. Most of the western countries have abandoned the 'fire brigade' approach in early 1980s (Goodger *et al.,* 1982) and have oriented their system to scientific system wherein involvement of the veterinarian is continuous, focused at meeting targets and detection of problems and performance inefficiencies at early stage (Cannon *et al.,* 1978). These countries have adopted a system that is targeted at keeping the groups of animals healthy and productive at a level that is most efficient and provides maximum economic returns to the animal owner (Schnurrenberger, 1979).

Ketosis, a disease of heavy lactating animals, occurs during peak milk yield causing a great economic loss to the milk industry. Diagnosis of bovine ketosis at an early (subclinical) stage is a must to prevent the economic loss to the farmers in terms of reduced milk yield. Some workers have considered the blood glucose (Gupta and Rai 1987, Vijay kumar *et al* 1987), others the blood ketones (Geishauser et al. 1998) and few to the non-esterified fatty acids (Simeonovet *et al.,* 1977) to be associated with ketosis of cows. No concerted efforts have been directed to correlate the blood biochemical parameters with blood ketones. Hence, the present research was taken to estimate and correlate some of the apparently changing biochemical parameters with the blood ketone levels to find out a suitable marker which sub subsequently can be used as a diagnostic test for impending subclinical ketosis in lactating dairy cows.

On the basis of published scientific literature, it has been observed that as there is no comprehensive data available in India for these diseases, except few isolated studies reported from some of the states (Sahoo *et al.,* 2009 and Thirunavukkarasu *et al.,* 2010).

Studies on minerals, biomarkers of oxidative stress and energy status during transition period in dairy cows was carried out with the following Objectives:-

1. To study minerals (Ca, P, Mg, Cu, Fe, Zn, Na and K) status during transition period in dairy cows
2. To study the levels of biomarkers of oxidative stress viz. Malondialdehyde (MDA) and reduced glutathione(GSH) during transition period in dairy cows
3. To study the levels of energy by estimation of non-esterified fatty acid (NEFA) and beta hydroxy butyric acid (BHBA) during transition period in dairy cows
4. To estimate glucose, cholesterol, triglycerides, total protein, albumin and globulin, gamma glutamyl transferase, blood urea nitrogen (BUN) and creatinine levels during transition period in dairy cows
5. To suggest suitable measures to reduce incidence and losses due to production diseases

3. MATERIALS AND METHODS

The following materials and methods were employed during the present studies on mineral, biomarkers of oxidative stress and energy status during transition period in crossbred dairy cows.

3.1 Animals:

The study was conducted in 123 crossbred cows during transition period which were in 3rd parity and above (Multiparus) in arid region (Jodhpur, Bikaner and Pali-Marwar) of Rajasthan. All the dairy animals were from private farms of study area. In every selected farm, crossbred cows which were in their transition period were selected for the study. Detailed history about age, milk yield, health status, body condition, any kind of disease condition, present and previous feeding history were collected for each animal. Body condition score (BCS) assessment was done as per the methods of Ferguson *et al.* (1994). The study was carried out between Auguest 2013 to January 2015.

3.2 Clinical examination:

Clinical examination of each dairy cow was done as per the methods described by Radostits *et al.* (2007). It includes temperature, respiration and pulse rate, feeding, watering practices, defecation, examination of visible mucous membranes, eyes, skin and physical condition. Auscultation of heart and lungs were also done each cattle. The date of service for each (natural/artificial insemination) was recorded.

3.3 Blood Collection:

For the estimation of various biomarkers, blood was collected from each cow selected for the sampling. Blood (25 ml approx.) was collected by jugular vein. 20 ml blood was transferred into stopper mineral free heparinised glass vials and remaining 5 ml was collected in plastic vials containing disodium EDTA as anticoagulant for oxidative biomarker and haemoglobin estimation. To prevent haemolysis, collected blood samples were immediately transported to laboratory on ice for analysis of parameters.

3.4 Sample Collection:

A total of 123 crossbred dairy cows in transition period were sampled during the study period. The samples were processed for the all parameters. From each animal blood samples were collected twice during different stages of transition period, viz:

I. **Stage-1-** Between 21 and 3 days prior to expected calving.
II. **Stage-2-** Between 3 to 21 days after calving.

Three days before and after parturition were excluded as these days are critical as most of the endocrinological changes occur during this period.

The subclinical ketosis were diagnosed on the basis of plasma BHBA > 0.6 mmol/L and NEFA> 0.4 mmol/L at the pre partum stage and > 1.2 mmol/L and >0.7 mmol/L in post partum, respectively (Nielan *et al.*, 1994; Whitaker, 1997 and Oetzel, 2004), along with the presence of other signs such as decrease in milk yield and body condition score. Inappetence was not observed in all subclinical ketotic animals. The sampling was carried out after approximately 4-5 hrs after feeding because after feeding BHBA increase.

3.5 Laboratory Analysis:

Blood samples were centrifuged at 3000 rpm for 20 minutes to separate the plasma immediately after collection to prevent haemolysis. Plasma samples were stored at -20ºC in deep freeze till further analysis.

3.5.1 BHBA AND NEFA:

Both BHBA and NEFA were estimated from the plasma samples with the help of kits provided by Elab Science Biotechnology Co., Ltd. china and Randox laboratories limited, U.K.

Assay Procedure for BHBA:

1. **Add Sample and Biotinylated Detection Ab:** Add 50 µl of standard, blank or sample per well. The blank well is added with references standard and sample diluents. Immediately add 50 µl of Biotinylated Detection Ab working solution to each well. Cover with the plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37 (solutions are added to the bottom of micro ELISA plate well, avoiding inside wall touching and foaming as possible.).
2. **Wash:** Aspirate each well and wash, repeating the process three times wash by filling each well with wash buffer (approximately 350 µl). After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
3. **HRP conjugate:** Add 100 µl of HRP conjugate working solution to each well. Cover with a new plate sealer. Incubate for 30 minutes at 37 ^{o}c.
4. **Wash:** Repeat the aspiration/wash process for five times.
5. **Substrate:** Add 90 µl of substrate solution to each well. Cover with a new plate sealer. Incubate for about 15 minutes at 37 ^{o}c. The reaction time can be shortened or extended according to the actual colour change.
6. **Stop**: Add 50 µl of stop solution to each well. Colour turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.
7. **OD Measurement:** Determine the optical density (OD value) of each well at once, using a microplate reader set at 450 nm**.**

Calculation of results :

Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the X-axis and draw a best fit curve through the point on the graph. The concentration of samples calculated from the standard curve for each test.

ASSAY PROCEDURE FOR NEFA:

Wave length: 550 nm
Cuvette: 1 cm light path
Temperature: 37 ^{o}c
Measurement: Against reagent blank

Pipette into Cuvette:

	Reagent Blank	Standard	sample	Sample blank
Distilled water	50 µL	-	-	-
Standard	-	50 µL	-	-
Sample	-	-	50 µL	-
Solution R 1	1.0 ml	1.0 ml	1.0 ml	1.0 ml

Mix and incubate at 37 ^{o}c for 10 minutes.

Solution R 2	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Sample	-	-	-	50 µL

Read absorbance of sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank at 550 nm.
NEFA (mmol /L) = ΔA Sample/ΔA Standard x concentration of standard

3.6 Biochemistry:

Biochemical analyses of samples were done to estimate plasma glucose, total protein, albumin, globulin, SGOT (AST), SGPT (ALT), gamma glutamyl transferase (GGT), Plasma urea nitrogen, creatinine, triglyceride, total cholesterol and plasma calcium, inorganic phosphorus, magnesium, sodium, potassium, zinc, copper and iron.

3.6.1 Estimation of determination of plasma glucose:

Plasma glucose was estimated by enzymatic GOD - POD method as recommended by Tietz (1976), with kits from Accurex Biomedical Pvt. Ltd, Mumbai.

Principle:

Glucose was oxidized by glucose oxidase to Gluconic acid and hydrogen peroxidase. In a subsequent peroxidase catalyzed reaction, the oxygen liberated was accepted by the Chromogen system to give a red coloured quinoneamine compound. The red colour so developed was measured at 505 nm and was directly proportional to glucose concentration. Reagents required, procedure and precautions were used as per the directives of the manufacturer of the kit.

1. Glucose + H_2O $\xrightarrow{\text{(Glucose oxidase)}}$ Gluconic acid + H_2O_2

2. H_2O_2 + 4 amino antipyrine + phenol $\xrightarrow{\text{(Peroxidase)}}$ Quinoneamine Complex

Calculation:

Glucose concentration mg/dl =Ab of test/Ab of standard X 100

3.6.2 Estimation of total protein in plasma:

Total protein in plasma was estimated as per modified Biuret and Doumas method (Doumas *et al.,* 1981) with kits from Accurex Biomedical Pvt. Ltd, Mumbai.

Principle:

Cupric ions of biuret reagent formed chelated with the peptide bonds of proteins in an alkaline medium to form a blue-purple complex. Sodium-potassium tartrate kept the cupric ions in solution. The intensity of the blue-purple colour thus formed was proportional to the number of peptide bonds, which, in turn, depended upon the amount of proteins in the specimen. Reagents required, procedure and precautions were used as per the directives of the manufacturer of the kit.

Calculation:

Plasma total protein in g/100ml = O.D. (test)/O.D. (Std.) X 6.0

3.6.3 Estimation of plasma albumin:

Plasma albumin was determined as per bromocresol green (BCG) dye binding method (Doumas *et al.,* 1971) with kits from Accurex Biomedical Pvt. Ltd, Mumbai. The reagents, procedure and precautions were followed as per the leaflet supplied by the manufacturer.

Principle:

Binding of a protein to an indicator changed its colour. Among plasma proteins, only albumin in plasma bounds itself with the dye bromocresol green (BCG) at pH 4.2, to form a green coloured complex, which was measured with spectrophotometer. The pH was maintained during the reaction using a buffer.

Calculation:

O.D (test)

$$\text{Plasma albumin} = \frac{\text{O.D (std.)}}{\text{----------}} \times 3.9$$

3.6.4 Estimation of plasma globulin:

Plasma globulin was estimated in g/100 ml as a difference between total protein and albumin.

3.6.5 Estimation of plasma aspartate aminotransferase (AST) and plasma glutamate oxaloacetate transaminase (SGOT)

Plasma AST level was estimated using semi auto-analyzer by 2, 4-DNPH method (Reitman and Frankel, 1957) with kits from Accurex Biomedical Pvt. Ltd, Mumbai.

Principle

Principle involves reaction of alpha-Ketoglutarate and aspartate resulting in formation of oxaloacetate by action of AST. Oxaloacetate so formed was coupled with 2, 4-dinitro- phenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gave brown colour in alkaline medium measurable semi auto-analyzer. Reagents required, procedure and precaution were as per the directives of the manufacturer of the kit.

L- Asparate + alpha -Ketoglutarate $\xrightarrow{\text{GOT* (AST)*}}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{\text{MDH}}$ L-Malate +NAD^+

The conversion of NADH to NAD^+ is proportional to the concentration of GOT in plasma and measured at 340 nm as rate of decrease in absorbance.

*Abbreviations

GOT= Glutamate Oxaloacetate Transaminase

AST= Aspartate Transaminase

Calculation:

Activity of SGOT in IU/L = Abs /min X 3339

3.6.6 Estimation of Plasma alanine aminotransferase (ALT) or plasma glutamate pyruvate transaminase (SGPT)

Plasma ALT level was estimated semi auto analyzer by 2, 4 DNPH method (Reitman and Frankel, 1957) with kits from Accurex Biomedical Pvt. Ltd, Mumbai.

Principle

Principle involved reaction of alpha-ketogluterate and L-alanine resulting in formation of pyruvate by the action of ALT. Pyruvate so formed was coupled with 2, 4 Dinitrophenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gave brown colour in alkaline medium measurable semi auto analyzer. Reagents required, procedure and precaution were used as per the directives of the manufacturer of the kit.

Calculation:

Activity of ALT in IU/L= Abs/min. X 2201

3.6.7 Estimation of Plasma Gamma- glutamyltransferase (GGT):

Gamma- glutamyltransferase (GGT) transfers the - glutamyl group of L--glutamyl-3-carboxy-4-nitroanillide to glycylglycine. The amount of 5-amino-2-nitrobenzoate liberated at 405 nm is proportional to the activity of GGT in plasma and is measured kinetically.

L--glutamyl-3- carboxy-4-nitroanillide to glycylglycine

$\downarrow$

L--glutamyl- glycylglycine + 5-amino-2-nitrobenzoate

Calculation: Activity of GGT in IU/L= Abs/min. X 2201

3.6.8 Estimation of Plasma triglycerides:

Glycerol released from hydrolysis of triglycerides by lipoprotein lipase is converted by glycerol kinase into glycerol-3- phosphate which is oxidised by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidise, hydrogen peroxide oxidizes phenolic chromogen to red coloured compound.

Triglycerides $\xrightarrow{\text{LPL*}}$ fatty acids + glycerol

Glycerol + ATP $\xrightarrow{\text{GK}}$ glycerol-3- phosphate + ADP

Glycerol-3- phosphate + O_2 $\xrightarrow{\text{GPO*}}$ Dihydroxyacetone phosphate + H_2O_2

H_2O_2 + phenolic chromogen $\xrightarrow{\text{POD*}}$ Red coloured compound

***Abbreviations:**

LPL- lipoprotein lipase
GK- glycerol kinase
GPO- glycerol phosphate oxidase
POD-Peroxidase

Calculation:

1. With standard
   cons. (mg %) =Absorbance of sample/ Absorbance of standard X 200
2. With factor for wavelength range : 500-510 nm
   cons. (mg %) =745 X Absorbance of sample

3.6.9 Estimation of Plasma total cholesterol:

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest -4-en-3-one and hydrogen peroxide. In presence of peroxidise, hydrogen peroxide oxidatively couples with 4-aminoantipyridine and phenol to produce red quinoneimine dye which has absorbance maximum at 510 nm (505-530 nm). The intensity of the red colour is proportional to the amount of total cholesterol in the specimen.

Cholesterol esterase $\xrightarrow{CHE^*}$ Cholesterol+ fatty acids

Cholesterol + O_2 $\xrightarrow{CHO^*}$ H_2O_2 + cholest -4-en-3-one

$2H_2O_2$ + 4-aminoantipyridine + Phenol $\xrightarrow{POD^*}$ Red quinoneimine Dye + H_2O

*Abbreviations:
CHE= Cholesterol esterase
CHO= Cholesterol oxidase
POD= Peroxidase

Incubation:

Incubate the assay mixture for 5 minutes at 37 or 10 minutes at room temperature (25-30). After incubation measure the absorbance of assay mixture against blank at 510 nm. The final colour is stable for two hour if not exposed to direct light

CALCULATION:

Total cholesterol in mg % = Absorbance of sample/Absorbance of standard X 200

3.6.10 Determination of plasma urea nitrogen

Urea nitrogen was determined by Diacetylmonoxime method (DAM method) as recommended by Coulambe and Favrean (1965) with kits from Accurex Biomedical Pvt.Ltd, Mumbai.

Principle

Urea is hydrolysed to ammonia and carbon dioxide by by urease. Ammonia produced reacts with alpha ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD^+ in this reaction, which is measured as decrease in absorbance at 340nm. The rate of decrease in absorbance at 340 nm is directly proportional to BUN concentration in the specimen.

Urea + H_2O $\longrightarrow$ $2NH_3$ + Co_2

$2NH_3$ + alpha ketoglutarate + NADH $\xrightarrow{GLDH^*}$ Glutamate+ NAD^+

Abbreviations
GLDH= Glutamate dehydrogenase

Calculation

Calculate the change in absorbance Abs. of standard and specimen.
Factor = Concentration of standard /Abs. of standard
= 20 / Abs. of standard
Concentration of BUN in mg/dl= Abs of specimen x Factor

3.6.11 Determination of creatinine in plasma:

Creatinine was determined by alkaline picrate method of Toro and Ackermann (1975) with kits from Accurex Biomedical Pvt. Ltd, Mumbai.

Principle

Creatinine in a protein free solution reacted with alkaline picrate and produced a red coloured complex measurable spectrophotometer.

Calculation

Plasma creatinine in mg/100 ml = O.D. test - O.D. Blank /O.D. Std - O.D. Blank X 2

3.7 Estimation of minerals:

3.7.1 Digestion procedure for mineral estimation of Plasma Samples:

Plasma sample was digested as per procedure described by Kolmer *et al.* (1951). Three ml of serum sample with equal volume of concentrated HNO3 was mixed in the digestion tube. The samples were kept overnight at room temperature followed by digestion on low heat (70-80) using heat bench (digestion bench), until the volume of samples was reduced to about 1 ml. To this 3 ml of double acid mixture (3 part concentrated HNO_3 and 1 part 70% $HClO_4$) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Further heating was continued to reduce the volume to approximately 0.5 ml. Final volume of filtrate was made up to 10 ml with triple distilled deionized water after luke warming the solution.

3.7.2 Procedure for Atomic Absorption Spectrophotometer (AAS):

Atomic Absorption Spectrophotometer was developed in mid 60s and is considered to be one of the modern and precise techniques for estimation of trace minerals in soils and biological materials like plants and animals. In AAS the ground state atoms from sample absorbs the light energy of a specific wavelength while entering the excited state. As the number of atoms in the light path increases, the amount of light absorbed also increases (i.e. Beer's Law). By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. Estimation of specific individual elements requires special light source and careful selection of the wavelength. AAS (PG instruments, United Kingdom) was used in present study. Sample analysis was done by attached computer and concentration of minerals was expressed in parts per million (ppm). At least 3 standards of known concentration were used for calibration and then the unknown test samples were analyzed. After sample analysis, sufficient distilled water flush was done for at least 10 minutes. The results were calculated according to formula given below. The standard working conditions of AAS for Cu, Zn and Fe estimation have been given in table 1.

Calculation:

a) **Element (ppm) in sample=** OD test /O D Std. X Conc. of Std. X Vol. made/ Wt. of sample

b) Element (ppm) in sample== Reading obtained (ppm) X Volume made/Wt. of sample

Analysis by Atomic Absorption Spectrophotometer:

Atomic Absorption Spectrophotometer AAS (PG Instruments, United Kingdom) was used for the estimation of plasma calcium, magnesium, sodium, potassium, zinc, copper and iron.

The instrument was set in order as per the specification of the AAS. Operating parameters for above estimations were as follows:

Table 1. Operating different parameters by AAS:

Parameters	**Zinc**	**Copper**	**Iron**	**Ca**	**Mg**	**Na**	**K**
Wavelength	213.9 nm	324.7 nm	248.3 nm	239.9 nm	285.2 nm	589.0 nm	766.5nm
Light source	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode
	Lamp	Lamp	Lamp	Lamp	Lamp	Lamp	Lamp
Flame type	Air-acetylene	Air-acetylene	Air-acetylene	Air-acetylene	Air-acetylene	Air-acetylene	Air-acetylene
	flame oxidising	flame oxidising	flame oxidising	flame oxidising	flame oxidising	flame oxidising	flame oxidising
	(lean, blue)	(lean, blue)	(lean, blue)	(lean, blue)	(lean, blue)	(lean, blue)	(lean, blue)
Operating current	5 mA	5 mA	5 mA	5 mA	5 mA	5 mA	5 mA

3.7.3 PREPARATION OF STANDARD SOLUTION FOR CALIBRATION OF AAS:

All the standards were prepared freshly every time during estimation of micro-minerals from samples.

3.7.3.1 Standardisation Procedure for Calcium:

An amount of 0.2497 gm analytical grade CaCO3, 20 ml triple distilled water was added. Then drop wise minimum volume of HCI (approx. 10 ml) was added to completely dissolve CaCO3 The final volume was made up to 100 ml to give a concentration of 1000 ppm (stock solution). This stock solution was used for further dilutions.

3.7.3.2 Standardisation procedure for Magnesium:

An amount of 0.8362 gm analytical grade $MgCI_2$ was dissolved in triple distilled water and the final volume of 100 ml was made to give a concentration of 1000 ppm (Stock Solution). This stock solution was used for further dilutions.

3.7.3.3 Standardisation procedure for Sodium:

Standard solution made up from stock solution of 1000 mg/L. Dilution was carried out in stages and acidified using nitric acid 7 % to avoid precipitation. From this working solution, standards of 0, 0.50, 1.0, and 1.5 ppm were prepared by dilution in 100 ml of volumetric flask. Standard curve was obtained against the reading of the AAS after setting the operating parameters and calibration of the instrument (AAS) as mentioned above.

3.7.3.4 Standardisation procedure for Potassium:

Standard solution made up from stock solution of 1000 mg/L. Dilution was carried out in stages and acidified using nitric acid 7 % to avoid precipitation. From this working solution, standards of 0, 0.75, 1.5, and 2.25 ppm were prepared by dilution in 100 ml of volumetric flask. Standard curve was obtained against the reading of the AAS after setting the operating parameters and calibration of the instrument (AAS) as mentioned above.

3.7.3.5 Standardisation procedure for zinc:

Hundred milligram of pure zinc metal was dissolved in minimum amount of dilute hydrochloric acid and made to 1 litre by adding double glass distilled water. This stock solution contained 100 µg zinc /ml. In six different 100 ml flasks, aliquots were taken and standard of 0, 0.5, 1.0, 1.5, 2.0 and 2.5-ppm zinc were obtained. Standard curve was prepared against the reading of the AAS after setting the operating parameters and calibration of the instrument (AAS) as mentioned above.

3.7.3.6 Standardisation procedure for copper:

A stock solution of 100 ppm copper was obtained by dissolving exactly 100 mg of pure AR grade metal in 5 ml of dilute (1: 1) HNO3 and finally diluting to 100 ml by adding double glass distilled water. From this working solution, standards of 0, 0.25, 0.5, 1.5, 2.0 and 2.5 ppm were prepared by dilution in 100 ml of volumetric flask. Standard curve was obtained against the reading of the AAS after setting the operating parameters and calibration of the instrument (AAS) as mentioned above.

3.7.3.7 Standardisation procedure for iron:

To prepare stock solution, 100 mg of AR grade iron powder was dissolve d in 5 ml of dilute HNO_3 and made to 100 ml by adding double glass distilled water. Standard s of 1, 2, 4, 6, 8 and 10 ppm were made in 100 ml volumetric flask by dilution. Standard curve was obtained against the reading of the AAS after setting the operating parameters and calibration of the instrument (AAS) as mentioned above.

3.8 Estimation of Serum Inorganic Phosphorus:

The Serum inorganic phosphorus was estimated by the method of Taussky and Shorr (1953).

Phosphorus in the form of inorganic phosphate was allowed to react with molybdic acid, producing the phosphomolybdate complex. This complex was reduced to a blue-coloured compound that is proportional to the phosphorus concentration.

Reagents:

1. Trichloroacetic acid (TCA) 20%
2. Ammonium molybdate- 5%
3. Sodium sulfite - 20%.
4. Hydroquinone 0.5%
5. Working phosphorus standard - 0.01 mg phosphorus/ml.

Procedure:

In a centrifuge test tube 3.5 ml distilled water was taken. Then 0.5 ml serum was added and mixed well. To this 1 ml 20% trichloroacetic acid was added, mixed well and kept for 10

minutes. This test tube was centrifuged at 3000 rpm for 10 minutes.

Then three test tubes were labelled Blank (b), Control (C) and Test (T) and the reagents were added as given below:

	(B)	(S)	(T)
Filtrate	-	-	2.5 ml
Distilled water	2 ml	1 ml	-
Working phosphorus standard	-	1 ml	_
Trichloroacetic acid (20%)	0.5 ml	0.5 ml	_
Ammonium molybdate (5%)	0.5 ml	0.5 ml	0.5 ml
Sodium sulfite (20%)	0.5 ml	0.5 ml	0.5 ml
Hydroquinone (0.5%)	0.5 ml	0.5 ml	0.5 ml

These were mixed well and allowed to stand for 30 minutes. The absorbance (A) of standard(s) and Test (T) were measured against blank on a spectrophotometer at 700 nm.

Calculation:

Serum inorganic phosphorus in mg % = absorbance of T/ absorbance of T x 4

3.9 Oxidative Stress Indices:

3.9.1 Estimation of Malondialdehyde:

Estimation of malondialdehyde was conducted by method developed by Okhawa *et al.* (1979). Malondialdehyde (MDA) a secondary product of lipid peroxidation, the reaction of lipid peroxides with Thiobarbituric acid (TBA) yields red pigment which can be measured on Colorimeter or Spectrophotometer at 532 nm. MDA was estimated in fresh blood samples collected from jugular vein of crossbreed dairy cow

Freshly collected blood 0.2 ml was taken in a test tube; 1.8 ml of 1.15% potassium chloride added to it and from this test tube 0.2ml was transferred to other test tube. A blank containing 0.2 ml of potassium chloride (1.15%) was also prepared in other test tube. After that 0.2 ml Sodium dodecyl sulphate solution (8.1%), 1.5 ml -20% Acetic Acid (pH adjusted 3.5 by NaOH), 1.5 ml -2 thiobarbituric acid (0.8% aqueous solution) were added , respectively to the test tube. The contents were thoroughly mixed and volume was made 4.0 ml with deionised distilled water, test tubes were capped with aluminum foil.

These test tubes were kept in water bath for 60 minutes. After that test tube were cooled under tape water immediately. One ml of distilled water was added to each test tube. After it each tubes were added 5 ml of n-butanol plus pyridine (15:1 v/v) and shaken the tubes vigorously and centrifuged at 4000 rpm for 10 minutes. Upper organic pink colour layer was collected from each test tube and absorbance was read at 532 nm against blank.

Solution of 5, 10, 15, 20, 25, 30 n moles / ml of tetramethoxy propane (TMP) were prepared by diluting it to 1.15% of potassium chloride solution. The calibration curve was prepared by taking 0.2 ml of this solution. Standard curve is depicted in figure 1.

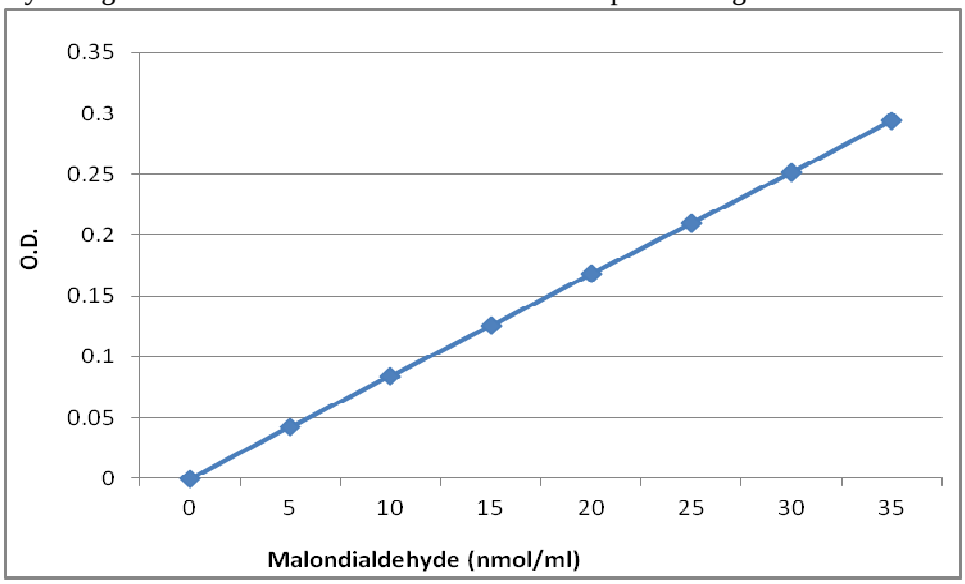

Fig. 1: Malondialdehyde standard curve

Calculation:
Concentration = (Ab. Sample/Ab. Standard) X Conc. Standard.

3.9.2 Estimation of Reduced glutathione in blood:

Estimation of reduced glutathione was done by method developed by Beutler *et al*. (1971). In a test tube 0.2 ml blood was added to 1.8 ml distilled water and after it, 3 ml of precipitating solution was added and mixed. The mixture was allowed to stand for 5 minutes. The mixture was filtered through course grade filter paper and 1 ml of clear filtrate was added to 4 ml of 0.3 M Na_2HPO_4 and optical density was read at 412 nm. A standard curve was obtained by using 0.2ml reduced glutathione solution 10, 20,40,60,80,100,120 mg percent. Standard curve is depicted in figure 2.

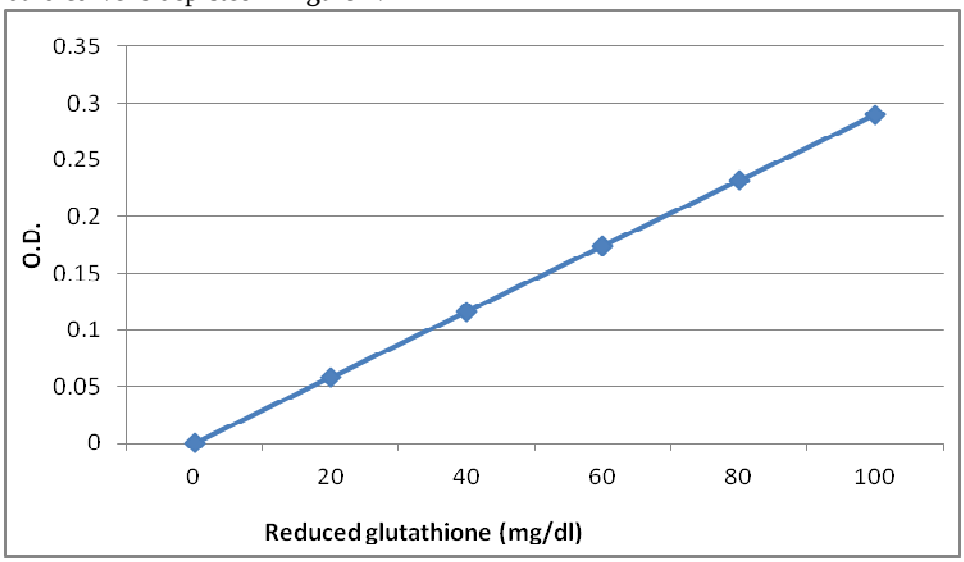

Fig.2: Reduced glutathione standard curve

Percipitating Solution:
Glacial Metaphosphoric acid -1.67 gm, Disodium EDTA- 0.20, Sodium Chloride- 30.0 gm, Distilled water- 100 ml.
DTNB reagent: 5, 5 Dithiobis- 20.00 mg, Sodium Citrate- 1.00 gm, Distilled water-100 ml.
Calculation: (Ab. Sample/Ab. Standard) X Conc. Standard.

3.10 Therapeutic Trials:

In the third phase, therapeutic trial was conducted in a group of animals in farmer dairies of Bikaner district. For this purpose prophylactic trial was conducted in crossbred cows to evaluate the efficacy of propylene glycol in treating cows affected with subclinical ketosis. Six cows which were diagnosed with subclinical ketosis based on results of biochemical test and on the reduced milk yield were selected for the present study. Cows were fed with Propylene glycol (PG) was fed to each cow with concentrate @ 200 ml per day and parental choline biocarbonate 100 mg by I/M for 5 days. The results of the therapeutic trial were evaluated. Milk yield was recorded as per milk record register.

3.11 Statistical Analysis:

The main parameters of the present investigation were plasma analyte in crossbred cows. The main effects were classified as overall. The subsets of were districts, health, stage and parity. For subset data were expressed as mean ± SE.

Mixed model least square and maximum likelihood computer programme PC-I (Copyright, 1987, Walter R. Harvey) were used to determine analyses of variance to test the significance of effects. The changes in the means were measured by using multiple mean comparison procedure. For this Duncon's new multiple range (Duncan, 1955 and Steel and Torrie, 1980) and paired T tests was used to estimate significance at different level of significance. The correlation was estimated by Microsoft excel.

4. RESULTS AND DISCUSSION

Negative energy balance (NEB) is a normal occurrence in dairy cattle as they transition from late gestation to early lactation. This transition period is often considered to occur from 3 weeks pre-partum to 3 weeks post-partum as during this time frame homeorhetic regulation of metabolic function is necessary in order to accommodate parturition and lactogenesis. For these reasons, the transition from late gestation to early lactation is a dynamic period for dairy cattle, during which most infectious and metabolic diseases are likely to occur. Cows unable to adapt to this challenging time are more prone to negative subsequent events. The economic impacts of maladaptation are important and include increased risk of metabolic disease, reduce milk production, early removal from the herd and poor reproductive performance. Keeping in view the importance of this transition period, the study was planned to establish the base line values for different parameters in high yielding crossbred dairy cows during transition period. These base values will serve as a reference values in the future for diagnosis of various metabolic diseases so that the cows calve down uneventfully and enter the lactation stage safely. The subclinical ketosis was diagnosed on the basis of plasma concentration BHBA > 0.6 mmol/L ;NEFA > 0.4 mmol/L at the pre-partum transition period and > 1.2 mmol/L ; > 0.7 mmol/L at post-partum transition period (within 3 weeks of parturition), respectively (Nielan *et al*., 1994; Whitaker, 1997 and Oetzel, 2004), along with the presence of other signs such as decrease in milk yield, appetite and body condition score (BCS). In this study total 123 animals were studied, out of which 94 animals were healthy and 29 were affected by subclinical ketosis according to standard value of NEFA and BHBA as mentioned earlier. This objective was accomplished by conducting a base line study from private organized dairy farms having high yielding crossbred dairy cows from three districts of Western Rajasthan. The ANOVA of liver enzymes, oxidative stress, minerals and biochemical parameters have been presented in appendix Table no. 24, 25 and 26.

4.1 Prevalence:

Based on the results of different biomarker (NEFA and BHBA values) of negative energy balance, prevalence for the subclinical ketosis was calculated. Out of the total 123 crossbred cows sampled during the study period, 29 animals were found positive with an overall prevalence of 23.57 per cent (Table 2).

Table 2: Prevalence rate of subclinical ketosis in crossbred cows in different district of Western Rajasthan.

Districts	No. of cows sampled	No. of SCK (Prevalence)
Bikaner	59	15 (25.42 %)
Jodhpur	41	8 (19.51 %)
Pali -Marwar	23	6 (26.08 %)
Total	123	29 (23.57 %)

The chi square statistics was 0.5681. The P-value is 0.9665. No significant difference in prevalence of SCK was observed between different districts.

4.1.1 Subclinical Ketosis (SCK):

District wise: The prevalence of subclinical ketosis in dairy cows of Bikaner, Jodhpur and Pali-Marwar districts was 25.42 (15/59), 19.51 (8/41) and 26.08 (6/23) per cent, respectively.

Parity-wise prevalence of subclinical ketosis was highest in above 5th parity (32.35 per cent), followed by 3rd to 5th parity (20.22 per cent). The incidence of SCK increased with age in present study. The results of the present investigation were similar to Bihani (2002) and Sharma (2006). Kumar (2011) reported 13.9 % and 3.4 %, prevalence of subclinical and clinical ketosis, respectively in dairy cows and significant difference found between region not in parity wise. The mean daily peak milk yield for clinical ketosis, subclinical ketosis and healthy cows in study were 28, 35 and 45 kg, respectively, suggesting a decline ranging from 22 to 38 %, compared to the healthy cows (Samiei *et al.*, 2013). Sato *et al.* (2005) reported 34.7% SCK in Japan and 57.7 % in Lithuania (Zilaitis *et al*., 2007).

SCK occurred at 5 days in milk (DIM), when 22.3% of cows had their first SCK-positive test. Peak prevalence of SCK occurred at 5 days in milk (DIM), when 28.9% of cows had a SCK-positive test (McArt *et al*., 2012a).

Marjan and Saman (2011) reported 7.2 % incidence rate of subclinical ketosis (per cent of cows with at least one positive test) in early lactation (0-70th day) period and the peak prevalence of subclinical ketosis occurred during the fourth week of lactation in Fars Province of Iran. The overall prevalence of subclinical ketosis was 6.9 per cent to 14.1 per cent in the first two months of lactation (Andersson and Emanuelson, 1985; Nielen *et al*., 1994; Duffield *et al.*, 1997).

The overall prevalence of ketosis was 9.38 per cent in cows and 2.92 per cent in buffaloes, observed in Tamil Nadu. They further observed low prevalence of ketosis in cows of Erode and Coimbatore districts of Tamil Nadu (Thirunavukkarasu *et al*., 2010b). It was due the improved feeding practices.

Garro *et al.* (2013) and Suthar *et al.* (2013) reported overall prevalence of 21.8 and 10.3 per cent, respectively in the dairy cows, using a threshold of >1.2 mmol/L for blood BHBA. However our studies having higher prevalence rate as compared to above mentioned study. The major cause of higher prevalence may be non scientific feeding and management practices adopted in the study area.

Duffield *et al.* (1998) reported an incidence of SCK 59.00 per cent and 43.00 per cent using a cutoff threshold for BHBA concentration of 1200 and 1400 mmol/L, respectively. Similarly Asl *et al.* (2011) observed a prevalence of 63.00, 68.00 and 59.00 per cent during the week 2nd , 4th and 6th post-partum, out of which 30.00 per cent of cows were found positive in all of the 2, 4 and 6 weeks post-partum.

McArt *et al.* (2012b) reported a prevalence of SCK 28.70 per cent, 26.30 per cent and 40.80 per cent in cows with parity 1, 2 and above 3, respectively. Higher prevalence reported in their study was due to the bi- weekly sampling (McArt *et al.* 2012b and Ospina *et al.* 2010) during the first two months, which resulted in increase in the detection rate of SCK, as the median time from first SCK positive BHBA test to first test <1.2 mmol/L was 5 days, (McArt *et al.*, 2012b) which helped them in detecting more number of SCK cases.

Highest prevalence of subclinical ketosis was found in cows from greater than 5th parity in comparison to 3rd - 5th parity. Possible reason for that might be the active haemostatic mechanisms in the young dairy animal to cope up with the negative energy balance during the early lactation period. Similar to the present findings, number of research workers recorded that

the prevalence of ketosis increases with the age and peak prevalence was observed between 3rd – 5th lactation (Overby *et al.*, 1974; Erb and Martin, 1978; Dohoo and Martin, 1984 and Grohn *et al.*, 1989).

4.2 Minerals status:

The ANOVA of minerals parameters have been presented in appendix Table no. 26.

4.2.1 Calcium:

4.2.1.1 Interaction (between districts and health; districts and stage) affecting calcium level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for calcium in plasma was 9.486±0.085 mg/dl. The differences among subclass means were non significant across districts (9.619±0.135 mg/dl in Bikaner, 9.194±0.143 mg/dl in Jodhpur and 9.643±0.175 mg/dl in Pali-Marwar). Highly significant difference was observed (P≤0.01) in healthy/SCK groups (10.095±0.095 in healthy and 8.876±0.146 mg/dl SCK) and non significant in parity groups (10.455±0.119 mg/dl 3-5th parity and 8.558±0.119 mg/dl above 5th parity). The effect of pre (10.582±0.102 mg/dl) and post-partum (8.834±0.112 mg/dl) stages were highly significant (P≤0.01)in pooled data. There were effects of two factor interaction indicating that difference among districts. Whereas, the interaction between districts and health status was highly significant. The trend is depicted in Fig. 3. This shows that the calcium level was lower in post- partum across all districts. The decrease in Ca level was significantly higher in (10.858±0.135 to 8.229±0.156 mg /dl) Jodhpur district and lowest (10.161±0.139 to 9.182±0.145mg/dl) in Bikaner districts. The interaction between districts and stage was also highly significant (P≤0.01). The trend is depicted in Fig. 4. This shows that calcium level was lower in SCK across all districts. The rise in their level was significantly higher in (10.832±0.212 to 8.454±0.298 mg/dl) in Pali-Marwar district and lowest (9.760±0.121 to 9.479±0.229 mg/dl) in Bikaner district.

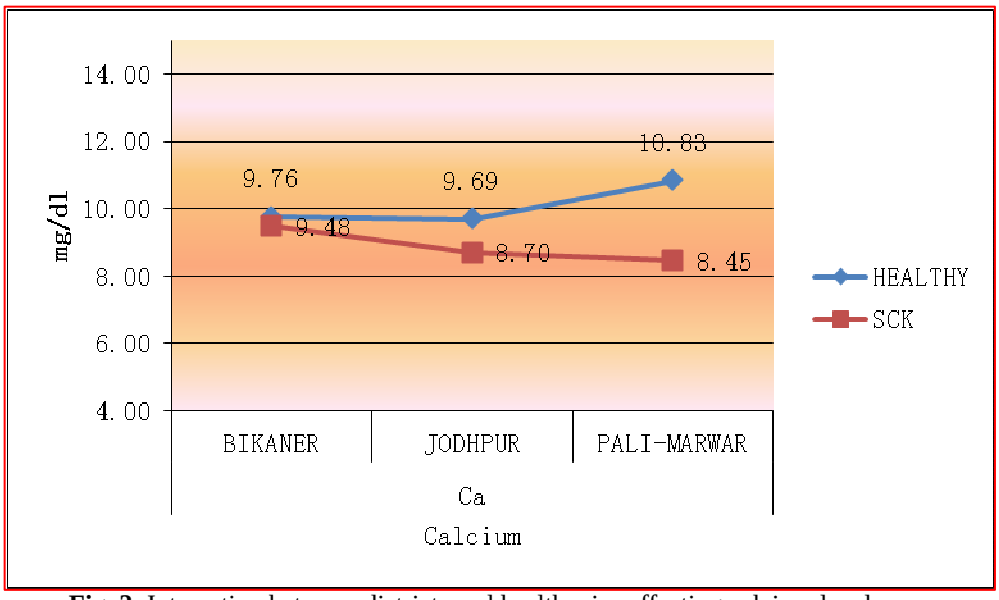

Fig. 3: Interaction between districts and health wise affecting calcium level.

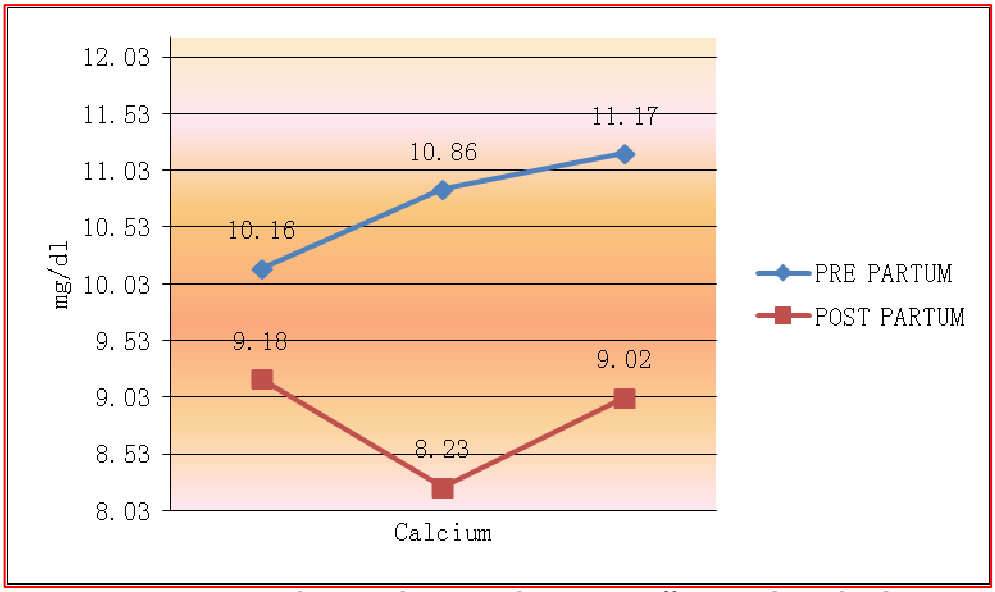

Fig 4. Interaction between districts and stage wise affecting calcium level.

Table 3: Overall macro and micro minerals status in crossbred cows during transition period.

Parameters	**Periods**	Over all		
		Healthy (n=94)	**SCK (n=29)**	Over all (123)
Ca (mg/dl)	Pre- Partum.	10.783±0.116	9.931±0.168	10.582±0.102**
	Post- Partum	9.003±0.127	8.287±0.206	8.834±0.112**
	Over all	10.095±0.095^{a}	8.876±0.146^{b}	9.486±0.085
Pi (mg/dl)	Pre- Partum.	5.287±0.117	5.156±0.163	5.256±0.097
	Post -Partum	4.501±0.106	4.100±0.087	4.407±0.085
	Over all	4.930±0.094	4.610±0.144	4.770±0.083
Mg (mg/dl)	Pre -Partum.	3.361±0.083	3.333±0.045	3.354±0.064**
	Post -Partum	3.457±0.085	3.743±0.033	3.525±0.066**
	Over all	3.475±0.068	3.526±0.105	3.500±0.061
Na (mmol/l)	Pre- Partum.	119.415±0.911	118.000±1.508	119.081±0.781**
	Post- Partum	115.191±0.704	114.586±1.432	115.049±0.633**
	Over all	116.566±0.741	117.085±1.137	116.825±0.661
K (mmol/l)	Pre- Partum.	4.873±0.094^{a**}	4.251±0.199^{b**}	4.726±0.089*
	Post- Partum	4.439±0.098^{a**}	5.436±0.264^{b**}	4.674±0.104*
	Over all	4.483±0.094^{a}	4.929±0.145^{a}	4.706±0.084
Cu (ppm)	Pre- Partum.	0.886±0.022	0.718±0.028	0.847±0.019**
	Post- Partum	0.738±0.018	0.587±0.023	0.702±0.016**
	Over all	0.754±0.014^{a}	0.633±0.021^{b}	0.694±0.012
Fe (ppm)	Pre- Partum.	2.253±0.073^{a*}	2.348±0.066^{a}*	2.276±0.058**
	Post- Partum	1.748±0.052^{a*}	2.166±0.051^{a}*	1.846±0.045**
	Over all	2.027±0.051	2.214±0.078	2.121±0.045
Zn (ppm)	Pre -Partum.	1.188±0.013	1.192±0.020	1.189±0.011**
	Post- Partum	0.988±0.013	0.974±0.020	0.985±0.011**
	Over all	1.067±0.011	1.069±0.016	1.068±0.009

NOTE: 1. Value bearing different superscript (a, b, c, d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). 3. No superscript means non significant within a row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

4.2.1.2 Calcium (Ca) concentration in healthy dairy cows:

In the present study, overall mean plasma calcium concentration from various districts was 10.783±0.116 mg/dl during the pre-partum period and 9.003±0.127 mg/dl during the post- partum transition period (Table 3), which was within the normal range i.e. 8.09-10.62 mg/dl as stated by Radostits *et al.* (2000). Highly significant (P≤0.01) decrease was observed from the pre to post-partum transition period. Non significant difference in Ca level was observed in between different districts.

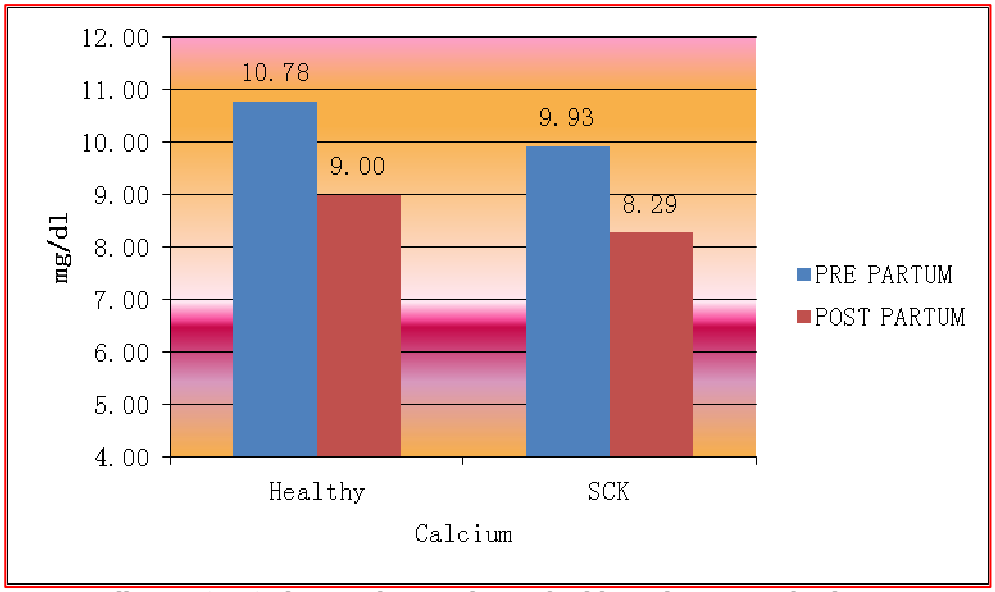

Fig. 5: Overall mean (±SE) plasma calcium values in healthy and SCK crossbred cows.

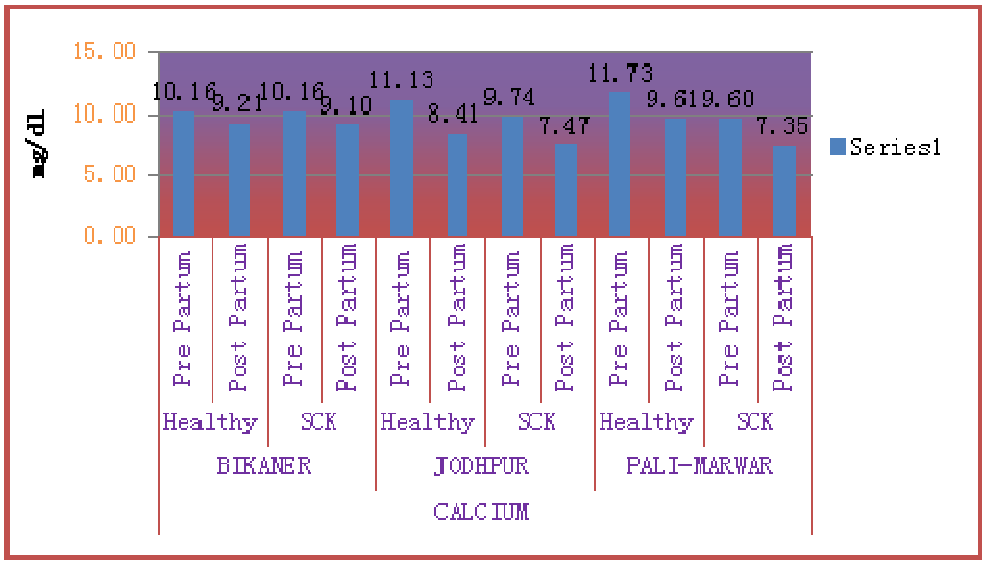

Fig. 6: Mean (±SE) plasma calcium values in healthy and SCK crossbred cows.

However, the overall mean plasma Ca concentration in healthy cows were highly significant (P≤0.01) higher in dairy cow in comparison to SCK. Changes in Ca metabolism induced by lactation are more significant. The lower plasma Ca level in lactating cattle might be observed due to high demand of absorbed Ca per liter of milk produced (approx. 50 g per day). Before calving, the approximate daily requirement for Ca is only 30 g, comprising 15 g for fecal and urinary losses and 15 g for fetal growth. This demand for Ca may only be satisfied by increasing absorption from the rumen or intestines, and increasing mobilization from tissue, especially bone reserves of Ca, as circulating blood Ca reserves are limited. As per NRC (2001) lactating cattle needs 1.37 gm of Ca/kg of milk produced in addition to maintenance requirement. This is probably because calcium requirement depending on the level of productivity and the physiological status (McDowell, 1992). This may also be attributed to dietary imbalances of calcium and phosphorus, higher requirement during pregnancy (3-8 months) and dietary interaction with other minerals, especially high intake of iron that results in formation of insoluble phosphate in acidic pH of abomasum affecting the bioavailability of phosphorus. This unavailable

phosphorus not only affects phosphorus, but also utilization of calcium by precipitation at intestinal level, thus preventing its absorption (Maynard *et al*., 1979).

District wise: The mean plasma Ca concentrations was 10.160± 0.162, 11.128±0.120 and 11.728± 0.254 mg/dl during the pre-partum period; 9.210±0.178, 8.414±0.175, 9.612± 0.329 mg/dl during the post-partum period in crossbred cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms respectively (Table 4 and Fig. 6). Lower values were observed in the cows of Jodhpur district as compared to the dairy cows from Bikaner and Pali-Marwar dairy farm during post-partum transition period. It was observed that no mineral supplementation in Jodhpur district. Districts wise health status and stage wise highly significant ($P \leq 0.01$) differences were observed in calcium values.

Table 4: Districts wise macro and micro minerals status investigated in crossbred cows during transition period in Western Rajasthan.

Parameters	**Periods**	Bikaner			Jodhpur			Pali-Marwar		
		Healthy (n=44)	**SCK (n=15)**	Over all (59)	**Healthy (n=33)**	**SCK (n=8)**	Over all (41)	**Healthy (n=17)**	**SCK (n=6)**	Over all (23)
Ca (mg/dl)	Pr-e Partum.	10.160± 0.162	10.162± 0.284	10.161±0.139**	11.128± 0.120	9.744±0.212	10.858±0.135**	11.728± 0.254	9.603±0.237	11.173±0.279**
	Post-Partum	9.210± 0.178	9.099± 0.234	9.182±0.145**	8.414± 0.175	7.469± 0.186	8.229±0.156**	9.612± 0.329	7.348± 0.131	9.021±0.323**
	Over all	9.760±0.121[a]	9.479±0.229[b]	9.619±0.135	9.694±0.136[a]	8.695±0.255[b]	9.194±0.143	10.832±0.212[a]	8.454±0.298[b]	9.643±0.175
Pi (mg/dl)	Pre-Partum.	4.921± 0.156	5.165± 0.264	4.983±0.134**	5.759± 0.196	4.807± 0.143	5.573±0.170**	5.315± 0.275	5.598± 0.348	5.389±0.221**
	Post-Partum	4.552± 0.163	4.409± 0.119	4.516±0.125**	4.203± 0.159	3.765±0.069	4.117±0.131**	4.949± 0.236	3.773± 0.023	4.642±0.205**
	Over all	4.710±0.120	4.731±0.226	4.721±0.133	5.050±0.134	4.353±0.251	4.702±0.140	5.029±0.209	4.745±0.294	4.887±0.173
Mg (mg/dl)	Pre-Partum.	3.302± 0.106	3.373± 0.038	3.320±0.080	3.555±0.174	3.381±0.109	3.521±0.141	3.135± 0.134	3.167±0.123	3.143±0.103
	Post-Partum	3.286±0.124	3.737± 0.040	3.401±0.096	3.536±0.144	3.787±0.081	3.585±0.117	3.747 ± 0.186	3.700± 0.073	3.735±0.138
	Over all	3.341±0.087	3.575±0.164	3.458±0.097	3.603±0.098	3.569±0.183	3.586±0.102	3.481±0.152	3.434±0.214	3.458±0.125
Na (mmol/l)	Pre-Partum.	118.932± 1.217	116.933± 2.092	118.424±1.049	121.030±1.719	117.625±3.273	120.366±1.521	117.529± 2.080	121.167± 2.880	118.478±1.712
	Post-Partum	115.636±1.013	113.733± 1.989	115.153±0.907	116.848± 1.159	114.875± 3.182	116.463±1.107	110.824± 1.423	116.333±2.813	112.261±1.350
	Over all	117.684±0.946[a]	114.700±1.781[a]	116.192±1.050	118.400±1.061[a]	116.962±1.981[a]	117.681±1.109	113.615±1.650[a]	119.593±2.318[a]	116.604±1.363
K (mmol/l)	Pre-Partum.	4.806±0.123	4.323±0.311	4.683±0.123	5.203± 0.161	3.943± 0.426	4.957±0.171	4.405± 0.227	4.485± 0.111	4.426±0.169
	Post-Partum	4.613± 0.139	5.221± 0.347	4.767±0.139	4.435± 0.172	5.383± 0.543	4.620±0.180	3.998± 0.206	6.043± 0.626	4.531±0.287
	Over all	4.644±0.120	5.028±0.227	4.836±0.133	4.707±0.135	4.603±0.252	4.655±0.141	4.099±0.210	5.156±0.295	4.627±0.173
Cu (ppm)	Pre-Partum.	0.962± 0.026	0.737± 0.042	0.905±0.026	0.923± 0.035	0.793± 0.034	0.898±0.030	0.619± 0.023	0.570± 0.023	0.607±0.018
	Post-Partum	0.832± 0.025	0.593± 0.038	0.771±0.025	0.711± 0.025	0.639± 0.023	0.697±0.021	0.546± 0.015	0.503± 0.022	0.535±0.013
	Over all	0.878±0.018[a]	0.656±0.034[a]	0.767±0.020[a]	0.795±0.020[a]	0.720±0.038[a]	0.758±0.021[b]	0.589±0.031[a]	0.524±0.044[a]	0.556±0.026[c]
Fe (ppm)	Pre-Partum.	2.500± 0.111	2.343± 0.101	2.460±0.087	1.961± 0.100	2.310± 0.098	2.029±0.085	2.182± 0.159	2.410± 0.169	2.242±0.125
	Post-Partum	1.855 ±0.075	2.197±0.078	1.942±0.062	1.591± 0.090	2.094± 0.085	1.689±0.081	1.776± 0.111	2.183± 0.117	1.883±0.094
	Over all	2.161±0.065	2.183±0.123	2.172±0.072	1.820±0.073	2.198±0.136	2.009±0.076	2.101±0.114	2.262±0.160	2.181±0.094
Zn (ppm)	Pre-Partum.	1.235± 0.021	1.209± 0.025	1.228±0.017	1.157± 0.016	1.194± 0.031	1.164±0.015	1.128± 0.024	1.150± 0.062	1.134±0.023
	Post-Partum	1.019 ±0.021	0.993± 0.031	1.012±0.018	0.983± 0.015	0.947± 0.022	0.976±0.013	0.916± 0.025	0.962± 0.054	0.928±0.023
	Over all	1.115±0.014	1.058±0.026	1.087±0.015	1.077±0.015	1.075±0.029	1.076±0.016	1.009±0.024	1.074±0.034	1.041±0.020

NOTE: 1. Value bearing different superscript (a, b, c, d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). 3. No superscript mean non significant with in row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

Mean Ca concentration was 10.765±0.135 and 9.008±0.137 mg/dl; 10.841±0.234 and 8.987±0.310 mg/dl in 3rd -5th parity and above 5th parity respectively during pre and post- partum transition period (Table 5). Overall non significant differences were observed in districts and parity wise. Horst *et al.* (2005) also observed a decrease in the plasma Ca concentration in the older cows and opined that one of the reason may be decreased number of receptors for 1, 25-dihydroxy Vitamin-D in the intestine resulting in decreased absorption of Ca. Ozukum (2011) also reported lower plasma Ca level in the cows of > 6 years of age. Most of the earlier studies have also indicated a slight decline in plasma Ca with advancing age (McAdam and O'Dell, 1982 and Shrikhande *et al.,* 1997). Our studies were not similar to the above cited author because most of the animals (71) belong to 3rd to 5th parity and only 23 animals were above 5th parity. There is no effect of parity in healthy dairy cows because all dairy cows were in healthy condition.

4.2.1.3 Calcium (Ca) concentration in SCK dairy cow:

Overall mean plasma Ca concentration observed in healthy and SCK affected cows was found to be 10.783±0.116, 9.931±0.168 mg/dl during pre-partum; 9.003±0.127, 8.287±0.206 mg/dl during post-partum period. A significant decrease was observed in the overall mean plasma Ca in pre to post-partum period, though values were within the normal physiological range (8.09-10.62 mg/dl) as stated by Radostits *et al.* (2000). Reduction in the concentrations of Ca and P observed in SCK cows could be due to decreased Ca uptake because of any illness such as ketosis which might affect the appetite and decrease its absorption from the intestine (Hove, 1978; Moore, 1997) and increased loss of base in the urine to compensate for the acidosis (Radostitis *et al*., 2007). Kumar (2011) also reported hypocalcaemia in ketosis and suspected that the lowering of blood calcium was due to reduced feed intake. Schultz (1971) recorded hypoglycemia accompanied with hypocalcaemia similar to that observed in present investigation. Additionally, Ca level can also be reduced due to increased loss of base in the urine to compensate for the acidosis in cows with ketosis (Radostits *et al.,* 2000). In present study showed that there was a problem of marginal Ca deficiency. In all three districts calcium level declined in stages levels; it was due to inadequate nutritional supplementation and feeding. Payne, (1972) reported hypocalcaemia is often associated with infertility, disappointing milk yield and ketosis. Sometimes it might be due to unbalanced maintenance diet or wrong choice of production ration.

Table 5: Macro and micro minerals investigated in crossbred cows depending upon parity wise in transition period (Mean± S.E.)

Parameters	Periods	3nd - 5th parity			>5thparity		
		Healthy (n=71)	**SCK (18)**	Over all (89)	**Healthy (n=23)**	**SCK(n=11)**	Over all (34)
Ca (Mg/dl)	Pre Partum.	10.765±0.135	10.166±0.233	10.643±0.120	10.841±0.234	9.546±0.182	10.422±0.198
	Post-partum	9.008±0.137	8.639±0.266	8.933±0.122	8.987±0.310	7.710±0.250	8.574±0.245
	Over all	10.017±0.089	9.026±0.212	10.455±0.119	10.174±0.167	8.808±0.221	8.558±0.119
Pi (mg/dl)	Pre Partum.	5.281±0.122	5.178±0.210	5.260±0.106	5.303±0.304	5.119±0.271	5.244±0.222
	Post-partum	4.503±0.131	4.234±0.128	4.449±0.108	4.497±0.160	3.880±0.053	4.297±0.120
	Over all	4.949±0.087	4.618±0.208	5.241±0.117	4.911±0.164	4.535±0.217	4.266±0.117
Mg (mg/dl)	Pre Partum.	3.306±0.095	3.328±0.047	3.310±0.076	3.530±0.169	3.341±0.093	3.469±0.118
	Post-partum	3.411±0.094	3.731±0.042	3.476±0.077	3.600±0.192	3.764±0.056	3.653±0.131
	Over all	3.380±0.064	3.498±0.152	3.341±0.085	3.569±0.119	3.554±0.159	3.660±0.085
Na (mmol/l)	Pre Partum.	119.887±1.044	118.389±2.007	119.584±0.924	117.957±1.873	117.364±2.348	117.765±1.458
	Post-partum	114.761±0.774	115.611±1.994	114.933±0.733	116.522±1.605	112.909±1.904	115.353±1.267
	Over all	116.984±0.693	119.165±1.647	118.742±0.926	116.148±1.297	115.005±1.721	114.909±0.926
K (mmol/l)	Pre Partum.	4.964±0.103	4.023±0.249	4.773±0.104	4.593±0.210	4.625±0.311	4.603±0.171
	Post-partum	4.501±0.116	5.266±0.329	4.656±0.117	4.247±0.179	5.714±0.448	4.721±0.220
	Over all	4.661±0.088[a]	4.627±0.210[b]	4.514±0.118	4.306±0.165[a]	5.232±0.219[b]	4.898±0.118
Cu (ppm)	Pre Partum.	0.904±0.027	0.734±0.042	0.870±0.024	0.831±0.035	0.690±0.028	0.785±0.027
	Post-partum	0.745±0.022	0.584±0.034	0.712±0.020	0.716±0.034	0.591±0.021	0.676±0.026
	Over all	0.778±0.013	0.631±0.031	0.755±0.017	0.730±0.024	0.636±0.033	0.632±0.017
Fe (ppm)	Pre Partum.	2.197±0.086	2.342±0.095	2.226±0.072	2.426±0.127	2.358±0.086	2.404±0.090
	Post-partum	1.761±0.063	2.192±0.069	1.848±0.055	1.709±0.092	2.123±0.077	1.843±0.075
	Over all	1.942±0.047	2.216±0.113	2.302±0.064	2.112±0.089	2.213±0.118	1.939±0.064
Zn (ppm)	Pre Partum.	1.193±0.015	1.221±0.025	1.198±0.013	1.176±0.029	1.145±0.028	1.166±0.021
	Post-partum	0.989±0.015	1.007±0.028	0.993±0.013	0.983±0.024	0.921±0.020	0.963±0.018
	Over all	1.076±0.010	1.112±0.024	**1.171±0.013[a]**	1.058±0.019	1.025±0.025	**0.964±0.013[a]**

NOTE: 1. Value bearing different superscript (a, b, c, d) in a row depict highly significant (P≤0.01).

2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).

3. No superscript mean non significant with in row or column.

4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

District wise: The mean level of Ca was 10.162± 0.284, 9.744± 0.212, 9.603± 0.237 in pre-partum and 9.099± 0.234, 7.469±0.186, 7.348±0.131 in post-partum period in Bikaner, Jodhpur and Pali-Marwar districts, respectively. A highly significant (P≤0.01) decrease was observed in the mean plasma Ca from the pre to post-partum transition period in the cows of all districts. However, the rate of decrease from pre to post-partum stage was more prominent in districts with lower Ca level in pre-partum stage. This suggested that pre-partum feeding must be supplemented to avoid sharp decrease in Ca after parturition. Non significant changes occur between the districts during pre-partum periods, while non significant changes were observed between the districts in post-partum periods. However, cows from Jodhpur and Pali-Marwar district showed marginal deficiency in the mean plasma Ca (7.469±0.186, 7.348±0.131 mg/dl) level during the post-partum period (Table 4). Reduced level of plasma calcium recorded in the cows of Jodhpur and Pali-Marwar district suffering from SCK during the post-partum period, might be due to the reduced dry matter intake and greater nutrient output in milk which reduces blood Ca concentration and increases lipomobilization (Ribeiro *et al.*, 2013). Similarly, Martinez *et al.* (2012) also observed reduced Ca level in cows with elevated NEFA and BHBA during the post-partum period.

4.2.2 Inorganic phosphorus (Pi):

4.2.2.1 Interaction between districts and stages affecting inorganic phosphorus level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for Inorganic Phosphorus in plasma was 4.770±0.083 mg/dl. The differences among subclass means were non significant across districts (4.721±0.133 mg/dl in Bikaner, 4.702±0.140 mg/dl in Jodhpur and 4.887±0.173 mg/dl in Pali-Marwar), non significant in healthy/SCK groups (4.930±0.094 in healthy and 4.610±0.144 mg/dl SCK) and non significant in parity groups (5.241±0.117 mg/dl 3-5th parity and 4.266±0.117 mg/dl above 5th parity). The effect of pre (5.256±0.097 mg/dl) and post-partum (4.407±0.085 mg/dl) stages were highly significant (P≤0.01) in pooled data. There were effects of two factor interaction indicating that difference among districts. Highly significant (P≥0.01) interaction was observed between districts and health status. The trend is depicted in Fig. 7. This showed that the Inorganic Phosphorus level was lower in post-partum across all districts. Rise in phosphorus level was significantly higher in (5.573±0.170 to 4.117±0.131 mg/dl) Jodhpur district and lowest (4.983±0.134 to 4.516±0.125 mg/dl) in Bikaner districts.

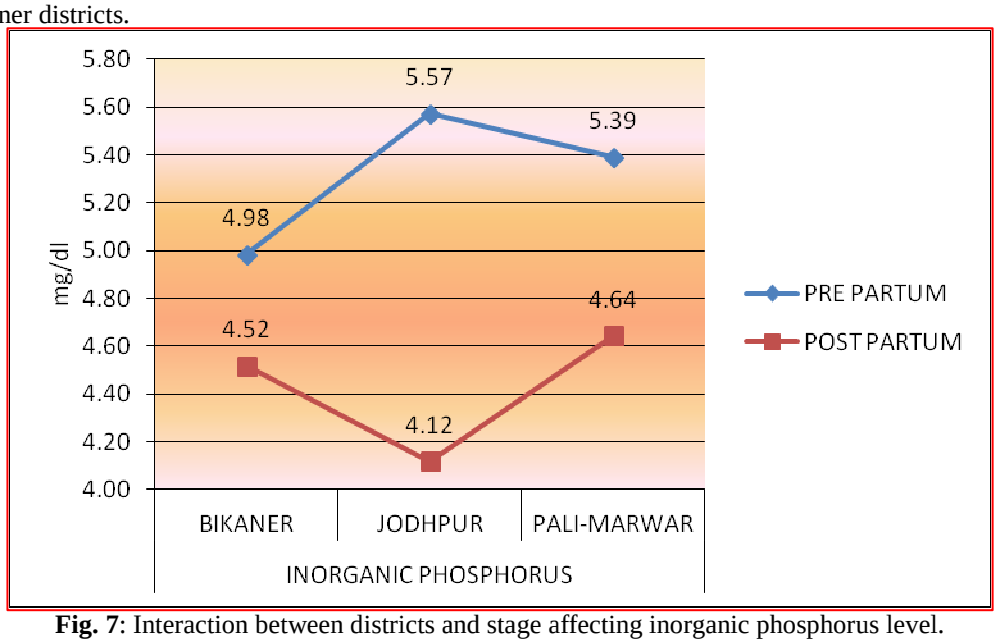

Fig. 7: Interaction between districts and stage affecting inorganic phosphorus level.

4.2.2.2 Inorganic phosphorus (Pi) in healthy cow:

The overall mean value of plasma inorganic phosphorus (Pi) in healthy cows was 5.287±0.117 mg/dl during pre-partum period and 4.501±0.106 mg/dl during post-partum period. Non significant differences were found in all the three districts and health. Highly significant (P≤0.01) difference was observed in the overall mean plasma Pi level from the pre to post-partum transition period (Table 3). The mean plasma Pi level showed similar pattern in all the districts, where it decreased from pre to post-partum transition period.

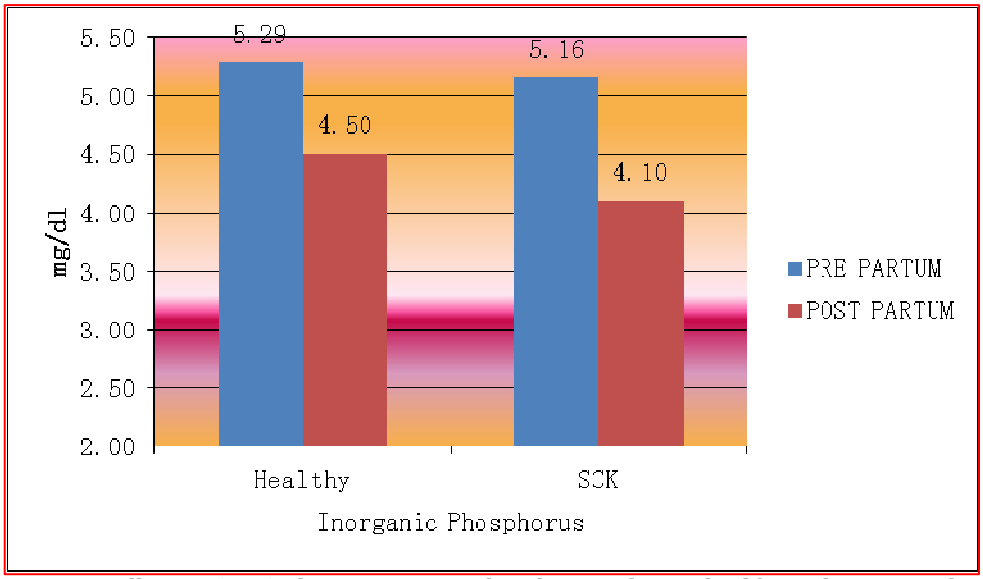

Fig. 8: Overall mean (±SE) plasma inorganic phosphorus values in healthy and SCK crossbred cows.

Larsen *et al.* (2001) also reported decrease in the plasma Pi parallel to that of plasma Ca with approaching parturition. The decrease in plasma Pi around parturition might be due to phosphorus mobilization across the placenta for the growth and development of fetal skeleton. Similar to our results, Kozlowska *et al.* (1981) recorded a decrease in plasma Pi and Ca level during the periparturient period. Although P concentrations are not as tightly regulated as Ca, both are closely related with plasma PO_4 concentrations regulated directly by 1, 25(OH) $_2D_3$ and indirectly by the PTH/Ca negative feedback loop (Goff, 1999). In cattle, there is evidence that a pre-calving diet high in P can have a negative impact on Ca homeostasis (Julien *et al.*, 1977; Kichura *et al.*, 1982; Barton *et al.*, 1987). During late gestation fetal skeletal development can withdraw up to 10 g P/day from the maternal P pools (House and Bell, 1993). About 0.3 g P is incorporated into each kg of body tissue (muscle) gained during growth of the animal. Production of milk removes about 1 g P from the extra cellular pool /kg of milk produced. Salivary secretions remove between 30 to 90 g P from the extra cellular P pool each day. Factors affecting salivary phosphate secretion include the time spent ruminating (chewing activity) and the PTH status of the animal. PTH stimulates parotid salivary P secretion (Wright *et al.*, 1984) and can increase salivary phosphate concentrations 2-3 fold. Salivary phosphate secretions help buffer the rumen and supply rumen microbes with a readily available source of P, which appears necessary for cellulose digestion.

Post-parturient haemoglobinuria characterized by intravascular haemolisis, anaemia, and haemoglobinuria is occasionally reported during the first 6 weeks of lactation. Many, but not all, cows developing this syndrome are hypophosphatemic. Severe hypophosphatemia is postulated to depress the ability of erythrocytes to produce ATP, as a key enzyme in glycolysis, glyceraldehyde-3-phosphate dehydrogenase, requires inorganic phosphate as a cofactor. Without sufficient ATP to power sodium pumps the intracellular sodium concentration rises, the cells become more rigid, and as a result, rupture as they pass through the capillary beds (Kaneko *et al.*, 1997).

District wise: The mean plasma Pi level showed highly significant ($P \leq 0.01$) difference in their mean values between the different districts during the pre to post-partum transition period. Highly significant difference ($P \leq 0.01$) was observed in the mean plasma Pi level between the cows of Bikaner, Jodhpur and Pali-Marwar farms during the pre and post-partum transition period (4.921± 0.156, 4.552± 0.163 in Bikaner, 5.759± 0.196, 4.203± 0.159 in Jodhpur and 5.315± 0.275, 4.949± 0.236 in Pali- Marwar mg/dl) (Table 4 and Fig. 9).

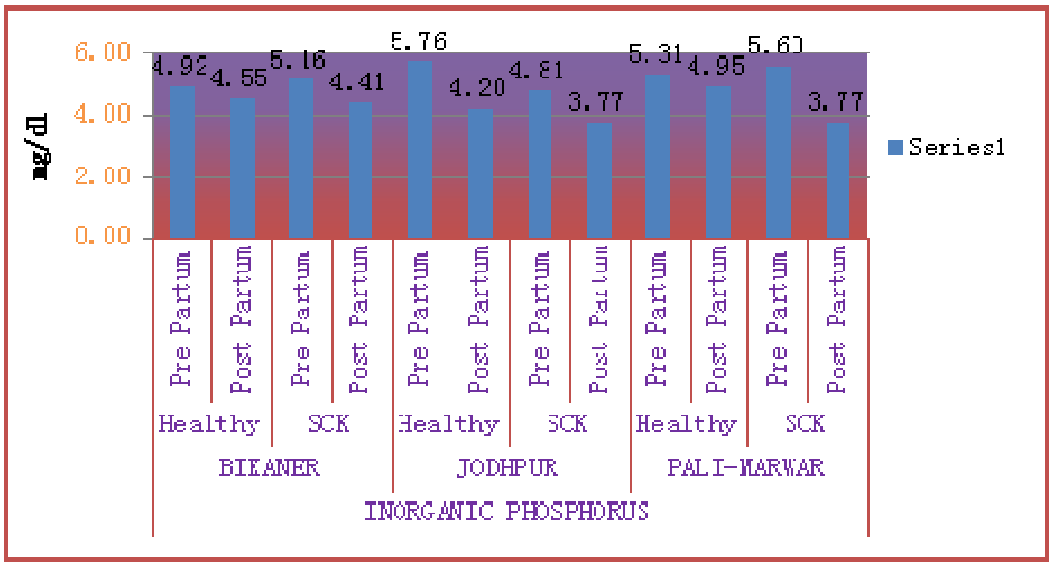

Fig. 9: Mean (±SE) plasma inorganic phosphorus values in healthy and SCK crossbred cows.

There are several contributing factors to lower Pi concentration in post-partum transition period. This post-partum decrease in plasma Pi level observed in the present study might be due to the fact that a large amount of Pi was drained from the blood for milk production and higher PTH level, induced by low plasma Ca, increased urinary loss and less excreted with faeces of phosphorus (Valk *et al.,* 2002). Moreover, lower feed intake in pre- partum cows reduced the amount of P absorbed from the intestinal tract (House and Bell, 1993).

Wilson *et al.* (1977) and Sahukar (1984) observed fall in phosphorus concentration during early lactation and on the day of parturition, might be due to enhanced carbohydrate metabolism and requirement of phosphorus for colostrum synthesis, which is known to be rich in phosphorus, (Rook and Thomas, 1983). Hypophosphetemia is common in high yielding cows grazing on pasture proposed by Payne (1972). In present study, the dairy animals were on stall feeding and no extra concentration (feed) were given during pre- partum transition periods; only few animals got the feed if lactating animals did not eat it than put to the advance pregnant cows.

The decrease of phosphorus level coincided with that obtained by Stockham and Scott (2002) and Ziogas *et al.* (2007). Insufficient phosphorus supply in the diet, prolonged anorexia, and increased urinary phosphorus excretion due to hyperparathyroidism could explain presence of hypophosphatemia in this condition. The primary site of P absorption is the small intestine (Care, 1994). Sanchez *et al.* (1994) study indicated that P uptake was reduced in cows under heat stress conditions. The primary route of P excretion is fecal (Hibbs and Conrad, 1983; Morse *et al.*, 1992). Plasma P concentration is normally between 1.3 and 2.6 mmol/L and 4 and 8 mg/dl. (Radostits, 2000).

High plasma Pi level was observed in cows from in 3 to 5th parity and non significant decrease was observed in all the two groups of parity from pre- partum to post- partum transition period. Similar to our results, Lane *et al.* (1968) also reported a negative correlation of age with phosphorus (Table 5).

4.2.2.3 Inorganic phosphorus (Pi) in SCK dairy cow:

The overall mean plasma Pi level in healthy and SCK affected cows were 5.287±0.117, 5.156±0.163 mg/dl during pre-partum period; 4.501±0.106, 4.100±0.087 mg/dl during post-partum period transition period, respectively (Table 3). Non Significant differences were observed between the healthy and SCK affected cows. Zhang *et al.* (2009) also reported non significant differences in Pi level in healthy and SCK affected cows.

The mean value of pre-partum and post-partum Pi level in Bikaner, Jodhpur and Pali –Marwar were 5.165± 0.264, 4.807± 0.143, 5.598± 0.348 and 4.409± 0.119, 3.765±0.069, 3.773± 0.023 mg/dl, respectively.

Significantly lower mean plasma Pi was in the cows from Jodhpur and Pali-Marwar (3.765±0.069, 3.773± 0.023 mg/dl) district during post-partum period as compared to those from Bikaner (4.409± 0.119 mg/dl) district (Table 5).

Ketz and Bergman (1966) reported low level of serum phosphorus due to lowered metabolic rate resulting from reduced feed intake in ketotic animals. This could have been the reason for increase in mean serum phosphorus level in the ketotic cows. In present study the levels of phosphorus differed non significantly among the healthy and subclinical ketosis. Radostits *et al.* (1997) and Kachhawaha and Tanwar (2010) observed low blood Pi in cases of parturient paresis and downer cow syndrome and credited these changes for the development of clinical signs.

4.2.3 Magnesium (Mg):

Interaction was not observed in magnesium level in transition periods of cross bred cow. The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for Magnesium in plasma was 3.500±0.061 mg/dl. The differences among subclass means were non significant across districts (3.458±0.097 mg/dl in Bikaner, 3.586±0.102 mg/dl in Jodhpur and 3.458±0.125 mg/dl in Pali-Marwar), non significant in healthy/SCK groups (3.475±0.068 in healthy and 3.526±0.105 mg/dl in SCK) and non significant in parity groups (3.341±0.085 mg/dl 3-5th parity and 3.660±0.085 mg/dl above 5th parity). The effect of pre (3.354±0.064 mg/dl) and post-partum (3.525±0.066 mg/dl) stages were highly significant in pooled data. There were no effects of two factor interaction indicating that difference among districts.

4.2.3.1 Magnesium (Mg) concentration in healthy dairy cow:

The overall mean plasma magnesium (Mg) concentration in crossbred cows from the various districts is presented in Table 3. The overall mean plasma magnesium concentration observed was 3.361±0.083 mg/dl during the pre-partum period; 3.457±0.085 mg/dl during the post-partum period, which was in accordance with the studies of Littledike *et al.* (1969), Phillipo *et al.* (1994) and Radostits *et al.* (1997).

The average value of plasma magnesium in all physiological groups of bovine was within the normal range. This may be attributed to adequate level of magnesium in soil and fodder. The concentration of magnesium in milk from cows in mid to late lactation was affected by dietary magnesium intake and the effect depended on the potassium level. The concentration potassium level will be low when the magnesium intake was high, but potassium level was high when the magnesium intake was low.

District wise: The mean plasma Mg concentration was 3.302±0.106, 3.555±0.174, 3.135±0.134 mg/dl during the pre-partum period and 3.286±0.124, 3.536±0.144, 3.747±0.186 mg/dl during the post-partum period in the cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. There was no significant difference in the cows of different districts during the pre and post-partum transition period. Plasma Mg concentration did not reflect any pattern in relation to parturition. However, general trend of increase was observed in all two districts. The elevated plasma Mg in cows along with the reducing calcium level observed in our study might be due to a combination of greater Mg mobilization from bone reduced Mg uptake by the cells and due to reduced urinary Mg loss as stated by Kincaid, 2000.

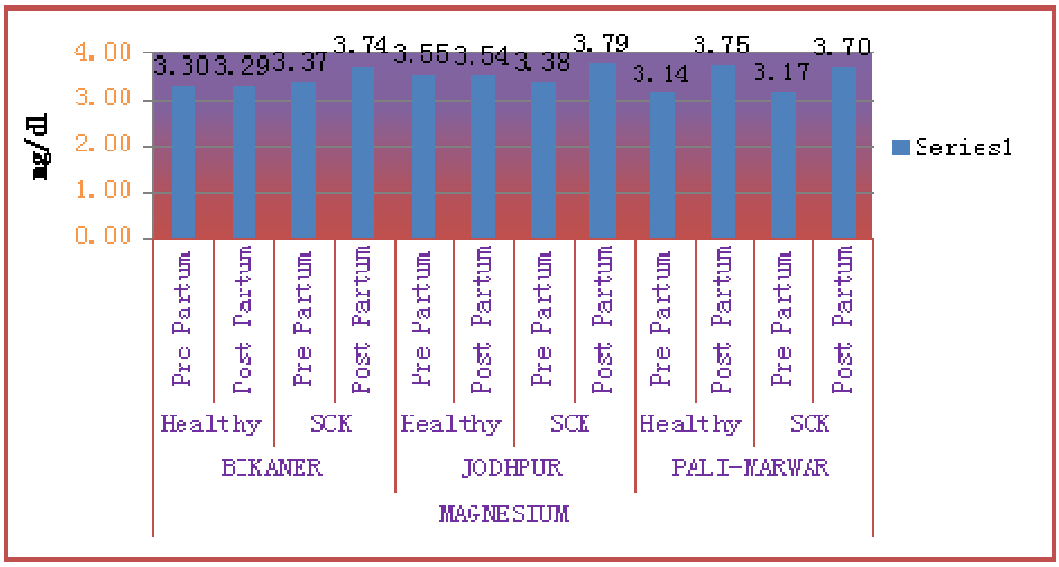

Fig. 10: Mean (±SE) plasma magnesium values in healthy and SCK crossbred cows.

No changes in Mg level were observed in cows of 3rd - 5th and above 5th parity. Singh (2013) recorded age related difference with significantly lower values recorded in group III (> 6 years) in buffaloes as compared to group I (<3 years) and group II (3-6 years). Randhawa (1999) and Singh *et al.* (2005) also detected decline in Mg level with advancing age. On the contrary, Carvalho (2013) reported non significant differences in the Mg level in the two age groups.

A transient increase in mean plasma Mg level was found after calving. This has also been recorded in other studies (Green *et al.*, 1981 and Goff and Horst 1997). Results of Goff *et al.* (2002) and Thilsing-Hansen *et al.* (2002) indicated that the increase in plasma Mg was related to the decrease in plasma Ca level after calving. The reason for this has been suggested to be the elevated renal threshold for Ca with parathyroid hormone being excreted as a response to the decrease in plasma Ca level resulting in a similar increase in the renal threshold for Mg (Goff 2008 and Taylor *et al.* 2008). Kronqvist (2011) reported that the peak in Mg level occurring at calving might depend on movements of Mg between the intracellular and extra cellular compartments rather than on decreased excretion. However, Verdaris and Evans (1976) and Rerat *et al.* (2009) reported decrease in mean plasma Mg concentration after calving. Mg is especially critical for ruminants, while non-ruminants absorb Mg primarily from small intestine, ruminants are able to absorb much of their Mg requirement from rumen via a Na-K-ATPase dependent process (Dua and Care, 1995). In fact, the reticulum and rumen can account for up to 80% of the Mg absorption along the entire digestive tract (Remond *et al.*, 1996). Green plants are an excellent dietary source of mg for animals because of the presence of Mg^{2+} in chlorophyll.

4.2.3.2 Magnesium (Mg) concentration in subclinical ketosis:

Overall mean plasma Mg concentrations observed were 3.361±0.083, 3.457±0.085 mg/dl in healthy cows and 3.333±0.045 and 3.743±0.033 mg/dl in SCK cows during pre and post-partum period, respectively. The differences were highly significant ($P \leq 0.01$) between pre and post-partum periods. District wise, mean plasma Mg level did not show any clear trend.

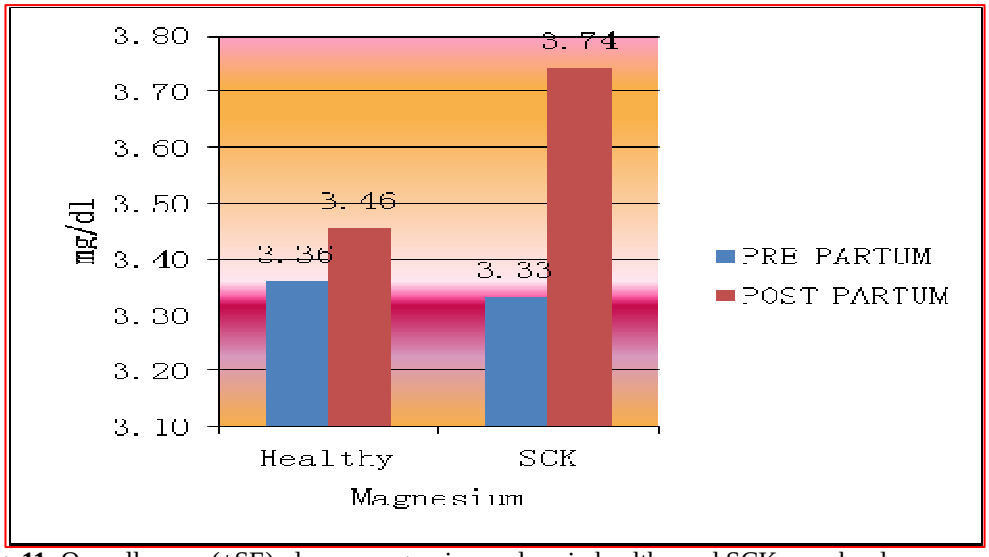

Fig. 11: Overall mean (±SE) plasma magnesium values in healthy and SCK crossbred cows.

Significant increased level of magnesium recorded in subclinical ketotic cows in post-partum period might be due to the shift of magnesium from the intracellular to the extra cellular compartment which might have caused transient increase in the Mg level. It could also be attributed to the increased tubular re absorption of magnesium due to the increased effect of serum PTH on kidneys (Riond *et al.,* 1995). Maynard *et al*., (1979) showed that Mg influenced the absorption of Ca and P and during high energy requirement, Mg requirement enhanced manifold.

4.2.4 Sodium (Na):

4.2.4.1 Interaction between districts and health affecting sodium level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for sodium in plasma was 116.825±0.661 mmol/l. The differences among subclass means were non significant across districts (116.192±1.050 mmol/l in Bikaner, 117.681±1.109 mmol/l in Jodhpur and 116.604±1.363 mmol/l in Pali-Marwar), non significant in healthy/SCK groups (116.566±0.741 in healthy and 117.085±1.137 mmol/l SCK) and non significant in parity groups (118.742±0.926 mmol/l 3-5th parity and 114.909±0.926 mmol/l above 5th parity). The effect of pre (119.081±0.781 mg/dl) and post-partum (115.049±0.633 mmol/l) stages were highly significant (P≤0.01) in pooled data. There were effects of two factor interaction indicating that difference among districts. The interaction between districts and health status was significant. The trend is depicted in Fig. 12. This showed that the sodium level was higher in healthy groups across all districts. The rise in their level was significantly higher in (119.593±2.318 to113.615±1.650 mmol/l) Pali-Marwar district and lowest (118.400±1.061 to 116.962±1.981 mmol/l) in Jodhpur districts.

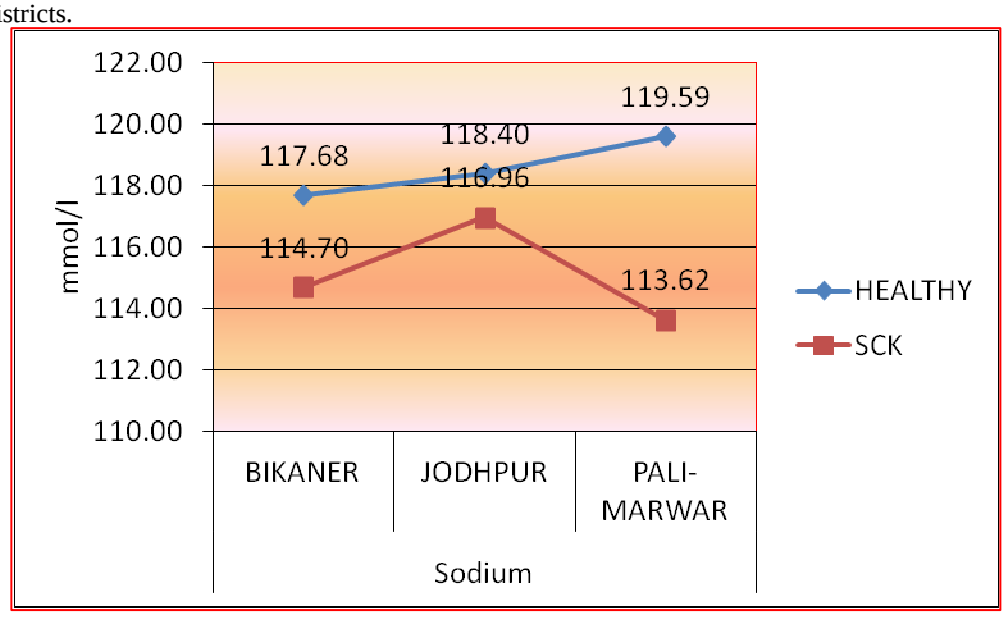

Fig. 12: Interaction between districts and health wise affecting sodium level

4.2.4.2 Sodium concentration in healthy dairy cow:

The overall mean plasma sodium (Na) concentration in crossbred dairy cows was 119.415±0.911 and 115.191±0.704 mmol/l during the pre and post-partum transition period. The overall mean plasma Na concentration was lower than the normal range (132-152 mmol/l) and a significant decrease was recorded from the pre to post- partum period.

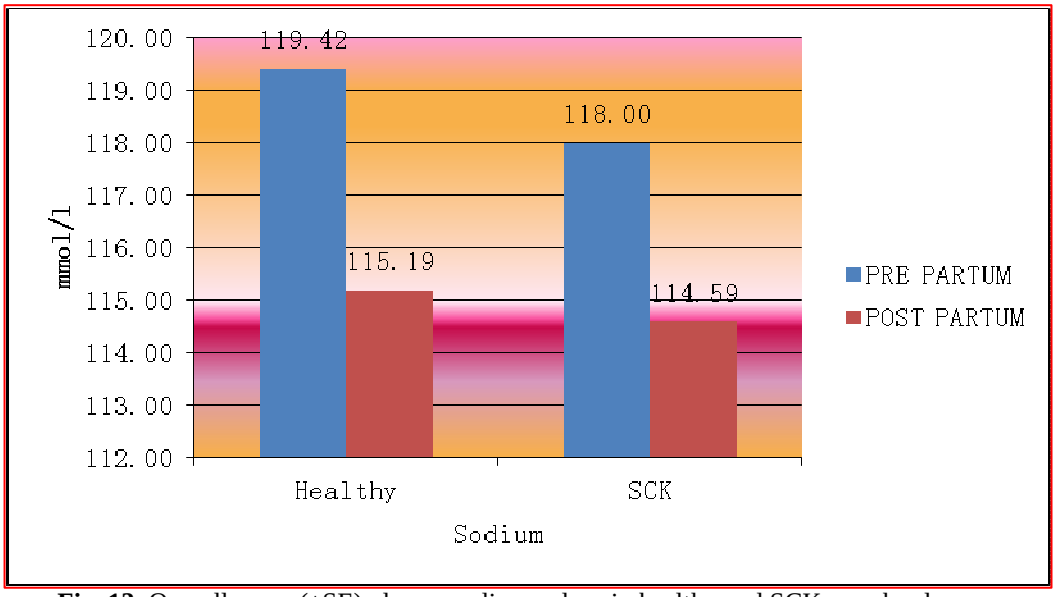

Fig. 13: Overall mean (±SE) plasma sodium values in healthy and SCK crossbred cows.

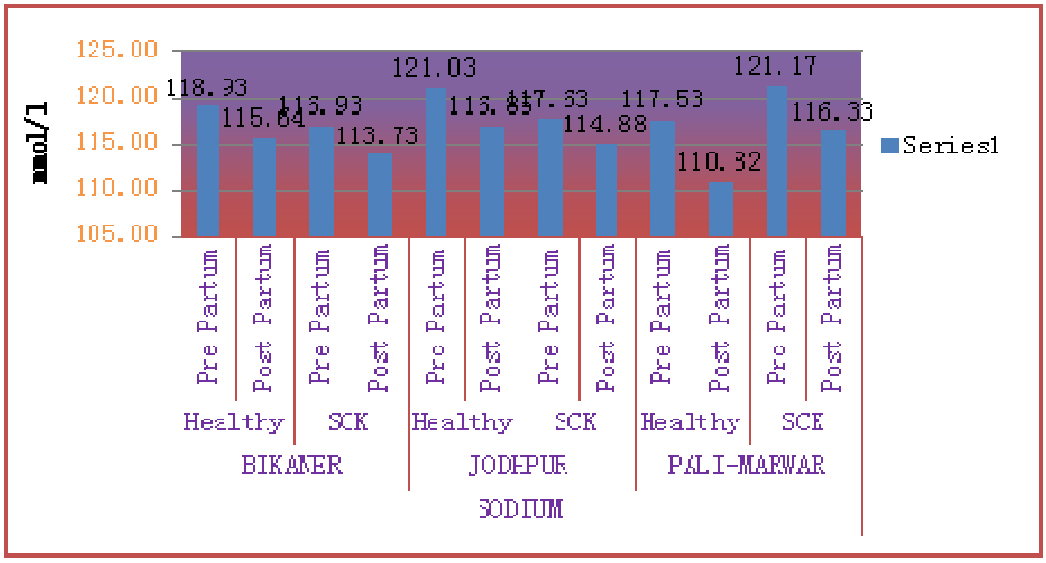

Fig. 14: Mean (±SE) plasma sodium values in healthy and SCK crossbred cows.

District wise: The mean plasma Na concentrations from various districts were 118.931± 1.217, 121.030±1.718, 117.529±2.079 mmol/l during the pre-partum period; 115.636±1.013, 116.848±1.158, 110.823±1.422 mmol/l during post-partum transition period in the cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. The mean plasma Na concentrations was no significantly different across districts (Table 4).

Parity wise, mean plasma sodium level was less than the normal range in the two groups and non significant decrease was recorded in 3rd to 5th and above 5th parity groups from pre to

post-partum transition period (Table 5). Non significant changes occurred between the groups.

Rowlands *et al.* (1975), Underwood (1981) and Deshpande *et al.* (1998) also recorded a progressive decrease in the plasma sodium level from late pregnancy to early lactation. They concluded that the most likely reason for the decrease was due to the drainage of sodium in milk during early lactation.

4.2.4.3 Sodium (Na) concentration in subclinical ketosis:

Overall mean plasma Na level recorded in both healthy and SCK affected cows were 119.415±0.911, 118.000±1.508 mmol/l during pre-partum period and 115.191±0.704, 114.586±1.432 mmol/l during post-partum period, which were lower than the normal physiological range (132-152 mmol/l) (Radostits *et al.*, 2000). Significant difference was observed in healthy and subclinical ketotic dairy cows in districts. A highly significant (P≤0.01) decrease was noticed in the mean plasma Na level in between the pre to post-partum transition period (Table 3). District wise analysis revealed a non significant decrease in the mean plasma Na level from the pre to the post-partum period in cows but between the districts non significant level was recorded. Zhang *et al.* (2011) reported no significant effect on plasma Na level in cows with sub clinical ketosis.

4.2.5 Potassium (K):

4.2.5.1 Interaction (between health and parity; health and stage) affecting potassium level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for potassium in plasma was 4.706±0.084 mmol/l. The differences among subclass means were non significant across districts (4.836±0.133 mmol/l in Bikaner, 4.655±0.141 mmol/l in Jodhpur and 4.627±0.173 mmol/l in Pali-Marwar), significant in healthy/SCK groups

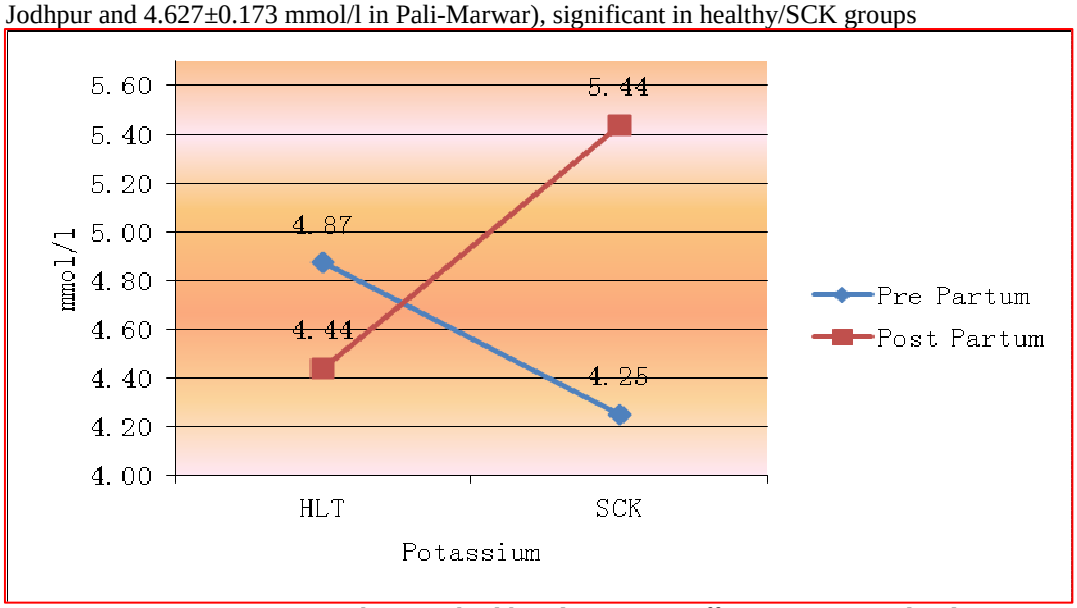

Fig. 15: Interaction between health and stage wise affecting potassium level.

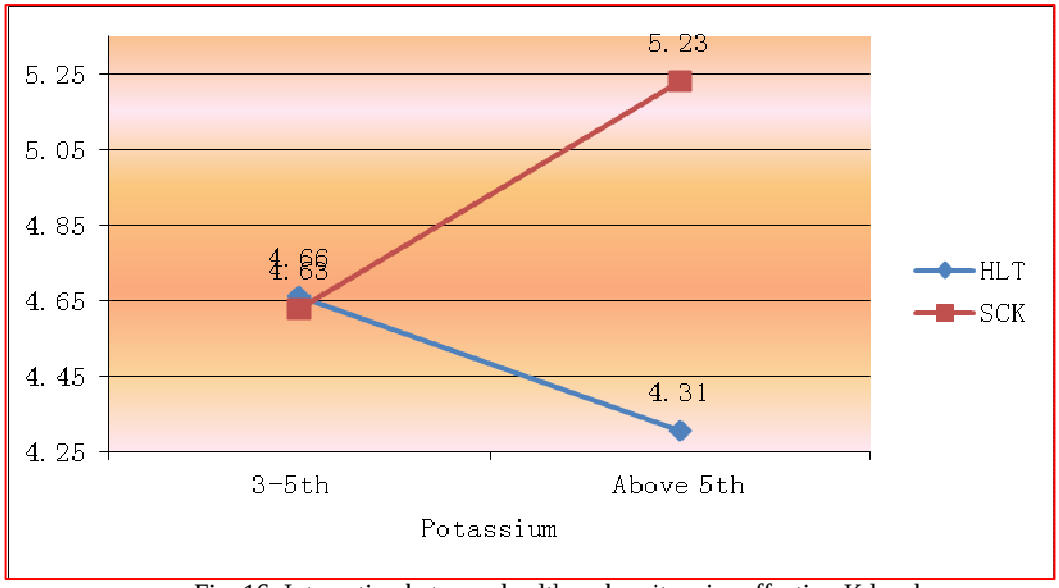

Fig. 16: Interaction between health and parity wise affecting K level

(4.483±0.094 in healthy and 4.929±0.145 mmol/l SCK) and non significant in parity groups (4.514±0.118 3-5th parity and 4.898±0.118 mmol/l above 5th parity). The effect of pre (4.726±0.089 mg/dl) and post-partum (4.674±0.104 mmol/l) stages were significant (P≤0.05) in pooled data. There were no effects of two factor interaction indicating that difference among districts. Whereas the interaction between health and stage group was highly significant (P≤0.01). The trend is depicted in Fig. 15. This showed that the potassium level was lower in healthy groups. The chances of SCK to ketosis will be more and animals were in dehydration due to excess loss of sodium and accumulation of K in blood causing depletion. In stage and health wise interaction, in pre- partum stage almost equal and post-partum stage high level of K level in SCK dairy cow. The interaction between parity and health was also highly significant (P≤0.01). The trend is depicted in Fig. 16. This showed the potassium level was higher in subclinical ketosis dairy cows of above 5th parity.

4.2.5.2 Healthy dairy cow potassium (K):

The overall mean plasma K concentration recorded was 4.873±0.094 mmol/l during pre-partum period; 4.439±0.098 mmol/l during post- partum period, which was well within the normal physiological range (3.9-5.8 mmol/l) for the cows. However a significant (P≤0.05) decrease was recorded in the overall mean plasma K level from the pre to post-partum transition period (Table 3).

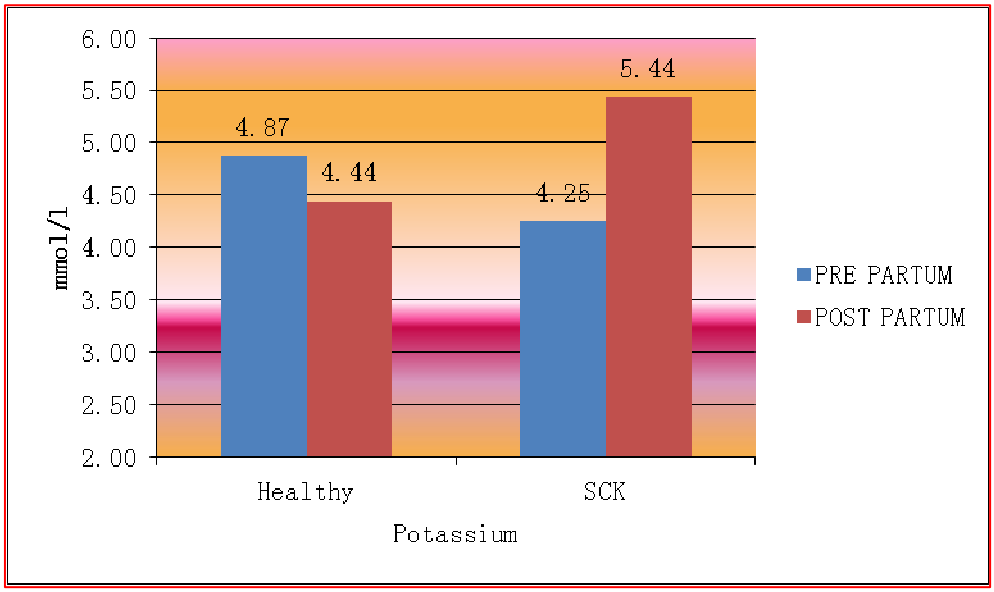

Fig. 17: Overall mean (±SE) plasma potassium values in healthy and SCK crossbred cows.

District wise: Mean plasma K concentration were 4.806±0.123, 5.203± 0.161, 4.405± 0.227 mmol/l during pre-partum period; 4.613± 0.139, 4.435± 0.172, 3.998± 0.206 mmol/l during

post-partum period in cows from Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. Non significantly lower mean K concentration was recorded in dairy farms during the pre to post-partum period (Table 4).

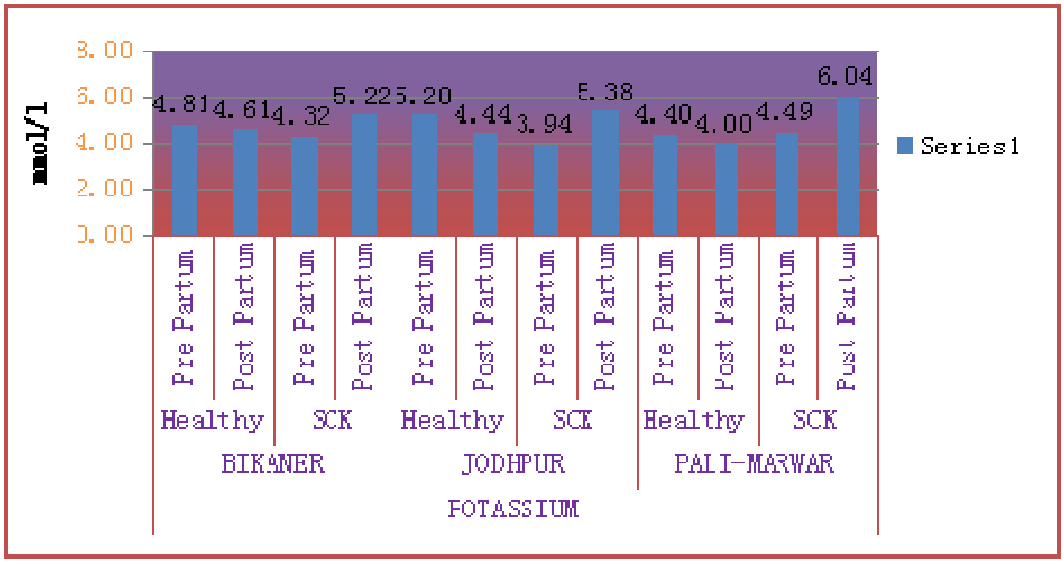

Fig. 18: Mean (±SE) plasma potassium values in healthy and SCK crossbred cows.

Overall Mean plasma K level recorded lower in cows in 3rd -5th parity (4.514±0.118 mmol/l) in comparison to above 5th parity cows (4.898±0.118 mmol/l) and there was no significant difference in parity wise in K level (Table 5). Parity wise, highly significant (P≤0.01) differences were recorded in healthy and subclinical ketotic dairy cows and stage wise (Pre to post-partum transition periods).

Plasma K level did not show any definite pattern during different parity; while a decrease was noticed after calving in farms during the early milking period. Different workers have proposed several hypotheses for these variable patterns. Some workers have concluded that the decrease in K level might be due to increased transfer of this cation into the milk (Jacob *et al.*, 2011). Similar results were reported by various other authors viz. Jacob (2000) in crossbred heifers and Sivaraman *et al.* (2003) in Jersey crossbred cows, Padodara *et al.* (2012) in triple crossbred cattle. Certain workers (Tainturier *et al.*, 1984, Deshpande *et al.* 1998 and Dodamani *et al.*, 2009) found non significant differences in serum K level during different stages of pregnancy. However, the increase in K concentration was recorded after pregnancy might be due to the fact that K is located mostly within the cells and is needed for maintenance of acid-base balance in the body and for normal tissue protein synthesis, Ca dependent big K channel (Bk (ca) channel) in protein depleted animals during the advanced pregnancy, which helps in the uterine relaxation at the time of labor (Choudhury *et al.*, 2011).

4.2.5.3 Potassium (K) concentration in subclinical ketosis dairy cow:

Overall mean plasma K concentrations recorded in healthy and SCK cows were 4.873±0.094, 4.251±0.199 mmol/l during pre-partum period; 4.439±0.098, 5.436±0.264 mmol/l during post-partum period, respectively. The differences between the healthy and sub clinical ketotic animal in pre-partum and post-partum transition period were significant. District wise, no significant variation was noticed in K concentration in cows from the entire district from the pre and post-partum period (Table 3). Findings of Zhang *et al.* (2011) revealed non significant differences in the plasma K level between healthy and SCK affected cows.

The higher concentration of ketone bodies in blood caused metabolic acidosis and thus, resulted in urinary loss of sodium and accumulation of potassium in plasma causing further dehydration. To compensate the loss of bicarbonate this normally occurs during metabolic acidosis, the excess of chloride ions got shifted from intracellular fluid (lCF) to extra cellular fluid (ECF) and, hence, its level increased in the serum of sub-clinical ketotic animals (Carison, 1997; Sharma and Kumar, 2001).

4.2.6 Copper (Cu):

4.2.6.1 Interaction between districts and health affecting copper level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for copper in plasma was 0.694±0.012 ppm. The differences among subclass means were

highly significant (P≤0.01) across districts (0.767±0.020 ppm in Bikaner, 0.758±0.021 ppm in Jodhpur and 0.556±0.026 ppm in Pali-Marwar), highly significant in healthy/SCK groups (0.754±0.014 in healthy and 0.633±0.021 ppm SCK) and non significant in parity groups (0.755±0.017 ppm 3-5th parity and 0.632±0.017 ppm above 5th parity). The effect of pre (0.847±0.019 mg/dl) and post-partum (0.702±0.016 ppm) stages were highly significant (P≤0.01) in pooled data. There were no effects of two factor interaction indicating that difference among districts. Whereas the interaction between districts and health status was significant (P≤0.05). The trend is depicted in Fig. 19. It shows that the Copper level was lower in healthy groups across all districts but the rise in their level was significantly higher in (0.878±0.018 to 0.656±0.034 ppm) Bikaner district and lowest (0.589±0.031 to 0.524±0.044 ppm) in Pali-Marwar district.

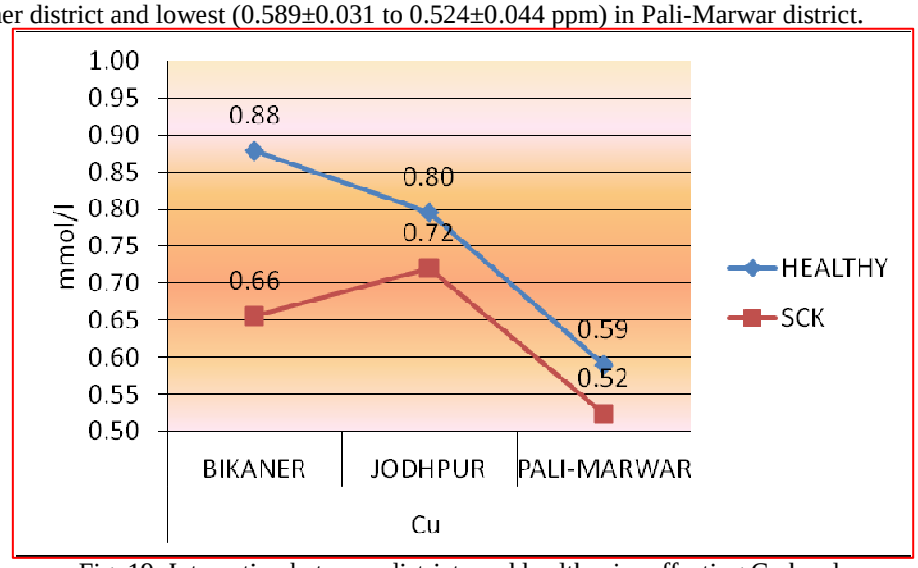

Fig. 19: Interaction between districts and health wise affecting Cu level.

4.2.6.2 Copper (Cu) concentration in healthy dairy cow:

The overall mean plasma copper (Cu) concentration recorded was 0.886±0.022 ppm during pre-partum; 0.738±0.018 ppm during post-partum transition period which was within the normal physiological range 0.60-1.50 ppm as stated by McDowell (1992). However, a highly significant (P≤0.01) decrease was recorded in Cu concentrations between pre to post-partum transition period (Table 3).

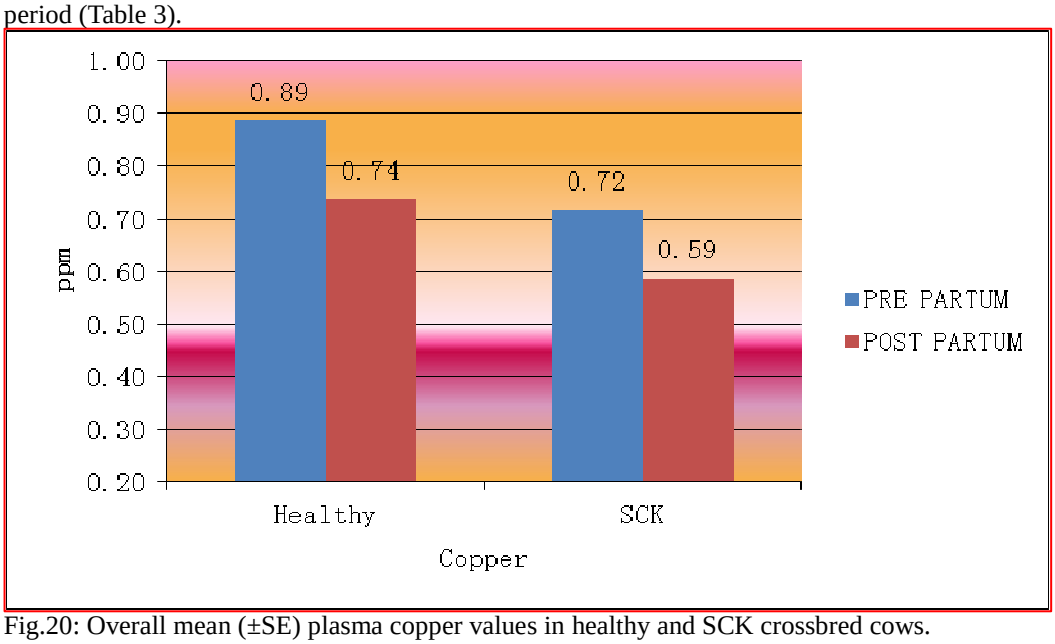

Fig.20: Overall mean (±SE) plasma copper values in healthy and SCK crossbred cows.

From the findings of Gooneratne *et al.* (1986a and 1986b), it appears that Cu is actively transported across the placenta from the dam to the foetus and storage of Cu by the growing foetus proceeds even when the dam is fed a Cu deficient diet, at the expense of maternal Cu reserves. The gradual reduction in plasma Cu concentration from 7-month of gestation period might have been due to increased foetal accumulation of Cu and the homeostatic mechanisms of the dam operating to maintain the dam plasma Cu concentration at a reasonable level and

conserved much needed Cu by minimizing losses via bile during that time. The variations in plasma Cu concentrations under the present different stage (pre and post transition period) are a reflection of the net result of foetal demand and/or lactation. Thus the plasma Cu concentrations in pregnant cattle observed in the present study was in agreement with those reported by Smart *et al.* (1986) and Gooneratne *et al.* (2013). The intra-ruminal complexing of the copper with insoluble sulphide and thiomolybdate leads to less copper availability in connection with development of rumen function (Suttle, 1975). The average value (ppm) of serum copper of cattle ranged from 0.58-0.67 ppm as against the critical limit of 0.65 ppm (McDowell, 1987). Similar finding was also reported by Das *et al.* (2003), Sharma *et al.* (2003b), Hussain (2006), Kumar (2006), Das (2007), Kumar *et al.* (2008), Sharma *et al.* (2009), Turkar (2010) and Bhat *et al.*, (2011) in hill zone of West Bengal, Haryana, Vidharba region of Maharastra, Tripura, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh and Ganderbal region of Kashmir, respectively.

District wise: The mean plasma Cu concentration recorded was 0.962± 0.026, 923±0.035, 0.619±0.022 ppm during pre-partum period and 0.832±0.025, 0.711±0.025 and 0.546±0.015 ppm during post-partum transition period from the various farms of the Bikaner, Jodhpur and Pali-Marwar dairy farm. District wise significantly (P≤0.05) lower values were recorded in all the district but in Pali-Marwar dairy cows copper level was low during the pre and post-partum period as compared to the cows from the other districts (Jodhpur and Bikaner) (Table 4). Non significant differences were observed between all districts during pre and post- partum transition period.

Mean plasma Cu level was highest in cows in 3rd to 5th parity (0.755± 0.017 ppm) and a non significant decrease was observed in cows from pre to post-partum transition period with the lowest (0.632± 0.017 ppm) level being observed in cows above 5th parity during the post-partum period (Table 5).

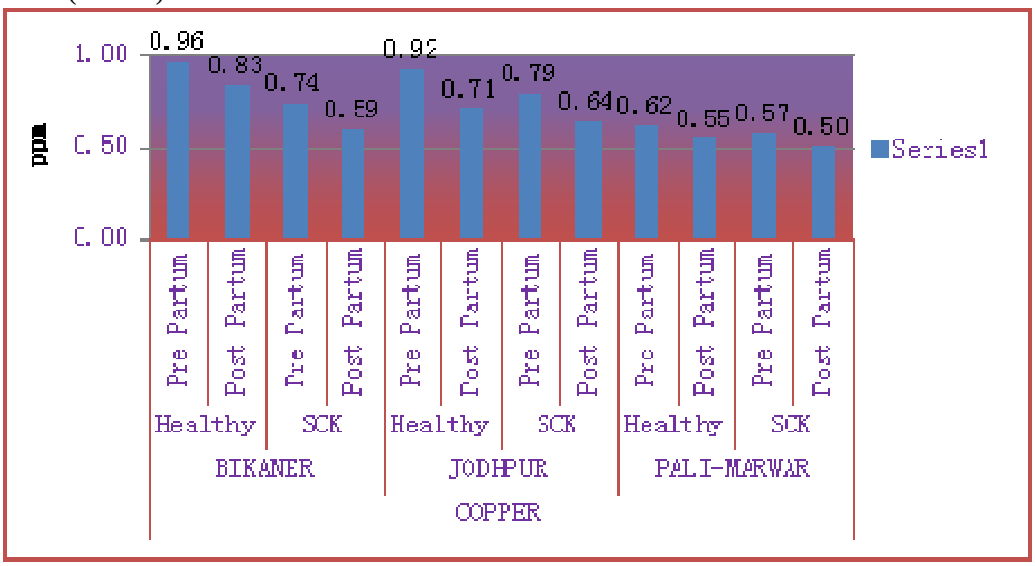

Fig. 21: Mean (±SE) plasma copper values in healthy and SCK crossbred cows.

The importance of Cu as an essential trace element has been recognized for over 70 years, with the early discovery that Cu was necessary for normal haemoglobin synthesis in young rabbits and rats. Since that time, the importance of Cu for normal growth, production and reproductive performance has been established. The biological role of Cu is exerted through a number of Cu-containing proteins including ceruloplasmin and superoxide dismutase (SOD) (Prohaska, 1991). The high serum Cu level in pregnant animals could be related to increased Cu in the form of ceruloplasmin enzyme in response to increased blood estrogen or progesterone (Sato and Henkin, 1973; Yokus and Cakir, 2006) or a decrease in serum Cu concentration during lactation because of being secreted out with milk (Underwood, 1971) and being stored in the liver before being secreted into milk (Adelstein and Vallee, 1962). Pathak *et al.* (1986) also reported that an increase in Cu concentration from the last 10 days of gestation towards parturition was a requirement to trigger on the endocrine glands related to the physiology of initiation of labor pain and process of parturition. One hypothesis for the decrease in Cu level during early lactation might be due to the decrease in albumin level during early pregnancy as albumin was required for the transport of Cu in the form of low-molecular-weight complexes (histidine) to target tissues, particularly the liver (Kaneko *et al*, 2008). Noaman (2013) concluded that reduced Cu level observed in his study may be due to the higher Fe level which caused inhibition of the intestinal

Cu absorption, which is similar to our study.

4.2.6.3 Copper (Cu) concentration in subclinical ketosis dairy cow:

Overall mean plasma Cu concentration was 0.886±0.022, 0.718±0.028 ppm during pre-partum period and 0.738±0.018, 0.587±0.023 ppm during post-partum period in healthy and SCK cows, respectively. Highly Significant (P≤0.01) differences across two stages were recorded in plasma Cu values between healthy and SCK cows.

Highly significant (P≤0.01) variation was recorded in mean plasma Cu concentration from pre to post-partum period in cows from Bikaner (0.737± 0.042 and 0.593± 0.038), Jodhpur (0.793± 0.034 and 0.639± 0.023 ppm) and Pali Marwar (0.570± 0.023 and 0.503± 0.022 ppm) district (Table 4), respectively. Contrary to the present findings, Zhang *et al.* (2010) did not observe any effect of subclinical ketosis on the mean plasma Cu level in the healthy and SCK affected cows. Puis (1994) reported that marginal copper deficiency causes reduced growth rates and reduced feed efficiency, fertility and increased incidence of retained placenta and chronic diarrhoea in buffalo calves (Kachhawaha, 2011). Phillipo *et al.* (1987) showed reduced conception rates and disrupted estrus activity in cattle suffering from a primary copper deficiency. Similarly in Pali district farmer reported repeat breeder and abortion in pregnant animal and chronic diarrhoea in young calf.

4.2.7 Iron (Fe):

4.2.7.1 Interaction between health and stage affecting iron level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for iron in plasma was 2.121±0.045 ppm. The differences among subclass means were non significant across districts (2.172±0.072 ppm in Bikaner, 2.009±0.076 ppm in Jodhpur and 2.181±0.094 ppm in Pali-Marwar), non significant in healthy/SCK groups (2.027±0.051 in healthy and 2.214±0.078 ppm SCK) and non significant in parity groups (2.302±0.064 ppm 3-5th parity and 1.939±0.064 ppm above 5th parity). The effect of pre (2.276±0.058 ppm) and post-partum (1.846±0.045 ppm) stages were highly significant in pooled data. There were no effects of two factor interaction indicating that difference among districts. Whereas the interaction between health and stage was significant (P≤0.05). The trend is depicted in Fig. 22, which showed that the iron level was significantly higher in healthy group in comparison to SCK.

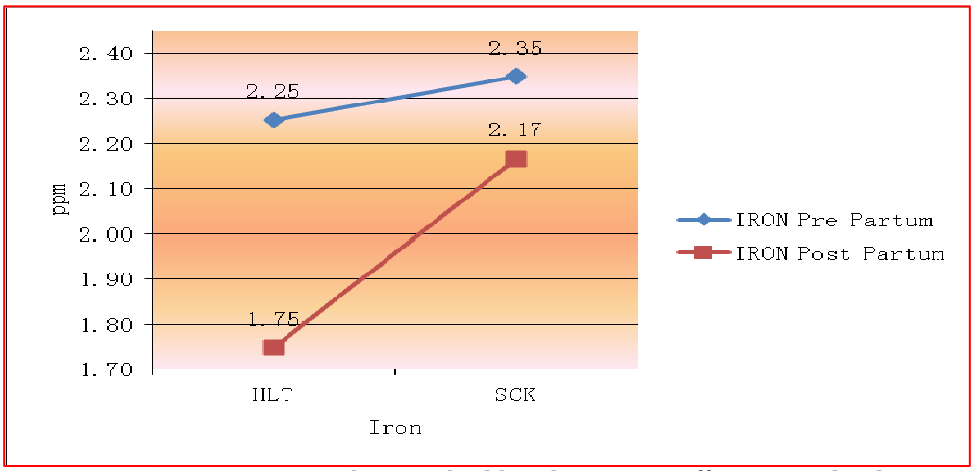

Fig. 22: Interaction between health and stage wise affecting iron level.

4.2.7.2 Iron (Fe) concentration in healthy dairy cow:

The overall mean plasma iron (Fe) concentration recorded was 2.253±0.073 ppm during the pre-partum period and 1.748±0.052 ppm during post-partum transition period. Highly significant decrease was noticed in the mean plasma Fe level from the pre-partum period to the post-partum period in the overall mean values. The overall mean values were higher than normal physiological range of 1-2 ppm (Radostits *et al.*, 2000). Pregnancy stimulates increased iron absorption from gastrointestinal tract to meet the requirement of foetus thus increasing serum iron. However, as pregnancy advances foetal demands overtake the rate of absorption and thus decreased value of serum and also due to increase in plasma volume resulting in decrease in haemoglobin and consequently iron concentration (Soliman and Amrousi, 1965). Hb level decrease during pregnancy due to transfer of Hb across the placenta and haemodilution (Singh *et al.*, 1992).This might be because of utilization of iron by mammary gland. Azab and Maksoud (1999) also reported similar findings in Baladi goat. The mean plasma iron concentration differed significantly (P≤0.05) among different groups.

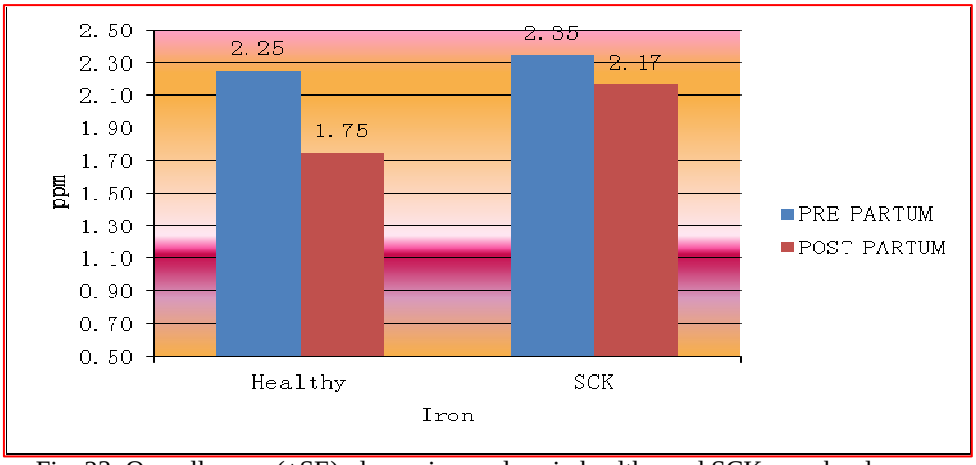

Fig. 23: Overall mean (±SE) plasma iron values in healthy and SCK crossbred cows.

District wise: Mean plasma Fe level recorded from the farms of the Bikaner, Jodhpur and Pali Marwar district dairy farm were 2.500± 0.111, 1.961± 0.100, 2.182± 0.159 ppm during pre-partum period and1.855 ±0.075, 1.591± 0.090 , 1.776± 0.111 ppm during post-partum transition period respectively. Non significant difference was recorded in the plasma Fe level in the cows of all district dairy cows from during the pre and post-partum period. However, the mean plasma Fe level were above the normal physiological range and it did not follow any clear trend along with parturition (Table 4).

Fig. 24: Mean (±SE) plasma iron values in healthy and SCK crossbred cows.

Mean plasma Fe level was recorded in cows in 3rd to 5th parity (2.197±0.086, 1.761±0.063 ppm) and in cows above 5th parity (2.426±0.127 and 1.709±0.092 ppm) in pre and post-partum transition period which were non significant in parity, healthy and stage wise. However, Singh *et al.* (2003) recorded comparatively lower mean values in adult cattle of 3-6 years of age groups (117.92±92µmol/l) than cattle of >6 years age groups (127.85±1.09µmol/l) and young cattle of <3 years of age (127.67±14.51µmol/l).

Iron plays an important role in many biochemical reactions such as anti-oxidant defense system, energy and protein metabolism, as a haem respiratory carrier, oxidation-reduction reactions and in electron transport system (Andreiu, 2008). Thus, it may be expected to have indirect effects on the animals during periparturient period. The present findings closely corroborated with the reports of Bostedt *et al.* (1974) and Rupde *et al.* (1993). The high Iron concentration during the late pregnancy and low during early lactation might be due to the increased transfer of this nutrient across the placenta and haemodilution during late pregnancy and at calving, together with the initiation of ovarian follicular activity postpartum, leading to high circulatory estrogen which stimulated the binding of iron with the proteins in liver and, thereby, increased concentration in plasma (Mehere *et al.*, 2002; Jacob *et al.*, 2003).

However, Akhtar *et al.* (2009) reported a decrease in the mean Fe concentration with approaching parturition and cited the possible reason for the decrease due to the utilization of Fe by mammary gland.

4.2.7.3 Iron (Fe) concentration in subclinical ketotic dairy cow:

Overall mean plasma Fe concentrations in healthy and SCK affected cows were 2.253±0.073, 2.348±0.066 ppm during pre-partum period; 1.748±0.052, 2.166±0.051 ppm during post-partum transition period, respectively. Significant differences were recorded between healthy and SCK and different stages.

District wise, mean value were 2.343± 0.101, 2.197±0.078; 2.310± 0.098, 2.094± 0.085 and 2.410± 0.169, 2.183± 0.117 in pre and post-partum transition period in Bikaner, Jodhpur and Pali –Marwar, respectively. Non significant decrease was observed in the mean plasma Fe level from pre to post-partum period in cows in the all districts. (Table 4).

4.2.8 Zinc (Zn):

There was no interaction recorded in zinc level in transition periods of cross bred cow. The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for zinc in plasma was 1.068±0.009 ppm. The differences among subclass means were non significant across districts (1.087±0.015 ppm in Bikaner, 1.076±0.016 ppm in Jodhpur and 1.041±0.020 ppm in Pali-Marwar), non significant in healthy/SCK groups (1.067±0.011 in healthy and 1.069±0.016 ppm in SCK) and significant in parity groups (1.171±0.013 ppm in 3-5th parity and 0.964±0.013 ppm in above 5th parity). The effect of pre (1.189±0.011 ppm) and post-partum (0.985±0.011 ppm) stages were highly significant (P≤0.01) in pooled data. There were no effects of two factor interaction indicating difference among districts.

4.2.8.1 Zinc (Zn) concentration in healthy dairy cow:

The overall mean plasma zinc (Zn) level recorded were 1.188±0.013 and 0.988±0.013 ppm during pre and post-partum period. Highly significant (P≤0.01) decrease was recorded in the overall mean Zn concentration from the pre-partum period to the post-partum transition period (Table 3).

In post-partum cows lower plasma zinc values were recorded as compared to pre-partum groups. This may be due to positive correlation of zinc of milk to dietary intake in case of neonatal feeding (Underwood, 1981).

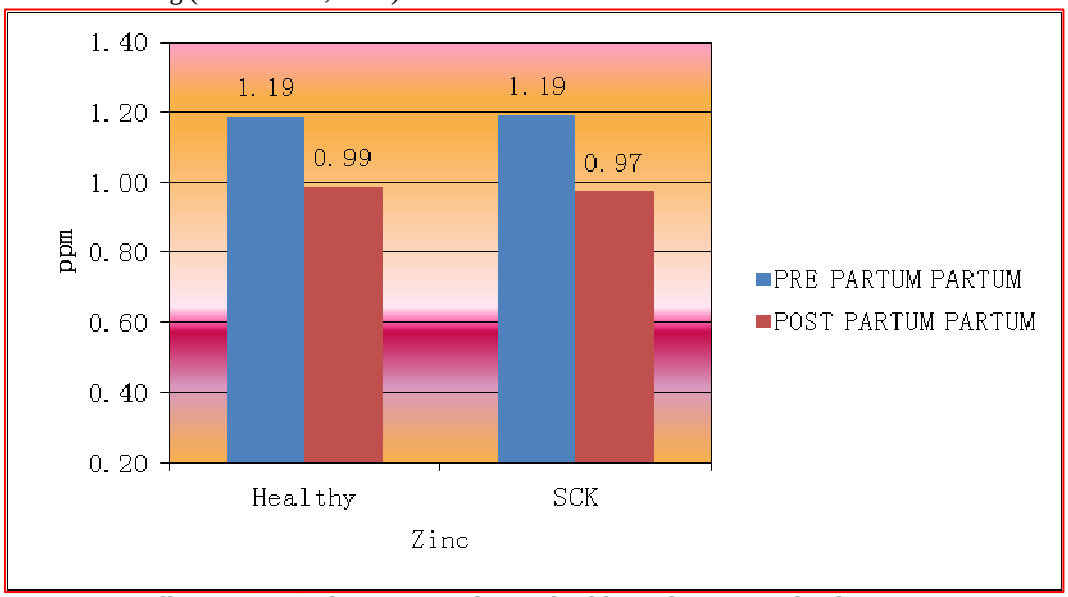

Fig. 25: Overall mean (±SE) plasma zinc values in healthy and SCK crossbred cows.

District wise: The mean plasma Zn concentration was 1.235± 0.021, 1.157±0.016, 1.128±0.024 ppm during the pre-partum period; 1.019± 0.021, 0.983±0.015, 0.916±0.025 ppm during the post-partum period in the cows Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. No significant difference was observed across the district during the pre and post-partum period a (Table 4 and Fig. 26).

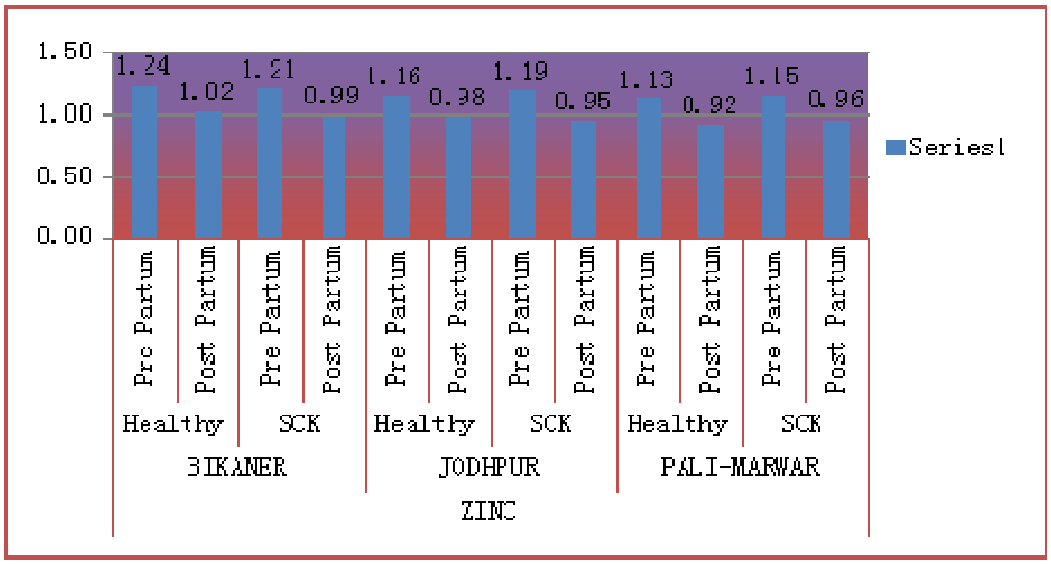

Fig. 26: Mean (±SE) plasma zinc values in healthy and SCK crossbred cows.

Overall parity wise significant difference was recorded in pre and post-partum transition period. Mean plasma Zn level were 1.193± 0.015, 0.989± 0.015 in 3rd to 5th parity and 1.176± 0.029 and 0.983± 0.024 in above 5th parity in pre to post transition period. No significant changes were observed in Zn level in healthy and SCK dairy cows during transition period in different parity.

Reduced mean plasma Zn concentration around the peripartum might be due to the fact that during the late term fetuses accumulate Zn at a rate of about 12mg/day (House and Bell, 1993) and also during early lactation Zn was required for the colostrum synthesis (Kincaid and Cronrath 1992), in addition glucocorticoids reduced Zn absorption combined with various stressors to stimulate metallothionoein synthesis, which pulled Zn into cells. Zinc is primarily involved in protein synthesis, carbohydrate and nucleic acid metabolism. The role in reproduction is pronounced in males. Reduced level of Zn in diet might also be the factor for reduced Zn level in plasma. Similarly, Alonso *et al.* (2000) suggested copper induced inhibition of Zn intestinal absorption for the inverse relationship observed between plasma concentrations of Zn and Cu in cows during the late pregnancy period. In our study, both the micro minerals (Zn and Cu) were low in post-partum transition periods and they had no inverse relationship.

4.2.8.2 Zinc (Zn) concentration in subclinical ketosis:

Overall mean plasma Zn level in healthy and SCK affected cows were 1.188±0.0131.192±0.020 and 0.988±0.013, 0.974±0.020 ppm, respectively from pre to post-partum period (Table 3). Non significant differences were observed between both groups. However, Zhang *et al.* (2010) reported a significant decrease in the mean plasma Zn level in SCK cows as compared to the healthy cows. A non significant decrease was observed in the mean plasma Zn level from the pre-partum to the post-partum period in both the groups (healthy and SCK cows), as it has been reported that decreased Zn concentration influenced the generation and metabolism of BHBA, by affecting the secretion of insulin in dairy cows with subclinical ketosis (Hayirli, 2006). However, a non significant effect was seen in all the cows from various districts in between pre and post-partum period (Table 4).

4.2.9 Prevalence of deficiencies status of various macro and micro mineral:

Mineral deficiency is a common problem in livestock farming affecting the animal productivity and thereby the economy of dairy farmers. Several studies have been carried out in different regions of the country revealed deficiencies of either one or more minerals (Sharma *et al*., 2006; Sharma and Joshi, 2004; Sharma *et al*., 2003b; Kumar, 2006; Prasad (1998), Hussain, 2006, Turkar, 2010; Sarkar *et al*., 2004; Ramana *et al*., 2000; Chaudhari and Gupta, 1983; Das *et al*., 1997, Baruah and Baruah, 1997; Garg *et al*., 2000; Yatoo, 2011). From available literature it is evident that no such study has been carried out in Rajasthan.

Based on the results of analysis, prevalence for the deficiencies for various minerals in crossbred cows was calculated in Bikaner, Jodhpur and Pali-Marwar districts of Western Raja.

4.2.9.1 Calcium:

The overall prevalence of Ca deficiency in cows of various districts was 34.15 per cent (42/123); which were marginally deficient (Ca= 5.17-8.15 mg/dl) (Table 6 and Fig. 27). Ribeiro *et*

al. (2013) reported a prevalence of 43.30 per cent for subclinical hypocalcaemia in cows during the first 10 days postpartum. However, overall calcium deficiency (34.15%) was higher than that for plasma phosphorus (31.70%) in the dairy cow of different district of western Rajasthan. Similarly, Sharma *et al.* (2006), Ozukum (2011), Siddique (2011) and Singh (2013) reported an overall prevalence of calcium deficiency of 22.89, 30.12, 22.45 and 16.60 per cent respectively in dairy animals. While Houe *et al*. (2001) reviewed several studies that recorded an incidence rate for subclinical hypocalcaemia between 23 and 39 %. Low Ca diets fed pre-partum are commonly used as a control strategy for milk fever. Calcium intake limited to less than 30 g of total Ca/ day (Sorensen *et al*., 2002). The mean plasma calcium level of all category dairy cow were close to the normal physiological range (8-10.5 mg/dl).

District wise: Analysis of results revealed that highest deficiency was shown by the animals of Jodhpur district with a deficiency of 43.90 per cent (18/41), and all the animals were marginally deficient in the Ca. Cows from the Bikaner district showed an overall deficiency of 25.42 per cent (15/59), which were marginally deficient. Similarly in Pali Marwar out of 23 cows, 14 animals (60.87 %) showed normal calcium level (>8.15 mg/dl) and 9 animals (39.13%) were marginal deficient in calcium (Table 6).

Parity wise: Maximal deficiency was observed in cows above 5^{th} parity, with a prevalence of 50 per cent (17 out of 34), followed by cows in 3^{rd} to 5^{th} parity with a prevalence of 28.09 per cent (25/89). However, Ozukum (2011) and Singh (2013) reported highest deficiency in dairy animals of 3-6 years age group followed by >6 years age group (Table 7).

4.2.9.2 Inorganic phosphorus (Pi) :

The overall prevalence of hypophosphataemia in the cows from all the districts was found to be 40.65 per cent (50/123) (Table 6 and Fig. 27). In the present study, among the major elements, the prevalence of plasma calcium and phosphorus deficiency was more in post-partum dairy cow as compared to pre-partum. This is probably due to the requirement of these minerals for productivity and the physiological status (Mcdowell, 1992). This may also be attributed to dietary imbalances of calcium and phosphorus, higher requirements due to production and dietary interaction with other minerals, especially high intake of iron resulted in formation of insoluble phosphate in acidic pH of abomasum affecting the bio-availability of phosphorus. This unavailable phosphorus not only affected phosphorus but also utilization of calcium by precipitation at intestinal level, thus preventing its absorption (Maynard *et al.*, 1979).

Similar to the present findings, Singh *et al* (2005), Sharma *et al.* (2006), Siddique (2011) and Singh (2013) reported phosphorus deficiency in 27.38, 22.07, 29.8 and 27.12 per cent in cows, respectively. However, Randhawa (1993) had recorded comparatively lesser incidence of hypophosphataemia (9.58%).

District wise: Highest deficiency was shown by the cows of Jodhpur district with a prevalence of 56.09 per cent (23/41), followed by cows from Pali-Marwar district showing a prevalence of 39.13 per cent, with 9 out of 23 cows having Pi level < 4 mg/dl. Similarly cows from the Bikaner district showed a hypophosphataemia of 30.51 per cent (Table 6).

Parity wise: Cows from the above 5^{th} parity showed maximal deficiency with a prevalence of 55.88 per cent (19/34), followed by cows in the 3^{rd} -5^{th} parity, with a prevalence of 34.83 per cent, respectively. However, contrary to our results, Ozukum (2011) and Singh (2013) reported age related fall in plasma Pi level in cattle, whereas, Randhawa *et al.* (2006) detected non-significant fall in the mean plasma Pi level with ageing (Table7). Regarding blood minerals, higher phosphorus concentrations in young cows were reported by McAdam and O'Dell (1982). This increase has been related to higher growth hormone activity, promoting intestinal phosphate absorption and renal phosphate re-absorption (Meyer and Harvey, 2004).

4.2.9.3 Magnesium (Mg):

The average values of plasma magnesium in all physiological groups of dairy cow were within the normal range. This may be attributed to adequate level of magnesium in soil and fodder. Similar finding was also reported by Sharma *et al.* (2003a) in dairy animals of Kumaon hills, Hussain (2006) in Vidharba region of Maharastra, Das (2007) in Tripura and Turkar (2010) in Madhya Pradesh and Bhat *et al.* (2011) in Kashmir. Randhawa *et al*. (2009) and Das *et al*. (2009) reported no significant deficiency of Mg in buffaloes of Punjab and cattle of high rainfall area of

Tripura.

4.2.9.4 COPPER (Cu):

The overall prevalence of copper deficiency in crossbred cows from various districts was found to be 33.33 per cent (41/123), with 29 out of 123 cows (23.57 %) marginally deficient (Cu - 0.5-0.6 ppm) and 12 out of 123 cows (9.76 %) were having copper levels lower than <0.5 ppm (Table 6 and Fig. 27). The overall prevalence recorded in the present study was comparable to those recorded in previous studies by various workers as 34.6 per cent by Randhawa (1999) and 46.9 and 40.4 per cent in summer and winter season respectively by Chhabra (2006). The overall prevalence of Cu deficiency in bovines of western Maharashtra was 33.77%. Kumar (2006) reported 44.37% prevalence of Cu deficiency in bovines of Bihar while Hussain (2006) revealed 36.20% prevalence of Cu deficiency in bovines of Vidarbha. However Noaman (2013), reported Cu deficiency in 69.00 per cent in the animals, reared in the industrial area of Iran. Molybdenum is geochemically in excess in soils and is abundantly taken up by legume crops causing its toxicity and conditioned copper deficiency (Underwood and Suttle, 1999). Besides, interactions of copper with molybdenum and sulphur interfere with its absorption resulting in its deficiency. Copper deficiency has been not reported earlier in Western Rajasthan. Perhaps it could due to excessive legume feeding which were reported during the sampling of dairy cows.

District wise: The prevalence of copper deficiency was highest in cow of Pali-Marwar district with a 82.60 per cent (19/23), with 12 out of 23 cows (52.17 %) having marginal Cu deficiency and 7 out of 23 (30.43%) cows having Cu level less than the <0.5 ppm. Similarly, cows from the Bikaner district showed a deficiency of 20.33 per cent (12/59); 8 cows (13.55%) having marginal Cu deficiency and 4 out of 59 (6.78 %) cows having Cu level less than <0.5 ppm. Cows from the Jodhpur district revealed a deficiency of 24.29 per cent (10/41), with 9 cows showing marginal Cu deficiency and 1 cows showing Cu level less than <0.5 ppm (Table 6). During the sampling period owner reported reproductive inefficiency characterized by depressed expression of oestrus behavior in cows and buffaloes. Administration of Cu orally or by injection has led to rapid improvement in breeding performance of the animals (Mahadevan and Zubery, 1993).

Parity wise: Highest deficiency was recorded in the cows in 3rd to 5th parity, with a prevalence of 33.70 per cent (30/89), followed by in above 5th parity cows, with a prevalence of 32.35 per cent (11/34). However in recent studies, Ozukum (2011) and Singh (2013) reported highest deficiency in animals of in the age group of <3 years (Table 7).

4.2.9.5 IRON (Fe):

Iron deficiency was not found in cows of all districts (Table 6). Iron deficiency in adult cattle is very rare because of their reduced requirements and the ubiquitous availability of Fe in nature and soil contamination of forages that ensures the fulfillment of Fe requirements of cattle (Underwood, 1981). Also the majority of Fe incorporated in tissues is effectively recovered and recycled thereby reducing the maintenance requirement for Fe (NRC, 2001). The higher plasma Fe concentrations in bovines of Western Maharashtra might be attributed to adequate Fe content in soils and fodder. Negligible deficiency of Fe in bovines has been reported by Kumar (2006) in Bihar (4.06%), Hussain (2006) in Vidarbha (7.9%). Fe concentration was reported adequate in cattle and buffaloes of Haryana, Delhi, Uttar Pradesh and Uttaranchal (Sharma *et al*., 2003b, c; 2005; Sharma and Joshi, 2004a, b).

4.2.9.6 ZINC (Zn):

Overall prevalence of Zn deficiency recorded in the cows from all the districts were 2.43 per cent (3/123) which showing marginal deficiency (0.6-0.8 ppm) (Table 6 and Fig. 27). The Zn content of milk is about 4 mg/kg and so lactational requirement of Zn may be very large in lactating animals compared to non-lactating bovines (Osis *et al*., 1972; Schwarz and Kirchgessner, 1975). NRC (1984) suggested that the recommended dietary level of 30 ppm with a range of 20-40 ppm commonly to be adopted to alleviate the zinc deficiency in animals. According to PuIs (1994), improved zinc status also improves fertility by reducing lameness, resulting in cows more willing to show heat. Zinc in the form of Zinc oxide, Zinc carbonate, Zinc sulphate or Zinc methionine in the diet has proved to be successful as good supplementation for cattle (Corah, 1996).

District wise: Highest prevalence of Zn deficiency was seen in the crossbred cows of Bikaner district with a prevalence of 3.39 per cent (2/59), which showed marginal deficiency. Similarly cows from the Jodhpur districts showed a prevalence of 2.50 % per cent Zn deficiency which showed marginal Zn deficiency. (Table 6).

Parity wise: Highest prevalence of deficiency was shown by the cows above 5th parity with a prevalence of 5.88 per cent (2/34), followed by cows in the 3rd to 5th parity with a prevalence of

1.13 per cent, which showed marginal Zn deficiency (Table 7).

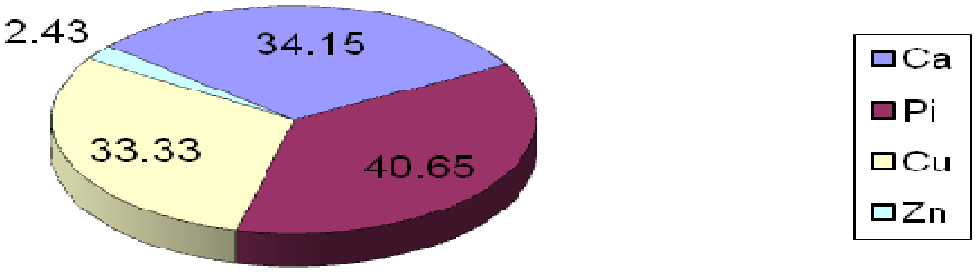

Fig. 27: Overall deficiency status of macro and micro minerals

Table 6: Status of Ca, Mg, Pi, Cu, Fe and Zn deficiencies from three districts of Western Rajasthan (n=123)

Plasma minerals	Status	Bikaner (n=59)	Jodhpur (n=41)	Pali-Marwar (n=23)	Overall (n=123)
Ca (mg/dl)	**Normal (>8.15mg/dl)**	44 (74.57 %)	23 (56.09%)	14 (60.87%)	81 (65.85%)
	Marginal (5.17-8.15 mg/dl)	15 (25.42 %)	18(43.90%)	9 (39.13%)	42 (34.15%)
	Low (<5.17 mg/dl)	0	0	0	0
	Deficiency (%)	25.42 %	43.90%	39.13%	34.15%
Mg (mg/dl)	**Normal (>1.8 mg/dl)**	59	41	23	123
	Low (<1.8 mg/dl)	0	0	0	0
	Deficiency (%)	0	0	0	0
Pi (mg/dl)	**Normal (>4.0)**	41 (69.49%)	18 (43.90%)	14(60.87%)	73 (59.34%)
	Low (<4.0)	18 (30.51%)	23 (56.09%)	9 (39.13%)	50 (40.65%)
	Deficiency (%)	30.51 %	56.09%	39.13%	40.65%
Cu (ppm)	**Normal (>0.6ppm)**	47 (79.66%)	31 (75.61%)	4 (17.39%)	82 (66.66%)
	Marginal (0.5-0.6ppm)	8 (13.55%)	9 (21.95%)	12 (52.17%)	29 (23.57%)
	Low (<0.5 ppm)	4 (6.78%)	1 (2.43%)	7 (30.43%)	12 (9.75%)
	Deficiency (%)	20.33%	24.29%	82.60%	33.33%
Fe (ppm)	**Normal (>1.0 ppm)**	59	41	23	123
	Low (<1.0 ppm)	0	0	0	0
	Deficiency (%)	0	0	0	0
Zn (ppm)	**Normal (>0.8 ppm)**	57 (96.61%)	40 (97.56%)	23 (100%)	120 (97.56%)
	Marginal (0.6-0.8 ppm)	2 (3.39%)	1 (2.43%)	0	3 (2.43%)
	Low (<0.6 ppm)	0	0	0	0
	Deficiency (%)	3.39%	2.50%	0	2.43%

Classification suggested as per Radostits *et al.*, 2007.

Table 7: Parity wise prevalence rate of Ca, Mg, Pi, Cu, Fe and Zn deficiencies among crossbred cows.

Plasma minerals	Status	3rd - 5th parity (n=89)	>5th parity (n=34)	Overall (n=123)
Ca mg/dl	Normal (>8.15mg/dl)	64(71.91%)	17 (50%)	81(65.85%)
	Marginal (5.17-8.15 mg/dl)	25 (28.09%)	17 (50%)	42(34.15%)
	Low (<5.17 mg/dl)	0	0	0
	Deficiency (%)	28.09%	50%	34.15%
Pi (mg/dl)	Normal (>4.0)	58 (65.16%)	15(44.11%)	73 (59.34%)
	Low (<4.0)	31(34.83%)	19(55.88%)	50 (40.65 %)
	Deficiency (%)	34.83%	55.88%	40.65%
Mg (mg/dl)	Normal (>1.8 mg/dl)	89	34	123
	Low (<1.8 mg/dl)	0	0	0
	Deficiency (%)	0	0	0
Cu (ppm)	Normal (>0.6ppm)	59(66.29%)	23(67.64%)	82(66.67%)
	Marginal (0.5-0.6ppm)	19(21.34%)	10(29.41%)	29(23.57%)
	Low (<0.5 ppm)	11(12.36%)	1(2.95%)	12(9.76%)
	Deficiency (%)	33.70%	32.35%	33.33%
Fe (ppm)	Normal (>1.0 ppm)	89	34	123
	Low (<1.0 ppm)	0	0	0
	Deficiency (%)	0	0	0
Zn (ppm)	Normal (>0.8 ppm)	88(98.87%)	32(94.11%)	120(97.56%)
	Marginal (0.6-0.8 ppm)	1(1.13%)	2(5.88%)	3(2.44%)
	Low (<0.6 ppm)	0	0	0
	Deficiency (%)	1.13%	5.88%	2.44%

4.3 OXIDATIVE STRESS INDICES:

The ANOVA of oxidative stress parameters have been presented in appendix Table no. 24.

4.3.1 Reduced glutathione:

4.3.1.1 Interaction between districts and parity wise affected GSH level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for GSH in plasma was 2.617±0.063 mg/dl per gm Hb. The differences among subclass means were highly significant (P≤0.01) across districts (2.411±0.101 in Bikaner, 2.439±0.107 in Jodhpur and 3.000±0.131 mg/dl per gm Hb in Pali-Marwar), highly significant in healthy/SCK groups (2.423±0.071 in healthy and 2.811±0.109 mg/dl per gm Hb in SCK), non significant in stage wise (2.456±0.072 in pre-partum and 2.249±0.076 mg/dl per gm Hb in post-partum) and

non significant parity groups (2.751±0.089 IU/L in 3-5th parity and 2.483±0.089 IU/L in above 5th parity). The effect of pre and post-partum stages were non significant in pooled data. There was significant effect of two factor interaction indicating that difference among districts and stage in reduced glutathione level. Values in healthy/SCK groups or stage groups remain unchanged, whereas the interaction between districts and parity status was significant (P≤0.05). The trend is depicted in Fig. 28.

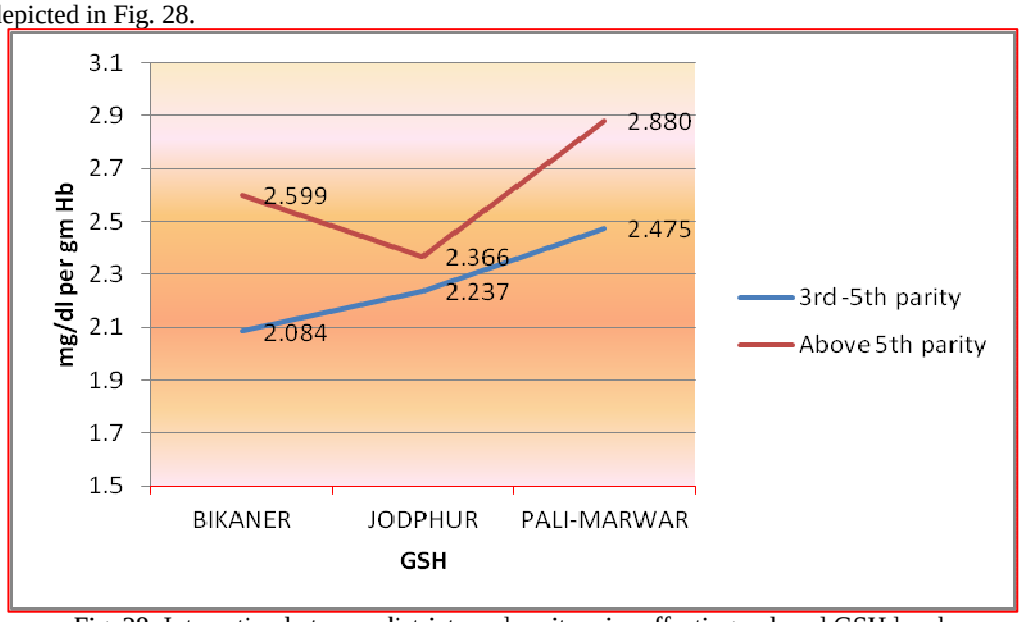

Fig. 28: Interaction between districts and parity wise affecting reduced GSH level.

4.3.1.2 Reduced glutathione (GSH) levels in healthy crossbred cows:

GSH present in reduced and oxidised form in the body. The oxidized form is very unstable. So we used reduced glutathione to measure the level of stress in whole blood. Reduced glutathione is GSH in its reduced form.

The overall mean GSH level recorded in cows from various districts was 2.333±0.085 mg/dl per gram Hb during pre parturient period and 2.181±0.090 mg/dl per gram during post parturient period. Highly significant (P≤0.01) differences were observed in overall healthy and subclinical ketotic dairy cows (Table 8 and Fig. 29).

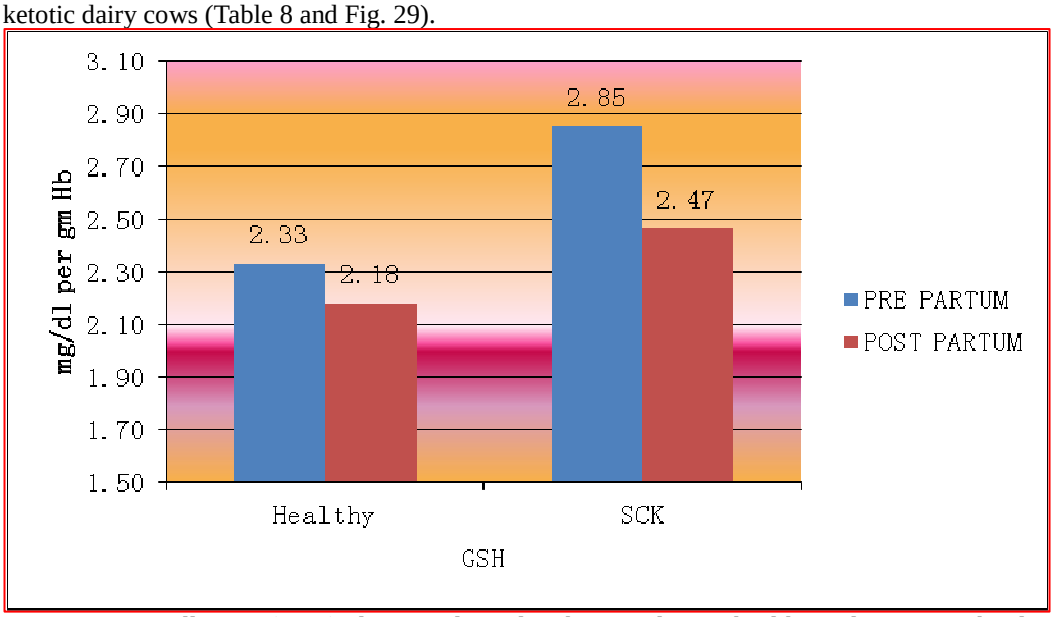

Fig. 29: Overall mean (±SE) plasma reduce glutathione values in healthy and SCK crossbred cows.

Table 8: Overall mean of oxidative stress biomarkers investigated under study during transition period.

Parameters	**Periods**	Over all		Total no. of dairy cows (n=123)
		Healthy (n=94)	**SCK (n=29)**	
GSH	Pre-partum.	2.333±0.085	2.853±0.103	2.456±0.072

(mg/dl per gm Hb)	Post-partum	2.181±0.090	2.468±0.129	2.249±0.076
	Over all	2.423±0.071[a]	2.811±0.109[b]	2.617±0.063
MDA (nmol/ml per gm Hb)	Pre-partum.	36.211±0.917[a*]	44.533±1.699[a*]	38.173±0.865**
	Post-partum	40.451±0.712[a*]	53.384±1.239[a*]	43.500±0.791**
	Over all	38.813±0.729[a]	49.753±1.118[b]	44.283±0.650

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). 3. No superscript mean non significant with in row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

The mean GSH level recorded from various districts was 2.152±0.136, 2.278±0.130, 2.911±0.092 mg/dl per gram Hb during pre parturient period and 2.036±0.160, 2.131±0.122, 2.654±0.083 mg/dl per gram Hb during post parturient period in the dairy cows of Bikaner, Jodhpur and Pali Marwar dairy farm. Lower values were recorded in the cows of Bikaner post-partum period.

Table 9: District wise oxidative stress investigated under study during transition period in Western Rajasthan.

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).

2. Value bearing same superscript (a, a) in a row depict significant (p≤0.05).

Parameters	**Periods**	Bikaner			Jodhpur			Pali-Marwar		
		Healthy (n=44)	**SCK (n=15)**	Over all (59)	**Healthy (n=33)**	**SCK (n=8)**	Over all (41)	**Healthy (n=17)**	**SCK (n=6)**	Over all (23)
GSH (mg/dl per gm Hb)	Pre Partum.	2.152±0.136	2.686±0.113	2.287±0.110	2.278±0.130	2.827±0.208	2.385±0.117	2.911±0.092	3.307±0.254	3.014±0.099
	Post-partum	2.036±0.160	2.316±0.186	2.107±0.128	2.131±0.122	2.370±0.243	2.177±0.109	2.654±0.083	2.977±0.173	2.738±0.080
	Over all	2.259±0.091	2.564±0.172	2.411±0.101[a]	2.236±0.102	2.641±0.191	2.439±0.107[b]	2.774±0.159	3.227±0.224	3.000±0.131[c]
MDA (nmol/ml per gm Hb)	Pre Partum.	34.244±1.304	44.321±2.655	36.806±1.305	35.813±1.508	39.248±1.751	36.483±1.271	42.077±1.893	52.108±2.019	44.694±1.746
	Post-partum	41.186±1.244	53.529±1.552	44.324±1.226	40.596±1.001	50.300±1.790	42.489±1.060	38.269±1.115	57.133±3.651	43.190±2.139
	Over all	38.406±0.930	48.751±1.751	43.578±1.032[a]	38.336±1.043	45.106±1.948	41.721±1.091[b]	39.699±1.622	55.404±2.279	47.551±1.340[c]

3. No superscript mean non significant with in row or column.

4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

Fig. 30: Mean (±SE) plasma reduce glutathione values in healthy and SCK crossbred cows.

4.3.1.3 Reduced glutathione (GSH) level in SCK crossbred cows:

The overall mean GSH level recorded in subclinical ketotic cows from various districts was 2.853±0.103 mg/dl during pre-partum period and 2.468±0.129 mg/dl during post-partum period. A non significant increase was noticed in the overall mean GSH level in between the pre-partum and post-partum period.

District wise: The mean whole blood GSH concentration recorded were 2.686±0.113, 2.827±0.208, 3.307±0.254 mg/dl per gram Hb during pre-partum period and 2.316±0.186, 2.370±0.243, 2.977±0.173 mg/dl per gram Hb during post-partum period from the various farms of the Bikaner, Jodhpur and Pali-Marwar dairy farm. Non significant differences were observed in healthy and subclinical ketotic dairy cows in three districts. Lower values were observed in the Bikaner dairy cows during the pre and post-partum transition period as compared to the cows from the other districts (Jodhpur and Pali-Marwar) (Table 9).

Reduced glutathione (GSH) plays a key role in scavenging t-butyl hydro peroxide, an agent which induces lipid peroxidation (Trotta *et al.*, 1982). Reduced Glutathione is a major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Scholz *et al.*, 1989). Reduced level of GSH was observed in the present study, which might be due to the decreased production or increased depletion. The activity of this enzymes increases before calving as an indirect compensatory response of cells to increased oxidant challenge around calving (Bernabucci *et al.*, 2005) to maintain homeostatic control system of cows. Reduced activity of these enzymes after calving may be due to reduction in availability of copper, zinc and iron in early postpartum period or reduction in antioxidant enzymes due to susceptibility to the oxidative reactive molecules (Bernabucci *et al.*, 2005). Similarly Kincaid (2000); Lohrke *et al.* (2005b) and Sharma *et al.* (2011) also recorded a significant depletion in the blood GSH level due to the increased production of ROM during the early lactation as compared to the advanced pregnancy, along with a significant positive correlation between the GSH and LPO (MDA) during the early lactation period. Bernabucci *et al.* (2002; 2005) reported highest levels of SH groups during the late pregnancy in comparison to the mid pregnancy and early lactation. Hogan *et al.*, 1993 found the GPx-P activity increased in cows towards parturition indicating more oxidative stress at the time of parturition in 2 different groups. Oxidative status of dairy cows was related to energy status. This indicated that post-partum energy status was lower than pre-partum. So dairy cows were more stress during post-partum. Various earlier workers have used reduce glutathione activity as an indicator to assess oxidative stress in animals (Kataria *et al.*, 2010, Joshi, 2012 and Panday, 2012).

4.3.2 Malondialdehyde (MDA):

4.3.2.1 Interaction between districts and stages of partum affecting MDA level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for MDA in plasma was 44.283±0.650 nmol/ml per gm Hb. The differences among

subclass means were highly significant (P≤0.01) across districts (43.578±1.032 in Bikaner, 41.721±1.091 in Jodhpur and 47.551±1.340 nmol/ml per gm Hb in Pali-Marwar), highly significant in healthy/SCK groups (38.813±0.729 in healthy and 49.753±1.118 nmol/ml per gm Hb in SCK), highly significant in stage wise (38.173±0.865 pre-partum and 43.500±0.791 nmol/ml per gm Hb post-partum) and non significant in parity groups (41.867±0.910 nmol/ml per gm Hb 3-5th parity and 46.700±0.910 nmol/ml per gm Hb above 5th parity). The effect of 3rd -5th and above 5th parity were non significant in pooled data. There was effect of two factor interaction indicating that difference among districts. Where in the interaction between districts and stage was highly significant (P≤0.01). The trend is depicted in Fig. 31. The interaction between health and stage wise was significant. This shows that the MDA level was higher in post-partum groups across all districts but the rise in their level was highly significantly (P≤0.01). In the pre-partum stage of Pali-Marwar dairy cows were more stressful condition due deficiency of Cu. The Cu level was low in Pali-Marwar in comparison to Jodhpur and Bikaner. The sampling of Pali-Marwar was taken in extreme hot season. In summer season animal were under more stress full condition in comparison to other seasons.

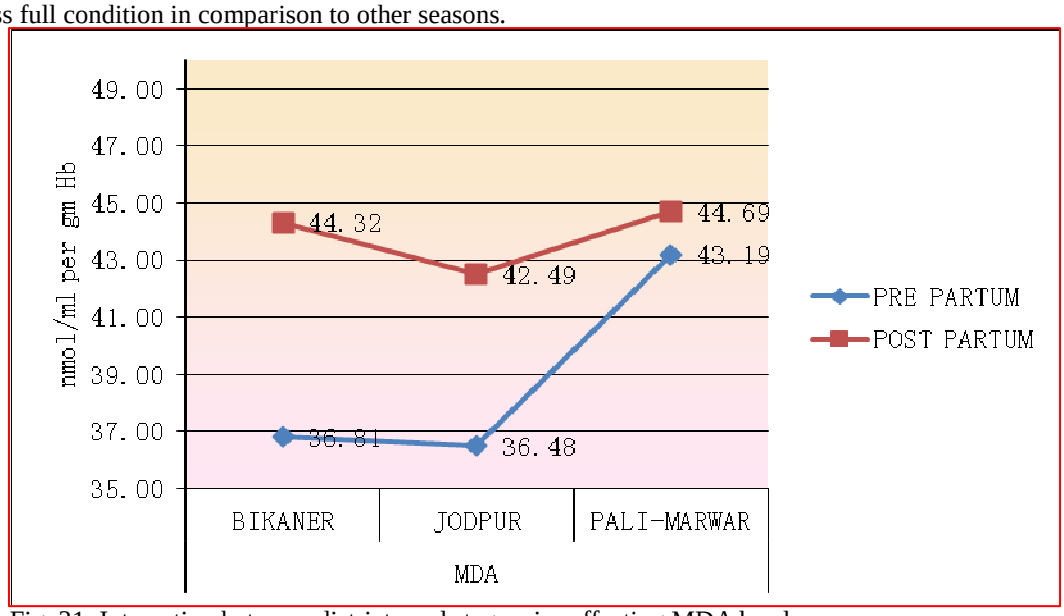

Fig. 31: Interaction between districts and stage wise affecting MDA level

4.3.2.2 Malondialdehyde (MDA) level in healthy crossbred cows:

The overall mean erythrocyic Malondialdehyde level measured during different stages of transition period was 36.211±0.917 and 40.451±0.712 nmol/ml per gram Hb during pre and post-partum period respectively. A highly significant (P≤0.01) increase was recorded in the overall mean malondialdehyde level from the pre to post-partum period (Table 8, Fig. 32).

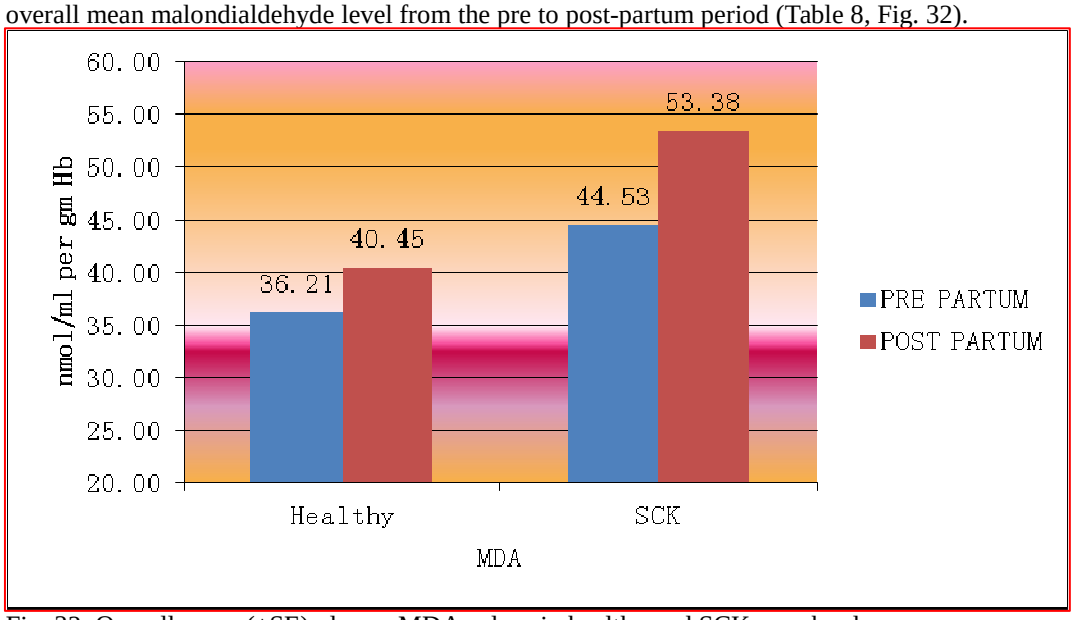

Fig. 32: Overall mean (±SE) plasma MDA values in healthy and SCK crossbred cows.

District wise: The mean erythrocyic malondialdehyde level in Bikaner, Jodhpur and Pali-Marwar dairy farm were 34.244±1.304, 35.813±1.508 and 42.077±1.892 nmol/ml per gram Hb during pre-partum period; 41.186±1.244, 40.596±1.001, 38.269±1.115 nmol/ml per gram Hb during post parturient period, respectively. Significantly (P≤0.05) higher value was noted in the cows of Pali-Marwar district as compared to the cows of Bikaner and Jodhpur during pre and post parturient period whereas, significantly (P≤0.05) higher values were observed in the cows of Bikaner district during the post-partum transition period (Table 9 and Fig. 33).

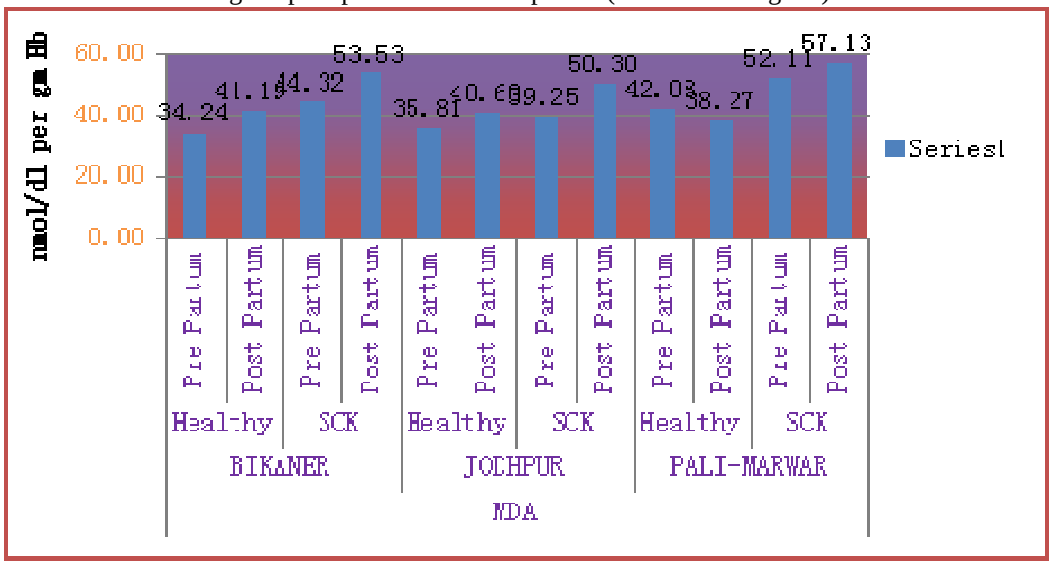

Fig. 33: Mean (±SE) plasma MDA values in healthy and SCK crossbred cows.

4.3.2.3 Malondialdehyde (MDA) level in subclinical ketotic crossbred cows:

The overall mean Malondialdehyde level recorded in subclinical ketotic cows from various districts was 44.533±1.699 nmol/ml per gram Hb during pre-partum period and 53.384±1.239 nmol/ml per gram Hb during post-partum period. Highly significant (P≤0.01) increase was noticed in the overall mean Malondialdehyde level in districts, healthy and SCK and between the pre-partum and post-partum period.

District wise: The mean whole blood Malondialdehyde concentration recorded was 44.321±2.655, 39.248±1.751, 52.108±2.019 nmol/ml per gram Hb during pre-partum period and 53.529±1.552, 50.300±1.790, 57.133±3.651 nmol/ml per gram Hb during post-partum period from the various farms of the Bikaner, Jodhpur and Pali-Marwar dairy farm. Significant differences (P≤0.05) were observed in healthy and subclinical ketotic dairy cows and stage wise. Lower values were observed in the Jodhpur dairy cows during the pre and post-partum transition period as compared to the cows from other districts (Bikaner and Pali-Marwar).

4.3.2.4 Oxidative stress parity wise in crossbred cows:

Mean value of GSH and Malondialdehyde level across parities was non significant.

Parameters	Periods	3rd - 5th parity			>5th parity		
		Healthy (n=71)	SCK (18)	Over all (89)	Healthy (n=23)	SCK (11)	Over all (34)
GSH (mg/dl per gm Hb)	Pre Partum.	2.266±0.100	2.841±0.136	2.383±0.088	2.540±0.149	2.873±0.165	2.648±0.116
	Post Partum	2.089±0.102	2.451±0.180	2.162±0.090	2.465±0.179	2.495±0.178	2.475±0.132
	Over all	2.293±0.067	2.925±0.159	2.751±0.089	2.553±0.125	2.696±0.166	2.483±0.089

MDA (nmol/ml per gm Hb)	Pre Partum.	35.607±1.071	44.492±2.372	37.404±1.045	38.078±1.746	44.599±2.377	40.188±1.487
	Post Partum	40.476±0.844	54.582±1.726	43.329±0.966	40.375±1.324	51.424±1.555	43.950±1.355
	Over all	38.500±0.681	51.059±1.620	41.867±0.910	39.127±1.275	48.448±1.692	46.700±0.910

Table 10: Oxidative stress profile in crossbred cows depending upon parity during transition periods (Mean± S.E.)

Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of reactive oxygen species (ROS). It leads to the creation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes and their physiological functions (Halliwell and Gutteridge, 1985). The most common of these intermediates are malondialdehyde (MDA) and 4-hydroxynonenal (Comporti, 1989). Malondialdehyde (MDA) is widely preferred for detection of free oxygen radicals in various pathological conditions (Lazzarino *et al.,* 1994), it is a stable by-product of cell membrane's lipid peroxidation and is an indicator of cell membrane damage (Otamiri, 1988) caused by oxidative stress. Blood levels of MDA were found significantly higher in lactating (post-partum transition period) dairy cows in comparison to pre-partum transition period indicating higher levels of oxidative stress in lactating dairy cows. Singh *et al*. 2013 also reported significant high level of MDA in pregnant camel in comparison to non pregnant and non lactating camels. Results of the present study have shown the pregnancy and lactation, both are associated with generation of free radicals and resultant oxidative stress in dairy cows. Pregnancy mostly because of the mitochondria rich placenta is a condition that favours oxidative stress. Transitional metals, especially iron, which are particularly abundant in the placenta, are important in the production of free radicals (Casanueva and Viteri, 2003). Lactating animals undergo substantial metabolic and physiological adaptations during the transition from pregnancy to lactation that contribute to dysfunctional host inflammatory responses (Sordillo, 2005). Physiological stresses associated with rapid differentiation of secretory parenchyma, intense mammary gland growth and the onset of copious milk synthesis and secretion are accompanied by a high energy demand and an increased oxygen requirement. This increased oxygen demand augments the production of oxygen derived reactants, collectively termed as reactive oxygen species (ROS). Accumulation of ROS can cause cell and tissue injury and can lead to a condition of oxidant stress (Sordillo and Aitken, 2009). Among the biomarkers malondialdehyde level in lactating animals were high and reduced glutathione were significantly low in lactating dairy cow, it shows that antioxidant defenses (Reduced glutathione) exhausts earlier than the rise in level of lipid peroxidation (Malondialdehyde). As a result, excess accumulation of ROS can cause cell and tissue injury and lead to a condition referred to as oxidative stress in periparturient dairy cows. Oxidative stress is thought to be a significant underlying factor leading to dysfunctional host immune and inflammatory responses particularly during times of increased metabolic stress. Indeed, several studies support the concept that oxidative stress can increase the susceptibility of periparturient dairy cattle to a variety of health disorders (Sordillo and Aitken, 2009).

Higher level of MDA in cows might be explained by higher level of glucocorticoids and adrenaline-induced pathways of aerobic energy production associated with parturition which generated reactive oxygen metabolites and lipid peroxidation. The findings of the present study are in corroboration with the results of Castillo *et al.* (2003), Saleh *et al.* (2007) and Sharma *et al.* (2011); they also used lipid peroxidation as a marker of oxidative stress in cattle and found an increase in the LPO level after calving, as lipids were most susceptible to peroxidative damage due to the presence of unsaturated bonds. The significant increase in the lipid peroxidation especially after calving could be due to the increased metabolic demands imposed on the cow by colostrum production and the onset of lactation that far exceeded the demands of the fetus. The metabolic adaptations to lactation were initiated in late pregnancy, especially during the near to parturition period. Also the highest LPO level observed in cows from the Pali-Marwar district might be linked to the decreased Cu and Zn levels, as their deficiency can reduce the activity of Cu-Zn SOD enzyme resulting in increased lipid peroxidation (Kaneko *et al.,* 2008). It might also reduce synthesis of metallothionoein, a metal binding protein that scavenge hydroxide radicals (Prasad *et al.,* 2004).

Significantly higher level of MDA and reduced level of reduced glutathione in whole blood observed in the present study, indicated decrease in antioxidant defense and oxidative damage in subclinical ketotic animals. Both zinc and copper are essential components of the antioxidant

enzyme. Thus, over utilization or sequestration of zinc and copper to neutralize the overproduction of reactive oxygen species (ROS) might be responsible for their lower erythrocyte concentrations in mineral deficient animals. It may be possible that multimineral deficiency might have reduced protective macromolecules (ceruloplasmin/albumin) or have created imbalances in transition metals (Cu^{++}/Fe^{++}) and zinc leading to membrane damage from free radicals via Fenton reaction and subsequent oxidative stress and rise in lipid peroxidation. Similarly, Sharma *et al.* (2011) studied significantly reduced activity of reduced glutathione in dairy cow in periparturient stage.

4.4 Biochemical parameters:

The ANOVA of biochemical parameters have been presented in appendix Table no. 25.

4.4.1 Total protein:

4.4.1.1 Interaction between (districts health wise and stage wise; parity and stage) affecting total protein

The overall mean of all the cows across three districts, stages, parity and healthy/SCK groups for total protein in plasma was 7.019±0.041 g/dl. The differences among subclass means were highly significant (P≤0.01) across districts (6.999±0.065 in Bikaner, 6.710±0.069 in Jodhpur and 7.347±0.085 g/dl in Pali-Marwar), highly significant in healthy/SCK groups (6.816±0.046 in healthy and 7.221±0.071 g/dl in SCK), non significant in stage wise (7.211±0.060 pre-partum and 6.557±0.040 g/dl post-partum) and highly significant in parity groups (7.016±0.056 g/dl, 3-5th parity and 7.021±0.066, g/dl above 5th parity). The effect of pre and post-partum stages were non significant in pooled data. There was no effect of two factor interaction indicating that difference among districts.Where as interaction between health status and stage wise highly significant and significant in parity and stage. In the post-partum stage of 3rd to 5th parity group were low levels of total protein in comparison to above 5th parity group. It shows that after the parturition young animals (3rd -5th parity) require more protein for production of milk and cope-out the negative energy balance.

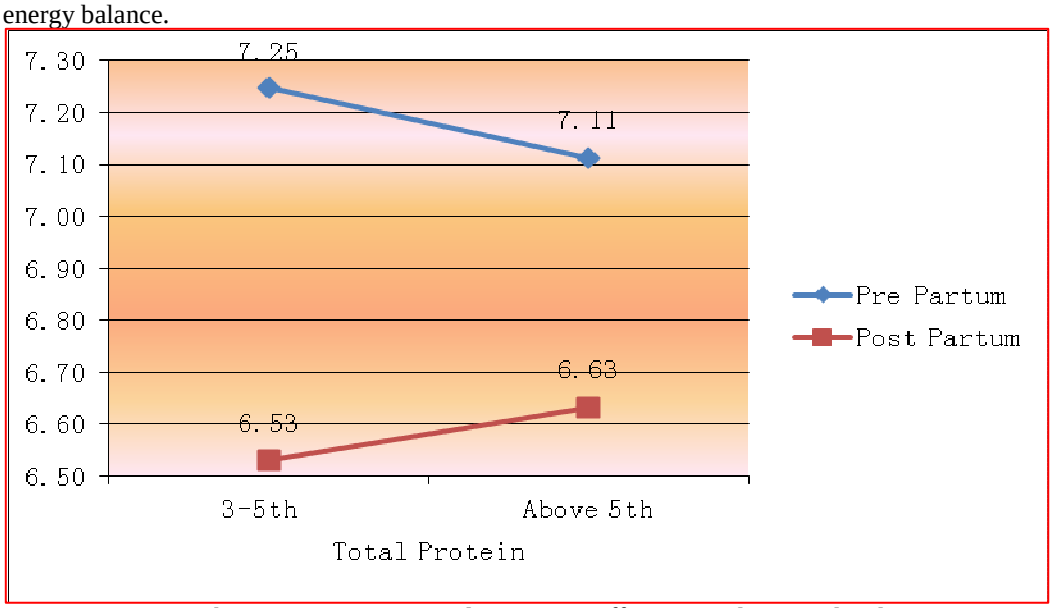

Fig. 34: Interaction between parity wise and stage wise affecting total protein level.

4.4.1.2 Total plasma proteins level in healthy dairy cows:

Table 11: Overall Plasma energy biomarkers and biochemical profile investigated in healthy and subclinical ketosis crossbred cows of three districts in Western Rajasthan during transition period.

Parameters	**Periods**	Over all		
		Healthy (n=94)	**SCK (n=29)**	Over all(123)
GLUCOSE (mg/dl)	Pre-partum.	60.233±0.731	45.612±1.323	56.786±0.850*
	Post-partum	53.641±0.608	39.167±1.349	50.228±0.790*
	Over all	56.293±0.611^{a}	42.768±0.937^{b}	49.530±0.545
TOTAL PROTEIN (g/dl)	Pre-partum.	7.045±0.049^{a**}	7.750±0.162^{b**}	7.211±0.060
	Post-partum	6.534±0.042^{a**}	6.633±0.098^{b**}	6.557±0.040
	Over all	6.816±0.046^{a}	7.221±0.071^{b}	7.019±0.041
AL (g/dl)	Pre-partum.	3.091±0.099	3.129±0.122	3.100±0.081*
	Post-partum	2.616±0.074	2.524±0.091	2.594±0.060*
	Over all	2.784±0.073	2.800±0.112	2.792±0.065
GL (g/dl)	Pre-partum.	3.954±0.108	4.621±0.207	4.111±0.099*
	Post-partum	3.919±0.073	4.109±0.109	3.963±0.061*
	Over all	4.032±0.081^{a}	4.420±0.125^{a}	4.226±0.072
AL/GL	Pre-partum.	0.960±0.082	0.744±0.060	0.909±0.065
	Post-partum	0.730±0.041	0.640±0.040	0.709±0.033
	Over all	0.807±0.053	0.677±0.082	0.742±0.047
PUN (mg/dl)	Pre-partum.	9.464±0.182	9.210±0.316	9.404±0.158
	Post-partum	11.994±0.155	12.245±0.354	12.053±0.144
	Over all	10.598±0.153	11.053±0.235	10.826±0.137
CR (mg/dl)	Pre-partum.	1.236±0.031	1.372±0.065	1.269±0.029
	Post-partum	1.070±0.024	1.081±0.052	1.073±0.022
	Over all	1.136±0.025^{a}	1.237±0.039^{a}	1.187±0.023
NEFA (mmol/l)	Pre-partum.	0.189±0.008^{a**}	0.518±0.021^{b**}	0.267±0.015
	Post-partum	0.333±0.015^{a**}	0.797±0.024^{b**}	0.442±0.022
	Over all	0.262±0.010^{a}	0.683±0.016^{b}	0.473±0.009
BHBA (mmol/l)	Pre-partum.	0.498±0.012^{a**}	0.795±0.024^{b**}	0.568±0.016
	Post-partum	0.947±0.020^{a**}	1.526±0.026^{b**}	1.084±0.027
	Over all	0.743±0.013^{a}	1.177±0.021^{b}	0.960±0.012
CHOL (mg/dl)	Pre-partum.	97.250±1.242^{a**}	82.541±1.589^{b**}	93.782±1.164
	Post-partum	101.168±2.118	68.390±1.686	93.440±2.087
	Over all	95.543±1.313^{a}	74.241±2.013^{b}	84.892±1.171
TRIG (mg/dl)	Pre-partum.	9.506±0.299	14.478±0.849	10.678±0.357
	Post-partum	13.401±0.622	17.159±1.071	14.287±0.555
	Over all	11.591±0.438^{a}	17.098±0.672^{b}	14.345±0.391

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). No superscript mean non significant with in row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

The overall mean total plasma proteins (TPP) level in the healthy cows from three districts of Western Rajasthan was 7.045±0.049 g/dl during the pre-partum period and 6.534±0.042 g/dl during the post-partum transition periods, respectively (Table 11), which were well within the normal physiological range (5.7-8.1 g/dl) as stated by Radostits *et al.* (2007). Non significant decrease was observed in the overall mean TPP level from the pre-partum period to post-partum transition period.

Kelly (1984) reported normal protein values in apparently healthy cattle to be 7.1 (6.7-7.5) g/dl. The blood biochemical like total protein decreased with approaching parturition due to their requirement for foetal development and colostrums synthesis (Rai, 1995). The total plasma concentration of total protein decreased from the peripartum to first week post-partum with decreased concentration of globulins largely responsible for the decline in total protein concentrations.

District wise: The mean TPP level were 7.052±0.034, 6.885±0.076, 7.338±0.195 g/dl during the pre-partum period and 6.568±0.050, 6.419±0.059, 6.671±0.150 g/dl during the post-partum transition period from the cows of Bikaner, Jodhpur and Pali- Marwar dairy farms, respectively (Table 12 and Fig. 36). A non significant difference was noted in the mean TPP level in cows in district wise and stage wise.

In cows, the total proteins level begins to increase at 2 months before parturition, reach maximum values at 1 month, and then rapidly decline toward parturition (Larson and Kendall, 1957), due to the transport of immunoglobulin from serum to the mammary gland that begins several weeks before parturition, reaching a peak 1 to 3 days before birth of the calf (Pathak *et al.,* 1986; Weaver *et al.,* 2000). The low level of total plasma proteins recorded in early pregnancy might also be due to the fact that plasma proteins in the late trimester of pregnancy was needed for the optimum secretion of gonadotropin releasing factors and number of other hormones needed in the culmination of pregnancy, and are also required for the increased foetal growth, and for the development of fetal muscles (Antunovic *et al.,* 2002), as well as for gluconeogenesis (Lone *et al.,* 2003). Similarly, Paquay *et al.* (1972) also reported decrease in plasma proteins, as the cow loses 37 pound of body proteins during the first two weeks of lactation in order to provide amino acid and glucose required for milk production. Similar results were observed by Bell *et al.* (2000), Roubies *et al.* (2006) and Mohri *et al.* (2007) in their respective studies as they also observed decrease in plasma proteins from late pregnancy to early lactation as compared to the mid lactation reflecting the maternal requirement of proteins for the onset of milking and providing immunoglobulin. Similar finding on total proteins showing decreasing trend with the advancement of parturition was reported in non descript cows (Mehta *et al.,* 1989) and Jersey and HF crossbred cows (Ghosh *et al.,* 1991 and Sivaraman *et al.,* 2003).

Table 12 : Energy biomarkers and biochemical profile investigated in healthy and subclinical ketosis crossbred cows of three districts of Western Rajasthan during transition period.

Parameters	**Periods**	Bikaner			Jodhpur			Pali-Marwar		
		Healthy (n=44)	**SCK (n=15)**	Over all (59)	**Healthy(n=33)**	**SCK(n=8)**	Over all (41)	**Healthy (n=17)**	**SCK (n=6)**	Over all (23)
GLUCOSE (mg/dl)	Pre-partum.	59.415±1.079	45.837±2.012	55.963±1.223	62.503±1.204	43.650±2.184	58.824±1.579	57.941±1.552	47.667±2.871	55.261±1.646
	Post-partum	54.605±0.807	39.577±1.820	50.784±1.141	55.109±1.002	36.775±3.062	51.532±1.512	48.294±1.124	41.333±2.459	46.478±1.209
	Over all	57.248±0.779^{a}	44.948±1.468^{b}	51.098±0.865	58.873±0.874^{a}	39.530±1.632^{b}	49.202±0.914	52.757±1.360^{a}	43.825±1.910^{b}	48.291±1.123
TOTAL PROTEIN (g/dl)	Pre-partum.	7.052±0.034	7.877±0.205	7.262±0.074	6.885±0.076	7.179±0.334	6.942±0.089	7.338±0.195	8.197±0.265	7.562±0.176
	Post-partum	6.568±0.050	6.533±0.078	6.559±0.042	6.419±0.059	6.416±0.117	6.418±0.052	6.671±0.150	7.172±0.343	6.802±0.146
	Over all	6.790±0.059	7.209±0.111	6.999±0.065	6.612±0.066	6.807±0.124	6.710±0.069	7.047±0.103	7.646±0.145	7.347±0.085
AL (g/dl)	Pre-partum.	2.883±0.148	3.237±0.138	2.973±0.117	3.433±0.146	2.775±0.231	3.305±0.132	2.966±0.243	3.332±0.352	3.061±0.201
	Post-partum	2.553±0.127	2.407±0.106	2.516±0.099	2.550±0.109	2.533±0.179	2.546±0.094	2.906±0.102	2.805±0.251	2.880±0.097
	Over all	2.565±0.093	2.646±0.176	2.605±0.103	2.965±0.105	2.651±0.196	2.808±0.109	2.823±0.163	3.104±0.229	2.964±0.134
GL (g/dl)	Pre-partum.	4.169±0.148	4.639±0.232	4.288±0.127*	3.452±0.163	4.404±0.508	3.637±0.172*	4.372±0.260	4.865±0.524	4.500±0.234*
	Post-partum	4.015±0.121	4.126±0.117	4.043±0.095*	3.869±0.104	3.884±0.198	3.872±0.091*	3.765±0.152	4.367±0.352	3.922±0.151*
	Over all	4.224±0.104	4.563±0.196	4.394±0.115^{a}	3.646±0.116	4.155±0.218	3.901±0.122^{b}	4.224±0.181	4.541±0.255	4.383±0.150^{c}
AL/GL	Pre-partum.	0.843±0.113	0.729±0.053	0.814±0.085	1.172±0.136	0.743±0.154	1.088±0.116	0.852±0.220	0.785±0.186	0.835±0.167
	Post-partum	0.721±0.073	0.600±0.045	0.690±0.056	0.703±0.058	0.684±0.093	0.700±0.050	0.803±0.055	0.680±0.107	0.771±0.049
	Over all	0.711±0.068	0.554±0.129	0.632±0.076	0.948±0.076	0.709±0.143	0.829±0.080	0.763±0.119	0.768±0.168	0.766±0.098
PUN (mg/dl)	Pre-partum.	9.625±0.273	8.537±0.255	9.348±0.222	8.900±0.277	9.205±0.629	8.960±0.251	10.141±0.420	10.900±0.843	10.339±0.377
	Post-partum	12.200±0.207	11.473±0.338	12.016±0.180	11.745±0.286	12.797±0.722	11.951±0.273	11.941±0.370	13.437±0.980	12.331±0.388
	Over all	10.758±0.196^{a}	9.988±0.369^{b}	10.373±0.217^{a}	10.290±0.220^{a}	10.947±0.410^{b}	10.618±0.230^{b}	10.746±0.342^{a}	12.224±0.480^{b}	11.485±0.282^{c}
CR (mg/dl)	Pre-partum.	1.237±0.044	1.421±0.109	1.284±0.044*	1.189±0.057	1.180±0.067	1.187±0.047*	1.327±0.065	1.507±0.087	1.374±0.055*
	Post-partum	1.128±0.038	1.115±0.061	1.125±0.032*	1.083±0.032	0.916±0.035	1.051±0.029*	0.895±0.039	1.217±0.184	0.979±0.061*
	Over all	1.198±0.033^{a}	1.263±0.062^{a}	1.230±0.036^{a}	1.063±0.069^{a}	1.125±0.037^{a}	1.094±0.038^{a}	1.086±0.057^{a}	1.386±0.080^{a}	1.236±0.047^{a}
TRIG (mg/dl)	Pre-partum.	8.868±0.346	12.019±0.878	9.669±0.382	10.018±0.602	16.169±1.388	11.219±0.669	10.161±0.713	18.372±1.920	12.303±1.041
	Post-partum	12.143±0.576	14.542±1.279	12.753±0.550	15.145±1.499	18.900±1.425	15.878±1.254	13.274±0.890	21.378±2.832	15.388±1.215
	Over all	10.870±0.559^{a}	13.121±1.053^{b}	11.996±0.621^{a}	12.271±0.627^{a}	17.941±1.171^{b}	15.106±0.656^{b}	11.632±0.976^{a}	20.233±1.371^{b}	15.933±0.806^{c}
NEFA (mmol/l)	Pre-partum.	0.220±0.012	0.467±0.029	0.283±0.018	0.172±0.013	0.541±0.033	0.244±0.026	0.145±0.017	0.615±0.018	0.267±0.046
	Post-partum	0.354±0.025	0.748±0.036	0.454±0.030	0.288±0.021	0.814±0.020	0.390±0.037	0.363±0.028	0.897±0.044	0.502±0.055
	Over all	0.297±0.013^{a}	0.616±0.025^{b}	0.456±0.014	0.248±0.015^{a}	0.669±0.028^{b}	0.459±0.015	0.242±0.023^{a}	0.764±0.033^{b}	0.503±0.019
BHBA (mmol/l)	Pre-partum.	0.474±0.020	0.725±0.022	0.538±0.021	0.527±0.020	0.784±0.029	0.577±0.023	0.501±0.024	0.987±0.015	0.628±0.049
	Post-partum	0.874±0.035	1.603±0.024	1.059±0.050	1.006±0.019	1.345±0.014	1.072±0.026	1.025±0.027	1.575±0.035	1.168±0.056
	Over all	0.693±0.017^{a}	1.183±0.032^{b}	0.938±0.019^{a}	0.782±0.019^{a}	1.058±0.036^{b}	0.920±0.020^{b}	0.753±0.030^{a}	1.289±0.042^{b}	1.021±0.025^{c}
CHOL (mg/dl)	Pre-partum.	101.241±1.810	82.927±2.232	96.585±1.793	96.276±2.051	83.363±3.033	93.756±1.918	88.812±1.815	80.483±3.898	86.639±1.811
	Post-partum	111.130±3.167	68.773±2.805	100.361±3.449	92.848±2.971	69.437±2.277	88.280±2.831	91.535±3.146	66.033±3.268	84.883±3.414
	Over all	103.313±1.674	74.785±3.153	89.049±1.859^{a}	93.027±1.878	76.171±3.507	84.599±1.964^{a}	90.288±2.921	71.768±4.104	81.028±2.413^{a}

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). No superscript mean non significant with in row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

4.4.1.3 Total plasma proteins level in subclinical ketosis:

The overall mean TPP level was in SCK cow 7.750±0.162 g/dl during pre-partum period and 6.633±0.098 g/dl during post-partum transition period. A highly significant (P≤0.01) decrease was observed in healthy and subclinical ketosis dairy cow from the pre to post-partum transition in the overall mean plasma proteins values and significantly higher value was observed as compared to the healthy cows during the pre-partum transition period (Table 11).

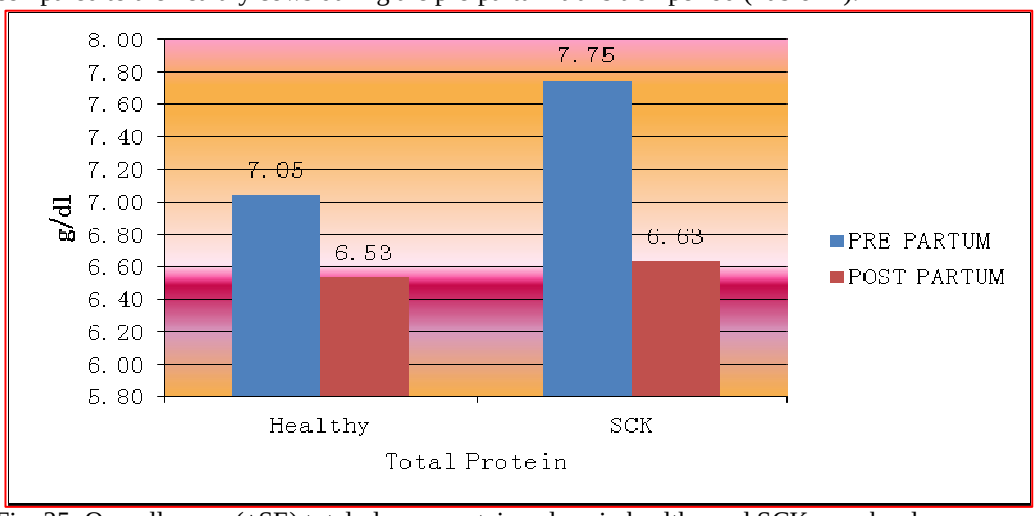

Fig. 35: Overall mean (±SE) total plasma protein values in healthy and SCK crossbred cows.

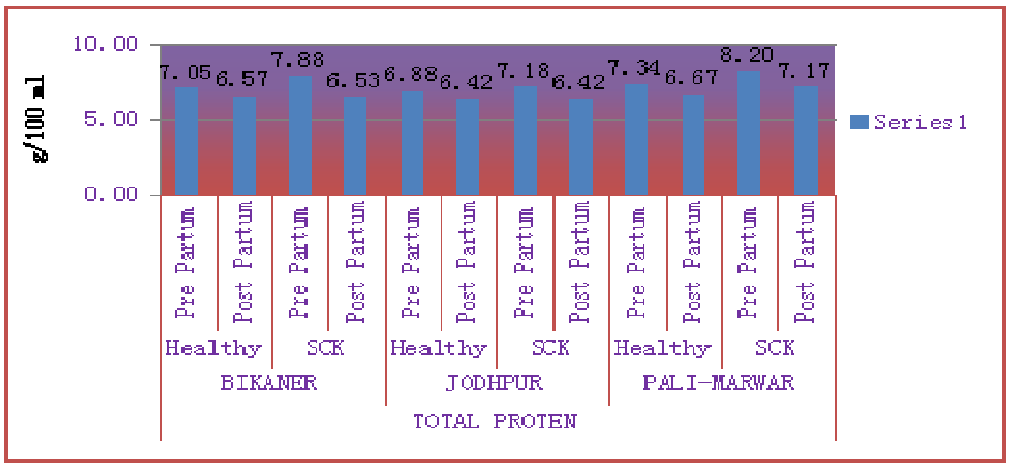

Fig. 36: Mean (±SE) total plasma proteins values in healthy and SCK crossbred cows.

District wise: Mean total protein values were 7.877±0.205, 7.179±0.334, 8.197±0.265 g/dl and 6.533±0.078, 6.416±0.117, 7.172±0.343 g/dl during pre and post-partum period. Though the mean TPP values were within the normal range during different periods, but a highly significant (P≤0.01) difference was observed from healthy and SCK dairy cow in two different stage.

In relation to parity, mean TPP level were (7.086±0.060, 6.545±0.043 g/dl) in 3rd -5th parity and above 5th parity (6.919±0.069, 6.501±0.110 g/dl) in healthy cows. Mean TPP level in SCK cows were 7.893±0.202, 6.469±0.075 g/dl in 3rd -5th parity and 7.516±0.266, 6.902±0.210 in above 5th parity from pre to post-partum period. Significant (P≤0.05) decrease was observed in the cows from all parity group from pre to post-partum transition period (Table 13). Overall highly significant (P≤0.01) differences were observed in two parity group.

Between districts, no significant difference was observed in the mean total plasma protein values (Table 12). Similar observations were also recorded by Zhabalanko (1976), Singh (1994), Gupta (1999), Youssef *et al.* (2010), Ghanem *et al.* (2010) and Elitok *et al.* (2010). On the

other hand Simenove *et al.* (1984) observed low concentration of total serum protein in ketotic cows. Our finding is similar to above mentioned author because significant decrease of total protein from pre-partum to post-partum transition period in subclinical ketotic dairy cow. Hyperproteinemia along with increased albumin and globulin accompanied by hypoglycemia might be due to energy deficient and protein rich ration given to the high yielders, though such a ration could provide enough energy and protein required to the low yielding cows (Schultz, 1968). According to Hibbit (1979) high protein intake worsen an energy deficit because of energy losses resulting from its metabolism and excretion. This energy deficit was responsible for development of ketosis.

Table 13: Plasma energy biomarkers and biochemical profile parity wise investigated in healthy and subclinical Ketosis crossbred cows of three districts of Western Rajasthan during transition period.

Parameters	**Periods**	**3nd - 5th parity**			**>5thparity**		
		Healthy (n=71)	**SCK (18)**	Over all (89)	**Healthy (n=23)**	**SCK (11)**	Over all (34)
GLUCOSE (mg/dl)	Pre-partum.	59.931±0.855	44.642±1.798	56.839±1.009	61.165±1.418	47.200±1.877	56.647±1.595
	Post-partum	53.569±0.701	37.425±1.587	50.304±0.942	53.861±1.251	42.018±2.263	50.029±1.462
	Over all	56.270±0.571	40.165±1.357	48.217±0.743	56.316±1.069	45.370±1.418	50.843±0.875
TOTAL PROTEIN (g/dl)	Pre-partum.	7.086±0.060	7.893±0.202	7.249±0.071	6.919±0.069	7.516±0.266	7.112±0.107
	Post-partum	6.545±0.043	6.469±0.075	6.529±0.038	6.501±0.110	6.902±0.210	6.631±0.104
	Over all	6.836±0.043	7.195±0.103	7.016±0.056	6.796±0.081	7.247±0.107	7.021±0.066
AL (g/dl)	Pre-partum.	3.203±0.105	3.230±0.155	3.208±0.089	2.747±0.231	2.965±0.198	2.817±0.168
	Post-partum	2.691±0.090	2.535±0.110	2.660±0.075	2.382±0.111	2.506±0.166	2.422±0.092
	Over all	2.963±0.068	2.915±0.163	2.939±0.089	2.605±0.128	2.686±0.170	2.646±0.105
GL (g/dl)	Pre-partum.	3.883±0.116	4.663±0.277	4.041±0.113	4.172±0.255	4.552±0.318	4.295±0.201
	Post-partum	3.853±0.083	3.934±0.138	3.870±0.071	4.120±0.146	4.395±0.146	4.209±0.110
	Over all	3.872±0.076	4.280±0.181	4.372±0.102	4.191±0.142	4.560±0.189	4.079±0.102
AL/GL	Pre-partum.	0.990±0.092	0.766±0.082	0.945±0.076	0.868±0.181	0.708±0.088	0.816±0.125
	Post-partum	0.768±0.051	0.677±0.058	0.749±0.042	0.613±0.049	0.579±0.046	0.602±0.036
	Over all	0.879±0.050	0.757±0.119	0.825±0.067	0.736±0.094	0.596±0.124	0.660±0.067
PUN (mg/dl)	Pre-partum.	9.635±0.228	8.611±0.234	9.428±0.193	8.935±0.211	10.191±0.656	9.341±0.270
	Post-partum	12.075±0.174	12.182±0.375	12.097±0.157	11.743±0.335	12.348±0.731	11.939±0.324
	Over all	10.876±0.143	11.046±0.341	10.961±0.187	10.319±0.269	11.061±0.356	10.690±0.220
CR (mg/dl)	Pre-partum.	1.258±0.035	1.399±0.096	1.286±0.034	1.171±0.065	1.328±0.074	1.222±0.051
	Post-partum	1.048±0.026	1.133±0.077	1.065±0.026	1.140±0.049	0.996±0.049	1.094±0.038
	Over all	1.146±0.024	1.283±0.057	1.215±0.031	1.126±0.045	1.191±0.060	1.159±0.037
NEFA (mmol/l)	Pre-partum.	0.180±0.009	0.493±0.027	0.243±0.016	0.218±0.020	0.560±0.030	0.329±0.032
	Post-partum	0.320±0.017	0.768±0.032	0.410±0.024	0.372±0.032	0.845±0.031	0.525±0.045
	Over all	0.248±0.009	0.676±0.023	0.462±0.012	0.277±0.018	0.689±0.024	0.483±0.015
BHBA (mmol/l)	Pre-partum.	0.491±0.013	0.748±0.027	0.543±0.016	0.520±0.030	0.873±0.033	0.634±0.036
	Post-partum	0.925±0.024	1.561±0.031	1.054±0.034	1.016±0.024	1.468±0.040	1.162±0.042
	Over all	0.723±0.012	1.170±0.030	0.946±0.016	0.763±0.024	1.184±0.031	0.974±0.019
CHOL (mg/dl)	Pre-partum.	98.277±1.386	81.572±1.968	94.899±1.372	94.078±2.679	84.127±2.732	90.859±2.151
	Post-partum	103.810±2.557	68.589±2.412	96.687±2.579	93.013±3.047	68.064±2.186	84.941±2.964
	Over all	98.523±1.227	72.930±2.917	85.727±1.597	92.563±2.296	75.552±3.046	84.057±1.880
TRIG (mg/dl)	Pre-partum.	9.327±0.320	13.519±1.042	10.175±0.373	10.058±0.722	16.047±1.379	11.996±0.812
	Post-partum	13.497±0.741	17.006±1.410	14.207±0.670	13.106±1.138	17.409±1.711	14.498±0.997
	Overall	11.619±0.409	18.072±0.974	14.845±0.533	11.563±0.767	16.125±1.017	13.844±0.628

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).

2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).

3. No superscript mean non significant with in row or column.

4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

4.4.2 Albumin (Al) :

4.4.2.1 Interaction between health wise and parity wise affecting albumin level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for albumin in plasma was 2.792±0.065 g/dl. The differences among subclass means were non significant across districts (2.605±0.103 in Bikaner, 2.808±0.109 in Jodhpur and 2.964±0.134 g/dl in Pali-Marwar), non significant in healthy/SCK groups (2.784±0.073 in healthy and 2.800±0.112 g/dl in SCK), significant in stage wise (3.100±0.081 in pre-partum and 2.594±0.060 g/dl in post-partum) and highly significant in parity groups (2.939±0.089 g/dl 3-5th parity and 2.646±0.105 g/dl above 5th parity). The effect of health status was not significant in pooled data. There was no effect of two factor interaction indicating that difference among districts. Where as in parity wise, albumin level was more in SCK dairy cow in comparison to healthy cows. The trend is depicted in Fig. 37. Perhaps it could be due to more consumption of leguminous feed after parturition.

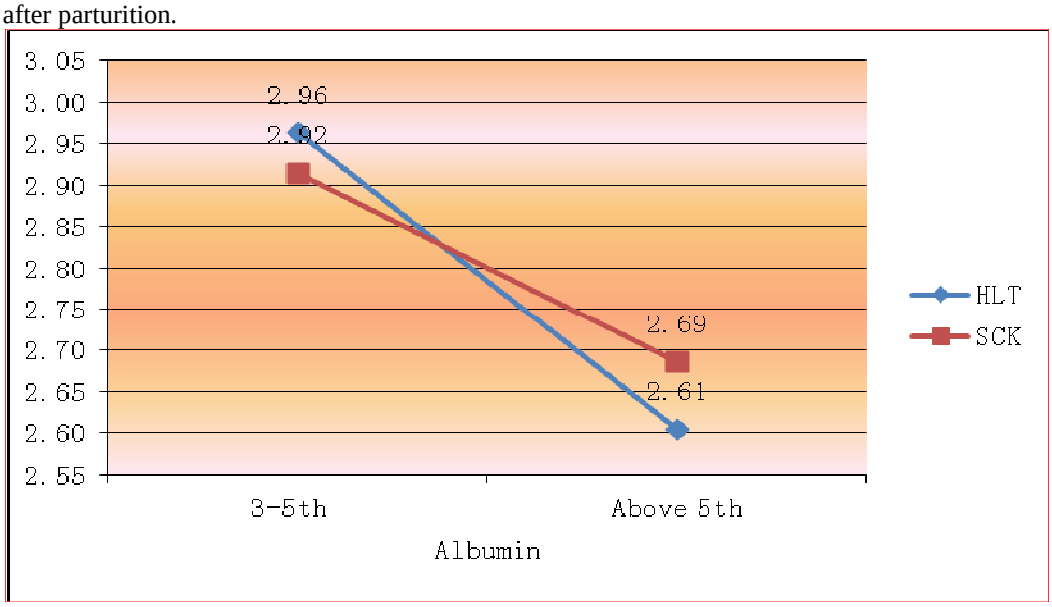

Fig. 37: Interaction between parity and health wise affecting albumin in healthy and SCK crossbred cows.

4.4.1.2 Albumin level in healthy dairy cows:

The overall mean plasma albumin value recorded in the healthy crossbred cows was 3.091±0.099 g/dl during the pre-partum and 2.616±0.074 g/dl during the post-partum period. A significant (P≤0.05) decrease was observed in the overall mean albumin values between pre to post-partum transition period (Table 11).

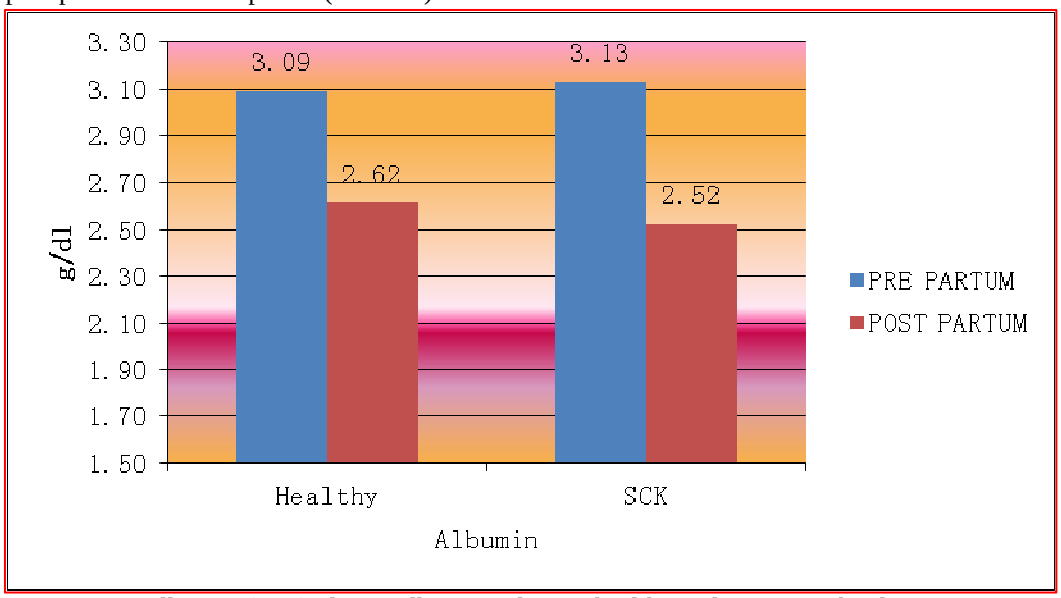

Fig. 38: Overall mean (±SE) plasma albumin values in healthy and SCK crossbred cows.

Districts wise: The mean albumin values recorded were 2.883±0.148, 3.433±0.146,

2.966±0.243 g/dl during the pre-partum period and 2.553±0.127, 2.550±0.109, 2.906±0.102 g/dl during the post-partum transition period from the cows of Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively (Table 12). Non significant difference was recorded in district wise in pre and post-partum period. A higher mean plasma albumin value was recorded in the cows from Jodhpur dairy cows as compared to other districts during the pre-partum and Pali –Marwar in post-partum transition period cows.

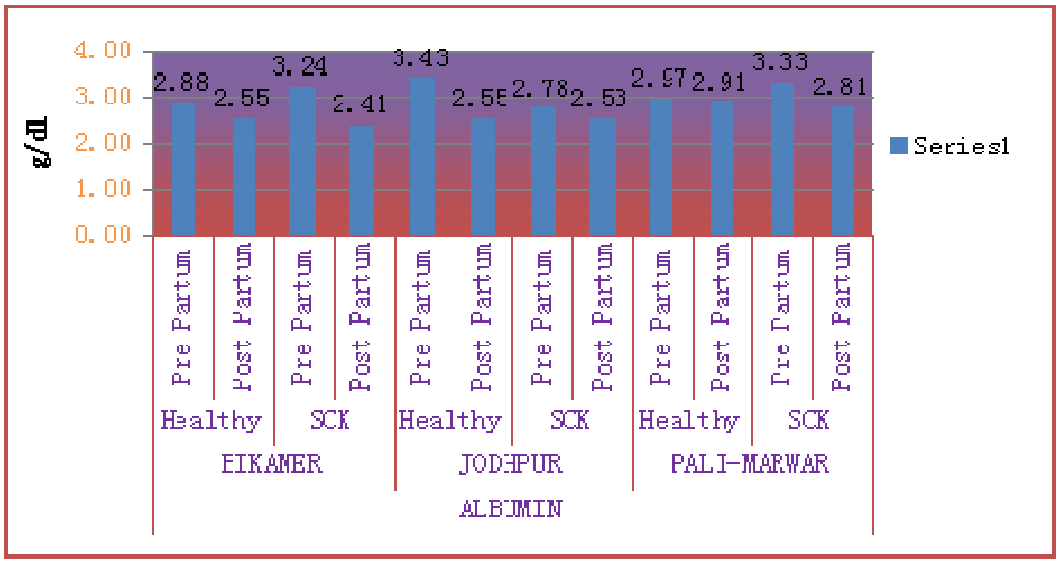

Fig. 39: Mean (±SE) plasma albumin level in healthy and SCK crossbred cows.

Due to the inability of the cows to consume sufficient dietary proteins to meet mammary and extra-mammary amino acid requirements, including a significant demand for hepatic gluconeogenesis, resulting in substantial, progressive mobilization of tissue proteins during the periparturient period. Along with the existing endocrine situation of the periparturient cow, major reductions in plasma levels of insulin and insulin-like growth factor-I together with insulin resistance in peripheral tissues, resulted in net mobilization of amino acids leading to decreased levels of plasma proteins and albumin (Bell *et al.*, 2000). Significant decreasing trend in serum albumin with advancing pregnancy had been reported in indigenous cows by Kumar *et al.* (2001), in Jersey crossbred cattle by Lone *et al.* (2003) and Sivaraman *et al.* (2003). However, Tainturier *et al.* (1984) reported no change in the serum albumin level during pregnancy. The fall in serum albumin towards the end of pregnancy might be due to the increasing nutrient requirement of the growing fetus (Lone *et al.*, 2003) and/or due to inadequate nourishment during advance stages of pregnancy (Sivaraman *et al.*, 2003). Mordak and Nicpon (2006) also observed lower albumin concentrations in the post-partum period. Lower albumin concentrations might also be due to gut malabsorption, malnutrition, dehydration, agammaglobulinaemia, glomerulonephritis as well as liver diseases (Stevens, 1975; Kupczynski and Chudoba-Drozdowska, 2001).

4.4.2.3 Albumin level in subclinical ketosis:

The overall mean plasma albumin recorded in crossbred cows from three districts was found to be within the normal range (2.1-3.6 g/dl) as proposed by Radostits *et al.* (2007), but overall significant decrease was noticed from the pre-partum-period to post-partum (3.129±0.122 and 2.524±0.091 g/dl) transition period respectively, and a non significant difference was noted in comparison to the healthy and SCK dairy animals but stage wise significant difference were observed (Table 11). Similarly, non significant decrease was noticed for the Bikaner (3.237±0.138 and 2.407±0.106 g/dl), Jodhpur (2.775±0.231 and 2.533±0.179 g/dl) and Pali-Marwar (3.332±0.352 and 2.805±0.251 g/dl) from pre to post-partum period (Table 12).

Overall parity wise highly significant ($P \leq 0.01$) differences were observed in dairy animal and also significant decrease occurred from pre to post-partum period. Overall albumin levels were recorded in 3^{rd} -5^{th} (3.203±0.105 and 2.691±0.090 g/dl) and above 5^{th} parity (2.747±0.231 and 2.382±0.111 g/dl) in healthy group and 3^{rd} -5^{th} (3.230±0.155 and 2.535±0.110 g/dl) and above 5^{th} parity (2.965±0.198 and 2.506±0.166) in SCK groups. Health wise, highly significant ($P \leq 0.01$) difference was observed in 2 different parity groups (Table 13).

Plasma level of total protein and albumin are indicator of hepatic function and decrease

in their concentration may indicate the fat infiltration into the liver. Infiltration of fat have deleterious effects on the metabolism in dairy cow and adversely affects milk production (Bobe *et al*., 2004; Djokovic *et al*., 2011; Piccione *et al*., 2011, 2012a, 2102b; Cincovic *et al*., 2012). Since albumin is indicative of the liver's synthetic function (West, 1990), the reduction in total protein and albumin in our study is an indicator for hepatic fat infiltration/dysfunction/ injury. Likewise, hypoalbuminemia is a common finding in chronic liver disease, occurring when the functional hepatic mass has been reduced to 20% or less (Dann *et al*., 2005). Djokovic *et al.* (2013) also reported a significant decrease in total plasma proteins and a non significant decrease in albumin level during early pregnancy in cows suffering from subclinical ketosis. Similarly, Gonzales *et al.* (2011) also observed a decrease in plasma albumin, total proteins and urea in cows with subclinical ketosis.

4.4.3 Globulin (GL) :

4.4.3.1 Interaction between districts and stage wise affecting Globulin level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for globulin in plasma was 4.226±0.072 g/dl. The differences among subclass means were highly significant across districts (4.394±0.115 in Bikaner, 3.901±0.122 in Jodhpur and 4.383±0.150 g/dl in Pali-Marwar), significant in healthy/SCK groups (4.032±0.081 in healthy and 4.420±0.125 g/dl in SCK), significant in stage wise (4.111±0.099 pre-partum and 3.963±0.061 g/dl post-partum) and non significant in parity groups (4.372±0.102 g/dl 3-5th parity and 4.079±0.102 g/dl above 5th parity). The effect of different parity groups were non significant in pooled data. There was effect of two factor interaction indicating that difference among districts. Districts and stage wise significant difference were recorded in globulin. Globulin level was higher in pre-partum stage in Pali-Marwar dairy cow. The trend is depicted in Fig. 40. The difference between pre to post-partum was higher in Pali-Marwar and lower in Jodhpur.

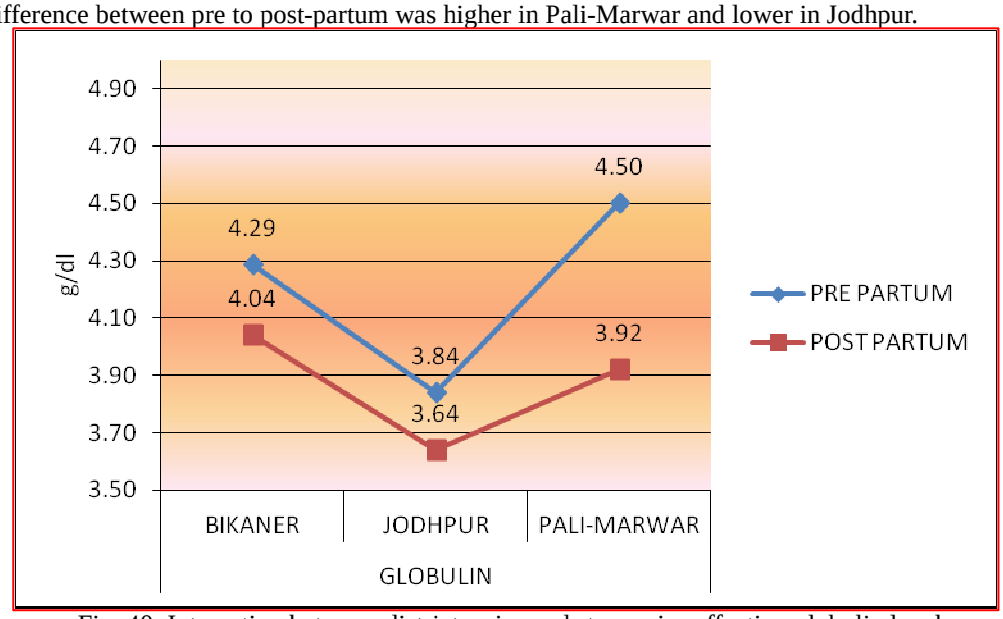

Fig. 40: Interaction between districts wise and stage wise affecting globulin level.

4.4.3.2 Plasma globulin level in healthy dairy cows:

The overall mean plasma globulin value in healthy cow was 3.954±0.108 g/dl during the pre-partum period and 3.919±0.073 g/dl during the post-partum transition period. The normal ranges for globulin to be 3.1-5.6 g/dl in cattle (Radostits *et al.*, 2000). Overall highly significant (P≤0.01) difference was observed in districts wise values. The overall significant differences were observed in healthy and in stage wise globulin level. (Table 11).

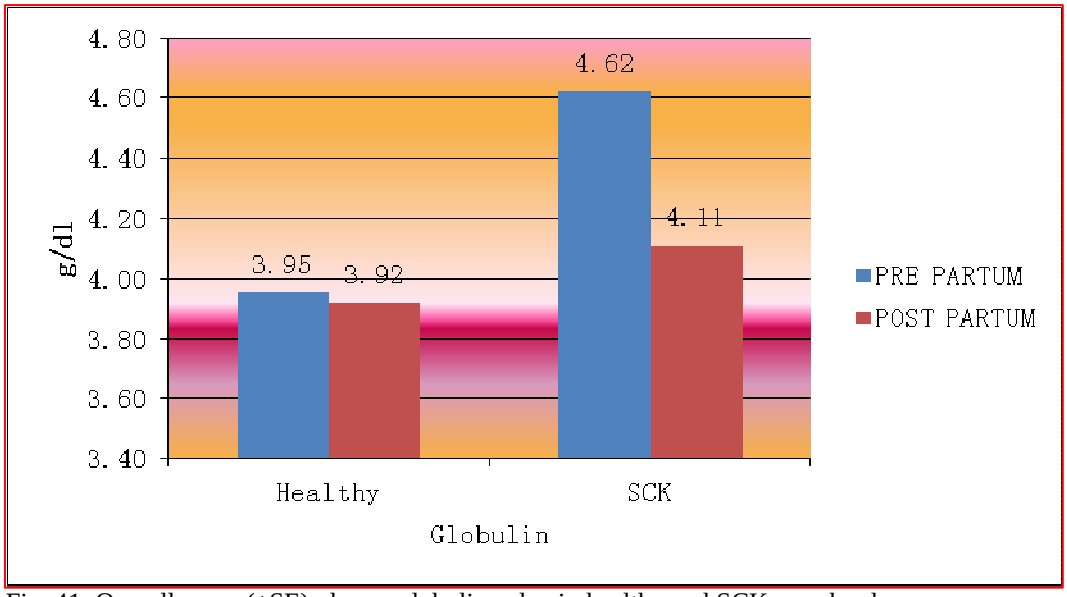

Fig. 41: Overall mean (±SE) plasma globulin value in healthy and SCK crossbred cows.

Districts wise: The mean globulin values recorded were 4.169±0.148, 3.452±0.163, 4.372±0.260 g/dl during the pre-partum period and 4.015±0.121, 3.869±0.104, 3.765±0.152 g/dl during the post-partum transition period in cows of Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively (Table12 and Fig. 42). Significant differences was observed district wise in pre and post-partum period. Higher mean plasma globulin value was observed in the cows from Pali-Marwar dairy cows as compared to other districts during the pre-partum and Jodhpur in post-partum transition period cows.

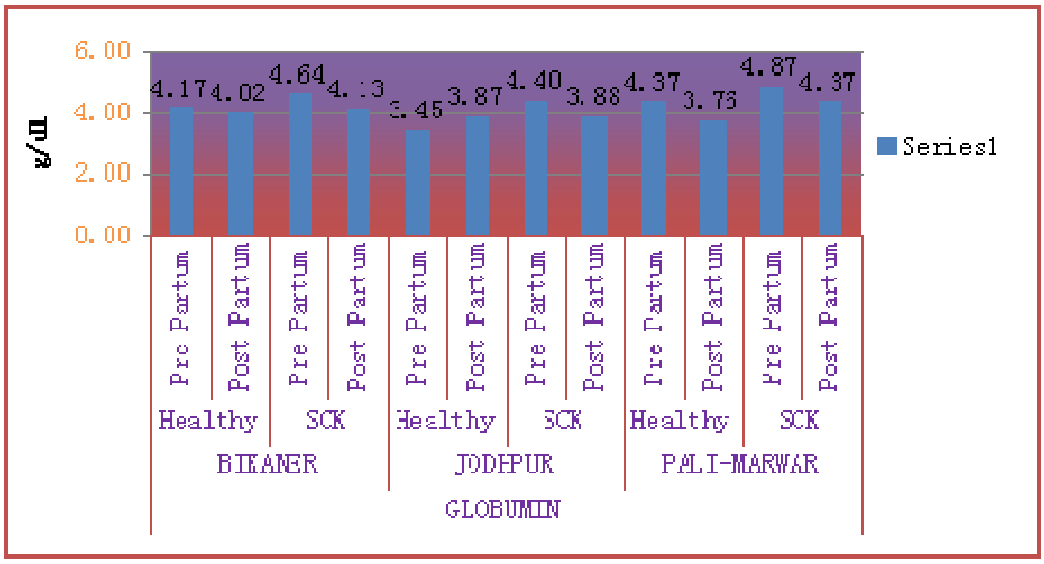

Fig. 42: Mean (±SE) plasma values of globulin in healthy and SCK crossbred cows.

4.4.3.3 Plasma globulin level in subclinical ketosis:

The overall mean plasma globulin recorded in crossbred cows from various districts was found to be within the normal range (3.1-5.6 g/dl) as reported by Radostits *et al.* (2007), but overall significant (P≤0.05) decrease was noticed from the pre-partum period to post-partum (4.621±0.207 and 4.109±0.109 g/dl) transition period, respectively and a significant difference was noted in between the healthy and SCK dairy animals (Table 11). Similarly, significant decrease was noticed for the Bikaner (4.639±0.232 and 4.126±0.117 g/dl), Jodhpur (4.404±0.508 and 3.884±0.198 g/dl) and Pali-Marwar (4.865±0.524 and 4.367±0.352 g/dl) from pre to post-partum period (Table 12).

Mean values of globulin were 3.883±0.116; 3.853±0.083 in 3rd -5th and 4.172±0.255; 4.120±0.146 above 5th parity in healthy pre and post-partum in cross bred dairy animal. Globulin level were in SCK cows from 3rd -5th parity (4.663±0.277; 3.934±0.138 g/dl) and in above 5th

parity (4.552±0.318; 4.395±0.146 g/dl) in pre to post-partum periods (Table 13). Non significant difference was observed in overall mean value of globulin in parity wise.

During gestation, albumin decreases and globulin increases. In cows, the total plasma protein, globulin begin to increase at 2 months before term, reach maximum at 1 month and then rapidly decline toward term (Larson and Kendall, 1957). The data indicated that the immunoglobulin rapidly leave the plasma during the last month of gestation when colostrum is being formed in the mammary gland. Lactation imposes further stresses on protein reserves and metabolism and changes similar to pregnancy also occur.

In all animals, there was a general increase in total protein, a decrease in albumin and an increase in globulins with advancing age (Forstner, 1968; Tumbleson *et al.*, 1972); and at very old, the total protein again decline.

The plasma proteins are sensitive to nutritional influences but the changes are often subtle and difficult to detect and interpret. Dietary protein deficiency results in a decreased turnover of serum albumin. Immunoglobulins are affected only on severe protein restriction (Benditt *et al*., 1949), but the effects are reversible on protein repletion (Wissler *et al*., 1946). The stress causes a decrease in total protein, decrease in albumin and often an increase in globulin.

High serum globulins are common in cattle with chronic infection (mastitis, cellulitis, liver abscesses, etc.) and high serum globulins will lower the A/G ratio (Russell and Russell, 2007). None of the cows sampled had evidence of chronic infection which would result in high serum globulins.

4.4.4 Albumin/ Globulin (A/G) ratio:

4.4.4.1 Plasma A/ G ratio in healthy dairy cows:

The overall mean plasma A/G ratio was recorded 0.960±0.082 during the pre-partum period and 0.730±0.041 during the post-partum in healthy dairy cow. Overall mean A/G ratio were within normal range 0.84-0.94 in dairy cows. Non significant effect was noted on the plasma A/G ratio with respect to transition period in healthy and SCK dairy cows (Table11).

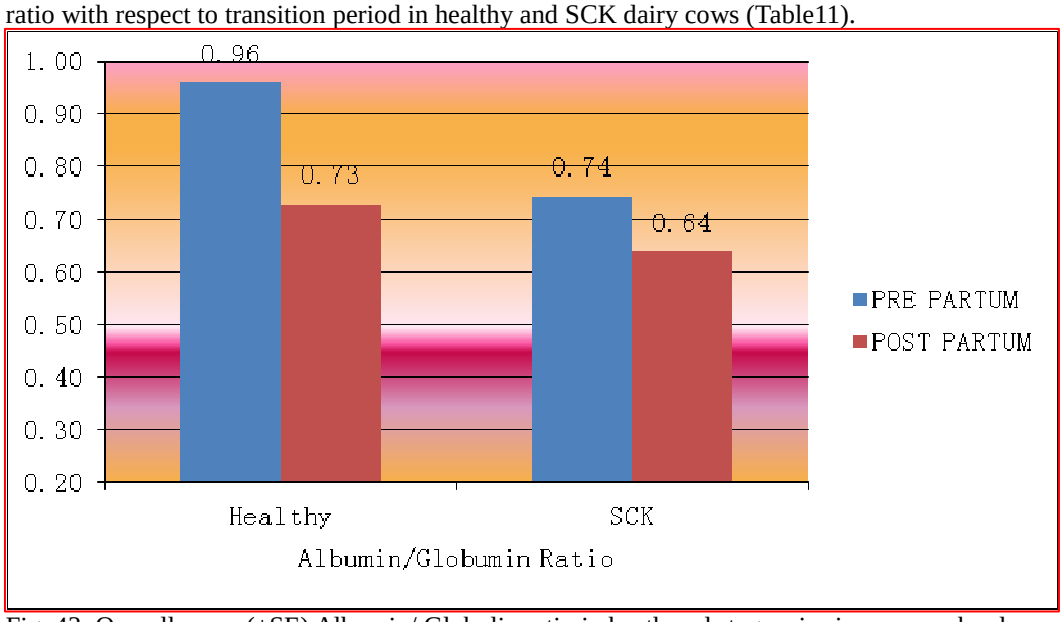

Fig. 43: Overall mean (±SE) Albumin/ Globulin ratio in heath and stage wise in cross bred dairy cows

District wise: The mean A/G ratio recorded were 0.843±0.113, 1.172±0.136, 0.852±0.220 mg/dl during the pre-partum period and 0.721±0.073, 0.703±0.058, 0.803±0.055 mg/dl during the post-partum transition period from the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms respectively. Non significant changes were observed in district, stage and health wise. (Table 12).

4.4.4.2 Plasma A/ G ratio in subclinical ketosis:

The overall mean plasma A/G ratio in SCK dairy cows were 0.744±0.060 and 0.640±0.040 in pre and post-partum periods which were non significant in health (healthy and SCK) and stage wise (pre and post-partum).

Overall parity wise non significant differences were observed in dairy animal from pre to post-partum period. Overall A/G ratio were recorded in 3rd -5th (0.990±0.092 and 0.768±0.051

g/dl) and above 5^{th} parity (0.868±0.181 and 0.613±0.049 g/dl) in healthy group and 3^{rd} -5^{th} (0.766±0.082 and 0.677±0.058 g/dl) and above 5^{th} parity (0.708±0.088 and 0.579±0.046) in SCK groups. Health wise, non significant differences were observed in 2 different parity group (Table 13).

Simple dehydration with water loss is essentially the only instance when a simple hyperproteinemia without change in profile or A: G occurs. In our study, all protein fractions increased proportionally, including albumin because only water has been removed from the system and without any change on A/G ratio.

4.4.5 Plasma urea nitrogen:

4.4.5.1 Interaction between districts and health affecting PUN level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for PUN in plasma was 10.826±0.137 mg/dl. The differences among subclass mean values were highly significant (P≤0.01) across districts (10.373±0.217 in Bikaner, 10.618±0.230 in Jodhpur and 11.485±0.282 mg/dl in Pali-Marwar), non significant in healthy/SCK groups (10.598±0.153 in healthy and 11.053±0.235 mg/dl in SCK), non significant in stage wise (9.404±0.158 pre-partum and 12.053±0.144 mg/dl post-partum) and highly significant in parity groups (10.961±0.187 mg/dl 3-5th parity and 10.690±0.220 mg/dl above 5th parity). The effect of pre and post-partum stages and healthy/SCK were non significant in pooled data. There was effect of two factor interaction indicating that difference among districts. Where as the interaction between districts and health status was highly significant. The trend is depicted in Fig. 44, which shows that the PUN level was lower in healthy group across all districts but the rise in their level was significantly higher in (10.746±0.342 to 12.224±0.480 mg/dl) Pali-Marwar district and lowest (10.290±0.220 to 10.947±0.410 mg/dl) in Jodhpur districts. The level of PUN in SCK dairy cow was high in Pali-Marwar. It was due to the more protein metabolism in negative energy balance animal in comparison to other districts. It showed that Pali-Marwar dairy cows were more in negative energy balance.

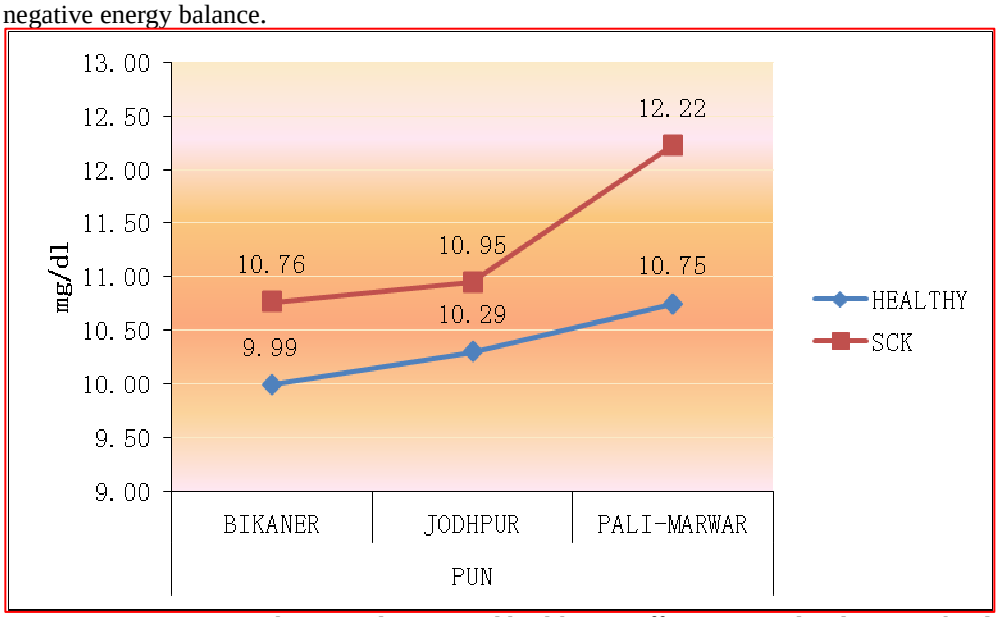

Fig. 44: Interaction between districts and health wise affecting PUN level in cross bred cows.

4.4.5.2 Plasma urea nitrogen (PUN) level in healthy dairy cows:

The overall mean plasma urea nitrogen (PUN) value in healthy cows was 9.464±0.182 mg/dl during the pre-partum period and 11.994±0.155 mg/dl during the post-partum transition period. The normal range for blood urea nitrogen is 6-27 mg/dl in cattle (Radostits *et al.*, 2000). Overall highly significant (P≤0.01) difference was observed in districts wise and non significant difference in stage wise in PUN level (Table 11).

District wise: The mean PUN values were 9.625±0.273, 8.900±0.277 and 10.141±0.420 mg/dl during the pre-partum and 12.200±0.207, 11.745±0.286 and 11.941±0.370 mg/dl during the post-partum transition period in crossbred cows from Bikaner, Jodhpur and Pali-Marwar dairy cows, respectively (Fig. 45). Highly significant (P≤0.01) PUN levels were observed in healthy and subclinical ketosis dairy cows. Higher value was observed in Pali-Marwar (10.141±0.420) dairy cows in comparison to the cows from Bikaner and Jodhpur dairy farms during the pre-partum and post-partum in Bikaner (12.200±0.207) (Table 12).

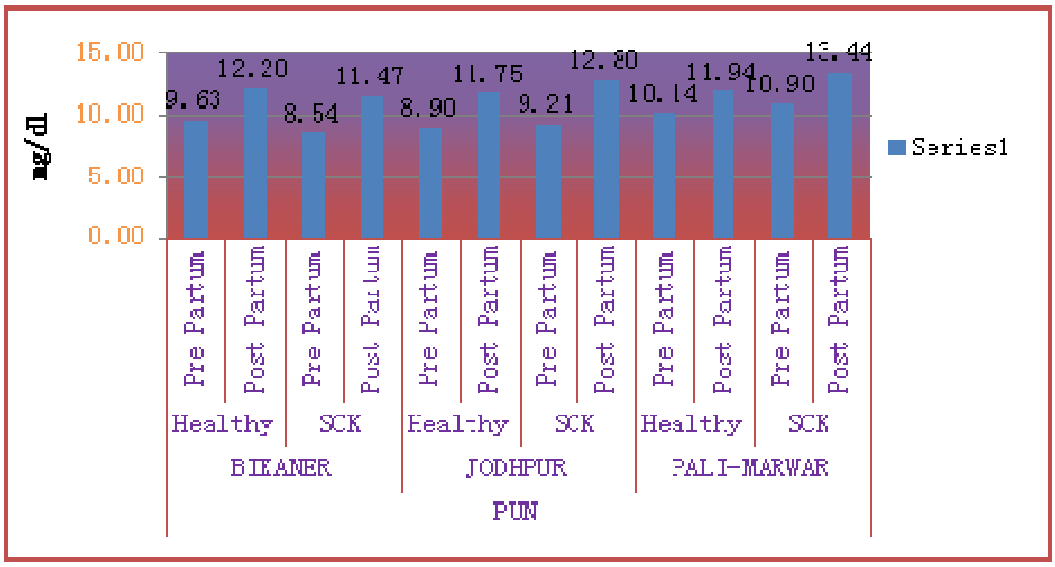

Fig. 45: Mean (±SE) PUN values in healthy and SCK crossbred cows.

Mean PUN level was significantly affected during the different physiological phases. The PUN (an end product of protein catabolism) concentrations are influenced by a wide variety of interrelated factors including dietary protein intake, rumen degradability, dietary amino acid composition, protein intake relative to requirement, liver/kidney function, muscle tissue breakdown, dietary carbohydrate amount and rumen degradability (Roubies *et al.*, 2006). Greater urea concentration in lactating animals could be a result of muscle protein catabolism when large amounts of body reserves are mobilized for meeting the lactation demands (Caldeira *et al.*, 2007 and Sreedhar *et al.*, 2013). Shwartz *et al.* (2009) and Fekry *et al.* (1989) reported that stressed cows had increased BUN level as compared to controls, which could be due to the higher utilization of amino acids as energy source. Also in late gestation, glucose availability for oxidation is supplemented by increased catabolism of amino acids at the expense of protein synthesis, thus increasing urea production. Similarly, Kulkarni *et al.* (2010) reported an increase in BUN level during the early lactation period. Plasma urea nitrogen concentration are metabolic breakdown constituients and will be elevated, depending on the severity of dehydration and decrease in circulating blood volume.

4.4.5.3 Plasma urea nitrogen (PUN) level in subclinical ketotic cow:

The overall mean plasma PUN level in cows from three districts was found to be 9.210±0.316 during pre-partum period; 12.245±0.354 mg/dl during post-partum transition period in SCK cows.

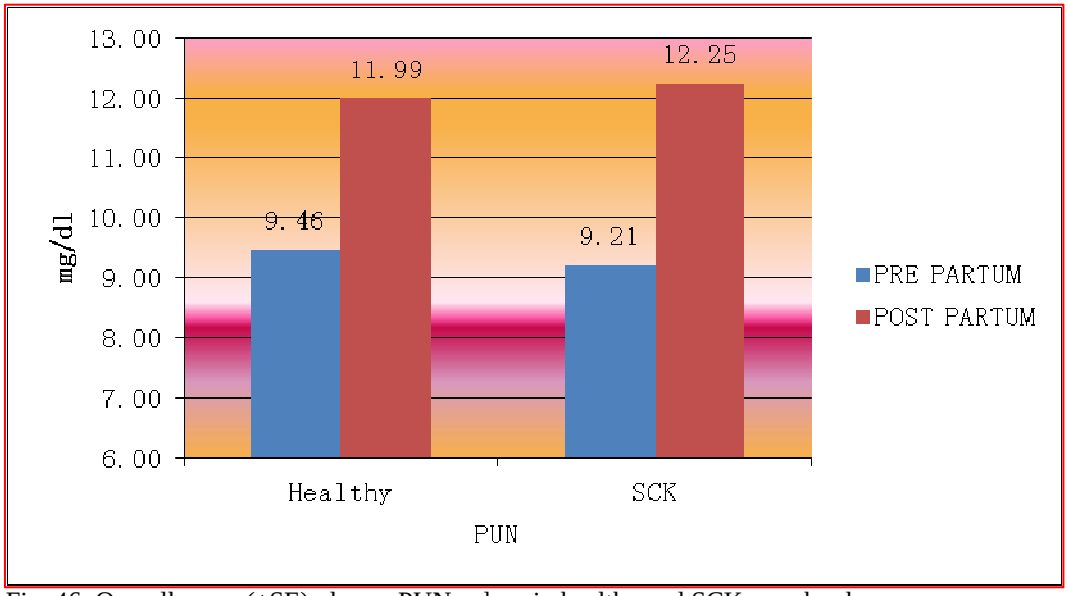

Fig. 46: Overall mean (±SE) plasma PUN values in healthy and SCK crossbred cows.

The mean PUN level was 8.537±0.255, 9.205±0.629, 10.900±0.843 mg/dl in pre-partum and 11.473±0.338, 12.797±0.722, 13.437±0.980 mg/dl in post-partum transition period in Bikaner, Jodhpur and Pali-Marwar district respectively. Highly Significant (P≤0.01) difference was observed between the healthy and SCK affected animals and a non significant increase was observed in the overall mean plasma PUN values in both the groups (healthy and SCK) from the pre-partum period to the post-partum transition period. Djokovic *et al.* (2013) also reported non significant decrease in urea level during early pregnancy in cows suffering from subclinical ketosis. District wise, highly significant (P≤0.01) increase was recorded in the mean plasma PUN values in the dairy cows of all district (Table 12). Gonzales *et al.* (2011) also observed a decrease urea in cows with subclinical ketosis.

Highly significant (P≤0.01) increase was noticed in the overall mean PUN level between two different parity group. Mean value were 9.635±0.228, 12.075±0.174 and 8.935±0.211, 11.743±0.335 in 3rd -5th and above 5th parity in healthy pre and post-partum dairy animal.

PUN level in SCK cows were from 3rd -5th parity (8.611±0.234 and 12.182±0.375 g/dl) and above 5th parity (10.191±0.656 and 12.348±0.731 g/dl) in pre to post-partum periods. (Table 13).

Higher plasma urea showed the increased activity of hepatocyte in synthesizing urea as observed in the results of present investigation during post-partum. Production stress extreme in transition period can influence the urea cycle in hepatocyte (Wotton, 1971).

PUN level might increase due to water deprivation, thirst, diarrhoea, urinary diseases, and acidosis, which were not applicable in case of cows of present study. However, individual differences in plasma concentrations among cows indicate that PUN varied from one cow to another, as they are also reflective of the body metabolism, amount of milk yield and the level of food consumption. Although the differences were statistically non significant in healthy and subclinical ketotic cow, as they were within normal range.

4.4.6 Creatinine (Cr):

4.4.6.1 Interaction between (districts and health wise; districts and stage wise) affecting Creatinine level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for creatinine in plasma was 1.187±0.023 mg/dl. The differences among subclass means were significant (P≤0.05) across districts (1.230±0.036 in Bikaner, 1.094±0.038 in Jodhpur and 1.236±0.047 mg/dl in Pali-Marwar), significant in healthy/SCK groups (1.136±0.025 in healthy and 1.237±0.039 mg/dl in SCK), non significant in stage wise (1.269±0.029 in pre-partum and 1.073±0.022 mg/dl in post-partum) and highly significant (P≤0.01) parity groups (1.215±0.031 mg/dl in 3-5th parity and 1.159±0.037 mg/dl in above 5th parity). The effect of pre and post-partum stages were non significant in pooled data. There were effects of two factor interaction indicating difference among districts. The interaction between districts and health status was significant

(P≤0.05). The trend is depicted in Fig. 47, which shows that the creatinine level was lower in healthy groups across all districts but the rise in their level was significantly higher in (1.086±0.057 to 1.386±0.080 g/dl) Pali-Marwar district and lower (1.125±0.037 to 1.063±0.069 g/dl) in Bikaner districts. In Pali- Marwar dairy cow creatinine level were due to high protein metabolism and fetal load of excretion in comparison to Bikaner and Jodhpur. High level of creatinine was observed in Pali-Marwar due to more protein metabolism in health wise in SCK cow in comparison to other district. It showed that effect of negative energy balance high in dairy cows of Pali-Marwar.

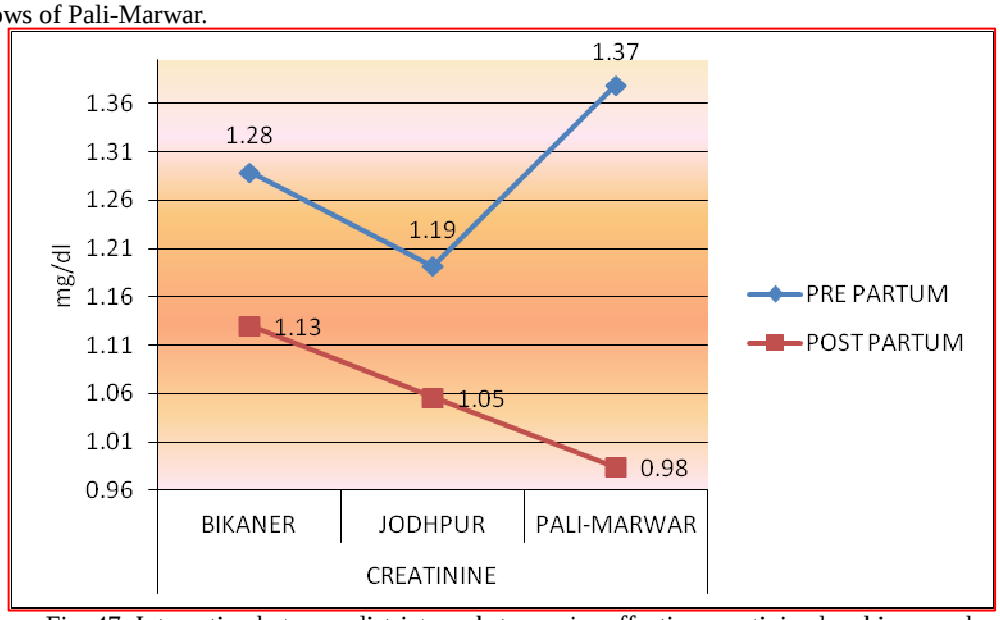

Fig. 47: Interaction between districts and stage wise affecting creatinine level in cross bred cows

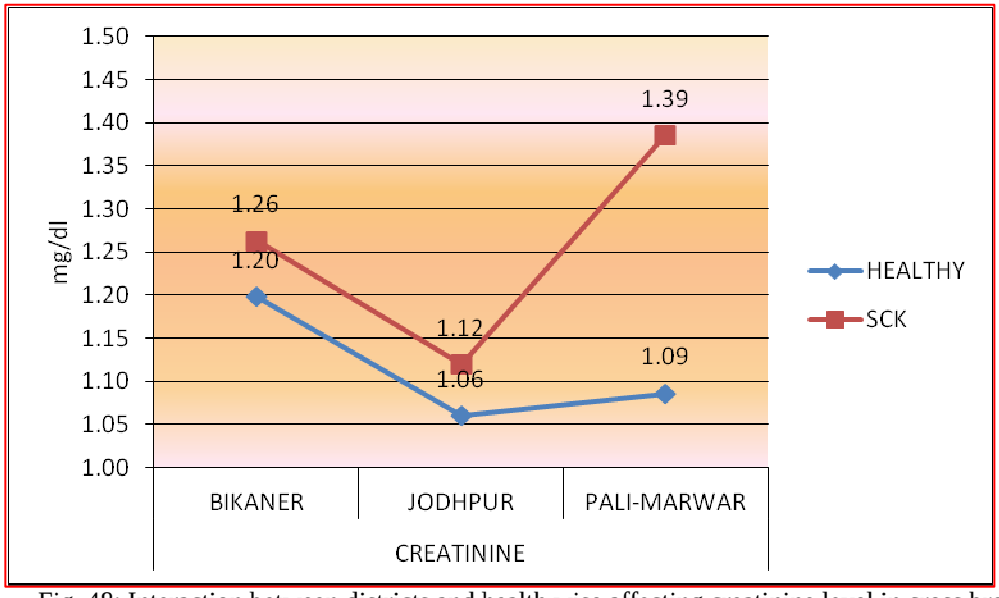

Fig. 48: Interaction between districts and health wise affecting creatinine level in cross bred cows.

4.4.6.2 Creatinine (Cr) level in healthy dairy cows:

The overall mean plasma creatinine level recorded were 1.236±0.031 mg/dl during the pre-partum period and 1.070±0.024 mg/dl during the post-partum in healthy dairy cows. Significant effect was noted on the plasma creatinine concentrations with respect to transition period in healthy and SCK dairy cows (Table11).

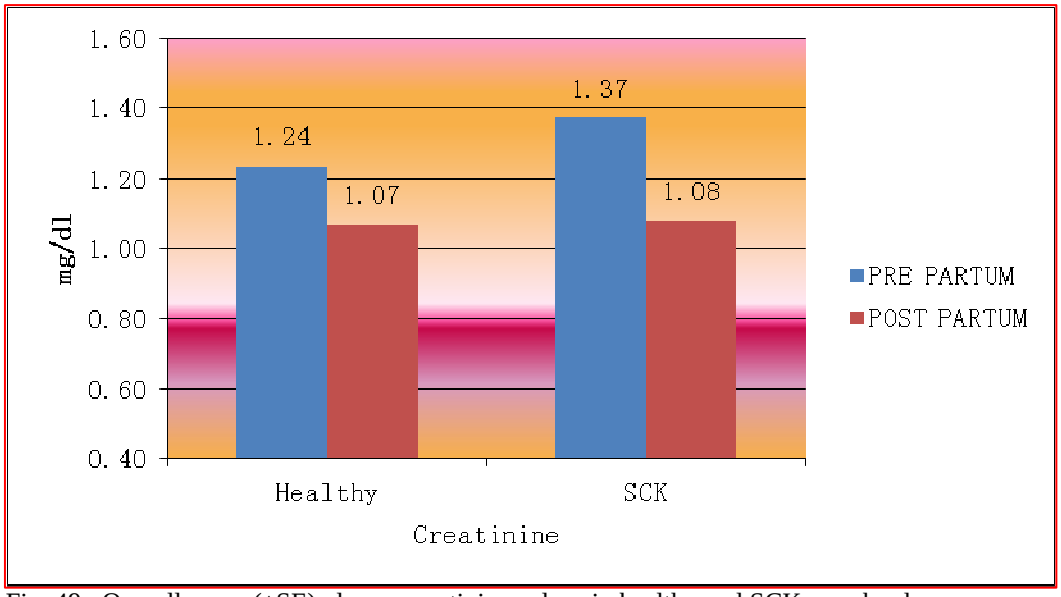

Fig. 49 : Overall mean (±SE) plasma creatinine values in healthy and SCK crossbred cows.

District wise: The mean creatinine level recorded were 1.237±0.044, 1.189±0.057, 1.327±0.065 mg/dl during the pre-partum period and 1.128±0.038, 1.083±0.032, 0.895±0.039 mg/dl during the post-partum transition period from the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. Significant (P≤0.05) differences were observed in district and health wise. Higher mean value of creatinine was observed in the cows of Pali- Marwar (1.327±0.065) dairy farms as compared to the cows from the other districts in pre-partum and Bikaner dairy farm during the post-partum transition period (Table 12).

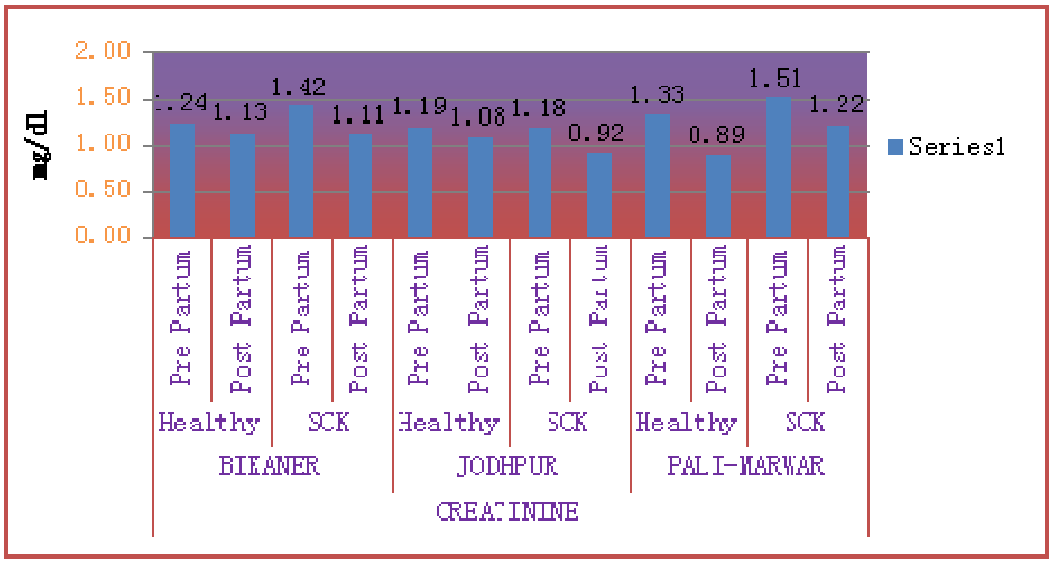

Fig. 50: Mean (±SE) plasma creatinine values in healthy and SCK crossbred cows.

Mean values of creatinine were 1.258±0.035; 1.048±0.026 and 1.171±0.065; 1.140±0.049 in 3rd -5th and above 5th parity in healthy pre and post-partum dairy animals.

Creatinine level in SCK cows was in 3rd -5th parity (1.399±0.096; 1.133±0.077 g/dl) and above 5th parity (1.328±0.074; 0.996±0.049 g/dl) in pre to post-partum periods (Table 13). Overall mean revealed highly significant (P≤0.01) difference, parity wise.

In domestic species, CK is mainly used as a specific marker of skeletal muscle injury (Hoffmann and Solter, 2008). No previous studies have reported on the effect of parity on CK activity of cattle. In horses, CK tends to be higher in younger and untrained animals (Harris *et al.,* 1998). The increase in CK observed in young cows (3rd to 5th parity) (Table 13) could derive from the physical stress caused by the competitive pressure when they are mixed with more experienced cows (Val-Laillet *et al*., 2009). Multiparous cows are generally dominant over primiparous ones and frequently show their social dominance through aggressive behaviors addressed toward subordinate cattle (Harris *et al*., 2007).

The mean creatinine level in our study showed significant patterns during pregnancy and lactation in districts and stage wise. The quantity of creatinine formed generally depends upon the total body content of creatine which in turn depends on dietary intake, rate of synthesis of creatine and muscle mass. Usually, the dry period has been thought to be necessary for replenishment of body reserves, regeneration of mammary tissue and for maximal benefits from lactogenic endocrine events (Annen *et al.,* 2004) which normally depends upon the mobilization of amino acids from the protein breakdown in skeletal muscles, although skin, uterine involution and myometral protein degradation may also have some contribution (Bell *et al.,* 2000). It is well recognized that during the late gestation, the mother, assumes the load of organic waste of the newborn for the foetal maternal circulation (Ferrell, 1991). So the increase in serum creatinine level observed in some farms could be attributed to the development of the foetal musculature which is well documented in sheep and ewes too (Roubies *et al.,* 2006). In dairy cows, plasma creatinine and muscle diameter start to decrease from one week before calving upto four weeks after calving. Moorby *et al.* (2002) also reported a decrease in longissimus dorsi muscle diameter before calving and reached a minimum between 4 to 7 week of lactation. Similarly, Doornenbal *et al.* (1988) found decreased serum creatinine concentration during lactation which increased during post weaning.

However, some researchers (Yokus and Cakir, 2006 and Gurgoze *et al.,* 2009) found no relationship between the serum creatinine level and reproductive status in cattle and sheep.

4.4.6.3 Creatinine (CR) level in subclinical ketosis dairy cow:

The overall mean plasma creatinine level recorded in the healthy and SCK cows were 1.236±0.031; 1.372±0.065 mg/dl during pre-partum period and 1.070±0.024 and 1.081±0.052 mg/dl during post-partum transition period, respectively. Significantly higher creatinine level were observed in SCK affected cows during pre and post-partum transition period in comparison to healthy cows and a non significant decrease was observed from pre-partum period to the post-partum period in both the groups (healthy and SCK) (Table 11).

The mean plasma creatinine values recorded were 1.421±0.109, 1.180±0.067, 1.507±0.087 mg/dl during pre-partum period; 1.115±0.061, 0.916±0.035, 1.217±0.184 mg/dl during post-partum period from the SCK affected cows of Bikaner, Jodhpur and Pali-Marwar districts, respectively. District wise, significant differences were observed in cows from three districts during different periods (stage). Li *et al.* (2011) recorded a decrease in the renal function in cows with subclinical ketosis.

The normal values of blood constituents can be affected by age of the animals (Gottam *et al*., 2005). Metabolic status of the animals is reflected by the level of creatinine. Most of the creatinine excreted originates from endogenous creatino. The amino acids arginine and glycine combine to form guanidinoacetate in the pancreas, kidney and small intestine. In the liver, methionine provides a methyl group for conversion of guanidinoacetate to creatine; creatine circulates in plasma and is taken up by muscle, where it stores energy in the form of phosphocreatine. This undergoes spontaneous crystallisation with the loss of inorganic phosphate to form creatinine. Creatinine undergoes no catabolic reaction other than decomposition to creatinine (Finco, 1999). Although creatinine estimation is not conventional component of hepatic function test but it is important to note that quality of creatinine formed each day depends also upon the rate of synthesis of creatine by the liver (Kaneko *et al*., 1999).

4.4.7 Glucose:

4.4.7.1 Interaction between (districts and health; health and parity wise) affecting glucose level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for glucose in plasma was 49.530±0.545 mg/dl. The differences among subclass means were non significant across districts (51.098±0.865 in Bikaner, 49.202±0.914 in Jodhpur and

48.291±1.123 mg/dl in Pali-Marwar), highly significant (P≤0.01) in healthy/SCK groups (56.293±0.611 in healthy and 42.768±0.937 mg/dl in SCK), significant (P≤0.05) in stage wise (56.786±0.850 in pre-partum and 50.228±0.790 mg/dl in post-partum) and highly significant in parity groups (48.217±0.743 mg/dl in 3-5th parity and 50.843±0.875 mg/dl in above 5th parity). The effect of districts was non significant in pooled data. There was effect of two factor interaction indicating that difference among districts. Where as the interaction between districts and health status was highly significant (P≤0.01). The trend is depicted in Fig. 51, which showed that the glucose level was high in healthy groups across all districts but the decrease in their level in SCK dairy cow which was significantly higher in (58.873±0.874 to 39.530±1.632mg/dl) Jodhpur district and lowest (52.757±1.360 to 43.825±1.910 mg/dl) in Pali-Marwar district. The glucose level was low in Jodhpur district in SCK dairy cow. It showed that dairy cows were more on negative energy balance during post-partum transition period in Pali-Marwar in comparison to Bikaner and Jodhpur. In parity wise, low level of glucose in SCK dairy cow in 3rd -5th parity in comparison above 5th parity.

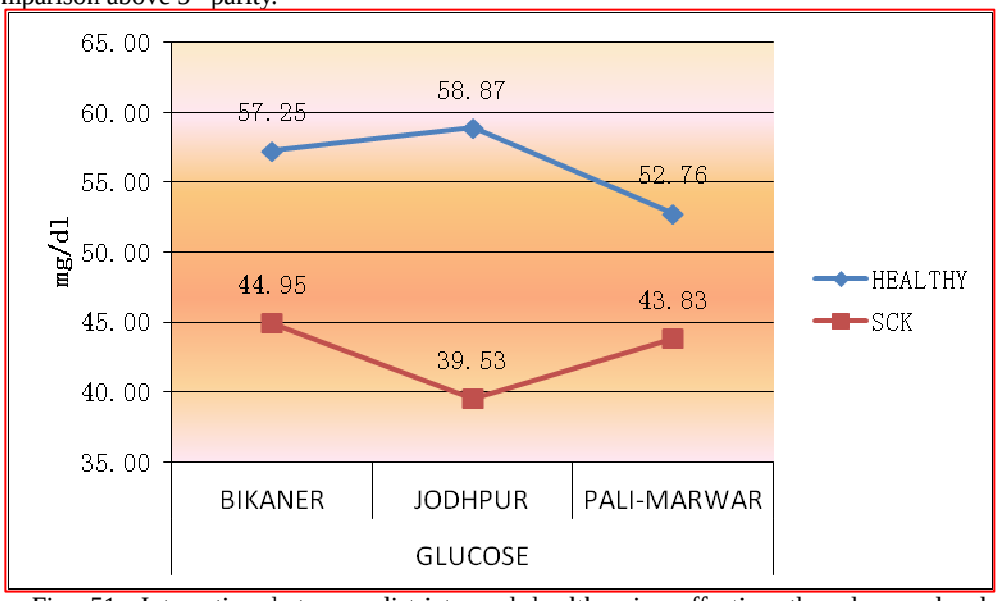

Fig. 51: Interaction between districts and health wise affecting the glucose level in crossbred cows.

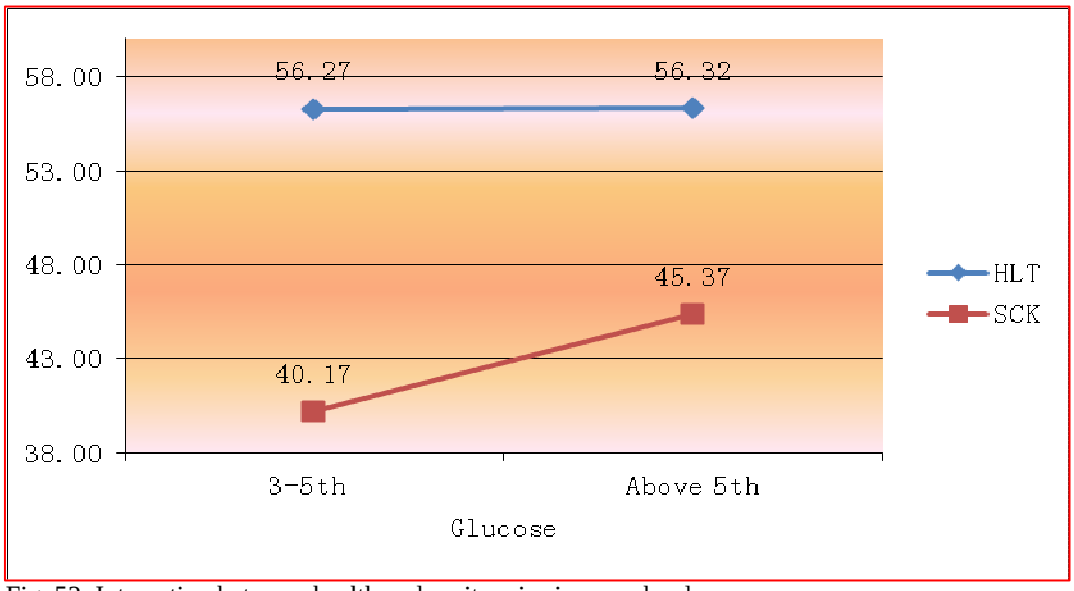

Fig. 52: Interaction between health and parity wise in cross bred cows.

4.4.7.2 Glucose level in healthy dairy cow:

The overall mean plasma glucose level was 60.233±0.731 mg/dl during the pre-partum period and 53.641±0.608 mg/dl during the post-partum transition period (Table 11). A highly significant (P≤0.01) difference was observed in the overall mean glucose level in healthy and

SCK dairy cows. Stage wise significant differences were found from the pre-partum to the post-partum transition period (Table 11).

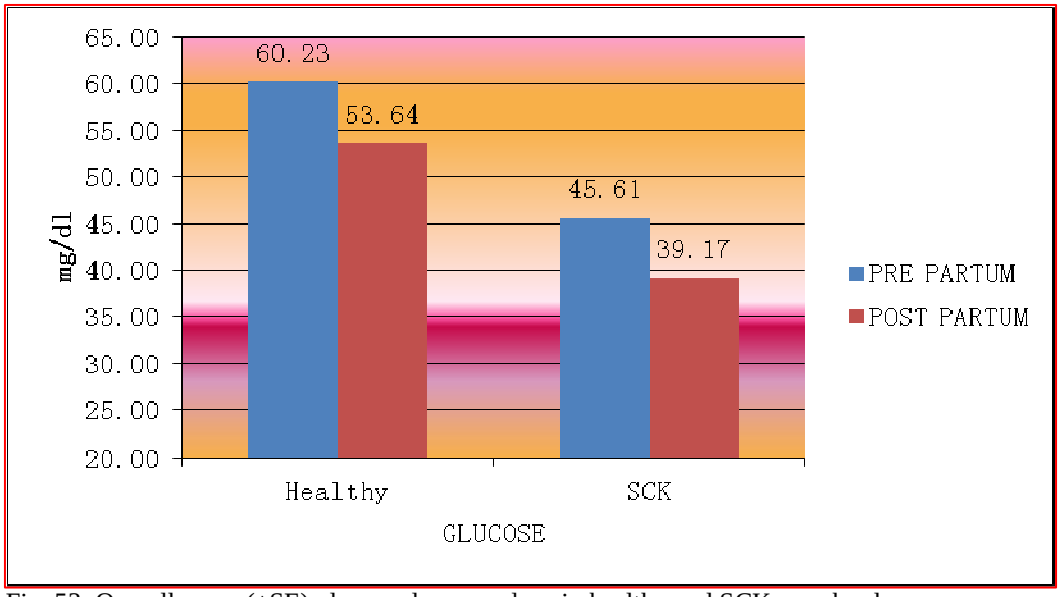

Fig. 53: Overall mean (±SE) plasma glucose values in healthy and SCK crossbred cows.

District wise: The mean glucose level was 59.415±1.079, 62.503±1.204, 57.941±1.552 mg/dl during the pre-partum period and 54.605±0.807, 55.109±1.002, 48.294±1.124 mg/dl during the post-partum transition period from the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. District wise highly significant (P≤0.01) differences were observed in healthy and SCK dairy cow. Higher values were observed for Jodhpur cows as compared to cows from Bikaner and Pali-Marwar dairy farm during pre and post-partum transition period similarly, lower value was noted for cows from Pali-Marwar (57.941±1.552) district as compared to the cows from Jodhpur and Bikaner dairy farm during pre-partum transition period (Table 12 and Fig. 54) which was non significant .

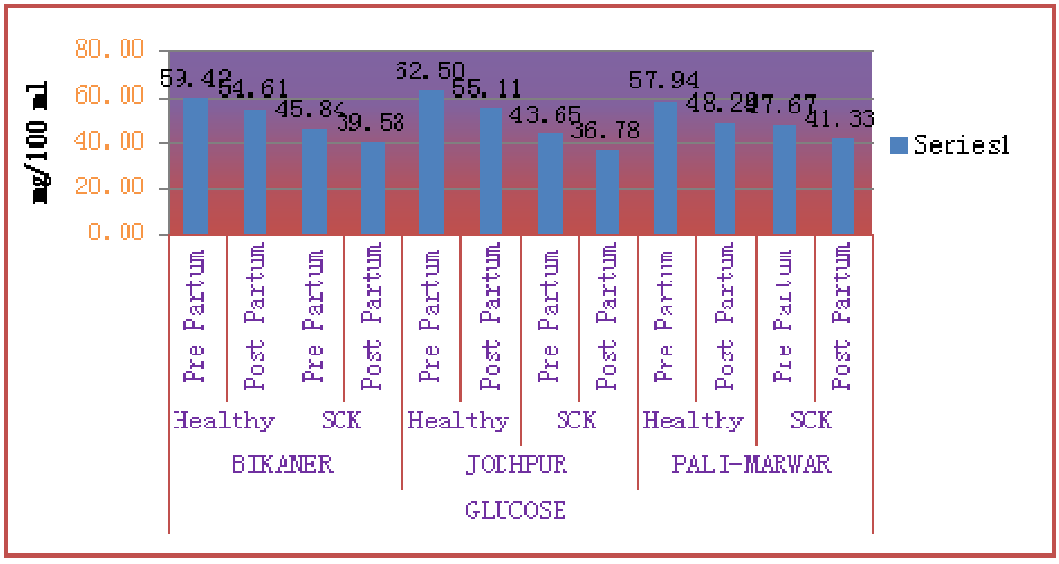

Fig. 54: Mean (±SE) plasma glucose values in healthy and SCK crossbred cows.

Glucose plays a fundamental role in energy metabolism. In the last weeks of fetal development, the fetus uses around 46 per cent of maternal glucose taken up by the uterus. Additionally, a cow producing 30 kg of milk per day uses at least 2 kg of blood glucose to synthesize lactose for milk. Thus, the end of pregnancy and the beginning of lactation, represents a time when there is a massive increase in glucose requirement. This poses an enormous challenge for the liver that has to synthesize all of this glucose from propionate and amino acids

as well as a challenge for other tissues and organs that have to adapt to a reduction of glucose utilization (Tabrizi *et al.*, 2007). Plasma glucose level increased before calving and then declined to a minimum value between 11 and 22 days post-partum. First lactation heifer had higher blood glucose level than cows in second or third lactation (Kappel *et al*., 1984). In the present study, the plasma glucose concentration showed decrease starting from the pre-partum to the post-partum transition period which could be associated with fetal development and mobilization of maternal glucose to fetal blood circulation (Jacob and Vadodaria, 2001) during advanced pregnancy and a high demand for lactose synthesis and/or insufficient gluconeogenesis during early lactation (Pambu-Gollah *et al.,* 2000). Blood glucose level was significantly decreased as the cow approached parturition as reported by Baird (1982), Doepel *et al.* (2002) and Bulent *et al.* (2006).

4.4.7.3 Glucose level in subclinical ketosis dairy cow:

The overall mean plasma glucose values recorded in the healthy and SCK affected cows were 60.233±0.731, 45.612±1.323 mg/dl during pre-partum period and 53.641±0.608, 39.167±1.349 mg/dl during post-partum transition period, respectively. Highly significantly ($P \leq 0.01$) lower mean value was observed in the SCK affected cows in comparison to the healthy cows during the pre and post-partum transition period. Similarly, a significant ($P \leq 0.05$) decrease was observed from the pre to post-partum transition period in the overall mean plasma glucose value in the SCK affected cows, and the mean value was less than the lower normal range during post-partum period in SCK dairy cows (Radostits *et al.,* 2000) (Table 11 and Fig. 53).

District wise: The mean plasma glucose values recorded were 45.837±2.012, 43.650±2.184, 47.667±2.871 mg/dl during pre-partum period; 39.577±1.820, 36.775±3.062, 41.333±2.459 mg/dl during post-partum period from the SCK affected cows of Bikaner, Jodhpur and Pali-Marwar districts, respectively. A highly significant ($P \leq 0.01$) difference was observed in the mean plasma glucose value in health wise, in all the three districts. Similar findings were recorded by several workers including Sharma *et al.* (2001), Dokovic *et al.* (2003), Sharma (2006), Radostitis *et al.* (2007), Nazifi *et al.* (2008), Youssef *et al.* (2010), Sarkar *et al.* (2011) and Kumar (2011) in clinical ketosis and healthy dairy cow.

Overall mean level of glucose was 59.931±0.855; 53.569±0.701 and 61.165±1.418; 53.861±1.251 in $3^{rd} – 5^{th}$ parity and above 5^{th} parity in pre and post-partum period in healthy cows. In SCK cows glucose level was 44.642±1.798; 37.425±1.587 and 47.200±1.877; 42.018±2.263 in $3^{rd} – 5^{th}$ parity and above 5^{th} parity in pre and post-partum period. Significant differences were observed parity wise in healthy and SCK cows in glucose level.

Lowest mean glucose level was observed in cows above 3^{rd} -5^{th} parity as compared to the cows from the other parity groups (above 5^{th}) and a non significant decrease was observed in the cows in both parity groups from pre to post-partum transition period (Table 13). Health wise, significant differences were observed in healthy and sub clinical ketosis dairy cows in both parity groups.

Glucose, a fundamental nutrient required for normal brain function in addition to use by other tissues, is under tight homeostatic control in order to allow for basic functioning of the animal. In ruminants, ingested carbohydrates are fermented to short-chain fatty acids by rumen microbes and thus most glucose must be synthesized by the liver (Reynolds *et al.,* 1988). As lactose is a major component in milk, gluconeogenesis is closely linked to lactogenesis as the amount of available glucose will determine the quantity of milk produced (Mepham, 1993).

After parturition, there is a decrease in insulin production by the pancreas (Drackley *et al.,* 2001) which results in decreased glucose utilization by insulin sensitive organs (e.g. adipose tissue and muscle). Coupled with a transient state of insulin resistance, associated with an increase in adipose tissue sensitivity to catecholamine and an exuberant lipolytic response (Herdt, 2000; Holtenius *et al.,* 2003), these mechanisms allow the mammary gland to have additional glucose for milk production (Komatsu *et al.,* 2005). Thus alternative fuel sources are needed for certain tissues in the body to maintain normal function during this period of increased milk production.

Carbohydrate being a major source of energy and got reduced in subclinical ketotic in lactating animals, as it is excreted in the form of lactose thus, the glucose become deficient with increasing milk yield. Increased ketogenesis is always associated with the increased rate of gluconeogenesis, which in turn depletes the oxaloacetate and further reduces the gluconeogenesis due to vicious cycle as indicated by the elevated levels of the pyruvate and lactate in subclinical ketotic animals (Asmare *et al.,* 1997).The monosaccharide glucose is used as an energy source in body tissue and also during lactation as a basis for producing lactose (i.e., milk sugar). The blood concentration of glucose is strictly regulated through homeostasis (Bauman & Currie, 1980).

Glucose concentrations decreases at parturition and is lower in the first weeks of lactation (Ingvartsen *et al.,* 2003). Hypoglycemia could develop in ketotic cows because of disorder of carbohydrate metabolism. Hypoglycemia in clinical cases are attributed to the large amount of glucose removed by mammary glands to make lactose coupled with insufficient food intake to replenish the supply of glucose (Baird, 1982) and may be due to the high productive and reproductive status of the animal (Radostitis *et al.,* 2007). Schwalm and Schultz (1976) and Hove (1978) studied the relationship of insulin concentration of blood to occurrence of ketosis in dairy cows. They reported that insulin concentration of blood first rises in response to hyperketonaemia and than falls once more as hypoglycemia become established. Baird *et al.* (1968) reported reduction in hepatic oxaloacetate levels in ketotic cows. Decrease of dry matter intake around parturition, increased demand for glucose and insufficient propionate production during the early postpartum period (Kronfeld (1971; Drackley 1999, Drackley and Dann 2005).

Gluconeogenesis is decreased significantly during ketosis (Mill *et al.*, 1986) and this is because of lack of gluco-corticoid hormone from adrenal insufficiency (Shaw, 1956) or decreased level of propionate or amino acids which are precursors of oxaloacetate.

According to Bergman (1996), dietary carbohydrates are fermented in the rumen to form volatile fatty acids (VFAs). Acetic acid, propionic acids and butyric acid are the most important VFAs, but propionic acid is the only VFA that can be converted in to glucose. In subclinical ketosis, less amount of propionic acid is produced. In normal condition about 70 per cent glucose formation is from propionic acid but in reduced appetite condition it is about zero, so in absence of this there is less formation of glucose resulting in hypoglycemia. The hypoglycemia in cows with SCK was ascribed to the decreased gluconeogenesis (Goff and Horst 1997). It has been recorded that decrease in glucose output by the liver in SCK cows causes lowered blood glucose concentrations and decreased insulin secretion, which, in turn, leads to increased lipid mobilization from adipose tissue and increased rate of hepatic fatty acid uptake and ketogenesis (Grummer, 1993). The results of the present study were in accordance with the number of similar studies (Oikawa and Oetzel, 2006 and Tabrizi *et al.,* 2007), where the authors reported a decrease in blood glucose in cows with subclinical ketosis.

4.4.8 Beta hydroxy butyric acid (BHBA):

4.4.8.1 Interaction between (districts and health; health and stage wise) affecting BHBA level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for BHBA in plasma was 0.960±0.012 mmol/l. The differences among subclass means were highly significant (P≤0.01) across districts (0.938±0.019 in Bikaner, 0.920±0.020 in Jodhpur and 1.021±0.025 mmol/l in Pali-Marwar), highly significant in healthy/SCK groups (0.743±0.013 in healthy and 1.177±0.021 mmol/l in SCK), non significant in stage wise (0.568±0.016 pre-partum and 1.084±0.027 mmol/l post-partum) and highly significant in parity groups (0.946±0.016 mmol/l in 3-5th parity and 0.974±0.019 mmol/l in above 5th parity). The effect of pre and post-partum stages were non significant in pooled data. There were no effects of two factor interaction indicating that difference among districts. The interaction between districts and health status was highly significant (P≤0.01). The trend is depicted in Fig. 55. This showed that the BHBA level was lower in healthy groups across all districts. Its level was significantly highest in (0.753±0.030 to1.289±0.042 mmol/l) Pali-Marwar district and lowest (0.782±0.019 to 1.058±0.036 mmol/l) in Jodhpur districts. The results showed that fat mobilization was higher in Pali-Marwar in comparison to Bikaner and Jodhpur.

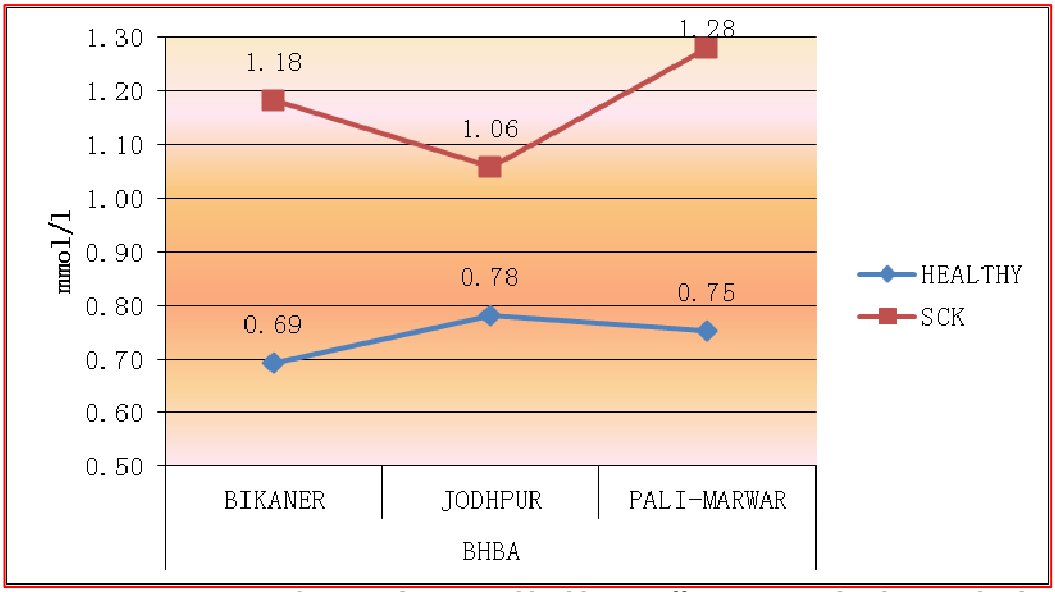

Fig. 55: Interaction between districts and health wise affecting BHBA level in crossbred cows.

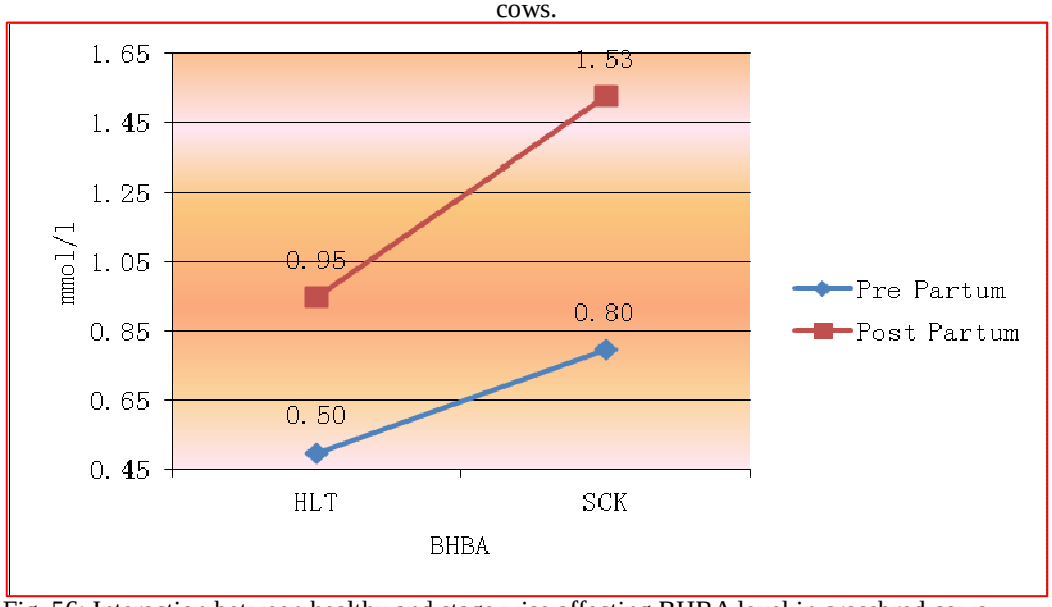

Fig. 56: Interaction between healthy and stage wise affecting BHBA level in crossbred cows.

4.4.8.2 Beta hydroxy butyric acid (BHBA) in healthy cows:

The overall mean plasma beta hydroxy butyric acid (BHBA) level recorded during the pre-partum period was 0.498±0.012 and 0.947±0.020 mmol/l during the post-partum period. Highly Significant (P≤0.01) difference was observed in the overall mean BHBA values in the districts (Table 11). The level of BHBA increased from pre-partum to post-partum period.

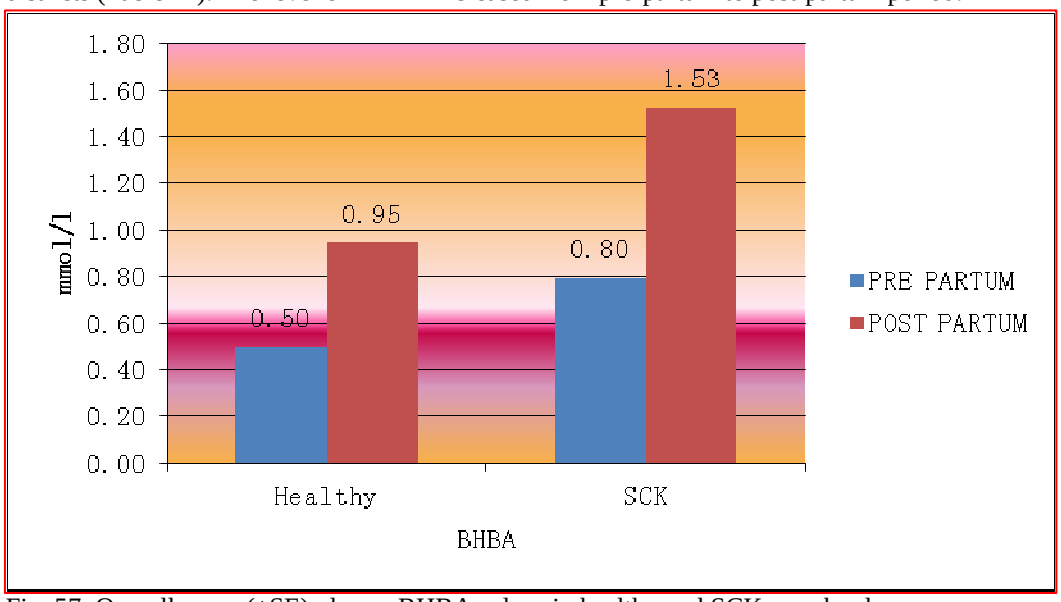

Fig. 57: Overall mean (±SE) plasma BHBA values in healthy and SCK crossbred cows.

District wise: The mean plasma BHBA values were 0.474±0.020, 0.527±0.020, 0.501±0.024 mmol/l during the pre-partum and 0.874±0.035, 1.006±0.019, 1.025±0.027 mmol/l during the post-partum transition period from the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively (Table 12 and Fig. 58). Highly Significant difference was observed in heath and stage wise in dairy cows. Higher values of BHBA were observed in Jodhpur dairy cows as compared to cows Bikaner and Pali- Marwar in pre-partum transition period. Perhaps it could be no concentrate feeding in dry period.

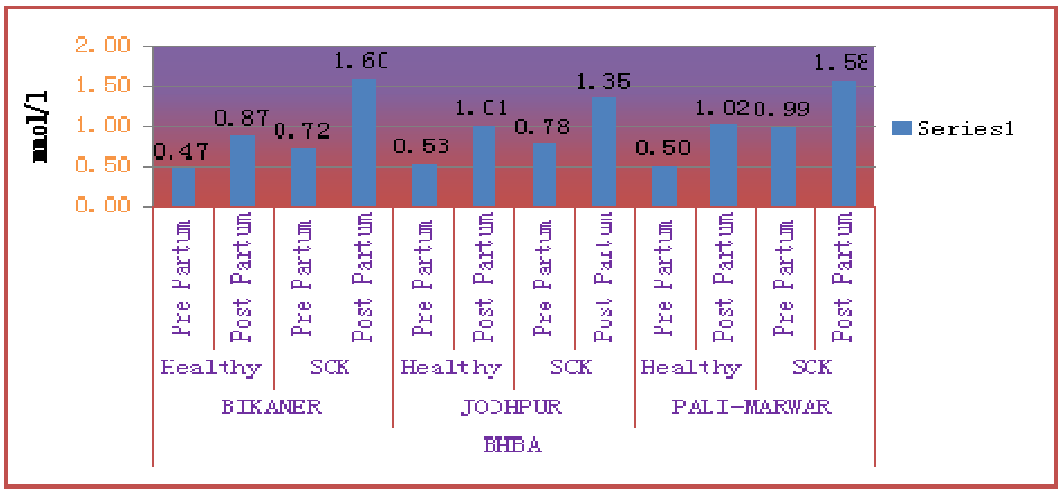

Fig. 58: Mean (±SE) plasma BHBA values in healthy and SCK crossbred cows.

4.4.8.3 Beta hydroxy butyric acid (BHBA) in subclinical ketosis:

The overall mean plasma BHBA concentration in the healthy and SCK affected cows was 0.498±0.012, 0.795±0.024 mmol/l during pre-partum 0.947±0.020, 1.526±0.026 mmol/l during post-partum period. Highly significant (P≤0.05) mean plasma BHBA value was recorded in SCK affected cows as compared to the healthy cows during different stages. Similarly, a significant increase was also recorded in the plasma BHBA value in all the affected cows of the three districts (0.725±0.022, 1.603±0.024 mmol/L for Bikaner; 0.784±0.029, 1.345±0.014 mmol/L for Jodhpur and 0.987±0.015, 1.575±0.035 mmol/L for Pali-Marwar) between pre and post-partum transition period, respectively (Table 12 and Fig. 58).

Overall mean level was recorded as 0.491±0.013; 0.925±0.024 and 0.520±0.030;

1.016±0.024 in 3rd – 5th parity and above 5th parity in pre and post-partum period in healthy cows. In SCK cows mean value of BHBA was 0.748±0.027; 1.561±0.031 and 0.873±0.033; 1.468±0.040 in 3rd – 5th parity and above 5th parity in pre and post-partum period. Highly significant (P≤0.01) differences were observed in parity wise in crossbred dairy cows. Highest mean plasma BHBA level was recorded in cows of above 5th parity (0.873±0.033 mmol/L) in SCK cows during pre-partum period (Table 13). District wise no effect of parity was observed in pre and post-partum period.

Blood BHBA is generally regarded as gold standard test for diagnosis of subclinical ketosis in cows and buffaloes in post-partum period (early lactation) because BHBA are being more stable ketone body than acetone or acetoacetate. A number of studies (Oetzel 2004; Tabrizi *et al.,* 2007; McArt *et al.,* 2012a; McArt *et al.* 2012b ; Ribeiro *et al.,* 2013) have also reported results similar to the present findings where they classified a cow affected with SCK on the basis of blood BHBA level above 1.2 mmol/L in post-partum period.

Near parturition feed intake is reduced and after parturition the demand for energy is progressively increased by the initiation of lactation. Concentrations of EFA increased after parturition and peaked at two week post-partum (Bell, 1980; Baird, 1982; Blum *et al.,* 1983; Kunz *et al.,* 1985) and reflected mobilization of body fat. In the case of excessive fat mobilization, associated with marked formation of acetyl-coenzyme A, the tricarboxylic acid cycle (TCA) cannot fully metabolize fatty acids. As a consequence, acetyl coenzyme A is converted to acetoacetate which is then reduced to BHBA by BHBA dehydrogenase or spontaneously decarboxylized to acetone (Baird, 1982; Brumby *et al.,* 1975). Hence, the presence of ketone bodies in body fluid is normal to a certain degree, whereas high concentrations of ketone bodies indicate that adaptability of metabolism is exceeded, i.e. that whole body homeostasis cannot be maintained (Aeberhard *et al.,* 2001 and Baird, 1982). BHBA concentration increased after parturition and peaked at four weeks postpartum later than that of NEFA which indicated that NEFA provide the substrate for BHBA synthesis. Increased BHBA concentration reveals incomplete oxidation of NEFA in TCA during negative energy balance (Grummer, 1993; Doepel *et al.,* 2002).

Cows can also have an excess of circulating ketone bodies without obvious clinical signs (SCK) (Andersson, 1988). The term hyperketonemia (i.e. blood BHBA 1.2 mmol/l) used throughout this to include animals with SCK. It is important to note that SCK and clinical ketosis are not different diseases, just variations in severity of a single disorder (BHBA). The distinction between SCK and clinical ketosis can either be based subjectively on clinical assessment or more objectively through measurement of BHBA. Clinical ketosis is generally associated with higher BHBA concentrations than SCK. Oetzel (2004) stated that cows with clinical disease generally had blood BHBA concentrations 3.0 mmol/L which is much higher than the BHBA threshold generally used to determine SCK.

Proportions of NEFA and BHBA outside reference ranges were used to evaluate energy balance or to find subclinical ketosis at the herd level (Oetzel, 2004). The reference ranges for NEFA in pre-partum period is <0.4 mmol/L and BHBA in post-partum is <1.2 mmol/l, respectively. Determination of BHBA is recommended to find herds with subclinical ketosis (Oetzel, 2004).

4.4.9 Non esterified fatty acid (NEFA):

4.4.9.1 Interaction between (districts and health; health and stage wise) affecting NEFA level:

The overall mean of all the cows across three districts, stages, parity and healthy/SCK groups for NEFA in plasma was 0.473±0.009 mmol/l. The differences among subclass means were non significant across districts (0.456±0.014 in Bikaner, 0.459±0.015 in Jodhpur and 0.503±0.019 mmol/l in Pali-Marwar), highly significant (P≤0.01) in healthy/SCK groups (0.262±0.010 in healthy and 0.683±0.016 mmol/l in SCK), non significant in stage wise (0.267±0.015 pre-partum and 0.442±0.022 mmol/l post-partum) and highly significant parity groups (0.462±0.012 mmol/l in 3-5th parity and 0.483±0.015 mmol/l in above 5th parity). The effect of pre and post-partum stages were non significant in pooled data. There was effect of two factor interaction indicating that difference among districts. Where as the interaction between districts and health status was highly significant (P≤0.01). The trend is depicted in Fig. 59. This showed that the NEFA level was lower in healthy groups across all districts. The level of NEFA level was significantly higher in (0.242±0.023 to 0.764±0.033 mmol/l) Pali-Marwar and lowest (0.297±0.013 to 0.616±0.025 mmol/l) in Bikaner districts.It indicate that lipid mobilization was

higher in Pali-Marwar in comparison to Jodhpur and Bikaner.

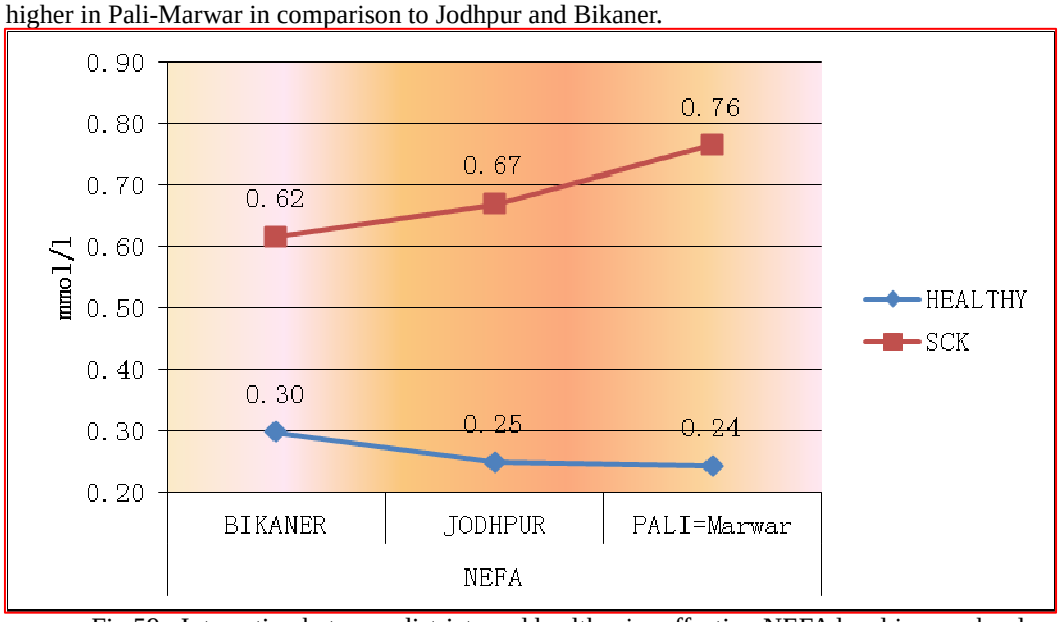

Fig.59 : Interaction between districts and health wise affecting NEFA level in crossbred cows.

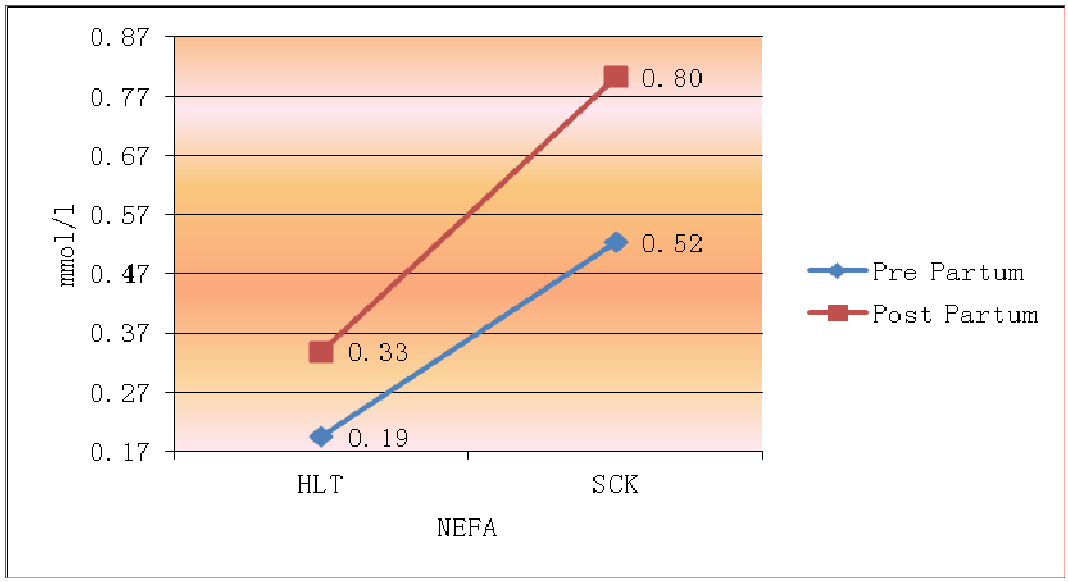

Fig. 60: Interaction between health and stage wise affecting NEFA level in crossbred cows.

4.4.9.2 Non esterified fatty acid (NEFA) in healthy dairy cow:

The overall mean plasma non esterified fatty acid (NEFA) values in the crossbred dairy cows from the different districts are shown in Table 11. The overall mean plasma NEFA value was 0.189±0.008 mmol/L during pre-partum and 0.333±0.015 mmol/l during post-partum period. A highly significant difference ($P \leq 0.01$) was observed in the mean plasma NEFA values in healthy and SCK dairy cow during transition period.

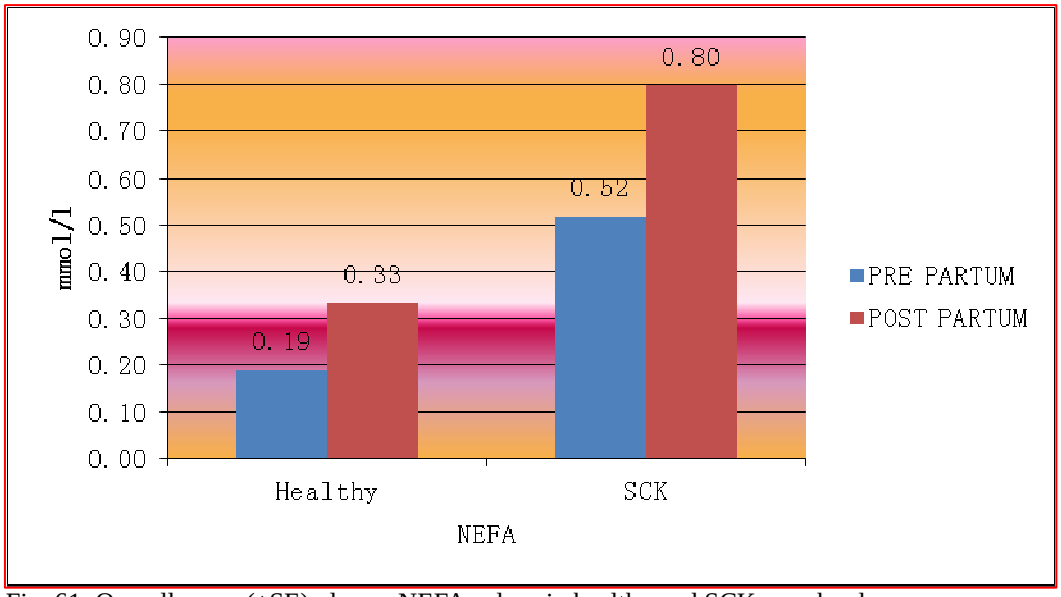

Fig. 61: Overall mean (±SE) plasma NEFA values in healthy and SCK crossbred cows.

District wise: The mean plasma NEFA values from the three districts were 0.220±0.012, 0.172±0.013, 0.145±0.017 mmol/L during pre-partum period and 0.354±0.025, 0.288±0.021, 0.363±0.028 mmol/L during the post-partum period in the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farm, respectively (Table 12). Highly Significant (P≤0.01) level was observed in district wise healthy and SCK dairy cow. Higher values were observed for the Bikaner dairy cows during the pre-partum and Pali- Marwar in post-partum period as compared to the cows from other districts (Table 12 and Fig. 62).

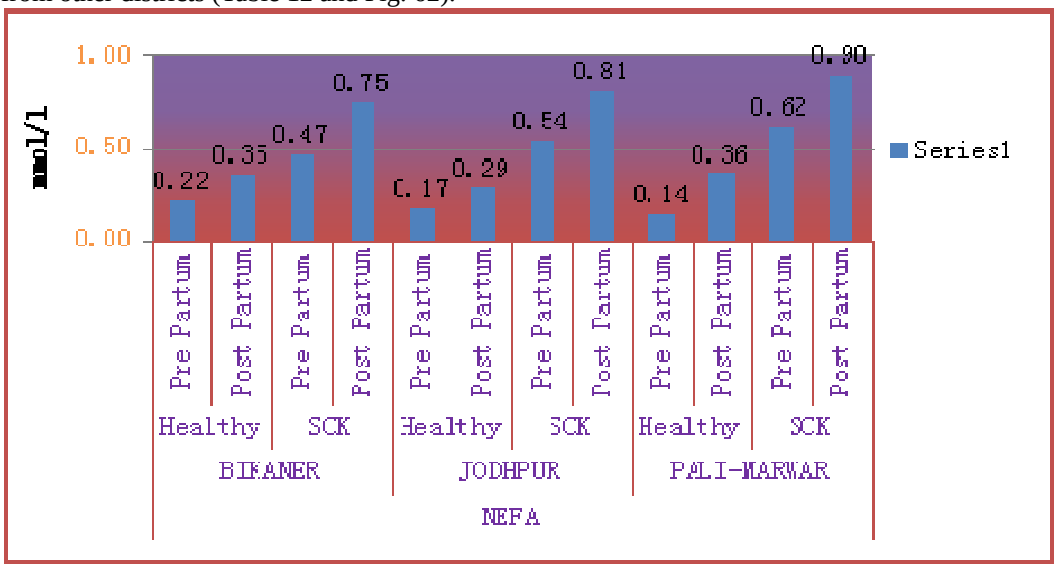

Fig. 62: Mean (±SE) plasma NEFA values in healthy and SCK crossbred cows.

4.4.9.3 Non esterified fatty acid (NEFA) in subclinical ketosis:

The overall mean plasma values for NEFA in healthy and SCK affected cows were 0.189±0.008, 0.518±0.021 mmol/l during pre-partum period; 0.333±0.015, 0.797±0.024 mmol/l during post-partum period. A highly significant increase (P≤0.01) was observed for the mean NEFA value in SCK affected cows and healthy dairy cows.

Energy status of dairy cows is variable during the peripartum period. It is generally based on the balance of energy intake and energy requirements (Oldick, 1999; Rukkwamsuk *et al.*, 1999). During the transition period, the energy requirements of the cow are compensated by intensive lipolysis i.e. break down of adipose tissue, leading to release of fatty acids, reversibly bound to albumin in the blood (Herdt,1997). In NEB, glucose level were decrease and increase in lipolysis releases non-esterified fatty acids (NEFAs), which circulate throughout the body in the blood (McNamara, 1991; Bertics *et al.*, 1992; Herdt, 2000). NEFAs can be used directly as a fuel source by various tissues such as muscle, used for milk fat synthesis by the mammary gland and taken up

by the liver (Palmquist *et al.,* 1969; Bell, 1995 and Drackley, 1999; Herdt, 2000). Ketone bodies released by the liver act as an alternate fuel source for tissues such as the brain and heart (Herdt, 2000; Drackley and Andersen, 2006). The elevated plasma NEFA and BHBA level are indicators of negative energy balance (NEB) in postpartum dairy cattle (Bell, 1995). Other indicators of NEB are decreased plasma glucose, insulin and insulin-like growth factor-1 (IGF-1) concentration (Grum *et al.,* 1996; Butler *et al.,* 2003) and a decreased body condition score (BCS) (Wildman *et al.,* 1982 and Oldick, 1999). Thus, a certain concentration of NEFAs and BHBA in the blood is a part of normal adaptation to NEB in early lactation. The liver removes 15–20% of NEFAs from the blood (Drackley and Andersen, 2006), which were completely oxidized. Excess production of NEFA partially oxidized to produce ketone bodies (acetone, acetoacetic acid, and beta-hydroxybutyric acid (BHBA), converted into triacylglycerol (TAGs) and packaged into very low density lipoproteins (VLDL)for transport back to the adipose tissue or stored as TAG (Spain and Scheer, 2001) . However, excessive concentrations of NEFAs or BHBA indicate an excess of NEB which is associated with detrimental to immune function (Hammon *et al.,* 2006; Contreras *et al.,* 2010; Ster *et al.,* 2012) and decrease appetite (Dale *et al.,* 1979). The latter may be the result of food intake being controlled by signals from the liver to the brain (Allen *et al.,* 2009) and production outcomes.

NEFA in excess may become toxic (Herdt, 1988; Emery *et al.,* 1992 and Overton *et al.,* 1998) as the bovine liver has a very limited capacity to metabolize NEFA into TAG. However, when the threshold is crossed, the TAG accumulates in the liver and acetyl CoA (resulting from oxidation of fatty acids) that is not utilized in the tricarboxylic acid cycle (TCA) and hence is converted into ketone bodies, such as acetone, acetoacetate and beta-hydroxy butyrate (BHBA) (Nelson and Cox 2005). Excessive accumulation of TAG in the liver impairs its normal function (Vanden *et al.,* 1996; Heuer *et al.,* 2000; Rukkwamsuk *et al.,* 2000 and Jorritsma *et al.,* 2001). Therefore, cows with lipolysis are at high risk to develop fatty liver syndrome (Grummer, 1993; Vanden 1995 and Bryers 1999). In addition, development of fatty liver has been found to impair the gluconeogenic activity of liver tissue, which lowers blood glucose and decreases insulin secretion. This, in turn, support greater lipid mobilization and increased rate of fatty acid uptake by the liver and increased ketogenesis (Grummer 1993). When ketones are produced in excess of peripheral tissue's capacity to use them, they accumulate in the blood stream, thus appearing in the blood, milk and urine; the resultant condition being referred to as ketosis (Goff and Horst, 1997). Ketosis is a major disease associated with negative energy balance. Increased energy demand because of lactation and/or fetal development and decreased energy intake because of depressed appetite before the disease results in ketosis, it result in quick blood glucose draw down and body fat mobilization (Xu *et al.,* 2008). Therefore, blood NEFA concentration is subsequently increased. The liver is the most active metabolic organ for cows (Melendez *et al.,* 2006). Therefore, metabolism of blood NEFA, in the liver, may be very important for NEB diseases such as ketosis. However, how metabolism of high concentrations of NEFA occurs in liver is not clear and little reported. Esterification and exportation of NEFA as VLDL in the liver can be enhanced through additional dietary ruminally-protected choline, but the storage of fat in liver was not relieved and ketosis was not prevented (Kristensen and Raun, 2007; Grummer, 2008). When ketosis occurred, synthesis and secretion of VLDL in the liver was also decreased (Oikawa, 1997; Yamamoto *et al.,* 2001).

District wise (subclinical): Mean plasma NEFA values in cows from the pre-partum to post-partum stage were 0.467±0.029, 0.748±0.036 mmol/l from Bikaner district; 0.541±0.033, 0.814±0.020 mmol/L from Jodhpur district and 0.615±0.018, 0.897±0.044 mmol/l from Pali-Marwar district, respectively. Significantly higher mean NEFA level was noted in Pali-Marwar in dairy cows (Table 11 and Fig. 62).

Overall mean level of NEFA was recorded as 0.180±0.009; 0.320±0.017 and 0.218±0.020; 0.372±0.032 mmol/L in 3^{rd} – 5^{th} parity and above 5^{th} parity in pre and post-partum period in healthy cows. In SCK cows NEFA values were 0.493±0.027; 0.768±0.032 and 0.560±0.030; 0.845±0.031 mmol/L in 3^{rd} – 5^{th} parity and above 5^{th} parity in pre and post-partum period.

Mean plasma NEFA values were highest in cows from above 5^{th} parity in comparison to 3^{rd} -5^{th} parity and a non significant increase was observed in the plasma NEFA values from the pre to the post-partum transition period in cows from 3^{rd} – 5^{th} parity in both healthy and SCK dairy cow (Table 13).

This increase in NEFA and BHBA in cows with SCK and increased fat mobilization from the adipose tissue during the early lactation period to support the negative energy balance, when blood glucose level was low due to the initiation of milk production, resulting in increased

production of acetyl-CoA, which resulted in increased production of ketone bodies (Wieland *et al.*, 1964). In multiparous cows, postpartum NEFA concentrations greater than about 0.7 mEq/L and BHBA concentrations greater than about 10 mg/dL were associated with lower predicted milk yield.

Excessively high circulating NEFA and BHBA in transition dairy cattle are associated with increased risk of clinical diseases, less milk production and reduced reproductive performance. The loss due to ketosis was estimated by Thirunavukkarasu *et al*., 2010b, who reported that Rs. 577.09 per affected cow, which included the cost of medicines (Rs. 262.99, 45.57 per cent), Veterinarian's fee including additional labour cost (Rs. 224.98, 38.99 per cent) and expenses on feed supplements (Rs. 89.12, 15.44 per cent). However, regardless of the accuracy of these estimates, when metabolic disease is considered at the herd level, it is considerably more costly than most clinical diseases, since subclinical disease is far more frequent. Cow-level risk factors are parity and body condition score. Herd variation in district for this disease problem is wide and herd level risk factors are poorly described. However, herd level risk factors most likely involve combinations of management, feed quality and nutritional programs, cow comfort, environment, and other variables that influence dry matter intake. Routine monitoring programme for subclinical ketosis is beneficial on many dairies.

4.4.10 Total cholesterol:

4.4.10.1 Interaction between health and stage wise affecting cholesterol level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for cholesterol in plasma was 84.892±1.171 mg/dl. The differences among subclass means were significant across districts (89.049±1.859 in Bikaner, 84.599±1.964 in Jodhpur and 81.028±2.413 mg/dl in Pali-Marwar), highly significant (P≤0.01) in healthy/SCK groups (95.543±1.313 in healthy and 74.241±2.013 mg/dl SCK) and highly significant parity groups (85.727±1.597 mg/dl 3-5th parity and 84.057±1.880 mg/dl above 5th parity). The effect of pre (93.782±1.164 mg/dl) and post (93.440±2.087 mg/dl) partum stages were non significant in pooled data. There was no effect of two factor interaction indicating difference among districts. Whereas the interaction between health and status group was highly significant (P≤0.01). The trend is depicted in Fig. 63. This showed that the cholesterol level was almost equal in healthy and SCK groups across pre-partum stage. Rise in their level was significantly higher in post-partum stage the level decreases in SCK dairy cow in comparison to healthy. In subclinical ketosis fat mobilization occur in liver and cholesterol was not produced in sufficient quantity by the liver and their level decrease in the blood.

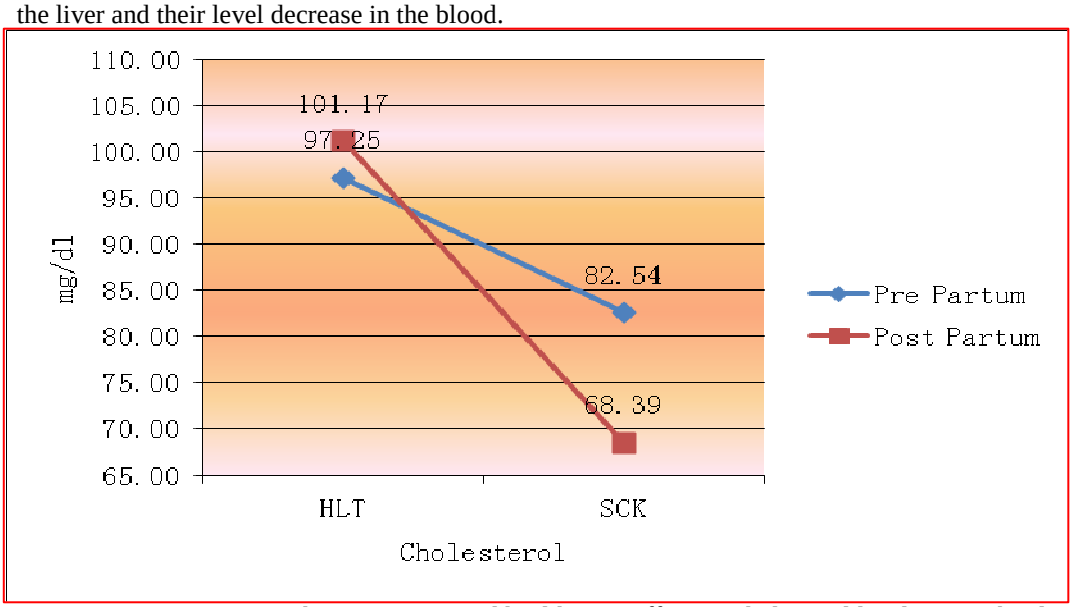

Fig. 63: Interaction between stage and health wise affecting cholesterol level in crossbred cows.

4.4.10.2 Total cholesterol in healthy dairy cow:

The overall mean plasma cholesterol level recorded was 97.250±1.242 mg/dl during the pre-partum period and 101.168±2.118 mg/dl during the post-partum transition period. A highly significant (P≤0.01) difference was observed in the overall mean cholesterol level in healthy and SCK dairy cow (Table 11).

District wise: The mean cholesterol level was 101.241±1.810, 96.276±2.051 and

88.812±1.815 mg/dl during the pre-partum period and 111.130±3.167, 92.848±2.971, 91.535±3.146 mg/dl during the post-partum transition period from the dairy cows of the Bikaner, Jodhpur and Pali- Marwar dairy farms, respectively. District wise non significant differences were observed in health, stage and parity wise.

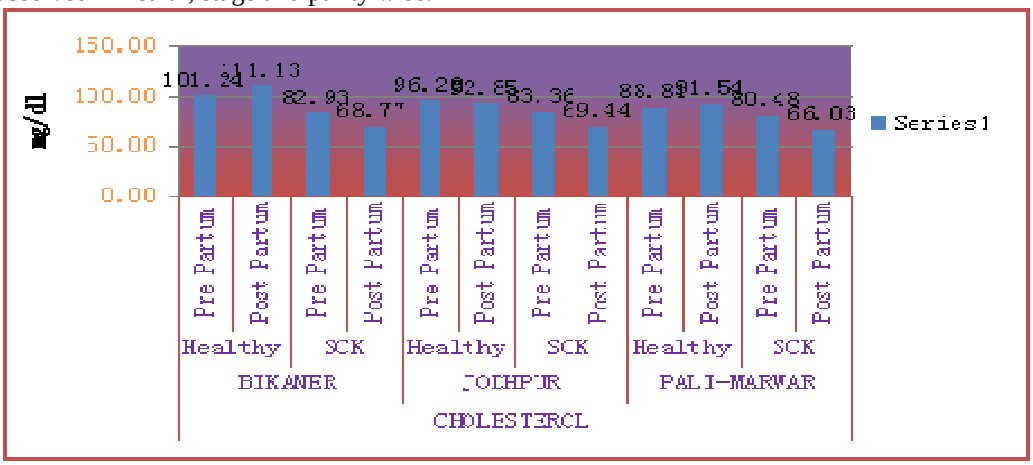

Fig. 64: Mean (±SE) plasma cholesterol values in healthy and SCK crossbred cows.

4.4.10.3 Total cholesterol in subclinical ketosis:

The overall mean plasma cholesterol concentration in the healthy and SCK affected cows was 97.250±1.242, 82.541±1.589 mmol/L during pre-partum and 101.168±2.118, 68.390±1.686 mmol/L during post-partum period. Significantly low mean plasma cholesterol value was recorded in SCK affected cows as compared to the healthy cows during different stage.

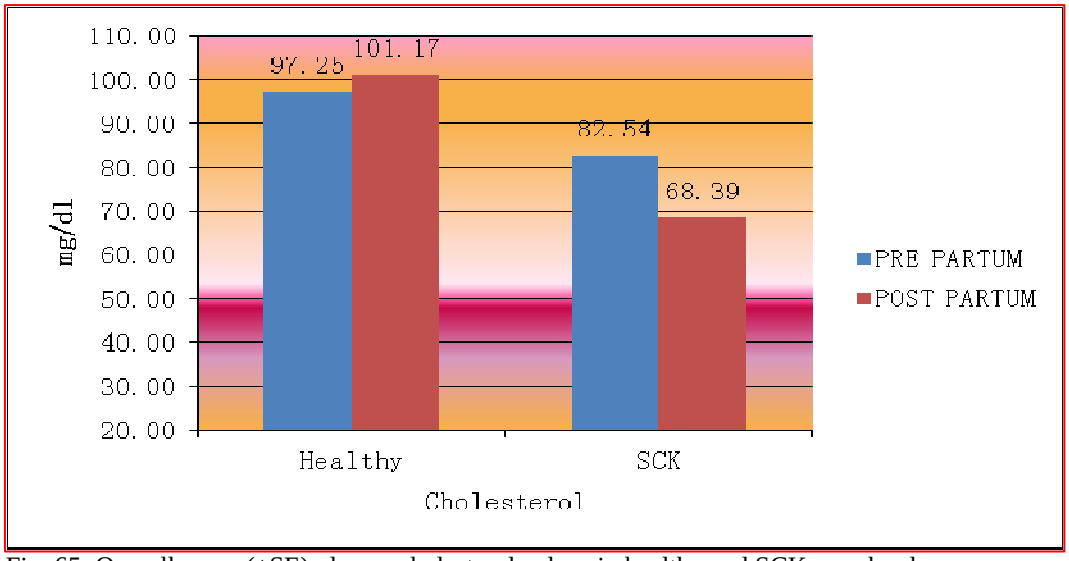

Fig. 65: Overall mean (±SE) plasma cholesterol values in healthy and SCK crossbred cows.

Similarly, a significant decrease was also recorded in the plasma cholesterol value in all the affected cows of the three districts (82.927±2.232, 68.773±2.805 mmol/L for Bikaner; 83.363±3.033, 69.437±2.277 mmol/L for Jodhpur and 80.483±3.898, 66.033±3.268 mmol/L for Pali-Marwar) between pre and post-partum transition period, respectively (Table 12 and Fig. 64).

Higher cholesterol level was reported by Setty and Razdan (1966) due to the gonadal steroid which have correlation with cholesterol metabolism, are produced in much greater amount during periparturient stages. Prakash and Tandon (1979) found increased level of serum cholesterol, which were due to the mechanism by which estrogens affected the complex interrelationship of pituitary-thyroid-adrenal function. The estrogen had an effect on the carbohydrate metabolism that in turn caused increased production of cholesterol in endocrine gland tissue from acetate The increased level of free cholesterol was of particular interest in the lactating animals for transport of free fatty acids to be incorporated in milk fat (Anantwar and Singh, 1993). In present study, the value of cholesterol was high in pre-partum stage which was similar as reported by the above mention author.

Overall Mean plasma cholesterol values revealed highly significant (P≤0.01) difference

in 2 parity group but district and stage wise no difference were observed in this study. Level of cholesterol was high in cows from 3rd -5th parity and a non significant increase was observed in the plasma cholesterol values from the pre-partum up to the post-partum period in cows from 3rd – 5th and above parity in healthy dairy cow (Table 13).

Cholesterol level showed a significant decrease in subclinical ketotic dairy cow compared with normal ones. These results were similar to previous report of Ghanem and El-deeb (2010) in buffalo. This could be attributed to mild liver function which causes reduction in cholesterol formation in the liver (Grummer, 1995). However, Anantwar and Singh (1993) reported that there was an increase in cholesterol level in ketotic buffalo. Marcos *et al.* (1990) reported decrease in serum cholesterol in liver injuries and fatty liver syndrome in cows. HDL-cholesterol level showed a significant decrease in subclinical ketotic dairy cow in comparison to healthy groups. These results coincide with those of Nasri and Baradaran (2004) and Turk *et al.* (2008). These results may be attributed to moderate reversible changes in liver (Steatosis), which causes reduction in cholesterol level. In the contrast, there was non-significant decrease in LDL-cholesterol levels in subclinical ketotic cow in comparison to the normal ones. Similar finding in cows was recorded by Van den Top *et al.* (2005).

Serum cholesterol was increased due to lipolysis occurring in adipose tissues of ketotic animals which in turn was responsible for elevated levels of free fatty acids in plasma along with other lipids (Brockman, 1979). This could have been the reason of increase in mean serum cholesterol level in ketotic cows.

The results were in agreement with the observations of earlier workers for the high plasma cholesterol. Some contradictory results were also collected from the literature. These discrepancies could be due to variation in age groups and season of collection of samples as growth and different age groups can affect the metabolic status as per the need of the body. High level of cholesterol in females could be due to estrogen which promotes cholesterol synthesis (Singh *et al*., 1994).

4.4.11 Triglycerides:

4.4.11.1 Interaction between districts and health wise affecting triglyceride level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for triglycerides in plasma was 14.345±0.391mg/dl. The differences among subclass means were significant across districts (11.996±0.621 mg/dl in Bikaner, 15.106±0.656 mg/dl in Jodhpur and 15.933±0.806 mg/dl in Pali-Marwar), highly significant (P≤0.01) in healthy/SCK groups (11.591±0.438 in healthy and 17.098±0.672 mg/dl SCK) and highly significant in parity groups (14.845±0.533 mg/dl 3-5th parity and 13.844±0.628 mg/dl above 5th parity). The effect of pre (10.678±0.357 mg/dl) and post-partum (14.287±0.555 mg/dl) stages were non significant in pooled data. There were no effects of two factor interaction indicating that difference among districts. The interaction between districts and health status was highly significant (P≤0.01). The trend is depicted in Fig. 66. This shows that the triglyceride level was lower in healthy groups across all districts. Rise in their level was significantly higher in (11.63 to 20.23 mg/dl) Pali-Marwar district and lower (10.87 to 13.12) in Bikaner districts. It means fat mobilization in Pali-Marwar dairy cow was more in comparison to other districts. It also confers with the level of NEFA and BHBA, which were high in Pali-Marwar dairy cow. These values were associated with fat mobilization.

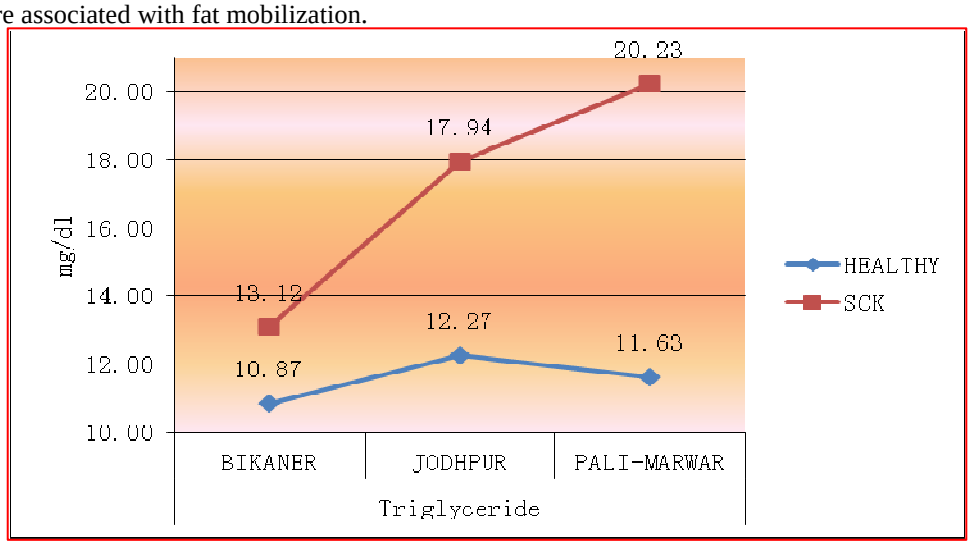

Fig. 66: Interaction between districts and health wise affecting triglyceride level in crossbred cow.

4.4.11.2 Total triglyceride level in healthy cows:

The overall mean plasma triglyceride values in the crossbred cows from the different districts are shown in Table 11. The overall mean plasma triglyceride value was 9.506±0.299 mg/dl during pre-partum and 13.401±0.622 mg/dl during post-partum. Highly significant increase (P≤0.01) was observed in the mean plasma triglyceride values in healthy and SCK dairy cows. Overall highly significant (P≤0.01) differences were observed in the districts.

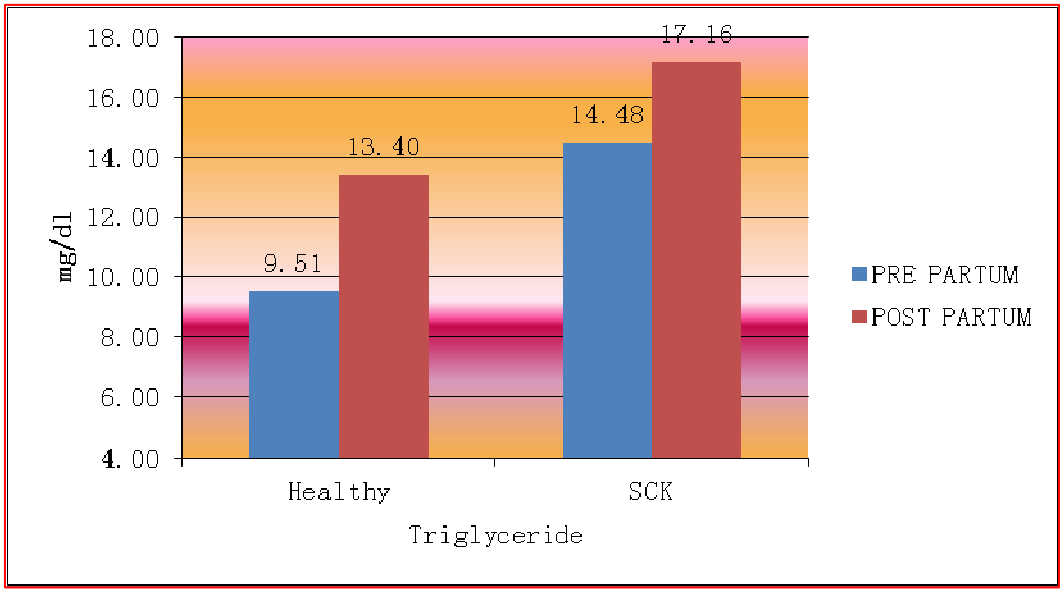

Fig. 67: Overall mean (±SE) health wise and stage wise triglyceride level in crossbred cows.

District wise: The mean plasma triglyceride values from the various districts were 8.868±0.346, 10.018±0.602 and 10.161±0.713 mg/dl during pre-partum period; 12.143±0.576, 15.145±1.499 and 13.274±0.890 mg/dl during post-partum period in the dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farm, respectively (Table 12). Significantly higher (P≤0.05) values were observed for the Pali-Marwar dairy cows during the pre-partum and Jodhpur in post-partum as compared to the cows of other districts (Table 12 and Fig. 68).

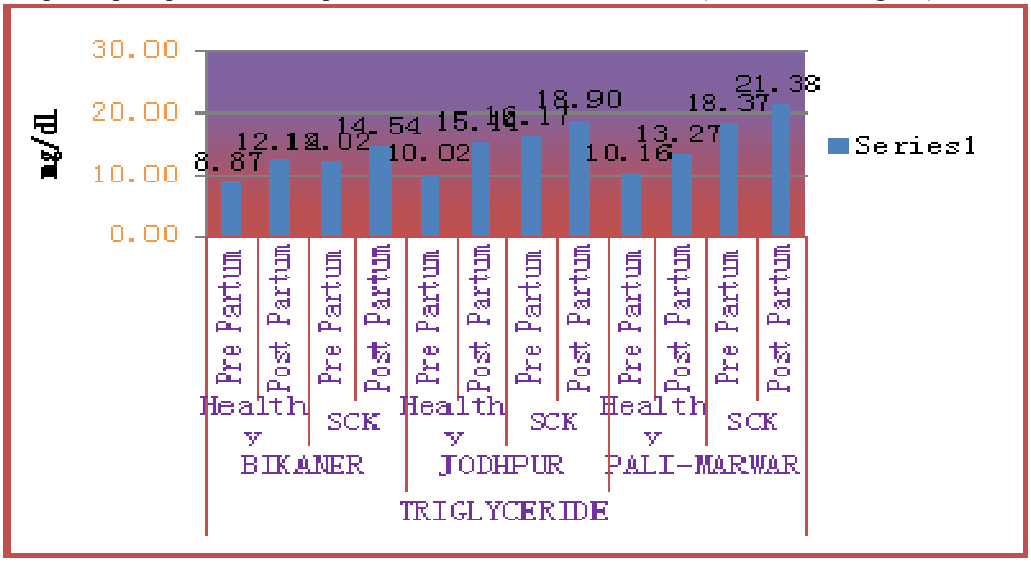

Fig. 68: Mean (±SE) plasma triglyceride values in healthy and SCK crossbred cows.

4.4.11.3 Total triglyceride level in subclinical ketosis:

Overall mean plasma triglyceride concentrations were 9.506±0.299, 14.478±0.849 mg/dl during pre-partum period and 13.401±0.622, 17.159±1.071 mg/dl during post-partum transition period. Highly significant (P≤0.01) differences were observed in the mean plasma triglyceride values between healthy and SCK cows.

No significant variation was observed in mean plasma triglyceride concentration from pre-partum to the post-partum transition period in cows from Bikaner (12.019±0.878, 14.542±1.279 mg/dl), Jodhpur (16.169±1.388, 18.900±1.425 mg/dl) and Pali-Marwar (18.372±1.920, 21.378±2.832 mg/dl) district, respectively.

Overall mean level was recorded 9.327±0.320; 13.497±0.741 and 10.058±0.722; 13.106±1.138 in 3rd – 5th parity and above 5th parity in pre and post-partum period in healthy cows. In SCK cows were 13.519±1.042; 17.006±1.410 and 16.047±1.379; 17.409±1.711 in 3rd – 5th parity and above 5th parity in pre and post-partum period. Overall mean plasma triglyceride level was highest significantly (P≤0.01) in dairy cows (Table 13).

Low triglycerides may be attributed to an influx of free fatty acids from adipose tissue near the time of parturition and a low output of lipoprotein by liver. The rapid increase in the triglycerides during lactation may be due to increased demand of the udder for fatty acid synthesis for milk fat, and also to lowest level of circulatory estrogen and thyroxin profile which influence the lipid metabolism (Tainturier *et al.*, 1984).

Nazifi *et al.* (2008) and Arya (2008) observed highly significant ($P < 0.01$) increase in serum triglycerides level in ketotic cows. Loor *et al.* (2007) also recorded increase serum triglyceride and decrease serum glucose level in induced ketosis in dairy cattle. In subclinical ketosis, triglycerides accumulate in liver cell, when there is a disturbance in the lipoprotein synthesis and ultimately animal develop fatty liver.

The study revealed that excessive lipid mobilization due to negative energy balance, dairy animal turn to increased triglyceride level in subclinical ketotic cows compared to normal healthy cows. Negative energy balance in dairy cows in post-partum transition period is mainly due to production stress and inappetence, which are typical during the peripartum period, are prerequisites for the onset of ketosis soon after parturition (Drackley and Dann, 2005). Ketosis is closely associated with liver lipidosis. Despite advances made over the last decades to understand the pathology and etiology of liver lipidosis and ketosis in dairy cattle, the molecular events associated with these diseases remain largely unknown. Ketosis and liver lipidosis develops when the hepatic uptake of nonesterified fatty acids (NEFA) exceeds their oxidation and secretion as triacylglycerol (TAG) in very-low-density lipoproteins (VLDL) by the liver. It occurs primarily during the first 4 wk postpartum. This disorder is associated with decreased health status, well-being, productivity, and reproductive performance of cows (Loor *et al.*, 2007).

4.5 Enzymatic status in transition period:

The ANOVA of liver enzymes have been presented in appendix Table no. 24.

The mean AST (Aspartate aminotransferase), ALT (Alanine aminotrans-ferase) and GGT (gamma-glutamyl transferase) were considered important parameters for assessing liver efficiency. Results are presented in Table 14.

4.5.1 Aspartate aminotransferase (**AST):**

4.5.1.1 Interaction between (districts and health; district and stage; parity and stage) affecting AST level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups plasma AST level was 56.789±0.947 IU/L. The differences among subclass means were significant ($P \leq 0.05$) across districts (56.958±1.504 IU/L in Bikaner, 60.004±1.589 IU/L in Jodhpur and 53.405±1.952 IU/L in Pali-Marwar), highly significant ($P \leq 0.01$) in healthy/SCK groups (53.393±1.062 IU/L in healthy and 60.185±1.628 IU/L SCK), non significant in stage wise (48.897±1.015 IU/L pre-partum and 60.590±1.216 IU/L post-partum) and highly significant in parity groups (AST level was lower in healthy groups across all districts but the rise in their level was 55.622±1.292 IU/l 3-5th parity and 57.956±1.520 IU/l in above 5th parity).

There was significant two factor interaction indicating that difference among districts. The interaction between (districts and health status; parity and stage) was significant and highly significant between (districts and stage). The trend is depicted in Fig. 69 and 70, which shows that the AST level was lower in healthy groups across all districts but the rise in their level to reach significantly higher difference in (50.430±1.354 to 63.485±2.550 IU/L) Bikaner district and lowest difference (58.986±1.519 to 61.021±2.837IU/L) in Jodhpur. It means AST enzyme was highly increased in subclinical ketosis animal of Jodhpur and hepatocyte more metabolized the fat. The AST level interaction with districts and stages was highly significant ($P \leq 0.01$) and raise their level in Pali-Marwar was higher. It indicates that lipid mobilization is more in Pali-Marwar dairy cows in comparison to Jodhpur and Bikaner. In parity wise, interaction shows that the liver enzymes are increases in old age group dairy cow.

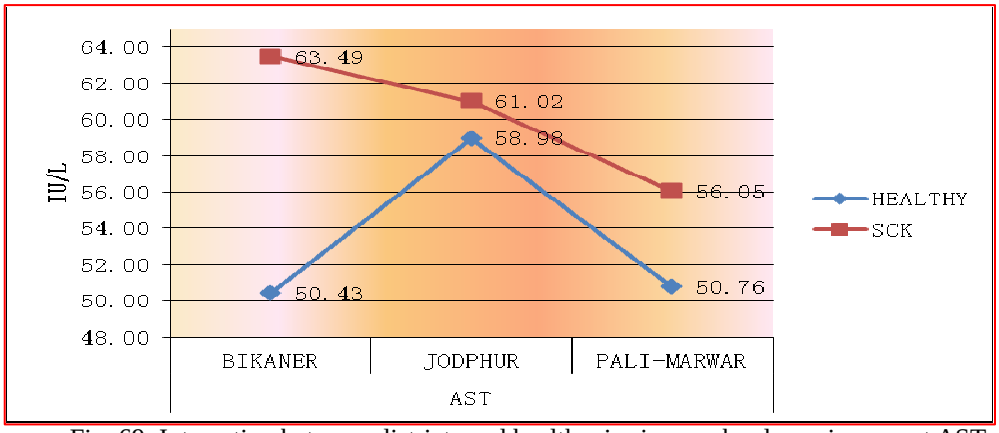

Fig. 69: Interaction between districts and health wise in crossbred cow in respect AST activity.

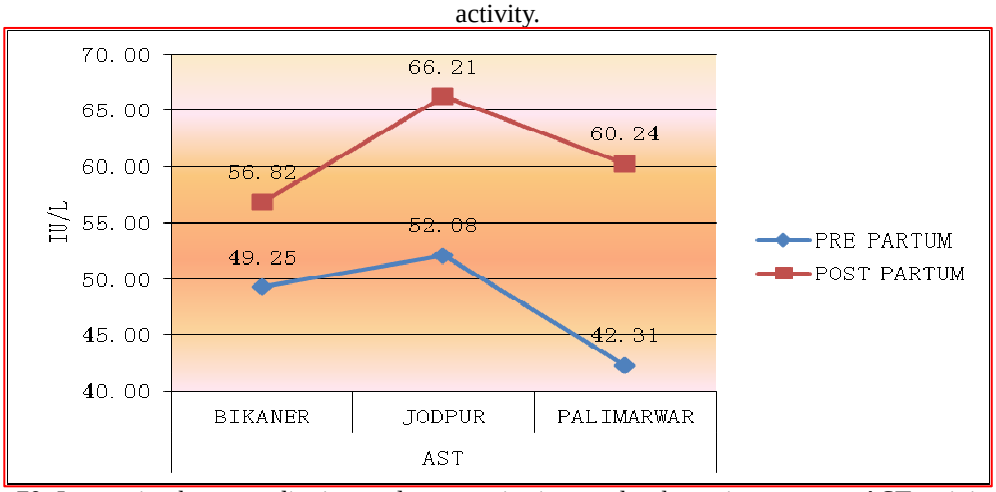

Fig.70: Interaction between districts and stages wise in crossbred cow in respect to AST activity

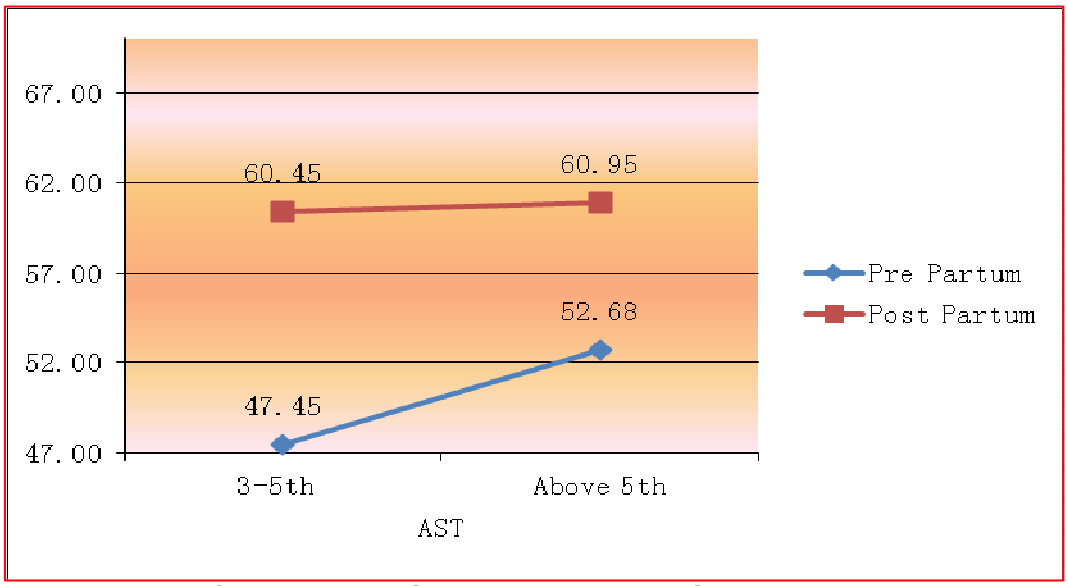

Fig. 71: Interaction between parity and stage wise in respect to plasma AST activity.

4.5.1.2 AST enzyme level in healthy dairy cows:

Overall mean plasma AST concentration from various districts was recorded to be 47.410±1.149 and 58.197±1.432 mg/dl during pre and post-partum transition period respectively (Table 14). A highly significant (P≤0.01) increase was observed in different districts from pre to post-partum period. The high plasma AST level in lactating cattle (post parturient) might be due to excess metabolism of fat and ultimately deposition of fat globule in the hepatocyte and leakage of enzyme in the blood circulation.

Table 14: Overall (Mean±SE) liver enzymes activity in dairy cows during transition period.

Parameters	**Periods**	Over all		Total no. of dairy cows (n=123)
		Healthy (n=94)	**SCK (n=29)**	
AST (u/l)	Pre-partum.	47.410±1.149	53.718±1.930	48.897±1.015
	Post-partum	58.197±1.432	68.347±1.556	60.590±1.216
	Over all	53.393±1.062[a]	60.185±1.628b	56.789±0.947
ALT (u/l)	Pre-partum.	23.229±0.904	23.099±1.243	23.198±0.748
	Post-partum	26.283±0.866	27.673±1.444	26.611±0.743
	Over all	24.160±0.760	25.452±1.165	24.806±0.677
GGT (u/l)	Pre-partum.	22.594±0.889	27.771±1.455	23.814±0.784
	Post-partum	29.611±0.947	35.155±1.550	30.918±0.835
	Over all	26.480±0.809[a]	31.232±1.240[b]	28.856±0.721

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01). 2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05). 3. No superscript mean non significant with in row or column. 4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

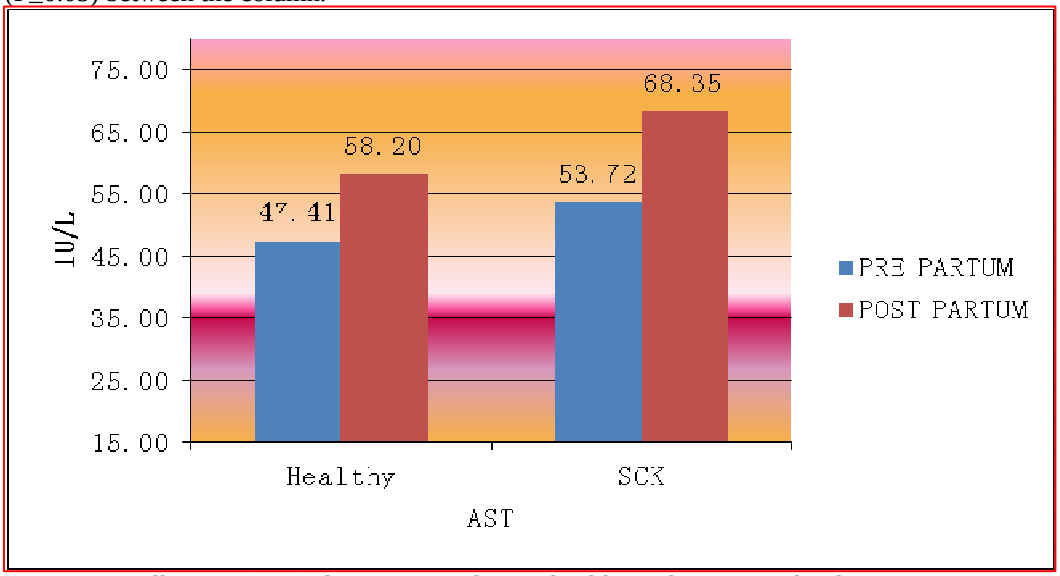

Fig.72: Overall Mean (±SE) plasma AST values in healthy and SCK crossbred cows.

District wise: The mean plasma AST concentrations were 46.573±1.218, 52.211±2.353, 40.256±2.123 IU/L during the pre-partum period and 52.855±1.988, 65.150±2.148, 58.528±3.172 IU/L during the post-partum transition period in crossbred dairy cows of the Bikaner, Jodhpur and Pali-Marwar dairy farms (Table 15 and Fig. 73).

4.5.1.3 AST enzyme level in SCK animal:

The overall mean plasma AST concentration in subclinical ketotic crossbred cows was 53.718±1.930 IU/L and 68.347±1.556 IU/L during pre to post-partum transition period. The overall mean plasma AST level increased from the pre-partum period to the post-partum transition period, the increase was highly significant (P≤0.01).

District wise: The mean plasma AST concentrations from various districts were 57.113±2.907, 51.556±2.976, 48.113±3.435 IU/L during the pre-partum period; 68.465±1.992, 70.582±3.859, 65.072±2.543 IU/L during post-partum transition period in the cows of the Bikaner, Jodhpur and Pali- Marwar dairy farms, respectively. The mean plasma AST concentrations were significantly higher in post-partum cows of Bikaner dairy farms as compared to the cows from other districts because lipid mobilization was more in comparison to other districts. (Table 15).

Table 15: Districts wise liver enzymes investigated under study during transition period in dairy cows in Western Rajasthan.

Parameters	**Periods**	Bikaner			Jodhpur			Pali-Marwar		
		Healthy (n=44)	**SCK (n=15)**	Over all (59)	**Healthy (n=33)**	**SCK (n=8)**	Over all (41)	**Healthy (n=17)**	**SCK (n=6)**	Over all (23)
AST (u/l)	Pre Partum.	46.573±1.218	57.113±2.907	49.253±1.305**	52.211±2.353	51.556±2.976	52.083±1.967**	40.256±2.123	48.113±3.435	42.306±1.914**
	Post Partum	52.855±1.988	68.465±1.992	56.823±1.796**	65.150±2.148	70.582±3.859	66.210±1.896**	58.528±3.172	65.072±2.543	60.235±2.483**
	Over all	50.430±1.354[a]	63.485±2.550[a]	56.958±1.504[a]	58.986±1.519[a]	61.021±2.837[a]	60.004±1.589[a]	50.763±2.363[a]	56.047±3.319[a]	53.405±1.952[a]
ALT (u/l)	Pre Partu.	21.862±0.909	24.647±1.822	22.570±0.828	27.038±1.782	19.798±1.881	25.625±1.540	19.375±2.282	23.630±2.791	20.485±1.849
	Post Partum	25.077±1.172	28.093±1.707	25.844±0.984	29.367±1.564	23.905±2.261	28.301±1.366	23.417±1.775	31.647±4.416	25.564±1.856
	Over all	23.435±0.969[a]	27.452±1.825[b]	25.443±1.076	21.949±2.030[b]	27.321±1.087[a]	24.635±1.137	21.723±1.691[a]	26.954±2.375[b]	24.339±1.397
GGT (u/l)	Pre Partum	23.219±1.338	28.227±2.088	24.492±1.157	20.499±1.587	22.880±2.066	20.964±1.338	25.043±1.475	33.153±2.560	27.159±1.461
	Post Partum	31.562±1.454	35.451±2.377	32.551±1.251	27.172±1.601	31.931±2.555	28.101±1.401	29.295±1.674	38.715±2.814	31.753±1.659
	Over all	26.914±1.031	31.360±1.943	29.137±1.145[a]	25.024±1.157	26.676±2.161	25.850±1.210[b]	27.503±1.800	35.661±2.528	31.582±1.487[c]

NOTE: **1**. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).

2. Value bearing same superscript (a, a) in a row depict significant (p≤0.05).

3. No superscript mean non significant with in row or column.

4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

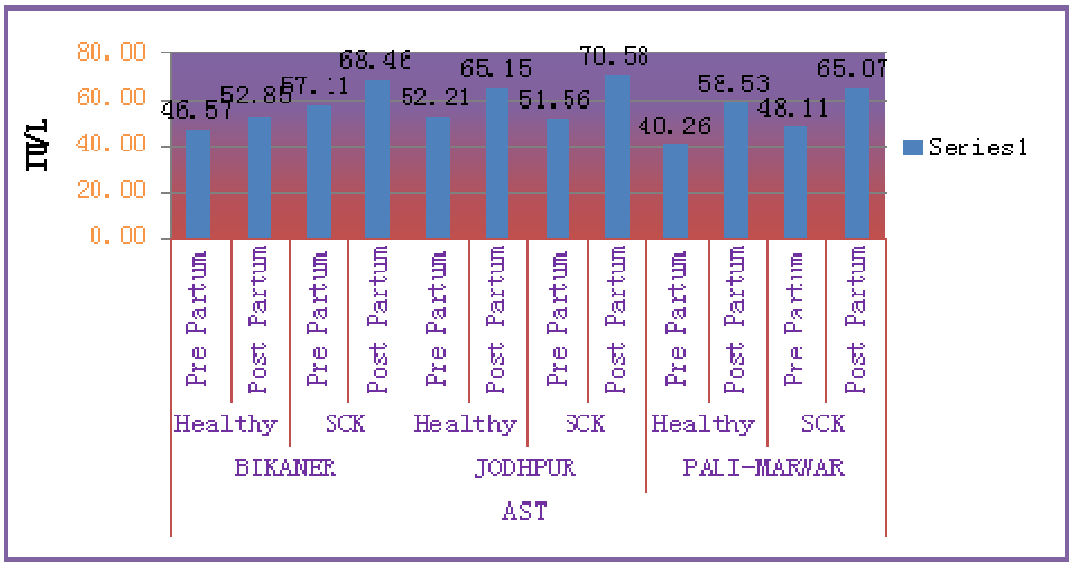

Fig. 73: Mean (±SE) plasma AST values in healthy and SCK crossbred cows.

4.5.1.4 AST level in different parity:

Overall AST level in SCK dairy cow districts were 53.316±2.360 and 69.383±2.022; 54.377±3.453 and 66.652±2.456 IU/L in 3rd to 5th and above 5th parity respectively, which were highly significant (P≤0.01) AST level in different parity groups.

. Mean plasma AST level in healthy dairy cows were 45.966±1.214, 58.187±1.610 and 51.869±2.672, 58.227±3.158 IU/L during pre and post-partum transition period in 3rd to 5th and above 5th parity respectively (Table 16).

Significantly increased AST activity was recorded during early lactation (<3 weeks) in our study in comparison to pre-partum transition period. Although AST is non-specific liver enzyme (Radostits *et al.*, 2007), estimation of its activity in dairy cows is most often associated with fatty liver syndrome (Cebra *et al*., 1997). AST has been found to greatly increase in ketotic cows compared with healthy ones (Steen *et al*., 1997). The infiltration of hepatic cells with fat increases cell membrane permeability with subsequent release of AST enzyme that serves as a good tool for metabolic diseases finding (Karasai and Schefar, 1984). Consequently, in the present study, the elevated serum AST in subclinical affected and healthy could be due to negative energy balance. Tainturer *et al*. (1984) found that AST activity in dairy cow changes irregularly during pregnancy and lactation, but that these changes were not statistically significant. El-Ghoul *et al.* (2000) established a significant increase in AST activity 6 weeks before parturition. Kauppinen (1984) and Kaneko *et al*. (1997) mention the value of AST activity in cows greater than the values in comparison to our study. Stojevic *et al.* (2005); Mordak and Nikpon (2006) and Ping Liu *et al.* (2012), reported that value of AST increased significantly from dry to early lactation period, which was similar to our study.

Table 16 : Mean (±S.E.) value of liver enzyme profile in crossbred cows under different parity during transition periods

Parameters	Periods	3nd - 5th parity			>5thparity		
		Healthy (n=71)	**SCK (18)**	Over all (89)	**Healthy (n=23)**	**SCK (11)**	Over all (34)
AST (u/l)	Pre Partum.	45.966±1.214	53.316±2.360	47.452±1.119**	51.869±2.672	54.377±3.453	52.680±2.105**
	Post Partum	58.187±1.610	69.383±2.022	60.452±1.427**	58.227±3.158	66.652±2.456	60.952±2.358**
	Over all	51.984±0.992	59.260±2.359	55.622±1.292[a]	54.802±1.857	61.110±2.464	57.956±1.520[b]
ALT (u/l)	Pre Partum.	23.498±1.053	22.411±1.871	23.278±0.918	22.400±1.788	24.224±1.205	22.990±1.267
	Post Partum	26.499±0.985	28.092±1.577	26.821±0.847	25.617±1.845	26.986±2.900	26.060±1.540
	Over all	24.659±0.710	24.408±1.688	24.534±0.924[a]	23.660±1.329	26.496±1.763	25.078±1.088[b]
GGT (u/l)	Pre Partum.	22.494±0.955	27.588±1.742	23.524±0.863	22.903±2.163	28.071±2.679	24.575±1.729
	Post Partum	29.203±1.111	34.316±2.039	30.237±0.997	30.870±1.805	36.528±2.420	32.700±1.502
	Over all	25.754±0.756	30.205±1.797	27.980±0.984[a]	27.206±1.415	32.259±1.877	29.733±1.158[b]

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).
2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).
3. No superscript mean non significant with in row or column.
4. **= highly significant (P≤0.01), *= significant (P≤0.01) between the column.

In ketotic animals permeability of hepatic cells increases due to which leakage of liver

specific enzymes more is in circulation (Agarwal *et al.*, 2002). Aspartate aminotransferase may also be high in cattle with hepatic lipidosis, passive venous congestion and diseases that cause distension of the fore stomach and abomasum (Moore, 1997; Kramer and Haffmann, 1997).

AST in liver, skeletal muscle and heart of cow is sensitive (Sattler and Furll, 2004) and increases of serum AST activities is an indicator of soft tissue damage, probably due to liver damage. Hoedemarker *et al.* (2004) indicated that increase of AST activity in postpartum is related to extensive muscle breakdown and increased amino acid catabolism.

4.5.2 Alanine aminotransferase (ALT):

4.5.2.1 Interaction between districts and health wise affecting ALT level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for ALT in plasma was 24.806±0.677 IU/L. The differences among subclass means were non significant across districts (25.443±1.076 IU/L in Bikaner, 24.635±1.137 IU/L in Jodhpur and 24.339±1.397 IU/L in Pali-Marwar), non significant in healthy/SCK groups (24.160±0.760 IU/L in healthy and 25.452±1.165 IU/L in SCK), non significant in stage wise (23.198±0.748 IU/L pre-partum and 26.611±0.743 IU/L post-partum) and highly significant (P≤0.01)in parity groups (24.534±0.924 IU/L 3-5th parity and 25.078±1.088 IU/L above 5th parity). The effect of pre and post-partum stages and healthy/SCK groups were non significant in pooled data.

There were significant effects of two factor interaction indicating that difference among districts. Whereas the interaction between districts and health status was highly significant (P≤0.01). The trend is depicted in Fig. 74. It showed that the ALT level was lower in healthy groups across all districts but the rise in their level was significantly lower in (23.435±0.969 to 27.452±1.825 IU/L) Bikaner district and higher (27.321±1.087 to 21.949±2.030 IU/L) in Jodhpur district.

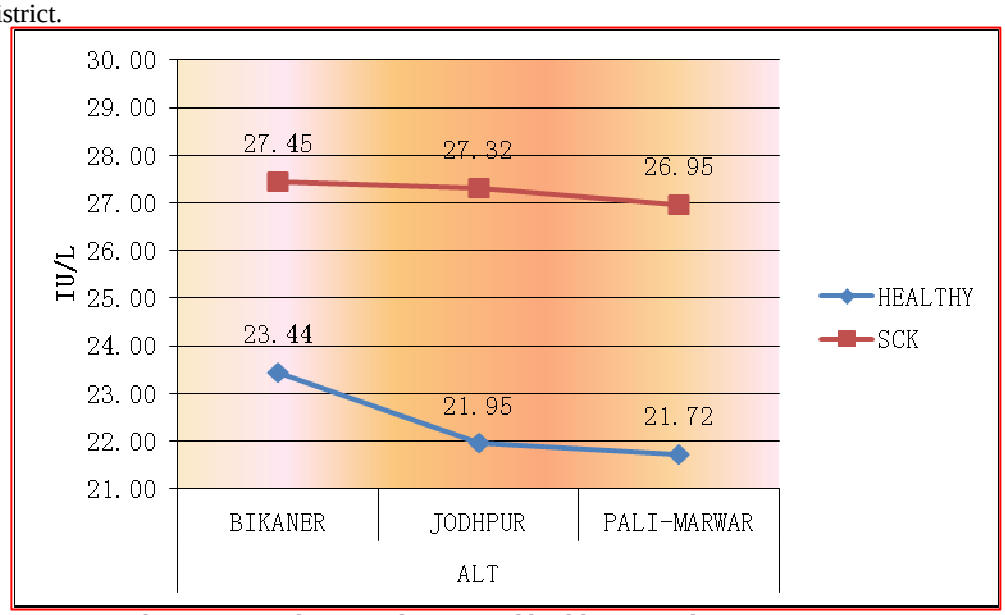

Fig. 74: The Interaction between districts and health wise with respect to ALT activity.

4.5.2.2 ALT enzyme level in healthy animal:

Overall mean ± SE values of ALT have been presented in Table 14. The overall ALT mean values were recorded to be 23.229±0.904, 26.283±0.866 IU/l during pre to post-partum transition period, respectively. The statistical analyses of the data of plasma ALT revealed non significant changes in pre to post-partum transition period.

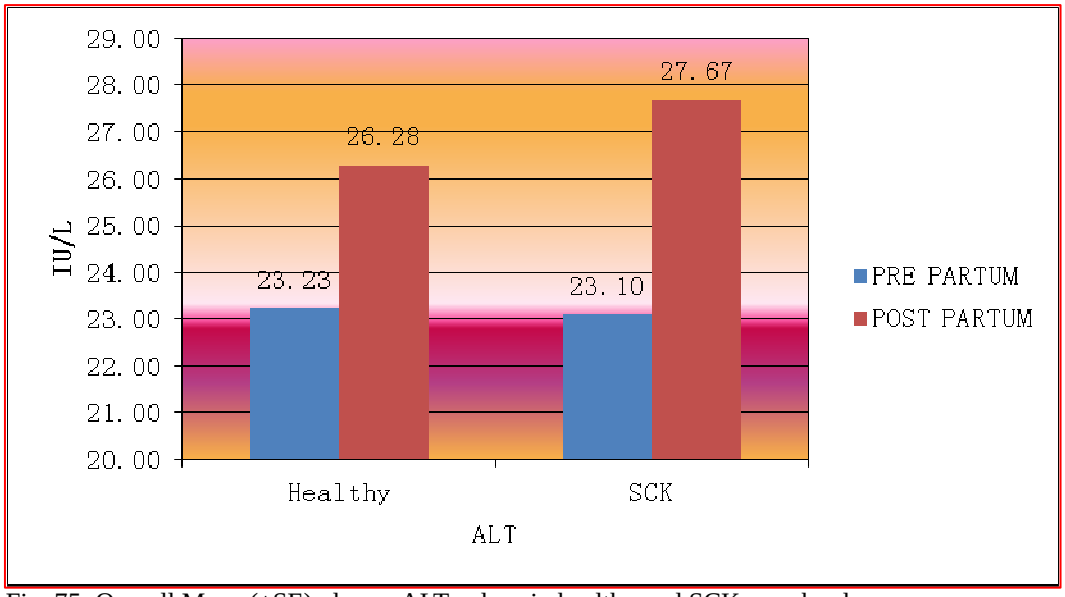

Fig. 75: Overall Mean (±SE) plasma ALT values in healthy and SCK crossbred cows.

The mean ALT level recorded from various districts were 21.862±0.909, 27.038±1.782, 19.375±2.282 IU/L during pre-partum and 25.077±1.172, 29.367±1.564, 23.417±1.775 IU/L during post-partum period in the dairy cows of Bikaner, Jodhpur and Pali- Marwar dairy farm. Non significant higher values were observed in the cows of Jodhpur district as compared to the cows from Bikaner and Pali-Marwar district during the post-partum period (Table 15).

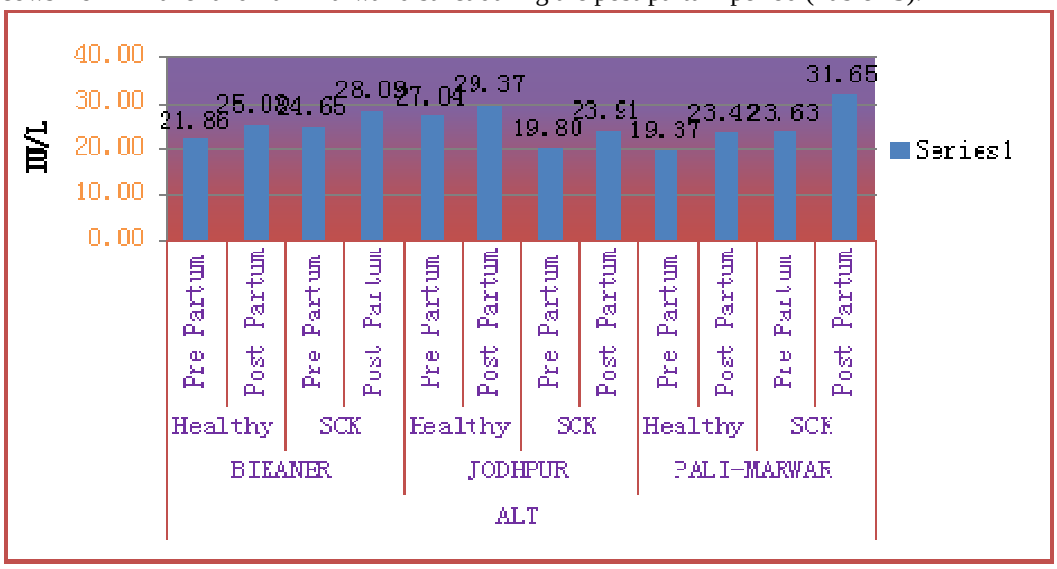

Fig. 76: Mean (±SE) plasma ALT values in healthy and SCK crossbred cows.

4.5.2.3 ALT enzyme level in SCK dairy cow:

The overall mean plasma ALT concentration recorded was 23.099±1.243 IU/L during pre-partum period; 27.673±1.444 IU/L during post-partum transition period. A non significant increase was observed in the overall mean plasma ALT level from the pre to post-partum period which was well within the normal physiological range for the cows (Table 14).

District wise: Mean plasma ALT concentration were 24.647±1.822, 19.798±1.881, 23.630±2.791 IU/L during pre-partum period and 28.093±1.707, 23.905±2.261, 31.647±4.416 IU/L during post-partum period in cows from Bikaner, Jodhpur and Pali-Marwar dairy farms, respectively. Non significantly difference was observed in districts and stage wise (Table 15).

4.5.2.4 ALT level in different parity:

Mean plasma ALT level in healthy dairy cows was 23.498±1.053 and 22.400±1.788 IU/L and 26.498±0.985, 25.617±1.740 IU/L during pre and post-partum transition period in 3rd to 5th and above 5th parity, respectively (Table 16).

The mean plasma values observed in subclinical ketosis dairy cow in these groups were 22.411±1.871, 24.224±1.205 IU/L during pre-partum and 28.092±1.577, 26.986±2.900 IU/L

during post-partum transition period in 3rd to 5th and above than 5th parity respectively. Overall highly significant differences were noticed in the plasma ALT value across the parity but there were no effect of health and stage.

ALT activity in cows differs during certain production periods. The highest ALT activity was measured during early lactation (post-partum transition period). Similarly, ALT has been found to increase in liver and bile duct malfunctions (Steen *et al.*, 1997). Consequently, in the present study, high AST and ALT support the occurrence of hepatic damage in subclinical ketotic dairy cow.

ALT activity indicated a statistically significant increase from the 5-7 weeks of lactation and activity in the 7th week postpartum periods significantly reached to the peak (Stojevic *et al.*, 2005; Ping Liu *et al.*, 2012). In the dry period enzyme activity was lower than early lactation, but it was still statistically much higher than Kauppinen, 1984 and Kaneko *et al.*, 1997 values (17.82 ± 11.51 U/L). In our finding the role of ALT in predicting liver damage in subclinical ketosis was significant. Tainturier *et al.* (1984) in their study presented information that ALT activity decreased in the 7th and 8th months of pregnancy and that it remained stable until the end of pregnancy, and in the first month of lactation. Our results confirm this only partially because in the period of pre-partum transition, we measured the lower concentrations of ALT in comparison to post-partum transition period.

The ALT activity in cattle is not specific for the liver in order to have a diagnostic significance (Kramer and Hoffman, 1997). However in our study, we found that ALT increase in the plasma was significant from pre to post-partum transition period. Tainturier *et al.* (1984) reported that ALT changes significantly in cows in the parturition period and ALT activity decreases in the last trimester and increases after one month of lactation period. The highest activity of ALT was found in the third period of lactation (Stojevic *et al.*, 2005).

4.5.3 Gamma glutamyl transference (GGT):

4.5.3.1 Interaction between districts and parity affecting GGT level:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for GGT in plasma was 28.856±0.721 IU/L. The differences among subclass means were highly significant (P≤0.01) across districts (29.137±1.145 IU/L in Bikaner, 25.850±1.210 IU/L in Jodhpur and 31.582±1.487 IU/L in Pali-Marwar), highly significant in healthy/SCK groups (26.480±0.809 IU/L in healthy and 31.232±1.240 IU/L SCK), non significant in stage wise (23.814±0.784 IU/L pre-partum and 30.918±0.835 IU/L post-partum) and highly significant in parity groups (27.980±0.984 IU/L 3-5th parity and 29.733±1.158 IU/L above 5th parity). There was significant (P≤0.05) effect of two factor interaction indicating that difference among districts. Whereas the interaction between districts and parity was significant. The trend is depicted in Fig. 77.

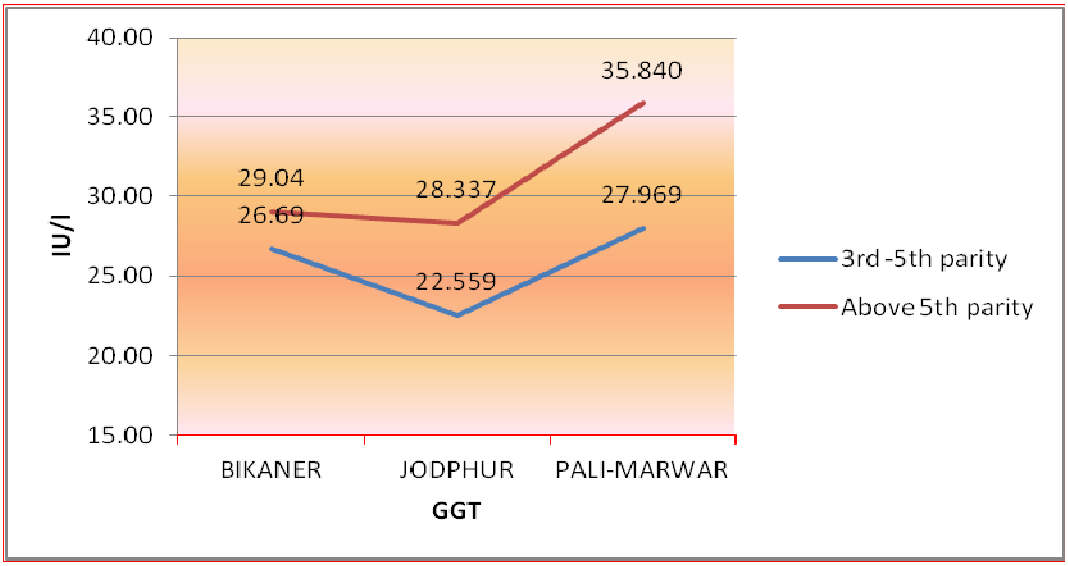

Fig. 77: Interaction between districts and parity wise affecting the level of GGT

4.5.3.2 GGT enzyme level in healthy animal:

The overall mean GGT level measured during different stages of transition period were 22.594±0.889 and 29.611±0.947 IU/l during pre and post-partum period respectively (Table 14).

District wise: The mean GGT level in Bikaner, Jodhpur and Pali-Marwar dairy farms were 23.218±1.294, 20.499±1.494, 25.042±2.082 IU/L during pre-partum period; 31.562±1.294,

27.172±1.494, 29.295±2.082 IU/L during post-partum period, respectively. Non Significantly higher value was noted in the cows of Bikaner district as compared to the cows of Jodhpur and Pali-Marwar during pre and post-partum period (Table 15 and Fig. 79).

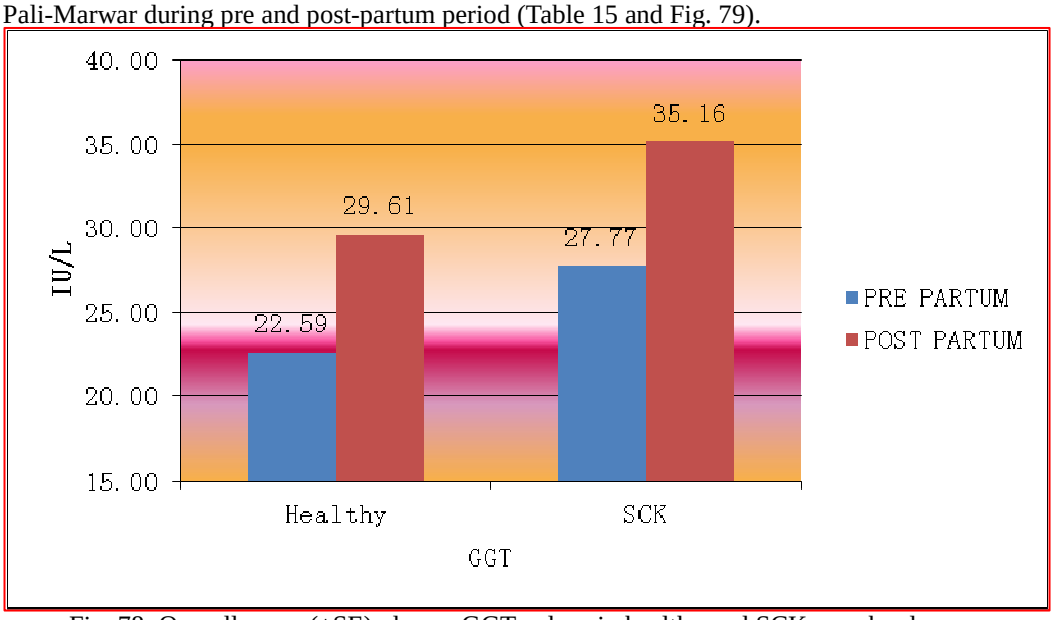

Fig. 78: Overall mean (±SE) plasma GGT values in healthy and SCK crossbred cows.

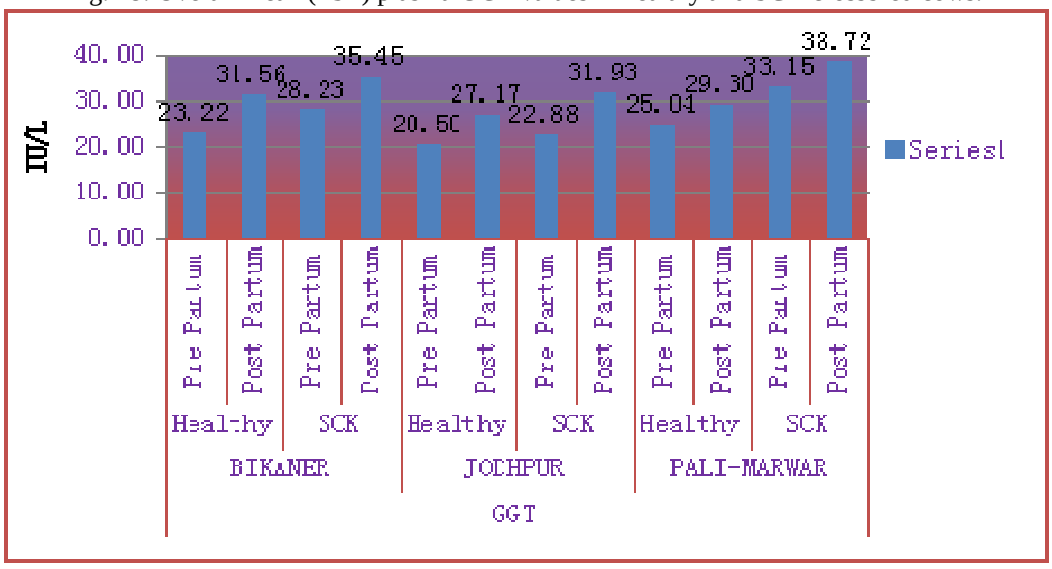

Fig. 79: Mean (±SE) plasma GGT values in healthy and SCK crossbred cows.

4.5.3.3 GGT enzyme level in SCK dairy cow:

The overall mean plasma GGT concentration recorded was 27.771±1.455 IU/L during pre-partum period and 35.155±1.550 IU/L during post-partum period which was higher than the normal physiological range 14-26 IU/L as stated by McDowell (1992). A highly significant (P≤0.01) increase was observed in GGT concentrations between healthy and SCK dairy cows in transition period (Table 14).

District wise: The mean plasma GGT concentration recorded were 28.227±2.088, 22.880±2.066, 33.153±2.560 IU/L during pre-partum period and 35.451±2.377, 31.931±2.555, 38.715±2.814 IU/L during post-partum period from the various farms of the Bikaner, Jodhpur and Pali-Marwar dairy farm. Non Significant changes recorded in districts and stage wise in GGT level (Table 15).

4.5.3.4 GGT level in different parity:

Mean plasma GGT level in healthy dairy cow were 22.494±0.955, 29.203±1.111 and 22.903±2.163, 30.870±1.805 during pre and post-partum transition period in 3rd to 5th and above 5th parity respectively (Table 16).

The mean plasma GGT values recorded in subclinical ketosis dairy cows in these groups were 27.588±1.742 and 28.071±2.679 and IU/L during pre-partum and 34.316±2.039 and

36.528±2.420 IU/L during post-partum transition period in 3rd to 5th and above than 5th parity respectively. Districts wise significant differences were observed in parity but health and stage wise no differences were observed (Table 16).

Higher values for Gamma-glutamyltransferase activity were measured in post-partum transition period in comparison to the pre-partum period. El-Ghoul *et al*. (2000) and Ping Liu *et al.* (2012) found that GGT activity in late pregnancy is much lower than early lactation (1 to 9 weeks). The GGT level measured in present study was higher than Kauppinen (1984) and Kaneko *et al*. (1997) and was low in comparison to Kataria and Kataria (2012). Increased GGT value in ketogenic cows (Steen, 2001) pointed towards a stressed liver as its activity is relatively high in liver (Tenant, 1997).

It could be related to negative energy status of high yielders particularly in late pregnancy, in the first weeks of lactation and during disease (Cebra *et al.*, 1997) as energy status have an influence on GGT values (Stojevic *et al.*, 2005).

The results showing with parity effects were more or less in agreement with earlier reports. Plasma liver enzyme activity is known to be concerned with metabolic process like mineral deposition in bones. Increase in the liver enzymes activity in more than 5th parity mediated by somatomedins in liver, which help in accelerating the osteoblastic activity for growth.

4.6 Body Score Condition and Milk Production:

The ANOVA of BCS and milk production have been presented in appendix Table no. 24.

4.6.1 Body condition score (BCS):

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for BCS in plasma was 3.187±0.025. The differences among subclass means were highly significant (P≤0.01) across districts (3.288±0.041 in Bikaner, 3.047±0.043 in Jodhpur and 3.228±0.053 in Pali-Marwar), highly significant in healthy/SCK groups (3.392±0.029 in healthy and 2.982±0.044 in SCK), highly significant in stage wise (3.486±0.034 pre-partum and 3.067±0.031 post-partum) and non significant in parity groups (3.406±0.036 IU/L 3-5th parity and 2.968±0.036 IU/L above 5th parity). There was no effect of two factor interaction indicating difference among districts.

4.6.1.1 BCS in cross bred healthy cows:

The overall BCS was 3.486±0.034 and 3.067±0.031 in pre and post-partum transition periods. A highly significant (P≤0.01) difference was observed in stage wise BCS score. The mean BCS was 3.569±0.039 and 3.154±0.033 in pre-partum and post-partum in crossbred healthy dairy cows.

The overall mean BCS changes in healthy and SCK cows were 3.569±0.039, 3.154±0.033 and 3.216±0.042, 2.784±0.042 in pre to post-partum transition period, respectively. There was highly significant difference in healthy and SCK dairy cows in pre and post-partum transition period.

The overall mean BCS recorded from different parity in healthy dairy cow was 3.362±0.027 in the 3rd to 5th parity cows, 3.422±0.050 in above 5th parity cows. BCS was recorded in the cows from the pre to post-partum transition period, as 3.547±0.043, 3.153±0.043 in 3rd to 5th parity and 3.620±0.090 , 3.174±0.087 in above 5th parity in two parity groups, there was no significant difference in BCS changes in both the parity group in healthy condition in two different stages but district wise, highly significant (P≤0.01) difference were observed. Bernabucci *et al.* (2005) showed reduction in the BCS during early pregnancy, as compared to the late pregnancy, and also the cows with higher BCS showed higher reduction in BCS from late pregnancy to first 30 days in milk, then the cows with low BCS and medium BCS. The result showed that loss of BCS in transition period is related to the nutritional management. It will increase the occurrence of production diseases.

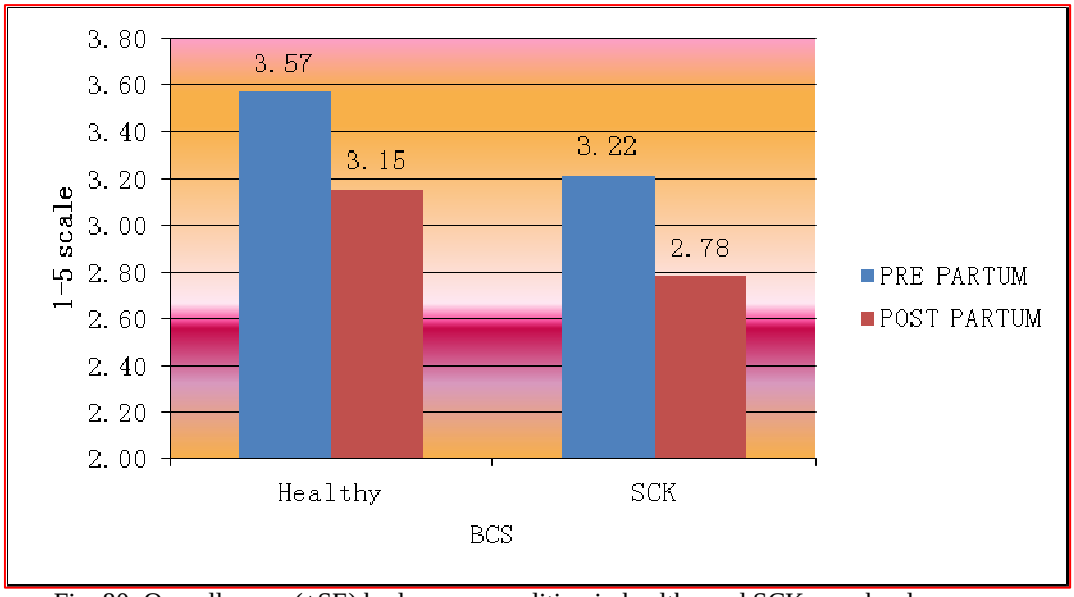

Fig. 80: Overall mean (±SE) body score condition in healthy and SCK crossbred cows.

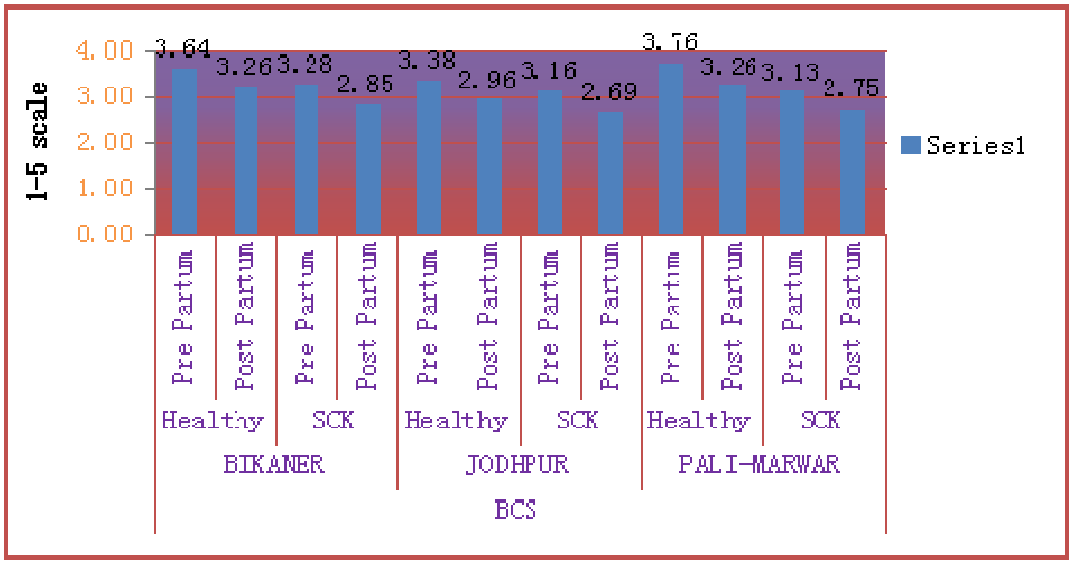

Fig. 81: Mean (±SE) body score condition in healthy and SCK crossbred cows.

The mean BCS in 3rd -5th and above 5th parity was 3.22±0.05; 2.79±0.05 and 3.20± 0.07; 2.77±0.07 in SCK dairy cow, respectively (Table 19). No significant difference was recorded in two parity groups.

The BCS in cows suffering from SCK was lower during the post-partum period in both the groups, and both were lower than healthy dairy cows.There was a reduction in milk yield in cows suffering from SCK; the average being 15.692±0.621 litres per day in cows from 3rd -5th parity cows, while it was 16.493±0.649 litres per day in cows from above 5th parity. The result shows that more reduction in BCS was associated with lower production of milk. Generally, body condition reflects the amount of sub cutaneous body fat of cows (Ferguson *et al.*, 1994) and BCS is usually done during dry and early lactation periods, because of the links with subsequent performance.

Table 17: Overall BCS and milk production (MP) investigated under study during transition period.

Parameters	**Periods**	Over all		Total no. of dairy cows (n=123)
		Healthy (n=94)	**SCK (n=29)**	
BCS (1-5)	Pre-partum.	3.569±0.039	3.216±0.042	3.486±0.034**
	Post-partum	3.154±0.033	2.784±0.042	3.067±0.031**

	Over all	3.392±0.029[a]	2.982±0.044[b]	3.187±0.025
MP (kg)	Post-partum	21.450±0.375[a]	16.093±0.583[b]	20.228±0.342

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).
2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).
3. No superscript mean non significant within row or column.
4. **= highly significant (P≤0.01), *= significant (P≤0.01) between the column.

Studer (1998) reported that cows with BCS <2.5 before calving were had increased risk of developing ketosis. In our study, in SCK dairy cows had mean BCS 3.25 and healthy cows. More BCS in pre parturient had more loss of BCS in post parturient transition period in both the stages. In SCK cows loss of BCS was more in comparison to healthy cow. Similar to the present study, Ribeiro *et al.* (2013) observed that cows with subclinical disease had higher BCS at first 7 days of calving, but looses more BCS during the 7-35 days in milk.

The BCS changes during the pre to post-partum transition periods in our study was consistent with previous reports (Ruegg *et al*., 1992; Walter *et al*., 1993, Pedron *et al*., 1993; Ruegg and Milton, 1995). The BCS changes were more in subclinical ketosis as compared to healthy animals. It showed more body condition loss in dairy cows which suffered to energy deficit from pre to post parturient transition period. More changes in BCS indicate that more quantity of fatty acids were metabolized in liver, after certain limit fatty acids are not metabolizing and ultimately hepatocytes are filled with fat and animal will develop fatty liver so that essential processes such as gluconeogenesis, detoxification of ammonia and stimulation of appetite etc remain at a sub-optimal rate. Poor feeding and management, high energy requirements in the post-partum period, lack of exercise, and housing conditions could explain it.

4.6.2 Milk production:

The overall mean of all the cows across various districts, stages, parity and healthy/SCK groups for milk production was 20.228±0.342 kg/day. The differences among subclass means were non significant across districts (19.864±0.539 kg/day in Bikaner, 20.415±0.519 kg/day in Jodhpur and 20.826±0.772 kg/day in Pali-Marwar), highly significant (P≤0.01) in healthy/SCK groups (21.450±0.375 kg/day in healthy and 16.093±0.583 kg/day in SCK), non significant in parity groups (20.584±0.416 kgs/day 3-5th parity and 19.294±0.567 IU/L above 5th parity). The effect of pre and post-partum stages, parity were non significant in pooled data. There was no effect of two factor interaction indicating that difference among districts.

Table 18: Districts wise BCS and milk production (MP) investigated under study during transition period in Western Rajasthan.

Parameters	**Periods**	Bikaner			Jodhpur			Pali-Marwar		
		Healthy (n=44)	**SCK (n=15)**	Over all (59)	**Healthy (n=33)**	**SCK (n=8)**	Over all (41)	**Healthy (n=17)**	**SCK (n=6)**	Over all (123)
BCS (1-5)	Pre Partum.	3.636±0.047	3.283±0.059	3.547±0.043	3.379±0.067	3.156±0.094	3.335±0.058	3.765±0.092	3.125±0.056	3.598±0.091
	Post Partum	3.256±0.044	2.850±0.059	3.153±0.043	2.962±0.052	2.687±0.103	2.909±0.049	3.265±0.066	2.750±0.000	3.130±0.068
	Over all	3.469±0.037	3.107±0.069	3.288±0.041[a]	3.179±0.041	2.914±0.077	3.047±0.043[b]	3.530±0.064	2.926±0.090	3.228±0.053[c]
MP (kg)	Post Partum	21.431±0.451	15.266±0.774	19.864±0.539	21.333±0.521	16.625±1.059	20.415±0.519	22.176±0.727	17.000±1.223	20.826±0.772

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).
2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).
3. No superscript mean non significant within row or column.
4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

Table 19: Body condition score and milk yield in crossbred cows from various parity (Mean± S.E.)

Parameters	Periods	3nd - 5th parity			>5thparity		
		Healthy (n=71)	SCK (18)	Over all (89)	Healthy (n=23)	SCK (11)	Over all (34)
BCS (1-5)	Pre Partum.	3.553±0.043	3.222±0.053	3.486±0.038	3.620±0.090	3.205±0.074	3.485±0.073
	Post-partum	3.148±0.034	2.792±0.054	3.076±0.033	3.174±0.087	2.773±0.071	3.044±0.070
	Over all	3.362±0.027	2.944±0.064	3.406±0.036	3.422±0.050	3.021±0.067	2.968±0.036
Milk (kg)	Post-partum	21.873±0.373	15.500±0.513	20.584±0.416	20.478±0.616	16.818±0.796	19.294±0.567
		21.873±0.373	15.500±0.513	20.584±0.416	20.478±0.616	16.818±0.796	19.294±0.567
		21.889±0.261	15.692±0.621	18.771±0.349	21.010±0.489	16.493±0.649	18.771±0.349

NOTE: 1. Value bearing different superscript (a,b,c,d) in a row depict highly significant (P≤0.01).
2. Value bearing same superscript (a, a) in a row depict significant (P≤0.05).
3. No superscript mean non significant within row or column.
4. **= highly significant (P≤0.01), *= significant (P≤0.05) between the column.

4.6.2.1 Milk yield:

The average milk yield recorded from the healthy cows was 21.889±0.261 l/day from the 3rd to 5th parity and 21.010±0.489 l/day from above 5th parity; which was almost similar in both the parity in healthy groups. In subclinical ketosis cows average milk production were 15.692±0.621 and 16.493±0.649 l/day in 3rd to 5th parity and more than 5th parity (Table 19). A significant association between subclinical ketosis and decreased milk production (P≤0.01) was recorded in dairy cows. Wondifraw *et al.* (2013) observed highest milk yield in cows from above 5th parity groups and lowest in cows from 1st parity groups in healthy dairy cow.

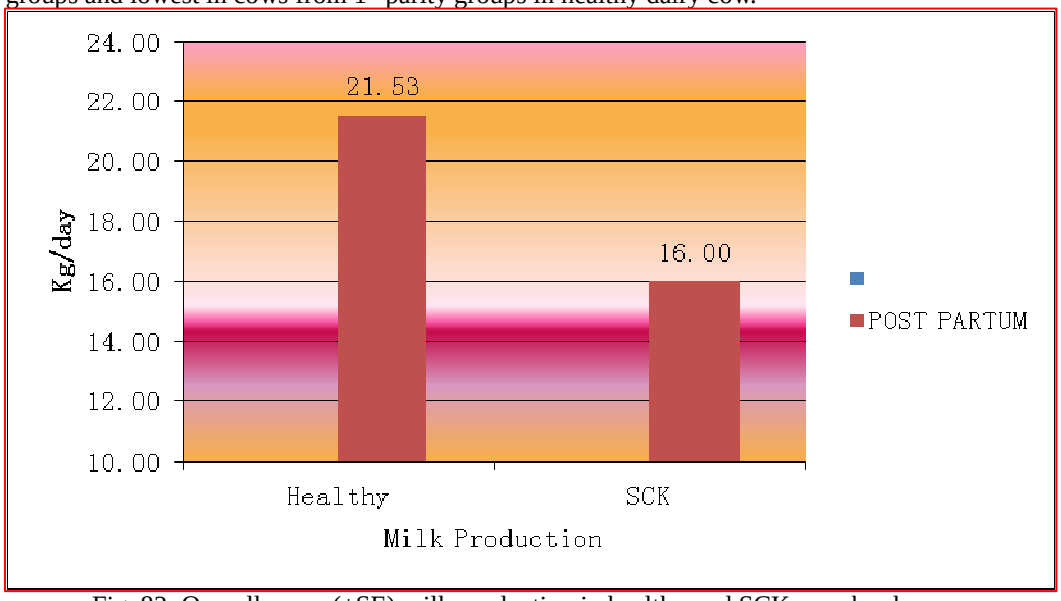

Fig. 82: Overall mean (±SE) milk production in healthy and SCK crossbred cows.

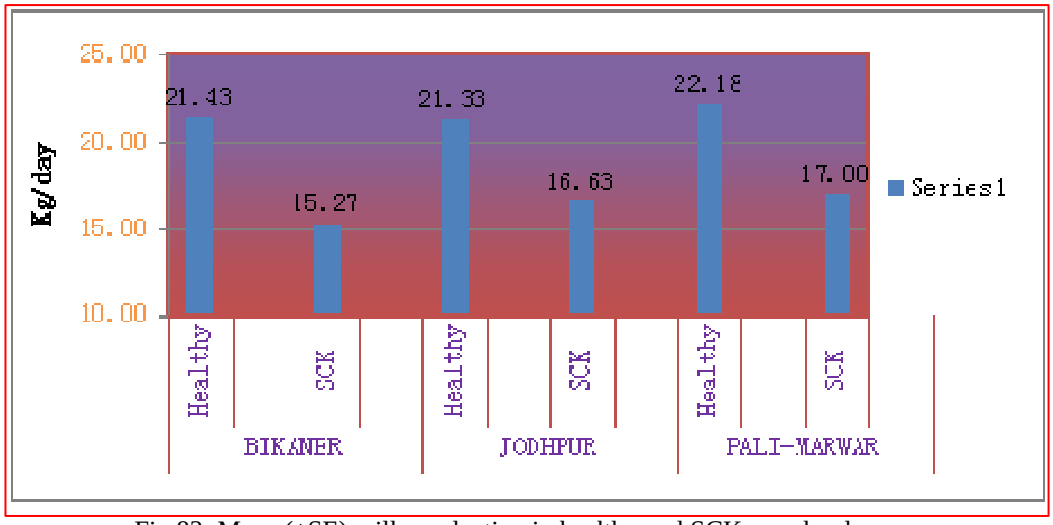

Fig.83: Mean (±SE) milk production in healthy and SCK crossbred cows.

Decreased milk yield observed in early lactation might be due to the hypoglycemia observed in SCK which caused drop in lactose synthesis and hence reduced milk production (Simensen *et al.,* 1990). Noticeable milk yield loss prior to actual diagnosis of clinical ketosis is a strong support for negative and unrealized impact of subclinical ketosis on milk production. Ketosis can cause economic losses through decreased milk production and association with pre parturient diseases (Ardvan Nowroozi *et al.,* 2011).

4.7 Correlation:

4.7.1 Healthy dairy cow:

In healthy crossbred cows, no significant correlations of glucose level was observed with plasma albumin, AST, ALT, cholesterol level, MDA, reduced GSH, Na, Fe and milk production, globulin and A:G ratio in healthy animals. However, Total plasma protein, BUN, Creatinine, NEFA, BHBA, GGT, Ca, K, Cu and Zn levels showed significant ($P \leq 0.01$) correlation with glucose level, while plasma triglycerides, P and Mg also had significant ($P \leq 0.05$) correlation with glucose level.

Total plasma (TPP) protein had no significant correlation with cholesterol, reduced glutathione, Mg, K, Cu and A: G ration. However, a significant ($P \leq 0.01$) correlation between TPP and albumin, BUN, BHBA, AST, plasma triglyceride, MDA, Ca, Fe, Zn, globulin and BCS were present. Significant ($P \leq 0.05$) correlation between TPP and creatinine, ALT, GGT, P, Na and milk production were also evident in healthy crossbred cows.

The plasma albumin concentration was significantly (P≤0.01) correlated with BUN, NEFA, BHBA, Ca, P, Zn, globulin, A:G ratio and milk production. However, no significant correlation was evident with creatinine, AST, ALT, GGT, Cholesterol, triglycerides, reduced glutathione, MDA, Mg, Na, K, Fe and BCS.

PUN had significant (P≤0.01) correlation with creatinine, NEFA, BHBA, AST, GGT, triglyceride, MDA, Ca, P, Cu, Zn, A:G ratio and BCS. Likewise, Na, K and Fe also had positive correlation (P≤0.05) with BUN. But, other biochemical parameters did not reveal any significant correlation with PUN.

Positive correlation (P≤0.01) of creatinine with BHBA, AST and Zn was recorded. However, ALT, GGT, cholesterol, reduced glutathione, MDA, P, Na, K, Cu, Fe, A: G ratio, milk production and BCS had no significant correlation with creatinine.

NEFA had significant positive correlation with BHBA, AST, Ca, P, Zn, milk production, BCS (P≤0.01), GGT, cholesterol, triglyceride, MDA, Mg, Na, Fe and A:G ratio (P≤0.05). No significant correlation of NEFA with ALT, reduced glutathione, K and globulin were evident.

BHB level had significant (P≤0.01) correlation with AST, ALT, GGT, triglyceride, MDA, Ca, P, Na, K, Cu, Fe, Zn, milk production and BCS; while no significant correlation was evident with reduced glutathione, globulin and A:G ratio.

AST activity was significantly (P≤0.01) correlated with ALT, triglyceride, Ca, P, Fe, Zn and BCS. However, no significant correlation of cholesterol, reduced glutathione, MDA, Na, K, Cu, globulin, A: G ratio and milk production was recorded.

ALT activity had significant correlation with Fe (P≤0.01), Ca and BCS (P≤0.05); while no significant correlation was evident with GGT, cholesterol, triglyceride, reduced glutathione, MDA, P, Mg, Na, K, Cu, Zn, globulin, A:G ratio and milk production.

GGT level was positively correlated (P≤0.01) with Ca, P, K and Zn concentrations, But, triglycerides, reduced glutathione, MDA, Mg, Na, globulin, A: G ratio, milk production and BCS had no significant correlation with GGT.

Cholesterol level had significant correlation (P≤0.05) with reduced glutathione, Cu, P, milk production and BCS. But, Triglyceride, MDA, Ca, Mg, Na, K, Fe, Zn, globulin and A: G ratio were not correlated with cholesterol level.

Plasma triglyceride was significantly (P≤0.05) correlated with Ca, P, Fe and Zn. On the other hand, MDA, reduced glutathione, Mg, Na, K, Cu, globulin, A: G ratio, milk production and BCS had no significant correlation with plasma triglyceride.

Reduced glutathione was significantly correlated with MDA, Cu and milk production (P≤0.01), Ca, Na and K (P≤0.05). However, no significant correlation was observed between reduced glutathione and P, Mg, Fe, Zn, globulin, A: G ratio and BCS.

MDA was significantly (P≤0.01) correlated with Ca, P, Cu, Zn and BCS. However, no significant correlation was evident with P, Mg, Na, K, Fe, globulin, A:G ratio and milk production.

Ca level was significantly correlated with P, K, Fe, Zn, BCS (P≤0.01), milk production and A: G ratio (P≤0.05). But, Mg, Na, Cu and globulin had no significant correlation with Ca.

Pi concentration had significant (P≤0.05) correlation with Zn, globulin, A: G ratio and BCS. However, Mg, Na, K, Cu, Fe and milk production had no significant correlation with P.

Mg concentration was significantly (P≤0.05) correlated with Zn and BCS. However, Na, K, Cu, Fe, globulin, A: G ratio and milk production had no significant correlation with Mg.

Na was significantly correlated with Cu (P≤0.01) and Zn (P≤0.05). But, K, Fe, globulin, A:G ratio, milk production and BCS had no significant correlation with Na.

K was significantly (P≤0.01) correlated with Cu, Zn, milk production and BCS. However, Fe, globulin and A: G ratio had no significant correlation.

Copper level was significantly (P≤0.05) correlated with Fe, Zn, A: G ratio, milk production and BCS. Globulin concentration was not significantly correlated with Cu.

Fe had significant (P≤0.05) correlation with Zn, BCS and globulin. But milk production and A: G ratio was not significantly correlated with Fe.

Zn was significantly (P≤0.01) correlated with A: G ratio and BCS. However, milk production and globulin concentration did not correlate significantly.

Milk production had significant (P≤0.05) correlation with BCS and A: G ratio. But, globulin level did not significantly correlate with milk production.

BCS did not have any significant correlation with globulin and A: G ratio. Globulin level was significantly (P≤0.01) correlated with A: G ratio.

Table 20: Correlation among various minerals, biomarkers of oxidative stress, energy and biochemical parameters investigated among all the healthy crossbred cows (n=188).

TRAITS	GLU	TPP	AL	PUN	CR	NEFA	BHBA	AST	ALT	GGT	CHOL	TRIG	RGSX	MDA	CA	P	MG	NA	K	CU	FE	ZN	MP	BCS	GB	ALGB
GLU	**1**	**0.197**	0.094	**-0.37**	**0.23**	**-0.392**	**-0.426**	-0.092	-0.083	**-0.184**	-0.116	**-0.135**	-0.014	-0.077	**0.226**	**0.16**	**-0.125**	0.096	**0.217**	**0.249**	0.06	**0.273**	0.011	**0.146**	0.02	0.055
TPP	**	**1**	**0.271**	**-0.296**	**0.157**	**-0.352**	**-0.464**	**-0.298**	**-0.16**	**-0.149**	0.013	**-0.305**	0.1	**-0.166**	**0.405**	**0.147**	-0.079	**0.127**	0.054	0.025	**0.212**	**0.354**	**0.126**	**0.319**	**0.306**	0.06
AL	NS	**	**1**	**-0.258**	-0.075	**-0.197**	**-0.241**	-0.117	-0.085	-0.047	0.008	-0.088	-0.06	-0.115	**0.204**	**0.251**	-0.083	0.104	0.044	**0.137**	-0.013	**0.266**	**0.197**	0.096	**-0.834**	**0.883**
PUN	**	**	**	**1**	**-0.229**	**0.358**	**0.5**	**0.287**	0.001	**0.251**	0.091	**0.178**	0.007	**0.233**	**-0.388**	**-0.231**	0.067	**-0.137**	**-0.136**	**-0.213**	**-0.141**	**-0.426**	0.021	**-0.229**	0.086	**-0.176**
CR	**	*	NS	**	**1**	**-0.165**	**-0.284**	**-0.307**	-0.053	-0.049	0.029	**-0.134**	0.029	-0.001	**0.153**	0.123	**-0.128**	0.116	-0.001	0.019	0.061	0.202	-0.059	0.085	**0.164**	-0.068
NEFA	**	**	**	**	*	**1**	**0.592**	**0.167**	0.095	**0.145**	**-0.136**	0.124	0.117	**0.145**	**-0.424**	**-0.161**	**0.15**	**-0.137**	-0.12	**-0.226**	**-0.158**	**-0.31**	**-0.277**	**-0.342**	-0.007	**-0.151**
BHBA	**	**	**	**	**	**	**1**	**0.368**	**0.194**	**0.295**	**-0.145**	**0.343**	0.124	**0.187**	**-0.478**	**-0.227**	**0.139**	**-0.166**	**-0.253**	**-0.374**	**-0.351**	**-0.529**	**-0.219**	**-0.477**	-0.028	-0.123
AST	NS	**	NS	**	**	**	**	**1**	**0.21**	**0.148**	0	**0.223**	-0.053	0.031	**-0.336**	**-0.223**	**0.146**	-0.115	0.055	-0.092	**-0.26**	**-0.308**	0.073	**-0.209**	-0.056	-0.047
ALT	NS	*	NS	NS	NS	NS	**	**	**1**	-0.044	-0.067	0.102	0.007	-0.052	**-0.15**	-0.043	0.042	-0.109	0.114	-0.01	**-0.216**	-0.088	-0.039	**-0.151**	-0.007	-0.056
GGT	**	*	NS	**	NS	*	**	*	NS	**1**	**0.158**	0.049	-0.008	-0.005	**-0.253**	**-0.203**	-0.041	-0.102	**-0.173**	**-0.143**	**-0.131**	**-0.23**	0.008	-0.116	-0.039	0.042
CHOL	NS	NS	NS	NS	NS	*	*	NS	NS	*	**1**	0.037	**-0.345**	-0.086	-0.008	**-0.16**	-0.108	-0.082	0.103	**0.364**	0.046	0.064	**0.268**	**0.216**	-0.001	0.015
TRIG	*	**	NS	**	*	*	**	**	NS	NS	NS	**1**	-0.007	0.088	**-0.146**	**-0.149**	-0.052	-0.039	0.045	-0.118	**-0.174**	**-0.258**	-0.002	-0.076	-0.088	-0.023
RGSX	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	**1**	**0.167**	**0.138**	0.043	0.098	**0.148**	**-0.162**	**-0.296**	0.004	-0.08	**-0.314**	-0.05	0.117	-0.047
MDA	NS	**	NS	**	NS	*	**	NS	NS	NS	NS	NS	**	**1**	**-0.21**	-0.118	-0.003	0.002	-0.036	**-0.36**	-0.065	**-0.296**	-0.08	**-0.342**	0.019	-0.089
CA	**	**	**	**	*	**	**	**	*	**	NS	*	*	**	**1**	**0.546**	-0.117	0.027	**0.181**	0.121	**0.21**	**0.342**	**0.165**	**0.515**	0.031	**0.126**
P	*	*	**	**	NS	**	**	**	NS	**	*	*	NS	NS	**	**1**	0.068	0.031	0.076	0.08	0.039	**0.225**	0.085	**0.144**	**-0.164**	**0.234**
MG	*	NS	NS	NS	*	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS	**1**	0.011	0.031	-0.108	-0.106	**-0.139**	0.008	**-0.144**	0.036	-0.065
NA	NS	*	NS	*	NS	*	**	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	**1**	-0.011	**0.229**	0.051	**0.147**	-0.069	-0.076	-0.029	0.078
K	**	NS	NS	*	NS	NS	**	NS	NS	**	NS	NS	*	NS	**	NS	NS	NS	**1**	**0.234**	-0.022	**0.257**	**0.2**	**0.167**	-0.013	0.026
CU	**	NS	*	**	NS	**	**	NS	NS	*	**	NS	**	**	NS	NS	NS	**	**	**1**	**0.261**	**0.384**	**0.157**	**0.279**	-0.122	**0.136**
FE	NS	**	NS	*	NS	*	**	**	**	*	NS	**	NS	NS	**	NS	NS	NS	NS	**	**1**	**0.239**	-0.084	**0.298**	**0.134**	-0.037
ZN	**	**	**	**	**	**	**	**	NS	**	NS	**	NS	**	**	**	*	*	**	**	**	**1**	0.011	**0.438**	-0.06	**0.19**
MP	NS	*	**	NS	NS	**	**	NS	NS	NS	**	NS	**	NS	*	NS	NS	NS	**	*	NS	NS	**1**	**0.19**	-0.123	**0.152**
BCS	*	**	NS	**	NS	**	**	**	*	NS	**	NS	NS	**	**	*	*	NS	**	**	**	**	**	**1**	0.088	0.105
GB	NS	**	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	*	NS	NS	NS	**1**	**-0.839**
ALGB	NS	NS	**	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	**	NS	NS	NS	*	NS	**	*	NS	**	**1**

The estimates of correlation coefficient ≤ 0.125 are significant at (P≤0.05), ≤ 0.166 are highly significant at (P≤ 0.01) and < 0.125 are non significant. NOTE: **= highly significant (P≤ 0.01). *= significant (P≤0.05), NS = Non significant

Table 21: Correlation among various minerals, biomarkers of oxidative stress, energy and biochemical parameters investigated among SCK crossbred cows (n=58).

TRAITS	GLU	TPP	AL	PUN	CR	NEFA	BHBA	AST	ALT	GGT	CHOL	TRIG	RGSX	MDA	CA	P	MG	NA	K	CU	FE	ZN	MP	BCS	GB	ALGB
GLU	**1**	**0.364**	**0.267**	**-0.257**	0.213	**-0.357**	**-0.355**	**-0.278**	-0.079	-0.22	0.188	0.004	0.168	-0.082	**0.302**	**0.278**	**-0.421**	0.169	0.004	0.159	0.009	0.141	-0.068	**0.371**	0.171	0.084
TPP	**	**1**	**0.344**	**-0.397**	**0.304**	**-0.398**	**-0.53**	**-0.435**	-0.031	**-0.251**	**0.277**	-0.076	0.156	-0.198	**0.42**	**0.368**	**-0.435**	**0.282**	-0.175	0.149	**0.32**	**0.414**	0.029	**0.503**	**0.746**	-0.197
AL	*	**	**1**	-0.232	**0.273**	**-0.31**	**-0.428**	**-0.251**	0.034	-0.159	**0.279**	-0.004	0.229	**-0.27**	**0.285**	**0.261**	**-0.416**	-0.074	-0.243	0.135	0.121	**0.328**	0.043	**0.364**	**-0.368**	**0.809**
PUN	*	**	NS	**1**	-0.23	**0.646**	**0.64**	0.238	0.167	**0.436**	**-0.47**	**0.322**	-0.02	**0.587**	**-0.532**	**-0.363**	**0.348**	-0.118	**0.329**	**-0.331**	-0.111	**-0.439**	0.08	**-0.517**	-0.23	-0.041
CR	NS	*	*	NS	**1**	**-0.279**	**-0.354**	-0.225	-0.246	-0.137	**0.288**	0.031	0.174	-0.126	**0.401**	**0.313**	**-0.387**	**0.25**	**-0.256**	0.06	**0.258**	**0.37**	0.104	**0.36**	0.108	0.135
NEFA	**	**	*	**	*	**1**	**0.749**	**0.375**	0.224	0.246	**-0.429**	**0.284**	**-0.278**	**0.344**	**-0.599**	**-0.415**	**0.551**	-0.143	**0.475**	**-0.389**	-0.102	**-0.509**	**0.289**	**-0.536**	-0.175	-0.132
BHBA	**	**	**	**	**	**	**1**	**0.536**	**0.331**	**0.475**	**-0.617**	**0.252**	**-0.257**	**0.523**	**-0.565**	**-0.514**	**0.622**	-0.174	**0.416**	**-0.545**	-0.192	**-0.656**	0.025	**-0.633**	-0.221	-0.189
AST	*	**	*	NS	NS	**	**	**1**	**0.304**	0.139	**-0.345**	0.135	**-0.377**	0.143	**-0.333**	**-0.477**	**0.465**	-0.136	0.153	**-0.333**	-0.235	**-0.482**	0.159	**-0.414**	**-0.253**	-0.092
ALT	NS	NS	NS	NS	NS	NS	**	*	**1**	0.181	-0.126	-0.05	-0.116	**0.266**	**-0.262**	**-0.262**	**0.262**	-0.089	0.195	-0.181	0.025	**-0.461**	-0.154	**-0.301**	-0.054	-0.066
GGT	NS	**	NS	**	NS	NS	**	NS	NS	**1**	-0.167	0.004	0.137	**0.479**	-0.234	**-0.265**	0.21	0.027	0.21	**-0.339**	-0.03	**-0.302**	0.008	-0.241	-0.136	-0.046
CHOL	NS	**	*	**	*	**	**	**	NS	NS	**1**	-0.15	0.182	**-0.427**	**0.534**	**0.371**	**-0.348**	0.091	**-0.32**	**0.258**	0.236	**0.333**	-0.156	**0.311**	0.076	0.124
TRIG	NS	NS	NS	*	NS	*	*	NS	NS	NS	NS	**1**	0.192	0.177	**-0.306**	**-0.292**	0.053	0.215	-0.095	-0.059	-0.099	**-0.35**	0.232	**-0.264**	-0.072	0.069
RGSX	NS	NS	NS	NS	NS	*	*	**	NS	NS	NS	NS	**1**	0.134	0.047	0.216	**-0.413**	0.055	-0.08	0.125	-0.121	0.104	-0.173	0.082	-0.008	0.185
MDA	NS	NS	*	**	NS	**	**	NS	*	**	**	NS	NS	**1**	**-0.355**	-0.209	0.217	0.065	**0.28**	**-0.38**	-0.069	**-0.35**	0.025	**-0.294**	-0.005	-0.21
CA	**	**	*	**	**	**	**	**	*	NS	**	*	NS	**	**1**	**0.61**	**-0.431**	0.055	**-0.567**	0.216	**0.325**	**0.528**	-0.221	**0.612**	0.214	0.068
P	*	**	*	**	*	**	**	**	*	*	**	*	NS	NS	**	**1**	**-0.527**	-0.071	**-0.333**	**0.334**	0.195	**0.531**	-0.13	**0.414**	0.18	0.12
MG	**	**	**	**	**	**	**	**	*	NS	**	NS	**	NS	**	**	**1**	**-0.317**	**0.372**	**-0.273**	-0.006	**-0.448**	0.045	**-0.44**	-0.136	-0.203
NA	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**1**	-0.139	0.022	0.084	0.059	0.12	0.2	**0.332**	-0.157
K	NS	NS	NS	**	*	**	**	NS	NS	NS	*	NS	NS	*	**	**	**	NS	**1**	-0.193	-0.153	-0.193	0.019	**-0.322**	-0.001	-0.155
CU	NS	NS	NS	**	NS	**	**	**	NS	**	*	NS	NS	**	NS	**	*	NS	NS	**1**	0.027	0.243	-0.135	**0.278**	0.052	0.115
FE	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	**1**	**0.273**	0.181	**0.355**	0.231	-0.013
ZN	NS	**	**	**	**	**	**	**	**	*	**	**	NS	**	**	**	**	NS	NS	NS	*	**1**	0.063	**0.639**	0.178	0.211
MP	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**1**	0.17	-0.002	0.062
BCS	**	**	**	**	**	**	**	**	*	NS	*	*	NS	*	**	**	**	NS	*	*	**	**	NS	**1**	0.241	0.118
GB	NS	**	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	**1**	**-0.769**
ALGB	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	**1**

The estimates of correlation coefficient ≤ 0.250 are significant at (P≤0.05), ≤ 0.325 are highly significant at (P≤ 0.01) and < 0.250 are non significant.
NOTE: **= highly significant (P≤ 0.01). *= significant (P≤0.05), NS = Non significant

4.7.2 Subclinical ketosis:

Total plasma protein, NEFA, BHBA, AST, GGT, cholesterol, reduced glutathione, Ca, P, Mg, BCS and globulin were significantly (P≤0.01) correlated with glucose level. Albumin , BUN, AST, Pi concentration were also significantly (P≤0.05) correlated with glucose. However, no significant correlation were observed between CR, ALT, GGT, cholesterol, triglyceride, reduce glutathione MDA, Na, K, Fe, Cu, Zn milk production, globulin and A:G ratio with glucose concentration.

Total plasma protein concentration was significantly correlated with CR, Na, Fe. Moreover, glucose, Al, PUN, NEFA, BHBA, GGT, AST, Cholesterol, Ca, Pi, Mg, Zn BCS, globulin were also correlated (P≤0.01) with total plasma protein. However, no significant correlation was observed between ALT, A/G ratio, MDA, Ca, K, reduce glutathione, triglyceride and milk production with total plasma protein.

Albumin concentration had strong correlation (P≤0.01) with TPP TPP, BHBA, Ca, P, Mg, Zn, BCS, globulin and A/G ratio. No significant correlation was observed between BUN, GGT, ALT, triglyceride, Na, Fe and milk production. However, Glucose, CR, Na, Fe, also had positive (P≤0.05) correlation with albumin.

BUN had strong significant (P≤0.01) correlation with TPP, creatinine, NEFA, BHBA, GGT, cholesterol, MDA, Ca, P, Mg, K, Cu, Zn and BCS. But, Al, CR, reduced glutathione, Na, Fe, milk production, globulin and A: G ratio had no significant correlation with BUN.

Creatinine concentration was strongly correlated (P≤0.01) with BHBA, Ca, P, Mg, Zn and BCS. But, no significant correlation was observed between creatinine and triglyceride, Cu, milk production and glucose, PUN, AST, ALT, GGT, reduce glutathione, MDA, globulin concentration.

NEFA was significantly (P≤0.01) correlated with glucose, TPP, PUN, BHBA, AST, cholesterol, MDA, Ca, P, Mg, Na, K, Cu, Zn, and BCS. However, Fe had no significant correlation with NEFA.

BHBA had strong significant correlation (P≤0.01) with glucose, TPP, AL, PUN, CR, NEFA, AST, ALT, GGT, cholesterol, MDA, Ca, P, Mg, K, Cu, Zn, and BCS. However, milk production, Fe, Na, Globulin, Al/Gl ratio had no significant correlation with BHBA.

AST activity was significantly (P≤0.01) correlated with TPP, NEFA, BHBA, cholesterol, reduced glutathione, Ca, P, Mg, Cu, Fe, Zn, BCS and glucose, AL, ALT, globulin also had significant (P≤0.05) correlation with AST. However, A: G ration had no significant correlation with AST.

ALT activity was strongly (P≤0.01) correlated with BHBA, Zn . However, cholesterol and milk production also had significant (P≤0.05) correlation with ALT.

GGT activity in subclinical ketotic cows had significant (P≤0.01) correlation with TPP, PUN, BHBA and Zn. MDA, Ca, Pi, Mg concentrations were showing significant (P≤0.05) with GGT activity. But, triglyceride, cholesterol, Na, K, Cu, Fe, A: G ratio and milk production did not show any significant correlation with GGT activity.

Cholesterol level had strong signification correlation (P≤0.01) with TPP, PUN, NEFA, BHBA, AST, reduced glutathione, MDA, Ca, P, Mg and Zn. Low but significant correlation (P≤0.05) of cholesterol was also present with Al, CR, K, Pi, Zn. However, Na, glublulin and A: G ratio had no significant correlation with cholesterol.

Triglyceride concentration had strong significant (P≤0.01) correlation with MDA, Ca, P, Mg, Zn. No significant correlation was recorded between Mg, K, Cu, Fe, globulin and A: G ratio with triglyceride.

Reduced glutathione level was significantly correlated with AST, Mg, (P≤0.01), BHBA, NEFA

(P≤0.05). However, no significant correlation of reduced glutathione with Ca, Na, K, Fe, Zn, BCS and globulin was present in the present study.

MDA level had strong significant (P≤0.01) correlation with PUN, NEFA, BHBA, Cholesterol, GGT, Cu, Zn, Ca. However, no significant correlation was present between MDA level and Na, Fe, globulin and milk production.

Pi concentration was strongly significant (P≤0.01) correlation with TPP, PUN, NEFA, BHBA, AST, Cholesterol, Ca, Mg, K, Cu,Zn, BCS and Significant with (P≤0.05) glucose, Al, CR, ALT, GGT and triglyceride.

Ca concentration in present study had strong significant (P≤0.01) correlation with glucose, TPP, PUN, CR, NEFA, BHBA, AST, Cholesterol, MDA, Pi, Mg, K, Fe, Zn and BCS. But, Na and A: G ratio had no significant correlation with Ca concentration.

Mg level was strongly significantly (P≤0.01)correlated with glucose, TPP, AL, PUN, CR, NEFA, BHBA, AST, Cholesterol, Ca, Pi, K, Zn, BCS. However, Fe and milk production had no significant correlation with Mg concentration.

Na concentration was significantly correlated with TPP, CR, Mg, Globulin (P≤0.05). K level had significant (P≤0.05) correlation with PUN, CR, NEFA, BHBA, Cholesterol, MDA,Ca, Pi, Mg, Globulin concentration had no significant correlation with K.

Cu concentration had no significant correlation with Fe, glucose, TPP, AL, CR, ALT, Triglyceride, reduce glutathione, Ca, Na, K, Fe, Zn Pi, Gb and AL/GB ratio. But correlation of Zn, milk production, MDA, GGT, AST, NEFA, BHBA, PUN and BCS with Cu were statistically significant (P≤0.05).

Fe level had strong significant (P≤0.01) correlation with TPP, CR, Ca, Zn, BCS. Zn concentration had significant correlation with BCS, albumin and A: G ratio. Milk production had significant (P≤0.01) correlation with BCS, while BCS was also correlated significantly (P≤0.01) with globulin.

In present study blood glucose concentration evidenced high correlation with NEFA, BHBA, TPP and body condition score. The high correlation of glucose with NEFA may be due to the fact that NEFA level increases during negative energy balance (Rezaeisaber *et al*. 2011). The NEFA levels in blood increases close to parturition and moves to liver, and can cause ketosis, abomasum displacement, metritis and fatty liver after parturition (Geelen and Wensing, 2006). However, it returns to normal values in healthy lactating cattle under the influence of positive energy balance (Rezaeisaber *et al*., 2011). BHBA level is also a sensitive indicator of subclinical ketosis in dairy cattle (de Roos *et al*., 2007).

In present study, high degree of positive correlation between NEFA and BHBA were observed. Results of the present study was in corroboration with the findings of Rezaeisaber and coworkers (2013), who after a abattoir based study reached to conclusion that elevating NEFA serum values, the LDH and BHBA values also increase in cattle. Likewise a significant negative correlation between BHBA and blood glucose concentration and lactation stage was recorded by Samiei *et al.* (2013). They further observed significant negative correlation between blood BHBA and peak milk yield.

In a study on non-pregnant ewes BHBA and urea was found to have significant effects (P ≤ 0.001) on the glucose concentration. Moreover, a negative correlation between glucose and urea concentrations and positive correlation between BHBA and urea concentrations, while no correlation

was observed between BHBA and glucose concentration were also recorded (Ramin *et al*., 2005). Likewise in present study, we also recorded significant correlation between BHBA and glucose, total plasma protein and NEFA.

Bogin *et al*. (1988) reported a highly significant ($P \leq 0.01$) negative correlation between the degree of fatty change in the liver and the concentration of serum albumin.

In present study AST had high significant correlation with TPP and NEFA. Likewise in a study on sheep high significant correlation between AST activity and energy metabolism indicators were recorded (Taghipour *et al.,* 2010). As observed in our study, authors also recorded significant correlation between NEFA and BHBA and glucose.

Bobe *et al.* (2004) and Xu *et al.* (1998) point out a correlation between elevated AST activity and postpartum NEFA accumulation into liver. The higher ALP activity observed in primiparous cows can be associated with the increased osteoblastic activity that occurs in young growing cattle (Meyer and Harvey, 2004).

In present study we observed high significant correlation of blood Ca, Pi with TPP, NEFA and BHBA. Nozad *et al.* (2012) recorded significant correlation between blood macrominerals, like Ca and P with milk yield and milk in lactating cows.

4.8 Therapeutic trial:

A therapeutic trial was conducted at Bikaner district, to evaluate the efficacy of propylene glycol (PG) and choline in treating cows affected with subclinical ketosis. Nine cows were selected on the basis of BHBA and NEFA value for subclinical ketosis. Cows were fed with PG @ 200 ml per day along with the concentrate ration and molasses fed separately from the forages and choline biocarbonate 100 mg I/M for 5 days. The results of the therapeutic trial were evaluated.

In present study, highly significant ($P \leq 0.01$) difference were observed in glucose, BHBA, NEFA, GGT, reduced glutathione, MDA and milk production. Significant differences were observed in cholesterol and Ca.

A highly significant ($P \leq 0.01$) increased mean value of glucose and milk production from pre to post treatment (40.961±2.143, 56.222±2.147 mg/dl and 15.000±0.471, 15.611±0.469 litre/day), respectively after feeding of propylene glycol. Similarly, highly significant decrease was noticed in the mean plasma BHBA (1.615±0.035 to 0.602±0.060 mmol/L) and NEFA (0.768±0.015 to 0.238±0.026 mmol/L) level after the PG feeding, whereas no significant change was recorded for other parameters viz. total protein, albumin, PUN, creatinine, AST, ALT and triglyceride) after the PG feeding (Table 22).

A highly significant ($P \leq 0.01$) increase mean value of reduced glutathione and decrease malondialdehyde from pre to post treatment (1.852±0.194, 1.931±0.211 mg/dl per gm Hb and 51.435±2.099, 49.551±1.774 nmol/ml per gm Hb), respectively. Similarly, highly significant ($P \leq 0.01$) decreased GGT value 32.127±3.078 to 27.426±2.140 IU/L and significant increase cholesterol level 68.666±4.091 to 74.973±2.139 mg/dl in pre to post treatment. Calcium level was increase significantly from pre to post treatment (9.396±0.266 and 10.181±0.155 mg/dl) of respectively, whereas all the other minerals (Pi, Mg, Na, K, Fe, Zn, Cu) did not show any change in their pre and post feeding values (Table 23).

Table 22: Mean (±SE) value of biochemical parameters studies in therapeutic trial of propylene glycol and choline in subclinical ketosis in dairy cows (n=9).

Sr.	Parameters	Pre treatment	Post treatment
1	Glucose (mg/dl)	40.961±2.143[a]	56.222±2.147[b]
2	Total protein (g/dl)	6.621±0.105	6.857±0.163
3	Albumin (g/dl)	2.452±0.167	2.701±0.135
4	Plasma urea nitrogen (mg/dl)	11.755±0.281	11.372±0.247
5	Creatinine (mg/dl)	1.121±0.060	1.136±0.025
6	NEFA (mmol/l)	0.768±0.015[a]	0.238±0.026[b]
7	BHBA (mmol/l)	1.615±0.035[a]	0.602±0.060[b]
8	AST (U/l)	67.867±2.966	64.753±1.630
9	ALT (U/l)	28.122±1.937	28.455±1.297
10	GGT(U/l)	32.127±3.078[a]	27.426±2.140[b]
11	Cholesterol (mg/dl)	68.666±4.091[a]	74.973±2.139[a]
12	Triglyceride (mg/dl)	16.473±1.799	15.782±1.408
13	Reduced glutathione (mg/dl per gm Hb)	1.852±0.194[a]	1.931±0.211[b]
14	Malondialdehyde (nmol/ml per gm Hb)	51.435±2.099[a]	49.551±1.774[b]

Note: 1. Value bearing different superscript (a, b) in a row depict highly significant (P≤0.01).

2. Value bearing same (a, a) in a row depict significant (P≤0.05).

3. No superscript mean non significant within row.

Table 23: Mean (±SE) value of macro and micro minerals studies in therapeutic trial of propylene glycol and choline in subclinical ketosis in dairy cows (n=9).

Sr.	Parameters	Pre treatment	Post treatment
1.	Ca (mg/dl)	9.396±0.266[a]	10.181±0.155[a]
2.	Pi (mg/dl)	4.618±0.138	4.353±0.272
3.	Mg (mg/dl)	3.744±0.062	3.531±0.118
4.	Na (mmol/l)	114.444±2.533	115.000±1.518
5.	K (mmol/l)	5.071±0.420	4.523±0.314
6.	Cu (ppm)	0.618±0.059	0.640±0.047
7.	Fe (ppm)	2.300±0.106	2.250±0.077
8.	Zn (ppm)	0.980±0.040	1.029±0.048
9.	Milk production (kgs/day)	15.000±0.471[a]	15.611±0.469[b]

Note: 1. Value bearing different superscript (a, b) in a row depict highly significant (P≤0.01).

2. Value bearing same (a, a) in a row depict significant (P≤0.05).

3. No superscript mean non significant within row.

Similar to the present finding, Cows fed a diet supplemented with oral PG had significantly elevated plasma glucose concentration. Grummer *et al.* (1994), Myoshi *et al.* (2001) and Juchem *et al.* (2004) found that cows fed with PG in the transition period exhibited significantly increased plasma glucose concentration. Stokes and Goff (2006) reported that milk yield of cows on dairy farm was increased by administration of PG on the first 2 day post-partum.

In present study, non significant effects of various hepatic enzymes were observed except GGT. GGT enzyme is specific to the ruminant. The GGT level decrease due to sufficient glucose precursor is available and burden of metabolism (gluconeogenesis) in liver is less in comparison to before treatment in subclinical ketotic cow. This may be due to the fact that these cows developed varying degree of fatty infiltration after parturition. Thus after parturition the mobilization of fat is even more intense due to lactation demands as was evident from biochemical parameters.

The palatability of PG was poor. Thus we employed PG mixed with molasses and concentrate. Propylene glycol is rapidly absorbed and metabolized by cows. In the rumen, PG is converted to propionic acid (Grummer *et al.*, 1994), then transported to the liver and metabolized into glucose through glyconeogenesis. PG rapidly supplies energy, this improved the degree of negative energy balance, resulting in decreased body fat catabolism and plasma NEFA levels (Miyoshi *et al.*, 2001). When degraded body fat releases large amounts of NEFA, as the quantity of NEFA exceeds the liver

burden or sufficient glucose is unavailable, NEFA is converted to ketone bodies. In ketosis oxaloacetate level is decrease in metabolism and not sufficient acetyl-CoA (co-enzyme A) produce in TCA cycle or ultimately ketogenesis (Krebs *et al.,* 1966 and Baird *et al.,* 1968 and 1972). When we supplemented propylene glycol, it helps to increasing oxidation of acetyl-CoA in TCA cycle by providing pyruvate and propionate. Propionate is major end product of PG. Most of the glucose synthesis from propionate. Thus, it helps to increasing the supply of glucose via gluconeogenesis. The increased production of glucose might have stimulated insulin secretion from the pancreas, which decreased mobilization of fatty acids from adipose tissues and hence, substrate for hepatic ketogenesis (Brockman and Laarveld, 1986). Similar to the present study, Chung (2007) also observed a decrease in the plasma BHBA concentration and subsequent decreased incidence of subclinical ketosis (SCK) from 39.00 to 13.00 per cent, and also the percentage of cows that experienced multiple episodes of subclinical ketosis was also reduced from 53.00 to 14.00 per cent during the first 3 weeks of lactation after the feeding of propylene glycol (PG).

Similar to our study, Dufva *et al*. (1983) and Ruegsegger and Schultz (1986) recommended feeding of choline and niacin to prevent the ketosis for high producing dairy cows. Niacin help in control fat metabolism, increase milk production and stimulate microbial protein synthesis. Thus the supplementation of niacin reduced the incidence of ketosis in lactating dairy cows because niacin is a component of two important co-enzymes namely nicotinamide adenine dinucleotide and nicotinamide adenine phosphate. These co-enzymes combine with hydrogen during degradation of food substrates and are essential for bacterial metabolism.

The present study parental choline was used in dairy cows. The net effect of choline supplementation was satisfactory in term of preventing ketosis. This might be due to the fact that major portion of choline is absorb by the parental route. Choline is used for phosphatidylcholine synthesis, a major phospholipid required for cell maintenance and replication. Phosphatidylcholine can also be synthesized from trans-methylation of Phosphatidyletanolamine by methyl donors such as methionine. In monogastric animals, choline deficiency has been shown to result in hepatic lipidosis. Choline can work as a methyl donor and spare methionine when amino acids supply is inadequate. Because choline is an integral component of the phospholipid phosphatidylcholine, it becomes a critical component for hepatic lipid metabolism. Phosphatidylcholine is needed for synthesis of very low density lipoprotein (VLDL), the lipoprotein responsible for export of triacylglycerol from hepatocyte.

Suitable measure for prevention of production diseases:

Prevention of ketosis is directed towards maximizing energy intake and providing adequate glucose precursors. It is suggested to feed well balanced ration and avoiding excessive fattening before calving. Avoid abrupt changes in the feeding program at calving time should be avoided, therefore, increase in concentrate intake should be done moderately in the late dry period but as rapidly after calving as possible and maintain intake. Provide adequate amounts (one third of the dry matter) of good quality roughage. Improving dry matter intake during last 3 weeks before and 3 weeks after calving should be the top priority. In addition, specific feed additives like choline may be added in feed to prevent ketosis/ fatty liver. Trial of propylene glycol administration of present study has proved to be effective in prevention of fatty liver and subclinical ketosis in dairy cows. Care must be taken to provide the pre-partum dairy animals with a stress free environment.

All the herd studied in present investigation for calcium level in pre-partum period showed marginal deficiency, which may be a cause for milk fever and can be prevented by restricted Ca feeding to less than 0.5% of the diet during dry period. Phosphorus intake must Negative energy balance in

bovines be less be less than 0.35 per cent of the diet during late pregnancy. Calcium deficient diet fed for at least 7-10 days before calving greatly reduces the risk of milk fever.

2. REVIEW OF LITERATURE

2.1 Prevalence:

Jonsson and Simesen (1973) noted that parturient paresis had never been reported in first calf heifers. Its incidence increases with parity up to at least 5^{th}-6^{th} calving. No specific seasonal occurrence or relationship to weather conditions was shown. Jerseys were more susceptible than other breeds. The morbidity rate was about 50 per cent higher in cows which had the disease previously.

Marquardt *et al*. (1977) reported that the incidence of parturient paresis in the young and aged cows were 0 (0/10) and 30 per cent (3/10), respectively.

Vlakhos and Tsaklof (1977) observed that the numbers of cases of paresis at the time of parturition were five times higher and further they reported that in the non- parturient paresis; there were smaller decrease in serum Ca and P, and smaller increases in serum Mg, than in parturient paresis.

Leengoed (1979) noted parturient paresis in 7 cows in a herd of 95 cows that was attributable to hypocalcaemia and hypomagnesaemia.

Kauppinen (1983) recorded a prevalence of 13 per cent for clinical ketosis and a prevalence of 34 per cent for subclinical ketosis in Finnish Ayrshire and Holstein cattle using blood acetoacetate levels.

Dohoo and Martin (1984) showed a prevalence of 12.1 per cent of ketolactia in Holstein cows in the first 65 days of lactation. The peak prevalence of hyperketonemia occurred in the third and fourth week of lactation and its herd prevalence in cows from 0 to 65 days in milk varied from 0 percent to 33.9 percent.

Markusfeld (1985) reported an 18 per cent rate of ketonuria in Israeli Holstein cows examined 7±24 days after calving.

Andersson and Emanuelson (1985), in a study on 3078 Swedish dairy cows with ketonemia from 126 herds, reported prevalence of 8.9, 4.7, and 1.1 per cent respectively, at the first three monthly production tests using a milk-acetone test. A significant influence on milk acetone was found for herd mean production, breed, herd, lactation number, cow, week of lactation, season and the interaction between lactation number and week of lactation. The highest individual milk yield and highest individual acetone values were significantly positively correlated. Significant correlation was also found between the prevalence of hyperketonaemia and herd means of the intervals from calving to first and last service.

Elevated NEFA concentration during the last 7 days before calving were associated with greater incidence of ketosis, displaced abomasums and retained fetal membrane but not with milk fever (Dyk *et al.*,1995).

Duffield *et al.* (1998), in his study on 507 untreated cows from 25 Holstein dairy farms over the first 9 weeks of lactation, reported the cumulative incidence of subclinical ketosis as 59 per cent and 43 per cent using cut off threshold beta hydroxy butyrate (BHBA) concentrations of 1200 and 1400 µmol/L and also the peak prevalence of hyperketonemia (BHBA concentration of > 1.2 mmol/L) occured at 1 week post-partum, respectively.

Geishauser *et al.* (1998) reported a prevalence of 7-41 per cent for subclinical ketosis and it

increased from primiparous to multiparous cows, and was highest during 2nd and 3rd week post parturition.

Jorritsma *et al.* (1998) studied 190 lactating cows and found that at a beta-hydroxy butyric acid concentration of 1.2 mmol/L in blood, the prevalence of ketosis was 14 per cent.

Al-Rawashdeh (1999) studied bovine ketonemia among 1155 dairy cows in various stages of lactation and parity on 25 Jordanian dairy herds, and showed that serum concentration of β-hydroxy butyrate (BHBA) <0.9 mmol/L between 0.9 and 1.7 mmol/lL and >1.7 mmol/L were considered to indicate normal, mild and severe ketonemia, respectively. The point prevalence of mild and severe ketonemia were 22 and 3.8 per cent, respectively and the prevalence of ketonemia decreased with increasing herd size. Further, a non significant association was found between the prevalence of ketonemia and parity, stage of lactation, metritis, somatic-cell count (SCC) and serum cholesterol levels.

Duffield (2000) reported that the early lactational incidence of subclinical ketosis was found to affect 40 to 60 per cent of cows in herds undergoing repeated testing and was much higher than 2-15 per cent incidence found with clinical ketosis.

Enjalbert *et al.* (2001) reported a prevalence of 19.2 per cent for subclinical ketosis from the 125 samples using a cut off of >1.2 mmol/L.

Bihani *et al.* (2002) studied 504 post-parturient cows and reported an overall prevalence of ketosis to be 9.90 percent in and around Bikaner. It was more during the second to fifth parity, during first and second month post partum, in cows in the age group 7-8 and 8-9 years and during month of November to January.

Akamatsu *et al.* (2007) recorded the highest prevalence of SCK of dairy cows during the first two months after calving.

Cheng *et al.* (2007) evaluated the effect of hypoglycemia on periparturient metabolism and lactation performance in 24 multiparous Holstein cows, revealed that the incidence of hypoglycemia was higher in pre partum than that of post partum (88% vs. 50%) and there was no effect on feed intake before and after calving. With the increase in postpartum feed intake, incidence of hypoglycemia decreased rapidly, even disappeared at 28 days of postpartum. Hypoglycemia had no impact on pre partum body weight (BW), but BW in hypoglycemic group was lower than that of normoglycemic cows at 1 day and 14 days of postpartum. Milk yield was not affected by hypoglycemia. However, cows in hypoglycemic group had higher plasma NEFA concentrations than cows in normoglycemic group. It reached a peak (1.4 mmol/L vs.1.052 mmol/L) at day 1 of postpartum; similarly, plasma BHBA concentration of cows in hypoglycemic group was higher than that in normoglycemic group, which reached a peak (2.01 mmol/L vs.1.34 mmol/L) at 14 days of postpartum.

Yameogo *et al.* (2008), in a study to establish the relationship between ketosis, milk production and biochemical blood metabolites; observed incidence of 33.57 per cent for subclinical ketosis and 6.43 per cent for clinical ketosis. Cows with subclinical ketosis had a decrease of 12.4 and 15.6 per cent in milk yield respectively for Montbeliard and Holstein, whereas, cows with clinical ketosis had a decrease of 18.6 and 26 per cent in their second month of milking. There was significantly lower average levels of blood glucose and significantly higher average levels of blood urea than cows with normal blood beta-hydroxy butyrate levels in ketogenic cows (subclinical and clinical). Also significant difference was recorded with concentration of total proteins and globulins, calcium and magnesium from one farm to another.

LeBlanc (2010) showed that approximately 75 per cent of diseases in dairy cattle occurred in the first month postpartum and 50 per cent of dairy cattle suffered from metabolic and infectious diseases in the transition period.

Ospina *et al.* (2010) reported a prevalence of 40 per cent in herds in which more than 15 per cent of sampled cows had increased post-partum BHBA concentrations of > 1.2 mmol/L, and the herds had an increased time to pregnancy and produced less milk compared to herds with fewer animals above the threshold.

Asl *et al.*(2011), in a study on the 100 clinically healthy multiparous Holstein cows from 16 dairy herds around Kazerun, Fars Province, Iran found that during the 2, 4 and 6 weeks post parturition 63, 68 and 59 per cent of the tested cows were sub-clinically ketotic. Overall, 97 per cent of tested cows (97/100) were sub-clinically ketotic in at least one sample period. Thirty percent of tested cows (30/100) were found suffering from subclinical ketosis in all of the 2, 4 and 6 weeks postpartum period.

Borchardt *et al.* (2012) found an overall prevalence of 30.7, 19.3, and 13.6 per cent, as determined by use of BHBA threshold concentrations of 1,000, 1,200, and 1,400 μmol/L, respectively in pooled serum samples for herd-based detection of subclinical ketosis (SCK) in dairy cows after calving.

Chapinal *et al.* (2012) in a study on 55 dairy herds in US and Canada, recorded a prevalence of 15 per cent in herds in which >25 per cent of sampled cows had increased post-partum BHBA concentrations of >1.4 mmol/L within a week post-partum.

McArt *et al.* (2012) in a study on 1717 cows on four large total mixed ration (TMR) fed free stall dairies in New York and Wisconsin, USA, found that the peak incidence and prevalence of SCK (BHBA concentration of 1.2–2.9 mmol/L) were found to occur at 5 DIM after intensive, three times per week monitoring .

Mir and Malik (2002) recorded 4.22 percent prevalence of ketosis in bovine. Highest prevalence was recorded in the age group of 8-9 years (47.36 percent), in the third lactation (45.10 percent) and during 1-2 months (42.10 percent) post-partum.

Singh (2002) described ketosis as a common metabolic disease of high producing animals occurring during the first 10-60 days after parturition.

Pourjafar and Heidari (2003) recorded the prevalence of sub-clinical ketosis to be 38 percent in dairy Holstein cows.

Sakha *et al. (*2007) determined the frequency of sub clinical ketosis in a dairy farm of Kerman (Iran) by measuring BHBA in blood during 3-6 weeks postpartum in 90 cows. Thirteen (14.4 percent) and five cows (5.55 percent) showed ketonemia with BHB level at >1.2 and >1.7 mmol/l, respectively.

Chakrabarti (2006) reported higher prevalence of ketosis in high milk yielding cows generally within a month after calving. He also observed the occurrence of ketosis at any time during the lactation period, sometimes during late pregnancy and more common in cows in their third lactation or following that.

Sharma (2006) reported the prevalence of ketosis in cows to be 10.20 percent among the suspected clinical cases.

Arya (2008) studied clinical cases of ketosis and reported that the prevalence was 9.85 percent in and around Bikaner.

Thirunavukkarasu *et al.* (2010a) revealed that a total 3774 cows in five milk shed districts of the State of Tamil Nadu, 516 (13.67 percent) were affected by milk fever; while 42 out of 342 buffaloes

(11.99 percent) suffered with milk fever. The average loss per animal due to the treatment of milk fever was higher in cow (Rs. 618) than a buffalo (Rs. 488) and the overall average loss was Rs. 608.

Thirunavukkarasu *et al*. (2010b) recorded overall prevalence of ketosis was 9.38 percent in cows and 2.92 percent in buffaloes, in Tamil Nadu in the year 2008. They, further observed low prevalence of ketosis in cows of Erode and Coimbatore districts of Tamil Nadu that could be attributed to the relatively better feeding management in these districts. The loss due to ketosis was estimated Rs. 577.09 per affected cow, which included the cost of medicines (Rs. 262.99, 45.57 percent), Veterinarian's fee including additional labour cost (Rs. 224.98, 38.99 percent) and expenses on feed supplements (Rs. 89.12, 15.44 percent). However, the loss per affected buffalo was slightly lesser at Rs. 510.80 of which Rs. 240.80 (47.14 percent), Rs. 187.50 (36.71 percent) and Rs. 82.50 (16.15 per cent) were contributed by medicine cost, veterinarian's fee (including additional labour cost) and cost of feed supplements, respectively.

Kumar (2011) studies clinical cases of ketosis, reported that the prevalence was 11.42 percent in and around Bikaner.

Thirunavukkarasu *et al.* (2011) reported that the prevalence of ketosis was significantly higher in cattle (9.38 percent) as compared to buffalo (2.92 percent). They further reported that the prevalence rate of ketosis is higher in rainy and summer season than winter. They recorded 37.01, 37.25 and 28.24 percent prevalence rate of ketosis in summer, rainy and winter season, respectively.

Samiei *et al.* (2013) reported prevalence of SCK and clinical ketosis were 13.9 and 3.4%, and significant between regions, but not parity; the mean daily peak milk yield for clinical ketosis, subclinical ketosis and healthy cows in study were 28, 35 and 45 kg, respectively, suggesting a decline ranging from 22 to 38 %, compared to the healthy cows.

2.2 Minerals:

The mineral requirements of animals vary depending upon many factors (Soetan *et al.,* 2010). However, concentration of these essential elements must be maintained within quite narrow limits, if the functional and structural integrity of the tissues are to be safeguarded and the growth, health and productivity of the animal are to remain unimpaired. Continued ingestion of diets that are deficient, imbalanced or excessively high in a mineral induces changes in the form or concentration of that mineral in the body tissues and fluids, so that it falls below or rises above the tolerable limits (Prasad and Gowda, 2005).

Despite the fact that the trace minerals are present in body tissues in very low concentrations (Arinola, 2008), they are very critical for normal body functions. They serve as components of metallo-enzymes and enzyme cofactors, or as components of hormones secreted by the endocrine system (Speer, 1996). Complex inter-relationships exist between certain micro-minerals, immune functions and disease resistance in cattle. Several micro-minerals have been shown to influence the immune responses. The relationship between deficiencies of some micronutrients and disease resistance is less clear. Immune cells, like all other types of cells, require an adequate supply of trace elements for the normal structure and function of metallo-proteins that participate in housekeeping processes such as energy production and protection against reactive oxygen species (Chew, 1995).

Intensification of production and increase of milk yield of cows requires full coverage and appropriate balancing of the mineral elements. Animal's feeds and fodder often do not include all the

requisite minerals and therefore, need to be supplemented. Increasingly, dairy farmers across the country recognize the benefits of supplements and are feeding recommended mineral mixtures with a marked improvement in milk production and breeding efficiency. Even within a state, feeds and fodder in different agro-climatic zones show variations in mineral content. National Dairy Development Board (NDDB) has undertaken a mineral profile mapping to develop area- specific mineral mixtures. Area specific mineral mixtures are more effective and economical in combating deficiencies. NDDB has found that while magnesium, potassium, iron, manganese, cobalt and selenium are more than sufficient in most areas of India, calcium, phosphorus, sulphur, sodium, copper and zinc levels vary greatly within states (Hosnedlava *et al.,* 2007).

The mineral composition of grasses and browse on grassland in India is highly variable due to seasonal variation in the maturity and chemical composition of forage, erosion of the soil etc. The common sources of minerals for the farm animals are minerals from the feedstuff, drinking water, in soil and mineral supplements. The leguminous fodders like berseem, lucern and cowpea contain adequate amounts of Cu, Fe, Mn, and Zn. However, a wide variation in the content of these elements exists not only from area to area but also even in the fodders of the same area. Many feeds and fodders of the western tract of Thar Desert of Rajasthan also have deficient mineral levels, leading to primary nutritional mineral deficiency in cattle (Singh, 1989).

Macrominerals calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), chloride (Cl) and sulfur (S) are of largest interest as to their status relative to their role in milk fever, alert downer cows and weak cow syndrome. Blood concentrations of these minerals are strictly regulated in the body through a variety of homeostatic processes; hence it does not reflect their dietary status when the homeostatic system is functioning properly. However, for phosphorus, K, Mg and S, blood concentrations are somewhat sensitive to dietary intake (Herdt *et al*., 2000).

Sodium and chloride concentrations are altered when renal or digestive function is compromised or in extreme dietary deficiency states. Assessment of Ca concentrations around calving time is a useful indicator of how well the Ca regulatory system is working and potential for clinical or subclinical hypocalcaemia problems. It is perceived that other than the 2 weeks prior to and following calving, blood Ca is not a very diagnostic value as a result of the intact regulatory system. Therefore, macro mineral blood concentrations need to carefully interpret in light of whether or not the homeostatic system is in proper operation (Oetzel, 2004).

Rowlands *et al.* (1975), in a study on 172 dairy cows in a herd, for various parameters of CMPT revealed that the most significant changes were confined to the periods up to 3 months either side of calving and the greatest changes occurred for Mg which increased during late pregnancy and for albumin which decreased at or near calving. Mean albumin, urea and glucose concentrations were lowest during the first month of lactation, whereas the globulin concentrations showed the reverse trend. Haemoglobin concentration decreased during late pregnancy and early lactation and was lowest in the period 30–120 days post- partum. Calcium concentration decreased during late pregnancy and increased during early lactation; whereas, sodium concentration increased during late pregnancy. However, with increasing milk yield, haemoglobin and K concentrations decreased and Mg concentrations increased.

2.2.1. **Macro Minerals**:

2.2.1.1 Calcium (Ca) :

Calcium is the most abundant mineral in the animal body. About 99% calcium in the body is present in the bones, which not only provide a strong frame for supporting and protecting delicate organs but also serves as a large reservoir of calcium for the body. Most of the remaining Ca (0.9%) is

sequestered in the plasma membrane and endoplasmic reticulum of cells. Extracellular fluid contains 0.1% of the body's Ca mass, with total Ca concentration of about 2.5 mmol/litre (Brown, 1994). Approximately 50% of the extracellular Ca is in the ionized form, which is the biologically active form of the Ca. Calcium functions in cell equilibrium, heartbeat and muscle contraction, and blood coagulation (Hurwitz, 1996). Leguminous species are good source of calcium than grasses. The calcium content of temperate and tropical legumes has been indicated as 14.2 and 10.1 g per kg of dry matter, whereas in corresponding grass it has been indicated as 3.7 g per kg of dry matter (Minson, 1990).

Calcium is absorbed from food in the gastro-intestinal tract, with the greatest absorption occurring in the duodenum (McDowell, 1992). In normal animals an equivalent amount of Ca is excreted primarily in urine, with small losses in sweat and intestinal secretion. The Ca released from bone by osteoclastic bone resorption and deposited in bone. Therefore, intestinal Ca absorption is the major determinant of the amount of ca excreted in the urine in adult animals. Two hormones, namely parathyroid hormone (PTH) and 1, 25- dihydroxycholecalciferol (Vit. D3), control the absorption. Parathyroid hormone is the principal hormone involved in the fine regulation of blood Ca. When ionic Ca concentration in ECF fluid decreases, the parathyroid gland will release PTH (Brown, 1994) and this activates vit. D3, which is hydroxylated in the liver to 25-OH-D3 and further to two compounds in the kidney, namely 24,25-(OH) 2 or 1,25-(OH)2D3 which opens Ca channel and facilitates Ca uptake and transfer with the help of Ca binding proteins calbindin (Hurwitz, 1996).

Calcium is the most important macro-mineral in terms of relative requirement and the diversity of functions in the body. Around 98 per cent of body Ca is located in the skeleton, which provides structural strength and hardness to bones. Remaining Ca is mainly found in extracellular compartment. Important functions of Ca in animals include formation of skeletal tissue, transmission of nerve impulses, excitation of skeletal and cardiac muscle contraction, blood clotting, and as a component of milk. Deficiency of Ca in young animals is characterized by failure to mineralize new bones with development of rickets and retardation of growth. Deficiency in older animals and high yielding cows forces to withdraw Ca from bones which in persisting deficiency lead to osteoporosis and osteomalacia making bones more prone to spontaneous fractures. Deficiency of Ca is a sporadic problem occurring in particular group of animals rather than specific geographic area (Radostits *et al.*, 2000). It is of two categories viz., metabolic deficiency due to sudden Ca losses in high milking dairy cows, also called as milk fever and other is nutritional deficiency associated with long term dietary deficiency of Ca. Calcium within mineral supplement is generally more bio available for absorption than Ca in forages and common feedstuffs (Hansard *et al.*, 1957). The absorption efficiency for Ca in animals decreases with the age (Horst *et al.*, 1978). As per NRC (2001), the availability of dietary Ca for absorption in cattle is around 50-60%. The normal blood plasma concentration of Ca in bovines is 9-10 mg/dl. Requirement of Ca is greatly increases during the last trimester of pregnancy for calcification of fetal skeleton, and during lactation. Deficiency of Ca may occur in animals on Ca deficient diet or in high producing dairy animals due to heavy drainage of Ca in milk. Verification of true nutritional Ca deficiency requires analysis of Ca in bones from suspected group of animals as plasma Ca concentrations are maintained by homeostatic mechanisms through bone resorption in animals on Ca deficient diets.

Mullick and Pal (1943) studied the blood mineral concentrations in 32 Haryana cows. The mean values for all the groups (lactating, lactating and pregnant and dry pregnant) included in the study were 4.32 mg %, 11.4 mg %, 2.54 mg % in respect of inorganic phosphorus (P), serum calcium (Ca) and serum magnesium (Mg), respectively.

Moodie (1960) proposed that a loss of calcium through the milk secretion is a necessary prerequisite for the lowering of blood Ca levels. The essential cause of the hypocalcaemia must, however, be attributed to a deficiency of available calcium in the bones, to impairment of absorption of Ca from the digestive tract or to a combination of these factors.

Cakala and Albrycht (1976) observed significant decrease in serum calcium and magnesium levels and increase in serum phosphorus level during starvation in cattle.

Pandiya *et al*. (1977) recorded the values of Ca and inorganic phosphorus (mg/100 ml) in 83 healthy crossbred cows of dairy herd of College of Veterinary and Animal Science, Bikaner to be 10.32 ± 0.106 and 5.70 ± 0.867, respectively.

Bostedt *et al*. (1979) estimated serum Ca, Pi and Mg of 314 parturient paretic cows. In 165 cases, serum Ca and Pi were lower, while Mg was higher than controls. Ca, Pi and Mg were within the normal limits in 21 cows; whereas in 128 cows Ca, Pi and /or Mg were low. Activities of aspartate amino-transferase, creatine kinase and isocitrate dehydrogenase were high in all groups of paretic cows.

Barton *et al*. (1981) found that in paretic aged cows, severe hypocalcaemia (5 mg/100 ml) and hypophosphataemia (3 mg/100 ml) developed on the day of calving; whereas hypocalcaemia and hypophosphataemia were transient in nonparetic aged and young cows at this stage.

Kulkarni *et al*. (1983) reported normal values of some biochemical constituents of blood in 6 crossbred lactating cows during the first three months of lactation. The mean values (M± S.E.) and ranges of concentration were: Ca (mg %) 9.19 ± 0.40 (7.65-10.65) and P (mg %) 5.36 ± 0.15 (5.03-5.86).

Kelly (1984) reported normal biochemical values in apparently healthy cattle to be calcium 2.3-3.0 mmol/l, inorganic phosphorus 1.0-2.5 mmol/l, and magnesium 0.8-1.2 mmol/l.

Waage (1984) recorded hypocalcaemia in cows, most of which had just calved, although a few had calved several weeks before.

Hejlasz (1985) observed reduction in Na, Ca and Pi, increase in Mg along with reduction in total protein (77.9 to 65 g/l) and albumin (36.9 to 26.95 g/l) in cows with clinical parturient paresis.

Singh (1985) reported normal serum calcium values in apparently healthy Rathi cattle to be 10.8 ± 0.23 mg/dl, which ranged between 10.8-12.4 mg/dl.

Prasad *et al*. (1987) studied haemato-biochemical profile of 21 normal crossbred cows in 3 physiological states (dry, above 8 months pregnant and recently calved) for prediction of downer cow syndrome. In recently calved cows, total protein, Ca, Pi and Mg were low. The values obtained in dry, advanced pregnant and recently calved cows (mean ± S.E.) were Ca (mg/dl) 12.28 ± 0.82, 10.57 ± 0.43, 13.65 ± 8.79, Pi (mg/dl) 7.41 ± 0.50, 6.50 ± 0.41, 5.47 ± 0.27 and Mg (mg/dl) 5.01 ± 0.39, 4.8 ± 0.29, 3.44 ± 0.18, respectively.

Pandey and Dwivedi (1988) observed a significant decrease in serum Ca from 10.05 ± 0.11 to 3.10 ± 0.31 mg/dl and Pi from 6.11 ± 0.56 to 4.32 ± 0.31 mg/dl during experimental hypocalcaemia in calves. No significant change was observed in the values of total serum protein and blood glucose. The study also indicated that clinical hypocalcaemic tetany occurs if serum Ca declines below the level of 3.5 mg/dl.

Pandey and Parai (1988) observed that occurrence of production disease was comparatively more in winter during December to March, when the climate was cold and dry. High milk yielder in their third or higher lactations suffered from the disease. They further conducted clinico-biochemical studies in 30 crossbred cows suffering from atypical form of production disease having a combined effect of parturient paresis and ketosis. The mean ± S.E. values of clinically ill cows for serum Ca (mg %), Pi (mg

%) and Mg (mg %) were 5.19 ± 0.06, 3.10 ± 0.07 and 2.98 ± 0.08, respectively. The mean ± S.E. values of normal control cows were 8.87 ± 0.08, 4.45 ± 0.11 and 3.09 ± 0.06, respectively.

Reinhardt *et al.* (1988) described that aging and nutrition can reduce the ability of intestine, bones and kidneys to respond rapidly to the hormone signals responsible for calcium (Ca), phosphorus (P) and magnesium (Mg) homeostasis in ruminants during rapid increase in demand for these minerals.

Caple (1989) reported that complex disorders arise when the calcium and magnesium requirements are not met during pregnancy and lactation. Hypocalcaemia and hypomagnesaemia occur when the losses of Ca and Mg in milk cannot be supplied from the diet or mobilized from body reserves. Older cows and ewes, which cannot mobilize enough minerals from bone or body fluids, are particularly susceptible when there is reduced absorption of Ca and Mg from the gut. This may arise simply from inadequate intakes, or as a result of complex interactions that decrease Ca and Mg absorption from the rumen.

Mishra (1991) recorded mean serum calcium 11.6 mg/dl in apparently healthy normal indigenous calves in and around Bikaner.

House and Bell (1993) showed that the amount of Ca, P and Mg needed for synthesis of 15 kg of Colostrum were 3.3, 2.5 and 7.5 folds higher as compared to the amount of nutrients transferred to the late term fetus.

Horst *et al.* (1994) noticed that most dairy cows experience some degree of hypocalcaemia during the peri-parturient period. There is, however a subgroup of dairy cows that experience a breakdown in its ability to maintain plasma calcium and consequently suffers from severe hypocalcaemia.

Parturient paresis is a metabolic disease occurring most commonly at about the time of parturition in adult females and is characterized by hypocalcaemia, general muscular weakness, circulatory collapse and depression of consciousness (Radostits *et al*., 1994).

Rajora and Pachauri (1994) concluded that serum albumin, Mg, Ca and P ratio did not differ significantly in pre-parturient, post-parturient cows and in milk fever. In milk fever, serum total proteins, globulins, iron and zinc increased and blood glucose, serum copper, A : G and Ca : Mg ratios decreased, when compared with pre-parturient cows.

Juneja (1996) reported biochemical values in healthy crossbred cattle in and around Bikaner. The values were: serum Ca 10.14±0.36 mg/dl (range 8.29-12.19 mg/dl), Pi 5.64±0.23 mg/dl (range 4.10-6.60 mg/dl) and magnesium 2.76±0.33mg/dl (range 1.6-4.8 mg/dl).

Joyce *et al.* (1997) revealed that one of the reasons for lower level of calcium in older cows is decreased number of receptors for 1, 25-dihydroxy Vitamin-D in the intestine resulting in decreased absorption of Ca.

Kamgarpour *et al.* (1999) observed episodes of subclinical hypocalcaemia in the first 6 weeks after calving in 6 out of 12 multiparous Friesian cows calved in the winter season and 7 out of 23 calving in the summer season. There was a significant decrease in mean plasma calcium concentration (<2.0mmol/L) on days 6, 27 and 36 after calving and these cows had a significantly higher mean body weight and higher mean milk production than normocalcaemic cows.

Gupta (1999) recorded serum calcium (mg/dl), inorganic phosphorus (mg/dl) and magnesium (mg/dl) of 10 normal healthy crossbred lactating cattle and indigenous Rathi lactating cattle in and around Bikaner area to be 10.25 ± 0.32 (8.23-11.70) and 9.77 ± 0.37 (7.82 - 12.12); 5.29 ± 0.22 (4.23-6.23) and 5.36 ± 0.26 (4.04-6.84) and 2.73 ± 0.30 (1.6-4.6) and 2.68 ± 0.25 (1.8-4.0), respectively.

Radostits *et al*. (2000) reported normal ranges in cattle for serum Ca, Pi and Mg to be 9.7-12.4 mg/dl, 5.6-6.5 mg/dl and 1.8-2.3 mg/dl, respectively.

Jacob *et al.* (2002), in their study to evaluate the serum profile of certain macroelements, such as Ca, Pi, Mg of healthy crossbred heifers in Kerala before and after conception at different stages of pregnancy and during early lactation, revealed that the mean serum Ca concentration (5.57 mg/dl) decreased significantly during second trimester of pregnancy in crossbred heifers as compared to controls and it again increased during the ninth month of pregnancy; whereas the mean serum Pi level (8.29 mg/dl) was significantly higher during the fifth month of pregnancy as compared to controls and then it decreased by the 9^{th} month of pregnancy. Further, the serum Mg level showed a significant increase (2.55 mg/dl) by the 9th month of pregnancy as compared to the control.

Roche *et al.* (2002) in a study on subclinical hypocalcemia in dairy cows recorded an incidence rate of 30–40 per cent on the day of calving for grazing New Zealand dairy cattle.

Oetzel (2003) studied low blood calcium concentration (< 2.0 mmol/L), immediately post calving as a risk indicator for subclinical hypocalcaemia. Blood urea nitrogen (BUN) and urine pH have also been advocated as potential indicators for assessing herd protein status and anionic salt responsiveness, respectively. These two values can be evaluated by using means of individuals.

Goff (2004) revealed that four macrominerals Ca, P, Mg and K have the distinction of being involved in the downer cow syndrome, which was unfortunately, often associated with parturition in cows. He indicated that inadequate concentrations of these minerals could cause a cow to lose the ability to rise on her feet as these minerals were necessary for nerve and muscle function; whereas, less severe disturbances of these minerals in blood could cause reduced feed intake, poor rumen and intestine motility, poor productivity, and increased susceptibility to other metabolic and infectious diseases.

Van Saun (2004 a, b) studied that macro-minerals (Ca and Pi) decreased up to parturition. Na decreased while K increased with approaching parturition; whereas the levels of both electrolytes decreased with the ongoing lactation.

Calcium demand is tremendous immediately after parturition and monitoring serum calcium in cows less than a week following calving may have some utility, but it is useless before or beyond this time period. Recently, low serum calcium concentrations (subclinical hypocalcemia) have been linked with increased risk of early lactation culling (Duffield *et al.*, 2005).

Yokus and Cakir (2006) revealed that physiological variations resulted in changes in cholesterol, calcium, LDH, and total proteins concentrations; whereas ALP, copper, magnesium, and potassium concentrations changed with physiological and seasonal conditions. Phosphorus varied only with seasonal but not physiological changes; the copper concentration was found to increase through the pregnancy and neither the seasonal nor the physiologic variations affected zinc, iron, sodium, chlorine, calcium, urea, creatinine, albumin, and globulin values in both groups in all periods.

Kimura *et al.* (2006) observed that 47 per cent of all cows in their second lactation had varying degrees of subclinical hypocalcemia which in some cases was severe enough to alter physiological and immune functions.

Kachhawaha and Tanwar (2010) observed significantly low values ($P < 0.05$) of calcium, phosphorus and potassium in downer cows. These minerals were associated with parturition. Hypokalemia occur due to rapid urinary excretion and diminished alimentary absorption of potassium associated with reduced feed intake. Downer cow syndrome generally occurred within a month after parturition.

Reinhardt *et al.* (2011), in dairy cows (n= 1462) within 48 hours of parturition and reported a prevalence of subclinical hypocalcaemia to be 25, 41, 49, 51, 54, and 42 per cent in 1^{st}–6^{th} lactation cows. Furthermore, a significant decline serum calcium concentrations declined significantly as the lactation number increased from 1^{st} to 4^{th} and the cows with serum calcium >2.0 mmol/l had lower serum NEFA concentrations postpartum then cows with serum calcium <2.0 mmol/l.In early lactation, synthesis and secretion of colostrum depresses systemic concentrations of Ca, which often results in reduced availability of ionized Ca (Ca^{2+}) for cellular metabolism. Surveys in the US indicate that 25 % of primiparous and more than 41 % of multiparous cows are sub clinically hypocalcaemic (Ca < 8.0 mg/dL) in the first 48 h after calving.

Pal and Bhatta (2013), observed glucose, BUN, creatinine, AST, ALT, total serum proteins and albumin levels close to standard reference values. However, mineral profiles, particularly calcium (6.76±0.20mg/dl) and inorganic phosphorus (3.03±0.18 mg/dl) levels were significantly low in HF cow.

Jordan and Lager (2013) reported that seasonal effects were present for Ca, Pi, Mg, albumin, BUN, glucose, cholesterol, NEFA, K and Cl levels. For cholesterol, glucose, albumin, BUN, there was lactation by week by season interactions. In addition to the seasonal changes, significant breed differences were noted between Holsteins and Jerseys for Ca, Pi, Mg, Na, K, albumin, BUN, cholesterol and NEFA; whereas no statistical differences between breeds were noted for BHBA, glucose or Na. Furthermore, lactation by week by breed effect existed for Pi, albumin, BUN, glucose, cholesterol and NEFA.

2.2.1.2 Phosphorous (P):

Phosphorous has a multitude of functions in animals. A primary role is the integrity and development of the skeletal system. Approximately, 80-85% of the total P in cattle is in the bones and teeth (Horst, 1986). Phosphorus is also involved with cellular energy transfer via the ADP, ATP system. Lipid metabolism is dependent on P, which is a component of phospholipids. Phosphorus is involved in a number of enzyme systems and is a constituent of saliva. The absorption of P in the dairy cow is affected by a number of factors (Horst, 1986; Miller, 1979). The primary site of P absorption is the small intestine (Care, 1994). One report indicated that P uptake was reduced in cows under heat stress conditions (Sanchez *et al.,* 1994). The primary route of P excretion is fecal (Hibbs and Conrad, 1983; Morse *et al.*, 1992). Plasma P concentration is normally between 1.3 -2.6 mmol/L or 4 -8 mg/dl.

During late gestation fetal skeletal development can withdraw up to 10 g P/day from the maternal P pools (House and Bell, 1993). About 0.3 g P is incorporated into each kg of body tissue (muscle) gained during growth of the animal. Production of milk removes about 1 g P from the extracellular pool /kg of milk produced. Salivary secretions remove between 30 and 90 g P from the extracellular P pool each day. Factors affecting salivary phosphate secretion include the time spent ruminating (chewing activity) and the PTH status of the animal. PTH stimulates parotid salivary P secretion (Wright *et al.,* 1984) and can increase salivary phosphate concentrations 2-3 fold. Salivary phosphate secretions help buffer the rumen and supply rumen microbes with a readily available source of P, which appears necessary for cellulose digestion.

Phosphorous deficiency is the most prevalent mineral deficiency in bovines across the globe (Wikse *et al*., 1992). It has highest known biological functions compared to other essential minerals. Around 80% of total body P is present in bones and teeth. Every cell of body constitutes P and almost all transactions of energy engage formation or breaking of high-energy bonds that link oxides of phosphate to carbon or to carbon nitrogen compounds (ATP). Other major roles of P in the body include acid-base

buffer system, cell differentiation, as component of cell contents like phospholipids, phosphoproteins, and nucleic acids. Ruminal microbes need P for digestion of cellulose (Burroughs *et al*., 1951) as well as for synthesis of microbial proteins (Breves, and Schroder, 1991). Effect of increasing dietary concentration of P on the growth and production of cattle has been extensively reviewed, which revealed increasing dietary P up to certain level have positive effects on growth and production parameters of cattle, but above which no increase in the performance was observed (Teh *et al.*, 1982; Miller *et al.*, 1987; Wu and Satter, 2000; Wu *et al*., 2000). Deficiency of P is common in animals grazing on forages on soils low in P or excessively mature forages with low P content. Chronic deficiency is characterized by non-specific signs like unthriftiness, inappetence, poor growth, and lactational performance as well as poor fertility. These signs are often accompanied by coincidental deficiencies of other nutrients like protein or energy. Severe deficiency of P in cattle can cause infertility or reduced reproductive performance (Alderman, 1963; McClure, 1994). During mobilization of 10 ions of Ca from bone, 6 ions of P are also released in to the blood and this resorption of bone P appeared to occur merely as a consequence of greater demand for Ca in periparturient period. Generally mineral stores in bone were mobilized in late pregnancy or early lactation, irrespective of rate of P absorption. Significance of Ca: P dietary ratio in nutritional diet formulation has been reduced over a time due to the fact that efficiencies of absorption of Ca and P vary greatly as per the source of element in the diet. Plasma inorganic phosphorus aids in the diagnosis of P deficiency in cattle. Dietary P or response to supplementation is a better indicator compared to tissue P concentrations, unless severe deficiency is present.

Yokus *et al.* (2004), in a study to evaluate the effect of seasonal and physiological variations on the serum major and trace element levels in sheep, observed that magnesium concentration in serum varied with seasonal variation but not physiological variations; whereas, iron and potassium concentrations in serum varied only with physiological variations. Copper concentration changed not only with pregnancy, but also with some hormonal changes not caused by pregnancy. Calcium, phosphorus and selenium concentrations varied with both physiologic and seasonal variations; whereas zinc, sodium and chloride was almost identical for both groups and altered depending on neither season of the year nor the physiologic status.

Van Saun (2005) showed that cows with serum Ca concentration < 2.0 mmol/l pre and post partum were at greater risk for mastitis (OR= 6), metritis (OR=6), ketosis (OR=9), retained placenta (OR=9.9) and udder edema (OR=8.5); whereas cows with post partum Mg concentration <0.925 mmol/l were at increased risk of metritis (OR=8.4). Similarly, cows with pre partum Cl concentration <101 mmol/l and pre partum K concentration >4.8 mmol/l were at increased risk of ketosis (OR=6) and udder edema (OR=5.5).

Enjalbert *et al.* (2006), in a study on 2080 dairy and beef cow herds to evaluate the relationship between trace-element status and production, reproduction and health in cows and their calves, revealed that inadequate copper status was not associated with adult disorders, but was an important risk factor for poor calf performance and health. However, zinc insufficiency was strongly associated with low milk production, impaired locomotion in dairy herds; and diarrhoea and poor growth in calves.

Goff (2006) stated that in dairy cows plasma inorganic phosphorus (Pi) concentration routinely falls below the normal range at parturition in dairy cows and in cows with milk fever, plasma Pi concentrations remains often below 0.8 mmol/l.

2.2.1.3 Magnesium (Mg):

Magnesium is the fourth most prevalent cation in the body and essential micronutrient required for all animals. Mg functions at three biochemical levels, as a cofactor at the enzymatic level, at the structural level in the assembly of ribosomes, and at the whole cell level as a stabilizing force in membranes (Gunther, 1986). Mg acts as a co-factor or an activator of many critical enzymes for the reactions involving ATP that energize all major metabolic pathways (Haeton, 1990). Extracellular Mg is vital to normal nerve conduction, muscle function and bone mineral formation. It is very important to the central nervous system because it competes with calcium in the excitation-secretion coupling process. This role is directly related to the most common symptoms of grass tetany, tetanic contraction of the muscle. The principal role of Mg is that it acts as calcium channel blocking agent and regulate heart and skeletal muscle function. Mg is especially critical for ruminants. While non-ruminants absorb Mg primarily from small intestine, ruminants are able to absorb much of their Mg requirement from rumen via a Na-K-ATPase dependent process (Dua and Care, 1995). In fact, the reticulum and rumen can account for up to 80% of the Mg absorption along the entire digestive tract (Remond *et al.*, 1996). Green plants are an excellent dietary source of Mg for animals because of the presence of Mg^{2+} in chlorophyll.

Van de Braak *et al.* (1987) revealed that blood magnesium concentration below 0.65 mmol/L in the periparturient cow was associated with increased susceptibility to hypocalcaemia and milk fever.

Saleh (1990) observed no significant change in serum magnesium level in lactating and dry cows.

2.2.1.4 Sodium (Na) and potassium (K):

According to Kelly (1984) approximately 50 per cent of the total sodium in the body is in the extracellular fluid. The major portion of the rest is present in bone from where it is not readily mobilized. The amount of sodium in the body is influenced by dietary intake and excretion which is mainly via urine, sweat, gastrointestinal secretions and milk, and in horses and cattle there may be considerable loss in faeces. The major proportion of the body potassium is located in the intracellular fluid so that the serum level does not reflect the overall status of this cation. The serum sodium and potassium values in apparently healthy cattle range between 135-150 mmol/l and 3.9-5.6 mmol/l, respectively.

According to Pattanaik (1998) the dietary deficiency of sodium is most likely to occur in milch animals fed predominantly low sodium, cereal based diets; under very hot environmental conditions; in animals engaged in intense physical work and in animals grazing pastures on sandy soils heavily fertilized with potash which depress forage sodium levels. The symptoms of deficiency in dairy animals include a marked polyuria, polydipsia, salt hunger, pica, drinking urine, loss of appetite and weight and a fall in milk production. Whereas, the potassium is related physiologically with sodium for maintenance of acid-base relationships and proper osmotic balance. Besides, it plays important roles in the transmission of nerve impulses, contractility of muscles and as co factor in several enzyme systems. Ruminants develop K deficiency as a consequence of excessive loss of K through milk. Reduced appetite is one of the first sign of K deficiency. Lower dietary concentration in conjunction with reduced feed consumption seems to be best indicator of K status.

Radostits *et al*. (2000) reported normal ranges for serum sodium and serum potassium in cattle to be 132-152 mEq/l and 3.9-5.8 mEq/l, respectively. Further the low levels of sodium occur in early lactation in cows grazing on summer pastures without supplementation with salt. Levels down to 135 mmol/l may be associated with depraved appetite, and polydipsia and polyuria. The potassium levels have been difficult to interpret because the levels of the electrolyte in serum are not necessarily indicative of potassium deficiency. Its normal serum concentration is such more variable than sodium and its

average concentration in roughages of all kinds is nearly in excess of requirements; any abnormalities are usually in the direction of excess.

Kulcu and Yur (2003), in a study found significant differences for K and Zn concentrations before and during pregnancy, and the lactation period in cows ($p<0.05$). In sheep significant differences was also observed between before pregnancy and the lactation period for serum Cu concentrations, and before pregnancy and during pregnancy for serum K concentrations; also pregnancy and the lactation period for serum Mg concentrations.

The significant associations between serum electrolytes (Na, Cl, and K) and periparturient disease suggest that acid-base status or fluid dynamics may play a role in periparturient disease pathogenesis. Further work is needed to validate the relationships identified in this preliminary study (Van Saun *et al*., 2004; 2005; 2006a, b).

The K requirement increases under heat stress. Dry cow diets will almost never need supplemental K, however, excess K is a substantial problem in practical diets. For dry cows, increasing K linearly increases the risk of milk fever (Lean *et al.*, 2006).

2.2.2 Trace mineral/ micromineral

Micro-minerals are mainly found as component of large number of proteins and enzymes involved in body metabolism, growth, immunity, production, and reproduction of animals (Kincaid, 1999). Micro-minerals like Fe (in catalase), Cu, Zn, Mn (in superoxide dismutase) and Se in glutathione peroxidase are essential to the structure and function of these antioxidant enzymes (Kleczkowski *et al.*, 2004). Micro-minerals are required for the differentiation, activation, and for performing various functions of immune cells. Directly or indirectly trace mineral supplementation has role in reducing infectious disease morbidity (Failla, 2003). Major trace minerals involved in the development of immunocompetence are Fe, Zn, Cu, and Se. Micro-mineral deficiencies involves several minerals as well as conditioning factors where deficiency symptoms of one micro-mineral may predominate and affect the performance of animal (Hidiroglou, 1979). Deficiencies of trace minerals like Cu, Zn, Co, Mn, Fe, I, Mo and Se have been implicated as risk factors for impaired production and health in cattle (Radostitis *et al.*, 2000; Enjalbert *et al.*, 2006). Different micro-minerals can influence reproductive performance of livestock animals. Reproductive problems or reproductive failures may be induced by single or combined micro-mineral deficiencies or imbalances (Hidiroglou, 1979).

2.2.2.1 Copper (Cu):

Copper is an important trace mineral in livestock health and production. It is a chief constituent of several enzyme systems of the body like cytochrome oxidase, which is required for electron transport during aerobic respiration; lysyl oxidase which catalyzes formation of desmosine cross links in collagen and elastin to strengthen bones and connective tissues. It is also a component of enzymes like ceruloplasmin, which has a role in iron absorption; tyrosinase, required for production of melanin pigment from tyrosine; and Cu-Zn superoxide dismutase (SOD) which protects the cells from toxic damage due to reactive oxygen species.

Copper is an important trace mineral. It is required for normal cellular homeostasis and structure and function of skeleton, cardiovascular nervous and immune system (Minatel *et al.*, 2001). Copper is an essential component of several important enzymes including fero-oxidase, cytochrome oxidase and super oxide dismutase (Bremner, 1980). Enzymes involved in protection from oxidative stress, pigmentation of hair. Connective tissue synthesis also requires copper for biological function. It is essential for haemoglobin formation and iron transport. Copper is the rate limiting element in the

synthesis of ceruloplasmin, an enzyme necessary for the oxidation of iron and permitting it to bind with iron transport protein transferring (NRC, 2001). Ceruloplasmin acts as an antioxidant defence by removing free ion and free radicles (Saenko *et al*., 1994). Copper also plays an important role in the immune system through the following ways: energy production, neutrophil production and activity, antioxidant enzyme production, development of antibodies and lymphocyte replication (Niederman *et al.*, 1994; Nockels, 1994). The importance of copper for maintaining the functions of the immune system has been demonstrated in several studies. Viral and bacterial challenges have been shown to increase serum ceruloplasmin and plasma copper in copper-depleted cattle indicating a major protective role of copper in infectious diseases (Stabel *et al*., 1993). Low copper status has resulted in decreased humoral and cell-mediated immunity (Jones and Suttle, 1981a and 1981b; Xin *et al*., 1991 and Genglebach *et al*., 1997), as well as decreased neutrophil bactericidal capability in steers.

Copper absorption in ruminants is lower (<1.0–10.0%) than non-ruminants (Underwood and Suttle, 1999). The low absorption of copper in ruminants is largely due to complex interactions that occur in the rumen environment. Before development of a functional rumen, copper absorption is high (70–85%) in milk-fed lambs, but decreases to <10% after weaning (Suttle, 1975). It is well documented that copper requirements vary greatly in ruminants depending on concentrations of other dietary components, especially sulfur and molybdenum. A three-way interaction between copper, molybdenum and sulfur has been recognized (Dick, 1953). This interaction can occur with concentrations of molybdenum and sulfur that are present naturally in feedstuffs and have shown to be responsible for the formation of thiomolybdates in the rumen (Gooneratne *et al*., 1989; Suttle, 1991). Thiomolybdates are formed by molybdate reacting with sulfide, which is produced by rumen microorganisms via reduction of sulfate and also degradation of sulfur amino acids. Thiomolybdates associated with solid rumen digesta (bacteria, protozoa and undigested feed particles) form insoluble complexes with copper that do not release copper even under acidic conditions (Allen and Gawthorne, 1987). Systemic effects on copper metabolism that are attributed to absorption of thiomolybdates include 1) increased biliary excretion of copper from liver stores; 2) strong binding of copper to plasma albumin, which results in reduced transport of available copper for biochemical processes; and 3) removal of copper from metalloenzymes (Suttle, 1991). Independent from its role in the molybdenum-copper interaction, sulfur reduces copper bioavailability. Increasing dietary sulfur in the inorganic (sulphate) or organic (methionine) form from 1.0 to 4.0 g of sulfur/kg of diet reduced copper bioavailability in hypocupremic ewes fed low-molybdenum diets by 30–56% (Suttle, 1974). Sulfur in the form of sulfide is believed to reduce copper bioavailability via formation of insoluble copper sulfide in the gut. Increasing dietary sulfur from 0.8 to 2.5 g of sulfur/kg of diet reduced omasal flow of soluble copper by 50% in sheep (Bird, 1970).

According to Bush *et al*. (1956) copper entering the blood plasma from the small intestine becomes loosely bound to serum albumin to form the small direct reacting pool of plasma copper, in which form it is distributed widely to the tissues and can pass readily into the erythrocytes.

Vankoetsveld and Boogaerdt (1960) reported mean copper content 76.8 ± 12.2 µg/dl in whole blood, 89.1±18.2 in plasma and 54.2±20.2 in erythrocytes from 100 clinically healthy dairy cows. Values below 60 were regarded as deficient. They stated that pregnancy had no influence on plasma copper but the levels were high after calving.

Hartmans (1962) studied hypocupraemia in Friesian cattle on 39 farms during two grazing seasons. He found that hypocupraemia was associated with depigmentation of black hair (particularly

around the eyes), a staring coat on the withers, chronic diarrhoea and lameness with swelling of epiphysis.

Claessens (1964) suggested that the optimum Cu level of the blood serum of cow was about 68 µg/dl. At higher levels Cu was eliminated by absorption in the hairs.

Ammerman (1970) commented that copper deficiency in ruminants under natural grazing condition occur in many countries including Australia, New Zealand, United States, Great Britain and Netherlands. He further stated that copper metabolism is influenced by many dietary factors, some of which include sulphates, sulphur, molybdenum, zinc, protein level and protein source. He further mentioned that dietary deficiency of copper may be the factor to cause deficiency leading to anaemia. The severely cobalt deficient areas were present in New England and lower Atlantic coastal planes and moderately deficient areas at Northern New York, Northern Michigan and part of central plains in United States of America. He also pointed out cobalt deficiency areas in countries including Australia, New Zealand, East Africa and Norway.

According to Claypool *et al*. (1975), plasma copper levels between 19 µg/dl and 57 µg/dl (3 and 9 µmol/lit), in general represent marginal deficiency and levels below 19 µg/dl (3 µmol/lit) represent functional deficiency or hypocuprosis. Plasma Cu levels of 49.9 µg/dl (7.85 µmol/l) or low are indicative of low liver copper levels. Plasma Cu levels above 90.2 µg/dl (14.2 µmol/l) are usually associated with liver levels above 38.1 mg/kg (0.6 m mol/kg) dry matter.

Copper is the component of enzyme superoxide dismutase. The copper levels were slightly higher in the buffaloes on the day of calving than during prepartum phase (Durand and Kawashima, 1979).

In Bikaner, Rajasthan Dwarkanath and Ghosal (1981) reported the blood copper to be 117.8 ± 11.0 µg/dl in Nagori cows and 67.38 ± 8.74 µg/dl in Rathi dairy cows. In four Rathi cows, blood levels were lower than 50.0 µg/dl.

Kelly (1984) reported normal levels in blood in apparently healthy cattle to be iron 26.5-35.0 µmol/l and copper 11.02-26.75 µmol/l, respectively.

Kappel *et al.* (1984) observed that the plasma Cu concentrations were within the normal laboratory limits and plasma Cu values increased around the time of calving, with the maximum level occurring on postpartum day 11. Moreover, mean plasma Cu concentration was significantly higher in the summer-calving cows and it was inversely related to milk production level.

Sanders and Sanders (1985) reported that copper deficiency is common in livestock under a wide range of soil and climatic conditions and in some part of world, it occurs as a dual deficiency with cobalt. They further reported copper-molybdenum-sulphur inter-relationship which reduces the copper availability to animal. Anaemia has been described as a manifestation of copper deficiency with other signs of deficiency.

Jain (1986) classified nutritional deficiency anaemia, as anaemia caused by (a) low dietary protein (b) mineral deficiency of iron, copper and cobalt and (c) vitamin deficiency of vitamin B_{12}, folic acid, niacin, pyridoxine, thiamine and riboflavin.

Phillippo *et al.* (1987) reported a negative interaction of copper to molybdenum and iron by their experiment on cattle. Both molybdenum and Iron fed calves developed copper deficiency.

Chugh *et al.* (1987) observed in ketosis, copper and/or selenium deficiency and poor antioxidant status were the factors that along with hypophosphatemia precipitated post parturient haemoglobinuria.

Major changes in copper metabolism occur in dam during pregnancy, which is aimed at increasing copper by absorption and preventing losses by excretory pathways in order to accommodate the high demand of fetus (Goonerathe *et al*., 1989).

Kleczkowski *et al*. (1990) studied copper deficient bulls and reported increased acid phosphatase activity and lactate and succinate dehydrogenase distributed on the periphery of the lobules of the liver.

Nazki and Rattan (1990) reported varying blood iron and copper in different seasons in sheep with corresponding increased and decreased erythropoiesis. They reported blood iron range as 60.0 to 300.0 µg percent and blood copper between 106.66 to 201.66 µg percent of blood in different seasons of a year.

Rajora and Pachauri (1994) showed that there is no variation in copper levels in cows during one week pre- and post-partum period; but, in milk fever cases the levels were lower than normal cows. They also observed beneficial effects of supplementation of copper during dry period on mastitis in dairy cows.

Copper, a part of superoxide dismutase, plays a role in cellular fight against infection by helping to protect the cell damage when harmful oxidants are produced during the process of phagocytosis (Frank, 1999).

Spear (2000) studied inter-relationship exist between certain micronutrients, immune function and disease resistance in cattle. Several micronutrients have been shown to influence the immune response. The relationship between deficiencies of some micronutrients and disease resistance is less clear. A number of studies have indicated that Cr supplementation may improve cell-mediated and humoral immune response as well as resistance to respiratory infections in stressed cattle. Deficiencies of Cu, Se, vitamin E and Co in cattle reduce the ability of isolated neutrophils to kill yeast and/or bacteria. Cu deficiency reduces antibody production, but cell-mediated immunity is generally not altered. However, Cu deficiency appears to reduce the production of interferon and tumour necrosis factor by mononuclear cells. Numerous studies have linked low vitamin E and/or Se status to increased susceptibility of dairy cows to intramammary infections. In contrast to findings in laboratory animals, marginal Zn deficiency does not appear to impair antibody production or lymphocyte responsiveness to mitogen stimulation in ruminants. Co deficiency has been associated with reduced resistance to parasitic infections. It is well documented that vitamin A deficient animals are more susceptible to various types of infections. B-carotene, possibly via its antioxidant properties, may affect immune function and disease resistance independent of its role as a precursor of vitamin A.

2.2.2.2 Zinc (Zn):

There are about two hundred zinc-dependent enzymes in all the major biochemical pathways in the body (Sharma and Joshi, 2005; Hosnedlava *et al.*, 2007). Zinc dependent enzymes are involved in macronutrient metabolism and cell replication (Arinola *et al.*, 2008). Zinc is an essential component of both DNA and RNA polymerase enzymes involved in protein biosynthesis and transfer of genetic information (Underwood, 1977). Zinc plays a structural role in the formation of the so-called zinc fingers. Zinc fingers are exploited by transcription factors for interacting with DNA and regulating the activity of genes. Another structural role of zinc is in the maintenance of the integrity of biological membranes resulting in their protection against oxidative injury (Bray and Bettger, 1990). Zinc may influence membrane structure by its ability to stabilize thiol groups and phospholipids. It may also

occupy sites that might otherwise contain redox active metals such as iron. These effects may protect membranes against oxidative damage. Zinc also comprises the structure of copper/zinc-superoxide dismutase (Cu/Zn-SOD) (Markiewicz *et al.*, 2005). Zinc may also have antioxidant activity via its association with the copper-binding protein metallothionein. It is vital to the activity of a variety of hormones including glucagon, insulin, growth hormone, and the sex hormones. Zinc is important for production of keratin, which lines the inside of the teat duct and helps to keep out microorganisms that can cause mastitis. Kruczynska (2004) reported positive correlation between zinc and epidermal health. Zinc is required for a number of immune functions, including T-lymphocyte activity (Cossack, 1989). Some of these effects may be accounted for by zinc's membrane-stabilization effect. This could affect signalling processes involved in cell-mediated immunity. Kinal (2005) reported increase in serum immunoglobulins by supplementing zinc. Zinc is known to be involved in such signalling processes. Zinc may also influence gene expression by structural stabilization of different immunological transcription factors. Zinc ions can induce blast formation of human peripheral blood monocytes (PBMCs). In PBMCs, zinc induces cytokines, including interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-alpha. The stimulation of T-lymphocytes by zinc appears to occur via monocyte released IL-1 and cell-cell contact. High zinc concentrations inhibit T-lymphocyte proliferation by blocking the IL-1 type 1 receptor-associated kinase (Ibs and Rink, 2003). Recent Studies of zinc deficiency (ZD) have demonstrated that nutritional imbalances can readily induce programmed cell death (PCD) or apoptosis in a variety of cells (Fraker, 2005). Research conducted by Carlson *et al*. (1998) and Hill *et al.* (2000) showed that feeding 3,000 ppm zinc, added as zinc oxide, enhances growth and health of nursery pigs. Zinc status influences several aspects of vitamin A metabolism, including its absorption, transport, and utilization. Two common mechanisms postulated to explain this dependence relate to 1) the regulatory role of zinc in vitamin A transport mediated through protein synthesis, and 2) the oxidative conversion of retinol to retinal that requires the action of a zinc-dependent retinol dehydrogenase enzyme (Christian and West, 1998).

In ruminants, normal plasma Zn level is 0.8 to 1.2 µg/ml. Concentration of Zn in plasma fluctuates with age, stress and infections. Zn content of colostrum and milk is 14 and 4 ppm, respectively (NRC, 2001). At parturition, due to increased colostrogenesis, there is diversion of Zn from plasma pool towards mammary gland. Drop in serum Zn level at calving is also associated with an acute phase response due to inflammatory reaction in uterus. Stress at calving induces synthesis of metallothionein, a protein associated with Zn distribution. As a result, Zn is redistributed from blood pool to other tissues such as liver (Meglia *et al*., 2001). During day 190 up to end of gestation, the foetus and uterus of cow retain about 12 mg Zn/day (NRC, 2001). Plasma Zn level of 0.90 µg/ml at 15 days prepartum decreased to 0.64 µg/ml on the day of parturition in dairy buffaloes, which increased to normal values at 15 days postpartum (Panda, 2003). Plasma Zn level of 1.51 µg/ml at 5 days prepartum decreased to 1.09µg/ml on the day of parturition in cross bred cows (Chandra and Aggarwal, 2010).

Dufty *et al.* (1977) revealed that following conception, the plasma zinc levels remained relatively constant until late pregnancy. A marked decline was noticed during the periparturient period, although the trend observed differed between animals normally calved and dystocia affected ones. The samples obtained from calves approximately 24 hours after delivery contained zinc at concentrations of more than double to that recorded for their dams. Damir *et al*. (1988) reported clinical cases of Zn deficiency in a cattle of Western Sudan after drought. The animals showed general weakness, stunted growth, infertility, parakeratosis and achromotrichia. There was macrocytic hypochromic anaemia.

Ghosal and Mathur (1988) revealed a significant difference between the serum zinc levels of farm cattle and that of village cattle. Higher zinc values in the farm was apparently due to the better management and nutrition. The farm and village means were 71.77 and 52.42 µg/100 ml of serum, respectively. There was no difference in the means in the two seasons. The overall mean of zinc was 64.50 µg/100 ml of serum.

Singh *et al*. (1994) studied a primary nutritional zinc deficiency in and around Bikaner under famine conditions. The overall blood serum zinc level was 58.4 ± 34.89 (range 14.9-183.7) µg/100 ml. 118 of 230 cattle tested were classified as either sub-clinically (n=66) or clinically deficient (n=52) animals. Clinical, primary nutritional zinc deficiency showed poor body condition, dull appearance, lowered appetite, low production and low reproduction. In addition to these signs, there was alopecia, parakeratosis and hyperkeratosis of skin. Haematological parameters recorded in deficient animals before treatment did not significantly change after the animals were treated.

Pattanaik (1998) reported that deficiency of zinc occurs in cattle and other ruminants and causes severe inappetance, growth depression, impaired reproductive performance, unthriftiness and parakeratosis, alopecia, abnormal hoof growth and lameness.

Radostits *et al*. (2000) reported poor growth, stiff gait, drooling copious amount of saliva when ruminating, parakeratosis around eyes, nose, feet and scrotum, shedding of hooves, dystrophy and shedding of wool in zinc deficiency in ruminants. Further, normal levels in blood serum range between 80-120 µg/dl in sheep and cattle. Calves and lambs on deficient diets have levels as low as 18 µg/dl in serum.

Singh and Pachauri (2001) studied use of zinc and copper supplementation in the prevention of bovine mastitis in cows. The supplementation with zinc had beneficial effect in controlling mastitis and further recommended that the periodical monitoring of serum minerals should be undertaken to evaluate the immune status of animals of mastitis prone herds and supplementation of immuno-compromised cows may prove as a breakthrough in control of mastitis.

2.2.2.3 Iron (Fe):

Iron is the most abundant trace mineral in the body and its value as dietary constituent has been recognized for over 200 years. Approximately two-thirds of body iron is present in hemoglobin in red blood cells and myoglobin in muscle, 20% is in labile forms in liver, spleen and other tissues with the remainder in unavailable forms in tissues such as myosin and actomyosin and in metalloenzymes. In hemoglobin, which contains 0.34% iron, an atom of ferrous iron in the center of a porphyrin ring connects heme, the prosthetic group, with globin, the protein. The iron in hemoglobin is essential for the proper function of every organ and tissue of the body. Iron also plays a role in other enzymes involved in oxygen transport and the oxidative process, including catalase, peroxidases, flavoprotein enzymes and cytochromes (Sharma *et al*., 2005). Iron in blood plasma is bound in the ferric state (Fe+++) to a specific protein called transferrin. Transferrin is the carrier of iron in the blood and is saturated normally only to 30-60% of it iron-binding capacity. The duodenum is the main site of iron absorption (Kaneko *et al*., 1997). Iron is very low in the milk of cows, goats and sows. It varies from 0.5 to 1.0 ppm. Since pigs depend heavily on mother's milk during the first two to three weeks of life, they need iron supplementation because their body Fe stores are unusually low. This magnitude of growth rate imposes a greater demand on iron needs than occurs with young ruminants (Radostits *et al.*, 2000). There is little evidence of an iron deficiency occurring in calves, lambs and kids raised under grazing conditions, except when blood loss or disturbance in iron metabolism occurs because of parasitic infection or

disease. This is because they start early to eat feed other than mother's milk. Iron supplementation is needed, however, when young ruminants are fed with exclusive whole milk diet. Young nursing calves and lambs receiving no supplemental source of iron have responded to intra-musculature injections of iron-dextran by improved hemoglobin levels and growth rate.

Maynard and Loosli (1969) mentioned that all over the world certain soils do not provide adequate amount of iron to plants. Such plant fodders having low iron contents are liable to induce anaemia in farm animals.

Iron in blood is present mainly as haemoglobin (Hb) in erythrocytes and as transferrin in the blood plasma. Pregnancy stimulates increased iron absorption from gastrointestinal tract to meet the requirement of fetus, thus increasing serum iron. But, as pregnancy advances fetal demands overtake the rate of absorption and thus decreased level of serum iron coupled with increase in plasma volume results into decrease in haemoglobin and consequently iron concentration (Soliman and Amrousi, 1965). Hb levels decrease during pregnancy due to transfer of Hb across the placenta and haemodilution (Singh and Fahim, 1992).

Underwood (1971) opined that the iron deficiency results in hypochromic microcytic anaemia with subnormal serum iron level in all species. He reported serum iron and TIBC to be 146 µg/dl (range 89-253) and 553 (383-724), respectively, in normal cows with mean saturation of 26 per cent.

Whitelock *et al*. (1974) reported that dairy cows over 9 year-old and 2-3 year-old were more prone to anaemia and prevalence reported were as 13.4 per cent and 12.9 per cent, respectively, of total cows (7075) screened. The higher prevalence of anaemia in 15.9 per cent cows giving more than 16 kg milk was recorded as compared to those giving 7-16 kg or less than 7 kg milk, where prevalence of anaemia was 9.8 per cent and 4.4 per cent, respectively.

Ghosal *et al*. (1976) reported mean serum iron values for cow, sheep and camel as 213.15 ± 14.08, 141.32 ± 8.26 and 101.32 ± 4.60 µg/100 ml, respectively, in the north western deserts of Rajasthan.

Ghosal and Mathur (1992) suggested that serum iron and copper levels are influenced by age, nutritional status, parasitism and reproductive state of the animal. They recorded 5 % animals having serum iron values less than 40 µg/ml indicating subclinical deficiency in Bikaner area of Rajasthan.

Gautam (1994) recorded serum iron values of normal healthy crossbred cattle in and around Bikaner to be 142.40 (127.0-158.0) µg%.

Rajora *et al*. (1995) found anorexia in cows suffering from microcytic hypochromic anaemia. The requirement of iron increases during gestation, lactation and active growth of animals and anorectic animals often suffer from iron deficiency anaemia due to poor availability of iron at the intestinal site and found significantly lower serum iron values in iron deficient anaemic cows as 12.38±0.25 mmol/l as compared to healthy control 30.74 ± 0.30.

Samanta *et al*. (1995b) recorded prevalence of anaemia in cattle in alluvial zone of Nadia District, West Bengal. Out of 712 grazing cattle, 420 (58.98 per cent) were found to be anaemic irrespective of the cause in natural grazing conditions. No relation of anaemia with season was found. In relation to age group, higher prevalence of anaemia 32.61 per cent was recorded in 2-3 year old cattle, followed by 24.28 per cent in 6 months to 1 year, 20.23 per cent in 1 to 2 years and 22.23 per cent in above 3 years old cattle. The higher prevalence in 2-3 year's old cattle was attributed to extra load in the process of first calving and lactation with insufficient feed supply. According to breed, the higher prevalence of anaemia was recorded in crossbred cattle (67.5 per cent) than indigenous cattle (48.59 per

cent). They also reported sex variation (F:M) in prevalence as 3.05: 0.95. The higher prevalence in female was due to the stress of production and reproduction.

Dhaliwal (1996) reported anaemia due to copper, cobalt and iron deficiency in crossbred cows in Bikaner, Rajasthan. The values for healthy cattle for serum copper 32.91±1.43 (11.04-55.52) µg/dl, cobalt 10.18±0.39 (6.09-18.28) µg/dl and iron 89.20±3.22 (44.44-141.17) µg/dl. Whereas, these values for healthy cattle to be serum copper 82.06 ± 2.71 (170-290) µg/dl, cobalt 22.45±1.56 (10.28-29.60) µg/dl and iron 214±12.67 (170-290) µg/dl.

Sarkar *et al*. (1996) reported low levels of serum copper in anaemic cattle in which cobalt deficiency caused decreased uptake of copper in the blood. Further the cobalt deficiency in soil was cause of cobalt deficiency in the cattle, which were anaemic in West Bengal. Further, lower serum iron, copper and cobalt values in anaemic cattle were 370.46±27.22 µg %, 69.44±4/81 µg % and 12.60 ± 3.01 µg % respectively as compared to healthy control cattle, where these values were 504.61±31.04, 110.79±6.80 and 31.40±4.17 µg %, respectively.

2.3 Minerals and oxidative damage:

Disturbance of the balance between the production of reactive oxygen species such as superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl, and peroxyl radicals and antioxidant defences against them produces oxidative damage (Gutterridge, 1995). The antioxidant defence system in most cells is composed of two components, the antioxidant enzymes component which includes enzymes such as superoxide dismutase (Cu, Zn, Mn), catalase (Fe) and glutathione peroxidise (Se), and the low molecular weight antioxidants component that includes vitamins A and E, ascorbate, glutathione and thioredoxin. These substances are the body's natural defense against endogenous generated ROS and other free radicals, as well as ROS generated by external environmental factors. Oxidative stress occurs when the production of ROS exceeds the bodys natural antioxidant defence mechanisms, causing damage to biomolecules such as lipids, proteins and DNA (Borek, 1997).

Zinc deficiency causes oxidative DNA damage (Oteiza *et al.*, 2000) and chromosome breaks have been reported in animals fed a zinc-deficient diet (Hainaut and Milner, 1993). The offspring of zinc deficient rhesus monkeys also have increased chromosome breaks (Olin *et al*., 1993). The chromosome breaks might be due to increased oxidative damage due to loss of activity of Cu/Zn superoxide dismutase or the zinc-containing DNA-repair enzyme, Fapy glycosylase, which repairs oxidized guanine (O'Connor *et al*., 1993).

Oxidative stress has been postulated to contribute to the pathology associated with dietary copper deficiency. In vivo erythrocytes are probable targets of oxidative damage because they are exposed to high concentrations of oxygen and contain heme iron that can autoxidize' which results in the formation of superoxide anions. Activity of the important antioxidant enzyme 'copper zinc superoxide dismutase' decreases markedly in erythrocytes during copper deficiency (Sukalski *et al.*, 1997). In a study Picco *et al.* (2001) reported that DNA and chromosome damage can be assessed by the Comet assay as a consequence of the higher oxidative stress suffered by hypocupremic animals.

2.4 Oxidative stress:

The role of intracellular SOD is to scavenge the superoxide ($\bullet O^{-2}$) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions (Fee *et al*., 1975).

Trotta *et al*. (1982) reported that lipid peroxidation and haemoglobin degradation were the two extremes of a spectrum of oxidative damage in red cells exposed to t-butyl hydroperoxide. The exact position in this spectrum depended on the availability of glucose and the ligand state of haemoglobin. Lipid peroxidation was not dependent on the rate or completion of t-butyl hydroperoxide consumption but rather on the route of consumption. Lipid peroxidation appears to depend on the balance between the presence of initiators of lipid peroxidation (oxyhaemoglobin and low concentrations of methaemoglobin) and terminators of lipid peroxidation (glutathione, ascorbate, high concentrations of methaemoglobin).

Miller (1992) reported direct effects include peroxidative changes in membranes and other cellular components. Indirectly, competitive consumption of reducing equivalents can interfere with important metabolic functions and divert glucose from other pathways by inducing the monophosphate shunt. Finally, peroxidative chain reactions initiated by reactive species that escaped enzymatic degradation are terminated by chain-breaking antioxidants, including water-soluble ascorbate, glutathione, and urate and lipid-soluble vitamin E, ubiquinone, and β-carotene. To optimize

performance, oxidative stress in high producing cows must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate reactive oxygen metabolites.

Cheeseman and Slater (1993) described that free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. They are produced continuously in cells either as accidental by-products of metabolism or deliberately during, for example, phagocytosis. The most important reactants in free radical biochemistry in aerobic cells are oxygen and its radical derivatives (superoxide and hydroxyl radical), hydrogen peroxide and transition metals.

Glutathione peroxidase is selenium dependent enzyme and it has also antioxidant property. It converts hydrogen peroxide to water. The protection offered to cellular membrane by Vit E may spare the requirement of GPx by oxidizing free radicals at the membrane, thereby preventing leakage of free radicals into cytosol and maintaining activity of cells at a high level thereby decreasing mastitis (Hogan *et al*., 1993).

Brezenska *et al.* (1994) observed that GPx tended to be higher in Vit E supplemented than Se offered or control animals (5.3, 5.0 and 5.1 U/ml).

Lactation is essential for survival of mammals and represents a substantial transfer of energy from mother to offspring. This transfer is facilitated by numerous endocrine and cellular adaptations. The transition from pregnancy to lactation is critically important for the health, subsequent production and reproduction in dairy cows. High reproductive and productive efficiency in dairy cow requires a disease free transition period. Both innate and acquired defense mechanisms are suppressed during pregnancy which gets aggravated during peripartum period and further during early postpartum (Cai *et al*., 1994).

Fridovich (1995) described that O^{2-} oxidizes the [4Fe-4S] clusters of dehydratases, such as aconitase, causing-inactivation and release of Fe (II), which may then reduce H_2O_2 to OH-. Superoxide dismutase (SOD) inhibits such OH-. Production by scavenging O^2-, but Cu-Zn SODs, by virtue of a nonspecific peroxidase activity, may per oxidize spin trapping agents and thus give the appearance of catalyzing OH- production from H_2O_2.

Antioxidant defences are diverse, can be either synthesized *in vivo* or derived from the diet, and are localized transiently throughout tissues and different cell types. The various antioxidant defense mechanisms also can be classified on the basis of several criteria, such as on their solubility in lipids and water or on their chemical and physical characteristics (i.e., enzymatic or non-enzymatic) (Mates and Sanchez-Jimenez, 1999).

McCall *et al*. (1999) reported that endogenous oxidative damage to proteins, lipids, and DNA is thought to be an important etiologic factor in aging and the development of chronic diseases such as cancer, atherosclerosis, and cataract formation. The pathology associated with these diseases is likely to occur only after the production of reactive oxygen species has exceeded the bodies or cell's capacity to protect itself and effectively repair oxidative damage.

Mates (2000) described that reactive oxygen species (ROS) are produced during normal cellular function. ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. They are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of DNA and proteins. Under normal conditions, antioxidant systems of the cell minimize the perturbations caused by ROS. When ROS generation is increased to an extent that overcomes the cellular antioxidants, the result is oxidative stress. It is now clear that several biological molecules,

which are involved in cell signalling and gene regulation systems, are very sensitive to redox statue of the cell.

Oxidative stress can contribute and/or lead to the onset of health disorders in cattle. They observed that during the transition period cows can experience oxidative stress which may contribute to periparturient disorders, and may be associated with metabolic diseases. The transition period is critical for the health of dairy cattle. They also reported that oxidative stress leads to peroxidative damage of lipids and other macromolecules with consequent alteration of cell membranes and other cellular components (Miller *et al*., 1993; Brezezinska-Slebodzinska *et al*., 1994; Drackley, 1999; Toyokuni, 1999).

Oxidative stress can contribute and/or lead to the onset of health disorders in cattle and during the transition period cows can experience oxidative stress which may contribute to periparturient disorders, and may be associated with metabolic diseases (Ronchi *et al*., 2000).

Spear (2000) studied inter-relationships that exist between certain micronutrients, immune function and disease resistance in cattle. Deficiencies of Cu, Se, vitamin E and Co in cattle reduce the ability of isolated neutrophils to kill yeast and/or bacteria. Cu deficiency reduces antibody production, but cell-mediated immunity is generally not altered. However, Cu deficiency appears to reduce production of interferon and tumour necrosis factor by mononuclear cells. In contrast to findings in laboratory animals, marginal Zn deficiency does not appear to impair antibody production or lymphocyte responsiveness to mitogen stimulation in ruminants. It is well documented that vitamin A deficient animals are more susceptible to various types of infections.

Oxidative stress resulting from increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defence, leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan *et al*. 2001).

Gitto *et al*. (2002) reported that an imbalance between increased production of ROS and reduced availability of antioxidant defences near the time of parturition increases oxidative stress and may contribute to periparturient disorders in dairy cows.

Bernabucci *et al.* (2002) carried out a study to assess whether hot season affected the oxidative status of transition dairy cows and found that, the cows exposed to moderate heat stress showed higher erythrocyte superoxide dismutase (SOD), erythrocyte glutathione peroxidase (GSH-Px-E), intracellular thiols (SH), and thio-barbituric acid reactive substances (TBARS) compared with spring cows, indicating a condition of oxidative stress in summer transition dairy cows.

Kirschvink *et al*. (2002) reported that oxidant/antioxidant imbalance in favour of oxidants has been identified as playing a decisive role in the pathogenesis of chronic inflammatory airway diseases. Nutritional antioxidant supplementation might reduce oxidative damage by enhancement of the antioxidant defence, thereby modulating inflammatory processes. In a placebo-controlled, blind study, it was tested whether a dietary antioxidant supplement administered for 4 weeks would improve lung function and reduce airway inflammation in heaves-affected horses.

Health problems during transition period can result in potential losses in subsequent peak yield and postpartum fertility. Impairment of the immune status during Peripartum period in cow increases the risk of uterine infections. Peripartum period is the most vulnerable period for the cows in terms of increased immunosuppression and susceptibility to infection (Drackley *et al*., 2001; Sheldon *et al*., 2002).

They suggested that when Se in the diet was adequate (2ppm), its supplementation had no effect on GPx concentration. Plasma glutathione peroxidase is considered as an indicator of oxidative

stress (Tuzun *et al.*, 2002).

Castillo *et al.* (2003), in a study to establish the values for plasma lipid hydroperoxides (LOOH) and total antioxidant status (TAS) in healthy cows and its relationship with milk yield, showed that the animals with a high milk yield presented higher levels of NEFA, triglycerides, AST, TAS, albumin and total proteins without any significant difference except for LOOH and urea which showed significant differences from lower producing cows. On the contrary, a higher level was observed for glucose and cholesterol in the low producing cows as compared to the high producing cows.

Wallace (2005) reported that radical group includes nitric oxide (NO-), superoxide (O2-) and hydroxyl radical (OH-). Oxidative stress can be defined as an excessive bioavailability of ROS, which is the net result of an imbalance between production and destruction of ROS. It is proposed that electrons leaking from the electron transport chain (ETC) produce ROS and that these molecules can then damage ETC components and mitochondrial DNA, leading to further increases in intracellular ROS levels and a decline in mitochondrial function.

Bernabucci *et al.* (2005) further recorded that before calving, cows showed an increase in the plasma levels of SH, SOD, GSH-Px, and a decrease in erythrocyte GSH-Px and plasma reactive oxygen metabolite (ROM). However, after calving cows showed a decrease in plasma and erythrocyte SH and SOD accompanied by an increase in ROM, TBARS and plasma GSH-Px. It was also observed that cows which showed higher BCS at the beginning and greater loss of BCS after calving, had higher plasma ROM, TBARS and SH, and lower SOD and erythrocyte SH in the postpartum period. Similarly cows with higher BHBA and NEFA showed higher ROM and TBARS plus lower levels of antioxidants.

Castillo *et al.* (2005), in a study on oxidative stress during late pregnancy and early lactation in dairy cows, revealed that the mean MDA levels did not differ significantly between pregnant cows (P) and late lactation (LL) cows at any stage, and further, it did not showed any clear trend within P-cow group. Similarly, mean TAS did not differ significantly between P cows and LL cows at any stage, but showed a clear and statistically significant increasing trend within the P-cow group, peaking one week after calving, and then possibly declining.

Lohrke *et al.* (2005a) reported that metabolic activity increases during the transition period, especially in the liver and mammary gland, the higher metabolic activity is accompanied by higher oxygen radical production which may cause greater concentrations of oxidative damage products if antioxidant status is inadequate.

Castillo *et al.* (2006) evaluated oxidative status in healthy cows during lactation using two parameters: (I) Plasma levels of malondialdehyde (MDA) and (II) Total antioxidant status (TAS). Results show that nutrition can influence the characteristic metabolic changes occurring between lactation onset and peak lactation. The most remarkable fact was the great inter-individual variations observed in MDA. When the animal reaches peak lactation, metabolic status is stabilized, and this is reflected by antioxidant status with mean values of 28.87+/-5.33 microm/L for MDA and 0.154+/-0.002 mmol/L for TAS values.

Pintea *et al.* (2006) carried out to assess the activity of antioxidant enzymes, the level of lipid peroxidation at the level of serum antioxidants, carotenoids and uric acid. GPX activity in cows was situated around 129 U/g Hb in the first week after parturition, and then increased to reach 166 U/g Hb in the sixth week after parturition, value comparable with that of cows in late lactation (160 U/g Hb). The maximum level of lipid peroxidation was observed in the first week after parturition (62 μmol/l),

comparing with the level of MDA in the cows in late lactation (35 μmol/l). In the second week after parturition the level of MDA decreased significantly, and remained at approximately same values during the experiment. The high level of MDA in the first week after parturition correlated with a low activity of antioxidant enzymes (SOD, GPX and catalase) and a low level of antioxidants.

Dairy cattle are more susceptible to a variety of metabolic and infectious diseases during the transition period. Increased incidence of disease during the periparturient period is related directly to numerous genetic, physiological, and environmental factors that can compromise the cow's immunological defenses ((Drackley, 1999; Sordillo, 2005; Goff, 2006).

A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in both humans and dairy cows. The possibility that oxidative stress during the transition period may be a major underlying cause of inflammatory and immune dysfunction in dairy cattle is supported by both *in vivo* and *in vitro* studies (Gitto *et al*., 2002; Bernabucci *et al*., 2005; Stefanon *et al*., 2005; Castillo *et al*., 2005; Sordillo *et al*., 2007).

ROS production during oxygen metabolism has necessitated the development of antioxidant defenses that can effectively trap reactive intermediates before causing oxidation to macromolecules or to reduce biomolecules that already have been oxidized. As such, antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecule (Halliwell and Gutteridege, 2007).

Kanna (2007) reported that after calving, HBCS cows had more lipid mobilization as indicated by higher NEFA levels and more pronounced alteration in oxidative status indicative of higher oxidative stress in HBCS cows. SOD activity was 4152.27 ± 71.19 and 4326.83 ± 81.85 (Units /g Hb/min) in medium BCS and high BCS cows, respectively.

Saleh *et al.* (2007) have reported reduction in antioxidant activity and increase in oxidative stress during periparturient periods.

Sathya *et al.* (2007) reported that MDA, the end product of lipid peroxidation in an important marker of oxidative stress.

Andrieu (2008) reported trace elements have a specific role in free radical control at the cellular level and influence the anti-oxidant/free radical balance. Dietary trace elements must be available for absorption throughout the digestive process until they reach the final site of absorption in the small intestine. Negative interactions between minerals can occur and, as the intestinal environment lowers the absorption of ionic minerals, chelation technology has been developed to increase mineral bioavailability. Organic trace elements have been used in dairy cow experiments, resulting in significant improvements in udder health, lameness and reproductive performance.

Oxidative stress is associated with various disease conditions in animals. Blood level of catalase, superoxide dismutase; malondialdehyde and reduced glutathione are the commonly used biomarkers of oxidative stresss (Cemek *et al.,* 2005; Esme *et al.* 2008).

Spears and Weiss (2008) studied the severity of immunosuppression which is exacerbated by factors such as negative energy balance (NEB), hypocalcaemia and increased circulating levels of cortisol for prolonged periods around calving. In addition, it has been established that dairy cattle subjected to the demands of late pregnancy, parturition or peak lactation may be subjected to oxidative stress or the production of reactive oxygen metabolites. Immune cells are sensitive to oxidative stress as their membranes contain high concentrations of polyunsaturated fatty acids that are vulnerable to lipid peroxidation and they produce large quantities of reactive oxygen metabolites when stimulated.

Sordillo and Aitken (2009) reported oxidation and the production of free radicals are an integral part of aerobic metabolism. A variety of reactive oxygen species (ROS) are produced by normal metabolic processes and by certain leukocyte populations during defense against disease. Unfortunately, considerably less is known about how oxidative stress can affect veterinary health and well-being, particularly during times of high metabolic activity. The performance of high producing dairy cattle can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transition cows. Innovative approaches are needed to enhance the antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands.

Chandra and Aggarwal (2010) found that cows supplemented vitamin E @1000 IU/day have higher glucose level in comparison to non supplemented cows during the transition period.

Dimri *et al.* (2010) studied the alterations in serum cortisol and erythrocyte lipid peroxides and superoxide dismutase activities in 28 pregnant water buffaloes supplemented with antioxidant nutrients, Vitamin E and selenium. Results suggested that pregnancy is associated with oxidative stress and supplementation of vitamin E and selenium may be beneficial by alleviating oxidative stress in water buffaloes.

Oxidative stress resulting from increased production of free radicals and reactive oxygen species (ROS), and/or a decrease in antioxidant defence, leads in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which, in turn, may be related to various health disorders (Trevisan *et al*., 2001; Abd Ellah, 2010).

Pedernera *et al.* (2010) evaluated the effect of diet, energy balance and milk production on oxidative stress in early lactation dairy cows and observed that high producing dairy animals had a lower degree of negative energy balance and also diet was found to have a indirect effect on the level of oxidative stress and the factors found to be responsible for high level of oxidative stress were severe negative energy balance and lower levels of milk production.

Waldron (2010) reported that immune function is weakened and dairy cows have a decreased capacity to fight disease. Factors suggested to be responsible for this immunosuppression include oxidative stress, nonesterified fatty acids, ketones and negative energy balance and calcium status during the transition period, approximately 3 weeks prior to calving until 3 weeks post-calving.

Sharma *et al.* (2011) studied on oxidative stress and antioxidant status during transition period in dairy cows considering plasma level of malonylaldehyde (MDA) as an indicator of lipid peroxidation and superoxide dismutase (SOD), catalase, glutathione(GSH) and glutathione peroxidise (GSHPx) as antioxidants. The lipid peroxidation was significantly ($p<0.001$) higher in cows during early lactation as compared to the cows in advanced pregnancy. A significant positive correlation ($r =+0.831$, $p<0.01$) was found between MDA and catalase in early lactating cows. In early lactating cows, blood glutathione was significantly lower than in advanced pregnant cows.

Zhang *et al.* (2011) found that in cows with subclinical ketosis, serum BHBA and NEFA concentrations were significantly higher, and glucose concentrations were significantly lower as compared to the values in healthy cows and no significant difference was observed in serum SOD, MDA, GSH-Px, catalase and TAS between the subclinical ketotic and healthy cows.

Festila *et al.* (2012) reported that both the average values of glutathione peroxidase and Superoxide Dismutase (SOD) were lowest during the early lactation in comparison to the advanced pregnancy and mid pregnancy concluding that dairy cows were under oxidative stress as the antioxidant defense capacity was reduced during postpartum period and during lactation than during advanced pregnancy.

Abuelo *et al.* (2013) observed an oxidative stress index (OSi) as a combined measurement through a ratio between pro-oxidants and antioxidants throughout the transition period in dairy farms in field condition. Serum samples of high-yielding dairy cows were taken, and markers of oxidative damage and antioxidant capacity were measured. With the joint evaluation through the OSi, differences were found that were not present with the separate evaluation of pro-oxidants or antioxidants, thus supporting our hypothesis that the OSi indicates more accurately the oxidative status of the animals. It was also confirmed that dairy cows undergo OS after parturition, and that antioxidant supplementation from 1 month before parturition until the peak of lactation may be needed to reduce the risk of OS.

The degree of lipid peroxidation is often used as an indicator of ROS mediated damage and concentration of malondialdehyde (MDA) in blood and tissue are generally used as biomarker of lipid peroxidation. The mechanism of action of most of natural products and chemical drugs is done through the antioxidant properties of these drugs by reducing the lipid peroxidation and stimulation of enzymatic and non enzymatic antioxidant system within the organism (Sabry and El-bahr, 2013).

Maurya *et al.* (2014) conducted the effect of vitamin E and zinc on oxidative stress and antioxidant enzymes during transition period in Karan Fries cows. The activity of plasma superoxide dismutase, catalase and glutathione peroxidase were significantly lower in treatment as compared to control cows. Plasma Zn was significantly higher in treatment group than control group. Plasma Zn level decreased at the time of parturition. The results indicated that supplementation of antioxidants like vitamin E and zinc have beneficial effects in improving the antioxidant activity and decrease oxidative stress to animals.

2.5 Biomarkers:

Paterson (1959) observed an increase in blood glucose and 17-hydroxy corticoids before calving and later two third of animals showed decline in blood glucose levels two to four weeks after calving.

Mylrea and Healy (1968) determined the serum protein concentration of 25 cows by Biuret method and SGOT by Reitman and Frankel method. The normal values ± 2 S.D. of total protein (gm/100 ml), Albumin (gm/100 ml), Globulin (gm/100 ml), A/G ratio and SGOT (µmoles/l/min) were 7.9 ± 0.64, 3.2 ± 0.43, 4.7 ± 1.01, 0.68 and 73 ± 12.3, respectively.

Treacher and Sansom (1969) recorded a variable rise in plasma SGOT activity in cows one week after calving.

Payne *et al.* (1970) designed the Compton Metabolic Profile Test (CMPT) first time for primarily to assist in the diagnosis of clinical problems in dairy herds by comparing the blood composition of specifically chosen groups of animals comprised of seven non lactating cows, seven cows giving maximum yields and seven cows giving average yields of milk on the day of the test. The test thus assesses the adequacy of both maintenance and production rations by associating levels of blood composition with milk yields. The components measured in the blood samples were packed cell volume (PCV), haemoglobin (Hb), urea nitrogen, calcium, inorganic phosphorus, glucose, albumin, total protein, magnesium, potassium, sodium, copper, iron, and globulin (measured as the difference between total protein and albumin).

Underwood (1971) reported that copper is required for the utilization of iron in haemoglobin formation.

Ghosal and Dwarkanath (1971) determined normal serum transaminase activities in domestic animals of Bikaner. Serum GOT and GPT activity of 30 lactating Rathi cows was 52.87 and 34.07 U/ml, respectively.

Hewett *et al*. (1975) observed in an experiment with three groups of cows receiving protein equivalent to 75, 100 and 125 % of Swedish feeding standard that the urea-N values were directly affected by protein level. They concluded that serum urea-N gives exact reflection of protein intake when energy and roughage remained constant.

Manston *et al*. (1975) in an experiment compared three diets containing 200, 133 and 82 % of recommended requirements for digestible crude protein and found mean serum urea concentration above, within and below the standard limits of Compton metabolic profile test, respectively.

Rowlands *et al*. (1975) reported that the globulin concentration before calving in a herd of dairy cows was 4.22 g/100 ml and it increased to 4.53 g/100 ml (normal value) within two weeks of parturition. Urea concentration decreased from 16.5 mg /dl before calving to 14.7 mg /dl after calving. The fall was most pronounced in summer month when normal urea-N concentration was higher due to grazing summer pasture. In winter, the fall during calving was not significant.

The concentration of albumin is affected by nutritional inadequacies, gut mal-absorption, dehydration or liver impairment (Stevens, 1975).

Kitchenham and Rowlands (1976) observed a downward trend of urea-N with increasing edge. They also found a breed difference in urea-N concentration.

Zhabolenko (1976) found low concentration of blood sugar, albumin and high concentration of total lipids, ketone bodies and total protein in hypocalcaemic cattle.

Rashid (1977) found a significant relationship between the mineral content, ketone bodies and glucose levels in blood. Similarly, significant positive relationship was found between the time of lactation, pregnancy and serum glucose and negative relation with ketone bodies. However, no correlation was observed between milk production and ketone bodies.

Copenhaver *et al*. (1978) reported that lipids form a diverse group of compounds (including fats, phospholipids, glycolipids and sterols) which are generally insoluble in water. Fats, which contain fatty acids linked to glycerol, are generally stored within cells in droplets of varying size. Certain lipids, particularly phospholipids and cholesterol, form important components of cell membranes. The steroid hormones are structurally very similar to the membrane component cholesterol and one of their important effects is to alter the permeability of the cell membrane. Cell lipids, called glycolipids, are found in combination with sugar molecules e.g. cerebrosides and gangliosides which are also utilized in the construction of the cell membrane. The lipid components form a major part of the membrane itself and the carbohydrate component constitute surface hydrophilic coat of the membrane.

According to Benjamin (1979) creatinine is formed in the metabolism of muscle creatine and phosphocreatine which is not affected by dietary protein, protein catabolism, age, sex or exercise. After being filtered by the glomerulus it is excreted in the urine. The normal values range between 1-2 mg/dl. The creatinine value increases in primary renal disease, pre-renal and post-renal uraemia. The normal BUN values range between 10-30 mg/dl in cattle.

Haraszti *et al*. (1979) determined the metabolic profile in 20 high yielding cows from 8-10 days before to 8-10 days after calving. The six cows that developed paresis had increased ALT, AST and LDH activities, and decreased serum Ca and P at calving.

Hayashi *et al*. (1979) found significantly increased plasma levels of Mg and significantly lowered plasma levels of Ca and K in cows affected with milk fever, as compared with normal post-partum cows. The mean values for 50 cows with milk fever and 13 control cows were : Ca (mg/dl) 4.2 ± 0.9 and 7.2 ± 1.0; P (mg/dl) 4.3 ± 1.8 and 5.4 ± 1.7; Mg (mg/dl) 1.9 ± 0.48 and 1.6 ± 0.3; GOT (U/ml) 105.3 ± 67.3 and 93.9 ± 33.9; GPT (U/ml) 19.4 ± 4.6 and 19.8 ± 7.4, respectively.

Morrow *et al.* (1979) reported higher total protein and albumin value and below 40mg/100ml glucose level in animals with fat cow syndrome.

The monosaccharide glucose is used as an energy source in body tissue, and also during lactation as a basis for producing lactose (i.e., milk sugar). The blood concentration of glucose is strictly regulated through homeostasis, Glucose concentration in blood can undergo short-term fluctuations in response to stress hormones as well as display diurnal variation (Bauman & Currie, 1980).

Murtuza *et al*. (1980) obtained the normal values of SGOT, SGPT and serum protein components under various physiological states in female Haryana cattle. SGOT and SGPT activities were highest in early lactating cows as compared to late pregnant cows, empty dry cows and heifers. No statistically significant differences were observed in the relative concentrations of albumin fraction and albumin: globulin ratio in different physiological states.

Blood glucose concentrations was related to the severity of fatty liver in periparturient cows and observed a low urea concentration in cows after calving and stated that it could be a reflection of the reduced anabolism of proteins due to fatty infiltration of liver (Reid *et al.* 1979, Reid, 1980) .

Roussel *et al*. (1982) conducted metabolic profile testing in jersey cows and suggested that total protein is usually used as an appraisal of the nutritive status of an animal reflecting intake and metabolism. They found that total protein increased with age due to increased protein utilization efficiency with maturity.

Doxey (1983) reported that elevated levels are indicative of renal damage which is not apparently manifested in large animals.

Gaal *et al.* (1983) stated that measurement of beta hydroxy butyric acid is of little value in the diagnosis of fatty liver.

Kulkarni *et al.* (1983) reported normal values of some biochemical constituents of blood in 6 crossbred lactating cows during the first three months of lactation. Mean values (±SE) and ranges of concentration were: Total proteins (g%) 6.53 ± 0.01 (6.41-6.63), Albumin (g%) 2.89 ± 0.29 (2.85-2.91), Globulin (g%) 3.46 ± 0.03 (2.96-3.68) and A:G 0.80±0.10 (0.78-0.83).

Reid *et al.* (1983) reported that activities of AST and NEFA differed significantly between moderate fatty liver and mild fatty liver.

Kelly (1984) reported that creatinine is the end product of muscle metabolism of creatine and phosphocreatine. Its concentration in the blood and urine of animals is neither significantly influenced by the diet, nor by catabolic factors such as fever, toxaemia, infection and drug administration. It is a non threshold substance for the kidney, hence is filtered freely by the glomeruli and neither excreted nor reabsorbed by the tubules. When there is severe renal damage, a rise in blood creatinine occurs. The normal serum creatinine values between 88.4-176.8 µmol/l and protein values of total protein 7.1 (6.7-7.5) g/dl, albumin 3.4 g/dl, A: G ratio 1.0 (0.9-1.1) urea nitrogen 1.6 - 3.4 mmol/l and glucose 2.11- 3.33 mmol/l (38-60 mg/dl) in apparently healthy cattle respectively.

Bogin *et al.* (1984) reported a highly significant negative correlation between the degree of fatty change in the liver and the concentration of serum albumin.

Gerloff and Herdt (1984) stated that AST activity was the only laboratory finding that was consistently correlated with hepatic fat infiltration due to a considerable lipid infiltration in the muscle as well as the liver.

Kappel *et al.* (1984) reported first lactation animals had higher glucose concentrations than cows in second or later lactations. Plasma glucose level increased before calving and then declined to a minimum value between 11 and 22 days post partum.

Kelly (1984) reported normal Haematological values in apparently healthy lactating cattle to be AST 20-26 U/l, ALT 4-20 U/l and alkaline phosphatase 2-48 U/l, respectively.

Tainturier *et al.* (1984), in a study on dairy cows during pregnancy and after calving period, recorded that serum iron decreased at the end of pregnancy while serum creatinine increased during the last six months. While there was decrease in blood glucose, cholesterol and alanine aminotransferase concentrations at the end of pregnancy; the triglycerides increased rapidly after drying-off and serum urea increased in the first month after calving.

Waage (1984) reported that the serum activities of AST, ALT and CK were lower in the healthy cows than at first treatment in cows suffering from milk fever. At second and subsequent treatments, serum AST and ALT were higher in the cows which failed to recover.

Hejlasz (1985) observed reduction in Na, Ca and Pi with increased Mg. There was a reduction in total protein (77.9 to 65 g/l), and albumin (36.9 to 26.95 g/l) in cows with clinical parturient paresis.

Singh (1985) reported normal total serum protein values in apparently healthy Rathi cattle to be 6.9 ± 0.37 g/dl which ranged between 5.8 and 8.2 g/dl.

Ghergariu *et al.* (1986) found an increase in urea-N in summer (26.1 mg/100 ml) than winter (16.9 mg/100 ml).

Cows with moderate and severe hepatic lipidosis have lower serum insulin concentrations, a high circulating NEFA and increased BHBA concentration (Gerloff *et al.* 1986).

Jain (1986) mentioned that good quality protein and strong positive nitrogen balance is necessary to produce new haemoglobin and plasma proteins.

Prasad *et al*. (1987) studied haemato-biochemical profile of 21 normal crossbred cows in 3 physiological states (dry, above 8 months pregnant and recently calved) for prediction of downer cow syndrome. In recently calved cows, total protein was low. The values obtained in dry, advanced pregnant and recently calved cows (mean±SE) were total protein (g/dl) 8.56 ± 0.30, 8.37 ± 0.34, 6.28 ± 0.08, respectively.

Bogin *et al*. (1988) observed significant increase in serum activities of AST in cows with severe fatty liver. But there was no correlation between the degree of fatty changes in the liver and serum AST activity.

Gnanprakasam (1988) observed normal range of AST in a group of 15 apparently healthy Friesian lactating cows to be 12 - 60 IU/l.

Pandey and Parai (1988) conducted clinico-biochemical studies in 30 crossbred cows suffering from atypical form of production disease having a combined effect of parturient paresis and ketosis. The mean ± SE value of clinically ill cows for serum total serum protein (g%) was 7.56 ± 0.12 and the mean ± SE value of normal control cows was 8.30 ± 0.12 mg %.

Pandey and Parai (1989) studied physiological changes in metabolic profile of cows at calving. The change in the blood metabolic profile of cows approaching calving appears to be related to the large turnover of fluids, salts and soluble organic materials. Earlier reports also indicated that the hormonal stimulation in cows at parturition was so strong that it resulted into a serious drain on the metabolites' reserve.

As a consequence of the extensive mobilization of adipose tissue in early lactation there is a manifold rise in plasma concentration of NEFA. Non-esterified fatty acids (NEFA) are one of the most sensitive metabolites to environmental stress. The increased NEFA concentration during early lactation in cows suggests mobilization of free fatty acids (NEFA) from adipose tissue due to negative energy balance to meet energy requirements (Pullen *et al.*, 1989).

West (1989) found that the plasma AST activities in cows after calving were significantly higher when compared with non pregnant non lactating cows.

Holtenius and Hjort (1990) in their study revealed that excessively high non-esterefied fatty acid (NEFA) concentrations due to negative energy balance resulted in fatty infiltration of the liver, which was associated with higher incidence of periparturient metabolic diseases.

West (1990) stated the value of AST as a diagnostic indicator was doubtful because some of the increase in AST would have been caused by muscle damage. The plasma glucose concentration was found to be a reliable indicator to grade the severity of hepatic damage in fat cow syndrome.

Adipose tissue in the cow is oriented towards mobilisation of NEFA in early lactation rather than lipid deposition. Stressors and poor nutritional management causing decreases in voluntary DMI (Dry matter intake) will result in large increases in NEFA immediately after calving (McNamara, 1991; Ingvartsen and Andersen, 2000).

Glucose levels decreased in cows with lipidosis (Reid *et al.,* 1979 and Andrews *et al*., 1991).

Andrews *et al.* (1991) observed insulin, elevated BHBA and NEFA concentration in early lactation.

Mishra (1991) recorded mean total serum protein 7.1 g/dl in apparently healthy normal indigenous calves in and around Bikaner.

Bahga and Gangwar (1992) reported that season of calving also influence blood levels

of free fatty acids. NEFA levels were significantly higher in animals parturated in summer compared to those parturated in winter during 6 to 57 days of lactation. Highest values were obtained on day 8 postpartum in both summer and winter seasons (55.38 and 33.81 mg/100ml) which declined consistently with number of days in both the seasons.

Bertics *et al.* (1992) found that plasma NEFA concentration and triglyceride concentration in the liver are positively correlated. Non esterified fatty acid concentrations were high after calving but decreased as lactation progressed.

Argenzio (1993) reported that most of the dietary fat is in the form of water-insoluble triglycerides, and these are emulsified by the action of bile salt. Large fat globules are released slowly into the duodenum because of feedback inhibition of gastric emptying by lipids in the duodenum. Lipid digestion in the intestinal lumen requires the participation of both pancreatic and salivary secretion. The fatty acids and monoglycerides are re-esterified to triglycerides inside the epithelium. The triglycerides are then associated with cholesterol, cholesterol esters, phospholipids and small amount of protein to form chylomicron which is analogous to the water soluble micelle facilitates the transport of water insoluble triglyceride. Without the protein coat, fat is unable to leave the cell.

The gradual **in**crease **in** plasma NEFA concentrations from wk-3 to wk-1 has been suggested as a feed **in**take effect, while the rapid **in**crease **in** the immediate precalv**in**g period may be hormonally regulated (Grummer,1993).

Gautam (1994) recorded serum glucose values of normal healthy lactating crossbred cattle in and around Bikaner to be 59.60 ± 2.07 mg/dl and the values ranged between 48.0-66.0 mg/dl.

Glucose concentration found maximum at calving, the peak at calving may be related to the release of glucocorticoids immediately before calving that stimulate glycogenolysis and gluconeogenesis. The decreased glucose concentrations postpartum are probably related to low DMI, and the concomitant reduction in propionate absorption, along with an increased glucose requirement for milk synthesis (Vazquez-Anon *et al.*, 1994).

Bell(1995) estimated that **in** the immediate postpartum period, approximately 50% of circulat**in**g NEFA are either oxidized or **in**corporated **in**to milk fat. The liver plays an important role in fat metabolism, removing NEFA from the blood. In early lactating cows, about 50% of NEFA are oxidized to ketone bodies or re esterified to triglycerides in the liver. The daily demands for fetal and placental growth in the last 3 wk of gestation are 360 g of metabolizable protein and 3 to 5 Mcal of net energy.

The concentration of plasma insulin continually declines in the transition period until calving and that of somatotropin increases rapidly between the end of gestation and the initiation of lactation. Concentration of plasma progesterone, which is high in gestation, rapidly falls at calving and there is a transitory elevation in estrogens and glucocorticoids in the periparturient period. These hormonal changes not only contribute to the decline in DMI, but also coordinate the metabolic changes that favour, if not force, the mobilization of body fat reserves from adipocytes. Resulting from this mobilization of lipids, he observed an increase in concentration of plasma non-esterified fatty acids (Grummer, 1995).

Rai (1995) studied on the metabolic profile of dairy cattle with particular reference to production diseases by using CMPT, revealed decreased haemoglobin and PCV with approaching parturition. Blood glucose decreased up to parturition indicating extra energy drain by the developing foetus. Blood biochemical viz total protein (TP), albumin, globulin and blood urea nitrogen (BUN) decreased with approaching parturition due to their requirement for foetal development and colostrums synthesis, but the values increased during lactation. Aspartate aminotransferase (AST) increased due to increased metabolism as the animal approached parturition and increased with lactation, whereas alanine aminotransferase (ALT) decreased with gestation and increased during lactation.

Samanta *et al*. (1995a) reported lower total serum protein value in anaemic cattle as 7.94 ± 0.58 gm % as compared to healthy control in which the respective value was 8.24 ± 0.17 gm %.

Juneja (1996) reported biochemical values in healthy crossbred cattle in and around Bikaner. The values were: Total protein 7.61 ±0.25 g/dl (range 5.93-8.48), albumin 3.57±0.16 g/dl (range 2.80-4.52), globulin 4.04±0.19 g/dl (range 3.13-5.05) and A: G ratio 0.89±0.06 (range 0.67-1.27). His studies on hypocalcaemia reported that serum Ca, Pi, Albumin and A: G ratio decreased and serum Mg, ALT and AST activity increased. Serum total protein and globulin were not affected significantly. Further, he recorded the biochemical values for ALT (27.7±3.96 U/ml) and AST (56.2±5.12 U/ml) in healthy crossbred cattle in and around Bikaner.

Pal (1996) reported plasma levels of NEFA to be around 534 to 299 mol/l up to day 19 of lactation and declined gradually till day 54 of lactation in buffaloes to 256 mol/l.

Dairy cows undergo tremendous changes during the transition from late gestation to early lactation. Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones. For example, growth hormone (GH) is increased around parturition and in early lactation. This increases responsiveness of adipose tissue to lipolytic signals such as nor-epinephrine. The resulting increase of NEFA from adipose tissue is used as alternate fuels for much of the rest of the body (Grum *et al*., 1996).

Aspartate aminotransferase (AST) is an enzyme that is released in blood with hepatic cell damage and may be elevated in cows with fatty liver disease. Although there have been associations between AST and subsequent occurrence of displaced abomasums, the test lacks both sensitivity and specificity (Geishauser *et al*., 1997).

Goff and Horst (1997) described first two weeks of lactation as the most susceptible period for the occurrence of most of the metabolic diseases such as milk fever, ketosis, retained placenta, displacement of abomasum and infectious diseases such as mastitis.

Kaneko *et al.* (1997) reported the creatinine; a waste product of creatine is distributed throughout the body. Conversion of creatine to creatinine depends upon its total body content, which in turn depends on its dietary intake, muscle mass and its synthesis from arginine, glycine and methionine.

Itoh *et al*. (1997) found an increase in plasma glucose concentration in cold exposed (^{0}C) cows. Plasma glucose concentrations were different between the hot (79.4 mg/dl) and cold (90.5 mg/dl) environments.

Whitaker (1997) described cut-off points for both plasma NEFA and BHBA at the end of pregnancy as 0.4 mmol/L and 0.6 mmol/L, respectively and for lactating cows in early lactation as 0.7 mmol/L and 1.0 mmol/L, and the glucose concentrations above 3.0 mmol/L for transition cows.

Blood BHBA originates from either the liver (due to incomplete oxidation of fatty acids) or from absorption of ruminal butyrate, which is easily converted to BHBA. Blood BHBA concentrations typically increase after feeding because of BHBA that came from the rumen. Consistent sampling at 4 to 5 hours after the start of feeding has been suggested in order to capture peak BHBA concentrations (Eicher *et al*., 1998).

Sevinc *et al.* (1998) have showed that cows with fatty liver had higher level of GGT and AST and decreased levels of cholesterol, triglyceride, glucose, calcium, albumin and inorganic phosphorus.

Mobilization of body fat is a normal process which enables the cow to augment the energy supply as the mobilized body fat avails energy for milk production to the cow. However, excessive mobilization of body fat elevates plasma non-esterified fatty acid (NEFA) concentration and increases its uptake by the liver, thereby leading to fatty liver syndrome and ketosis (Drackley, 1999).

Gupta (1999) recorded serum glucose (mg/dl) of 10 normal healthy crossbred lactating cattle and indigenous Rathi lactating cattle in and around Bikaner to be 58.88 ± 1.03 (53.50-65.32) and 55.05 ± 1.43 (49.19 - 61.77), respectively. ALT (U/l), AST (U/l) and alkaline phosphatase (U/l) recorded in 10 healthy crossbred lactating and indigenous lactating cattle of Bikaner area to be 24.9 ± 2.13 (17-39) and 21.8 ± 1.93 (12-32); 53.8 ± 4.53 (29-72) and 48.7 ± 4.46 (28-75) and 15.4 ± 1.79 (8-25) and 18.5 ± 1.29 (11-25), respectively.

Radostits *et al*. (2000) reported that the plasma urea nitrogen are metabolic breakdown constituents and will be elevated, depending on the severity of dehydration and decrease in circulating blood volume. The normal ranges for blood urea nitrogen, creatinine, serum glucose, ALT, AST and alkaline phosphatase values to be 6-27 mg/dl, 1-2 mg/dl, 47-75 mg/dl, 11-40, 78-132, and 0-500 U/l respectively in cattle.

The measured concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) the success of adaptation to negative energy balance. NEFA reflects the magnitude of mobilization of fat from storage. BHBA indicates the completeness of oxidization ("Burning") of fat in the liver. Ketone bodies (BHBA, acetone and acetoacetate) are intermediate metabolites of oxidation of fatty acids; as the supply of NEFA to the liver exceeds the ability of liver to completely oxidize the fatty acids to supply energy, the amount of ketone body production increases. Ketone bodies can be used by muscle as an alternative fuel source to glucose, sparing glucose for milk production (Herdt, 2000a).

Herdt (2000b) reported that glucose is the primary metabolic fuel and is absolutely required for vital organ function, foetal growth and milk production. In dairy cows, the massive energy demand to support milk production is largely met through gluconeogenesis. Glucose concentrations are under tight homeostatic control. Therefore, although glucose has a central role in metabolism, it is a poor analyte for monitoring or investigating herd problems.

Glucose production in liver is controlled by the availability of substrates for glucose, the metabolic capacity of the primary cells of the liver (Hepatocyte) that synthesize glucose and hormonal status of the animal particularly insulin and glucagon. Controlled studies have demonstrated that fat infiltration in liver cells impairs their ability to synthesize glucose and to detoxify ammonia (Zhu *et al*.,

2000).

Bhuvnesh Kumar and Pachauri (2001) recorded plasma creatinine levels in 120 crossbred dairy cattle under different physiological stages viz. Non pregnant heifers in the age of 12-15 months, pregnant heifers under 4-6 months of pregnancy, empty dry cows in the age group of 5-8 years, pregnant lactating cows under 4-6 months of pregnancy, early lactating cows between 4-8 weeks of lactation yielding 8-12 litres milk/day and early lactating cows between 4-8 weeks of lactation yielding 13-18 litres milk/day. The samples were taken during May-June and December-January. All the groups showed significant seasonal variations in plasma creatinine and the values being higher in summer. This study indicated that physiological status of animals as well as season, influence the creatinine levels.

Ruminal and hindgut fermentation result in production of volatile fatty acids (VFA), with propionate being the major gluconeogenic precursor. Propionate contributes an estimated maximum of 32 to 73% to hepatic gluconeogenesis, whilst amino acids from dietary intake and skeletal protein mobilization contribute 10 to 30%. However, propionate supply during the transition period is limited (Seal and Reynolds, 1993; Drackley *et al.*, 2001).

Jorritsma *et al.* (2001) studied in 218 dairy cows in nine dairy herds to find out the prevalence of post-partum fatty infiltration in the liver and its relationship to subsequent body condition scores, blood variables and milk production. The prevalence of fatty liver was 54.1%. Serum non-esterified fatty acids, urea and blood glucose concentrations appeared to be significant indicators of hepatic lipidosis between 6 and 17 days post partum (R^2 0.33). High milk production and large losses of body condition score (BCS) in early lactation were significant indicators of hepatic lipidosis from a retrospective point of view (R^2 0.22). It was concluded that fatty liver seems to be fairly common in early lactating dairy cows.TAG in the liver than combined measurements of blood glucose, serum urea and NEFA concentrations or the observation of milk production and BCS loss. This is probably useful as the first approach for diagnosing fatty liver herds in practice. Biopsy is more accurate, but also more invasive and effectivein diagnosing high TAG concentrations in the liver.

Serum bile acid, GGT, AST and albumin concentration were helpful for liver function test in cows with fatty liver (Sevinc *et al.*, 2001).

Singh (2001) observed correlation of albumin with postpartum disease and hence can be used to predict disease risk in close-up and fresh periods (Fresh cows that could maintain serum albumin concentrations $\geq$ 35 g/l were less likely to have postpartum disease. Serum albumin concentrations $\leq$ 32.5 g/l in close-up dry cows resulted in a three-fold greater risk for postpartum disease. In spite of concerns about variables confounding albumin interpretation, it seems to be a good disease risk indicator, possibly reflecting availability of amino acids from the labile protein pool.

Advancement in the field of genetics and breeding for maximizing milk production completely ignored the fundamental laws of physiology and animals are considered to possess unlimited production potentials. There is always the danger of crossing the barriers of physiological limits in an animal in most upgrading activities. Health and performance management systems for dairy animals focus on the early identification and subsequent prevention of production diseases by either treating the affected animals or by improving the herd diet (Enjalbert *et al.*, 2001; Ingvartsen *et al.*, 2003).

Buckley *et al.* (2003) reported that only 30% of cows with a BCS of 3.25 at calving lost more than 0.5 units of BCS in early-lactation, while 50% of cows with a BCS of 3.5 at calving lost 0.5 units of BCS or more in early lactation. Therefore, the higher the BCS at calving, the more BCS will be lost

in early-lactation. It is important to remember that this BCS loss means that the liver is presented with large quantities of nonesterified fatty acids for metabolism and the liver of the dairy cow is not capable of metabolising large quantities of fat.

Ingvartsen *et al.* (2003) observed that over-conditioned dry cows are more likely to suffer from ketosis and fatty liver, both of which may suppress immunity directly or through an excessive negative energy balance route. Glucose concentrations decrease at parturition and are lower in the first weeks of lactation than before calving or later in lactation.

Kida (2003) reported that an increase in NEFA concentration directly indicated a negative energy balance and on the other hand, no significant relationship between NEFA and total digestible nutrients was observed in the milking cows.

Kim and Suh (2003) investigated the effect of body condition loss from the dry to near calving periods on the subsequent body condition change, the occurrence of postpartum diseases (including abomasal displacement, milk fever, ketosis). Cows were categorized based on body condition loss from the dry to near calving periods into two groups. The triglyceride, glucose, and urea nitrogen concentrations did not differ ($P > 0.05$) from the dry period to month 4 of lactation between the two groups.

Osbome (2003) evaluated 136 transition cows, 24 had BHBA concentrations ≥ 1400 μmol/L of serum in the first week post-calving (17.6%). There was a significant association between NEFA concentration in the week prior to calving and BHBA concentration in the first week post-calving. A nearly 5-fold increased risk of subclinical ketosis was noted when the NEFA concentrations in the week before calving were greater than 0.7 mmol/L (*OR*=4.8, *P*=0.04).

Towards lactation the negative energy balance is already initiated due to a decline in dry matter intake together with an increased energy demand of the fetus. Leptin levels reflect the energy balance of the cow. Adipose lipogenesis is essentially shut down, and the sensitivity to lipolytic signals (epinephrine and norepinephrine) is greatly enhanced. Increase in blood NEFA in response to an intravenous epinephrine challenge was significantly greater in early lactation compared to any other stages in lactation (Hayirli *et al*., 2002; Liefers *et al*., 2003; Theilgaard *et al*., 2002; Underwood *et al*., 2003).

Bobe *et al*. (2004) opined that diagnosis of fatty liver is possible only by minor surgery. Ultrasonic techniques offer a potential non-invasive tool to detect fatty liver. Future gene-array and proteomic studies may provide means to detect early molecular events in the pathogenesis of fatty liver plus their connection with immune function and reproductive performance so that more effective treatment and preventative measures for fatty liver can be developed. Such advances hopefully will make the fatty liver, a problem of the past.

Carrier *et al.* (2004) evaluate the performance of 3 cowside diagnostic tests for detection of subclinical ketosis, defined as a serum β-hydroxybutyrate (BHBA) concentration ≥1400 μmol/L. On average, use of the Ketostix at the "small" cut-off point or the KetoTest at 100 μmol/L would result in no more than 3 or 4 false positives per 100 cows screened, with prevalence levels ranging from 5 to 30%, whereas the number of false negatives would range from one false negative at 5% prevalence to 7 or 8 false negatives at 30% prevalence. Finally, given their relative imprecision, use of any of these individual cowside tests to estimate herd prevalence must be done cautiously, especially when only a small number of animals are sampled.

Duffield (2004) revealed that elevated pre fresh nonesterified fatty acids (NEFA) concentration (≥ 0.4 mEq/L) and post fresh β-hydroxybutyrate (BHB) concentration (≥14.4 mg/dl)

were recognized risk factors for ketosis and left displacement of abomasum.

Nielsen and Ingvartsen (2004) reported propylene glycol (PG) increases insulin by 200–400% within 30 min after drenching, indicating that PG is absorbed rather quickly. Allocation of PG also increases plasma glucose, although the response is limited, probably because of the large increase in insulin. PG decreases plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB), especially in early lactating cows with relatively high levels of NEFA. PG also reduces the triacylglycerol (TG) content of the liver and the concentrations of ketone bodies in milk and hence, has anti-ketogenic properties.

Oetzel (2004) studied 18 to 35% of cows have NEFA >0.4 mmol/l in the last week before calving. He suggested an "alarm" threshold of 10% prevalence of subclinical ketosis, based on serum BHB. Prevalence of subclinical ketosis was found around 15% studied in Canada in comparison to average prevalence of 20%. Adjusting for cow-side test performance, a threshold of 10% true prevalence of subclinical ketosis corresponds to an apparent prevalence (proportion of tests that are positive) of 25% when using the Keto-Test with a 100 μmol/l cut-point, or 11% at the 200 μmol/l cut-point.

Van saun (2004a), in a study to establish diagnostic relationship between prepartum blood metabolite concentrations and postpartum health status, revealed that all the sick cows had lower albumin, BUN, glucose and cholesterol and higher AST, BHBA and NEFA compared to healthy cows in the fresh period. Cows, with close up (CU) period albumin concentrations < 3.25 g/dl and fresh (FR) period < 3.30 g/dl were 1.46 and 1.79 times more likely to experience a disease event. Within fresh cows, cholesterol concentrations increased with increasing albumin concentrations. Similarly, the cows in which NEFA values were >0.4 mEq/l in either CU or FR samples, were 1.57 and 1.47 times more likely to have a disease event, respectively and the disease risk was greater if NEFA concentration was >0.6 mEq/l at CU and FR periods.

Van Saun (2004b) studied B-hydroxybutyrate (BHB) concentration is most commonly used for ketosis. Concentrations of BHB < 26 mg/dl and > 14.5 mg/dl represent animals with subclinical ketosis. The concentrations ≥ 26 mg/dl are defined with clinical ketosis. Prior to calving, BHB concentrations generally do not exceed 6-8 mg/dl, unless the animal is in negative energy balance or consuming ketogenic silage. Following calving, BHB concentrations can become greatly elevated. Cows with BHB concentrations above 10 or 14 mg/dl are 3.2 and 4.3 times at greater risk for postpartum disease.

Castillo *et al.* (2005), in a study on dairy cows during late pregnancy and early lactation in healthy cows revealed that four parameters (Serum glucose, NEFA, albumin and alkaline phosphatase) did not differ significantly between late lactation (LL) and pregnant (P) at any stage, and remained roughly constant in the P-cows group. Mean serum triglyceride content showed an apparent increase in the P-cows group in the week before calving, and dropped after calving, whereas cholesterol levels showed an apparent declining trend between six weeks before calving and one week after calving. Mean serum urea content dropped significantly two weeks before calving and the mean serum creatinine content was most of the time significantly higher in P cows than in LL cows; Mean serum AST level showed an increasing trend in P cows, with values at one and two weeks after calving being significantly higher than previous values and/or the LL value.

The ketone bodies i.e., BHB, acetoacetate, and acetate, are formed by incomplete oxidation of NEFA in the liver. Another source of BHB in blood is ruminal butyrate that is oxidized to BHB in the rumen wall. Ketogenesis is part of the normal energy metabolism in ruminants. If glucose

concentrations are low, more ketone bodies are produced in the liver to meet the energy needs of body tissues. Concentrations of BHB increase pp and peak 2–4 weeks pp (Cavestany *et al*., 2005; Ingvartsen *et al*., 2003).

Dann *et al.* (2005), determined the effect of pre partum intake, postpartum induction of ketosis, and periparturient disorders on metabolic status in dairy cows whereby they found that pre partum intake did not affect postpartum metabolic status or milk yield. However cows in which ketosis was induced, had lower intake, milk yield, and serum glucose concentration but higher concentrations of non esterified fatty acids and β-hydroxy butyrate in serum and total lipid and triacylglycerol in liver in comparison to control cows.

Kokkonen *et al.* (2005) reported increased level of NEFA in lactating cows as compared to non-lactating cows. Plasma NEFA concentrations were in the range of 100 to 2000 µeq/litre in cows and were low in low producing cows.

High level of NEFA >0.4 mmol/L in the last 7-10 days before expected calving is associated with 2-4 times increased risk of left displacement of abomasum, 2 times increased risk of retained placenta, 2 times increased risk of culling before 60 days in milk and 1.5 times increased risk of culling over the whole lactation (Leblanc *et al*., 2005).

The increase in NEFA is part of the physiological response necessary to meet higher energy demands at the onset of lactation, but excess NEFA concentrations are detrimental; for example, high NEFA concentration is toxic to peripheral tissue and negative affects fertility (Adewuyi *et al*., 2005; Leroy *et al*., 2005).

Stojevic *et al.* (2005) examined the activities of aspartate aminotransferase (AST), alanine aminotrans-ferase (ALT) and gamma-glutamyltransferase (GGT) in the plasma of 120 dairy HF cows. Animals were divided in 4 groups. The first, second, third and fourth group consisted of animals from the 10^{th} - 45^{th}, 46^{th}- 90^{th}, 91^{st} day of lactation until the end of milk production. Cows in the dry period comprised the fourth group. The highest activity of AST was determined in the first production period, while enzyme activities in the second and third periods were higher than in the dry period. ALT activity showed a statistically significant increase from the 46^{th} day of lactation until the dry period, and activity in the second and third periods were statistically higher than in the dry period. GGT activity in the first production period and in the dry period was statistically higher in comparison with the second and third periods.

The serum creatinine level was also significantly affected by the physiological phase and showed the higher levels during the late pregnancy and early lactation. It is recognized that during the late gestation, the mother, for the foetal maternal circulation, assumes the load of organic waste of the newborn. So, the increase in serum creatinine levels could be attributed to development of the foetal musculature, which is well documented in sheep and ewes too (Roubies *et al*., 2006).

Mordak and Nikpon (2006) compared the values of selected blood parameters in 30 clinical healthy cows in periparturient period that did not develop any pathological signs of disturbance just before, during or after calving. They found significant differences in the value of total bilirubin, SGOT, inorganic phosphorus, calcium and chloride before and after calving. However, large differences were observed in the values of total proteins, glucose, and creatinine without any significant difference.

Kalaitzakis *et al.* (2006) observed a strong correlation between serum AST activity and triglyceride and total lipid concentration, serum bile acids, total bilirubin in cows with fatty liver. The authors also stated that there were no changes in serum ALP activity.

Blood concentrations of NEFA, beta-hydroxybutyrate (BHB), glucose, insulin, and

cholesterol have been associated with energy metabolism in dairy cows and may be used as indicators of NEB (Macrae *et al*., 2006; Oetzel, 2004; Agenas *et al*., 2003; Ingvartsen *et al.,* 2003; Kim & Suh, 2003; Kronfeld, 1972).

LeBlanc *et al.* (2006) reported elevated prefresh nonesterified fatty acids (NEFA) concentration (≥ 0.4 mmol/L) and post fresh β-hydroxybutyrate (BHB) concentration (≥1200-1400 μmol/L) are recognized risk factors for ketosis and left displaced abomasums.

Kalaitzakis *et al.* (2007) found that the serum biochemical variables have relatively high negative predictive value, since the enzyme activity has low positive predictive value.

Kanna (2007) reported glucose levels were significantly (P<0.1) higher in high BCS than medium BCS cows (54.03 ± 3.02 vs 43.88 ± 2.33 mg/dl).

Radostits *et al.* (2007) mentioned a mini metabolic profile test which measures levels of blood glucose, serum urea nitrogen and albumin in cows between 4 and 10 weeks after calving that can serve as a sufficient test to assess the adequacy of energy and protein intake. The sampling were done at intervals of 4-6 weeks. The time of sampling can affect the results. The values will change with the season and stage of lactation.

Valde *et al*. (2007) found that cows in a fatter condition at calving lost more BW and body condition over a longer period of time than cows in a thinner condition at calving.

Glucose uptake in cells is mediated by glucose transporters. Glucose uptake in skeletal muscle, adipose tissue, and cardiac muscle is dependent on insulin, but in the udder, glucose uptake occurs independently of insulin (Zhao & Keating, 2007a; Zhao & Keating, 2007b; Joost & Thorens, 2001).

Cholesterol is secreted into the blood stream as VLDL, and low concentrations of cholesterol may impair the transport of TAG from the liver. Cholesterol concentrations are low at calving and increase slowly over the first weeks after calving (Cavestany *et al*., 2005; Van Knegsel *et al*., 2007).

Kaneko *et al.* (2008) studied glutamate dehydrogenase (GD), an enzyme present in hepatocyte mitochondria, is necessary for urea synthesis in the conversion of glutamate to alpha ketoglutarate. In cattle, GD is liver specific and used to detect liver cell damage. When there is cell damage or necrosis of fat infiltrated hepatocytes, the enzyme can leak into the blood stream

and increased serum activity of GD may be detected. Increased GD concentration has been associated with hepatic lipidosis.

The metabolic diseases of the dairy cows are manifestation of the cow's inability to cope with the metabolic demands of high production and they continue to be a cause of economic loss to the dairy industry and an animal welfare concern (Mulligan and Doherty, 2008).

Stengarde *et al.* (2008) conducted metabolic profiling in five high-producing Swedish dairy herds having history of abomasal displacement and ketosis and found that all herds had over conditioned dry cows that lose body condition substantially during the first 4–6 weeks postpartum, two herds had elevated levels of NEFA prepartum and three herds had elevated levels postpartum, while one herd had low levels of insulin prepartum and low levels of cholesterol postpartum.

Djokovic *et al.* (2009) reported that glucose levels were significantly lower (P<0.01) in the puerperal cows than in the cows examined during the maximum lactation period (90-100 days), which suggested a decreased gluconeogenesis in the liver. Significantly lower blood levels of total protein (P<0.01), albumin (P<0.01), urea (P<0.01) and triglyceride (P< 0.05) were recorded in the puerperal cows, which suggested the reduced synthetic capacity of liver cells during early lactation in cows. Blood

bilirubin levels in the puerperal cows were significantly higher (P<0.05), which clearly indicated the reduced excretory capacity of the liver. Significantly increased (P<0.01) AST, GGT and LDH activities in the blood in the puerperal cows suggested the disturbed morphological and functional integrity of liver cells and the release of these intracellular enzymes into the blood. Duffied *et al.* (2009) reported subclinical ketosis (BHB > 1200 to 1400 µmol/l) in the first or second week after calving is associated with 3 to 8 times increased risk of left displacement of abomasums (LDA), 3 times greater risk of metritis when serum BHB in week 1 was > 1200, 4 to 6 times increased risk of clinical ketosis increased probability of subclinical endometritis at week 4 postpartum and increased duration and severity of mastitis but not with the incidence of mastitis. Milk yield at first test was reduced by 1.9 kgs /day when BHB was >1400 µmol/l in week 1 and by 3.3 kgs /day when BHB was >2000 mol/l in week 2. Cows with serum BHB >1800 µmol/l in week 1 had >300 kgs lower projected production for the whole lactation.

Hammon *et al.* (2009) studied the elevated liver fat content in high-yielding dairy cows during the transition period. They observed that high fat mobilization, high liver fat content, and severe NEB in high-yielding dairy cows may not always be a consequence of impaired farm management, but might be a consequence of individual cow factors that cannot be easily alleviated by the farmer when using feeding management. Individual cow factors were responsible for differences in energy metabolism during the transition period.

Iwersen *et al.* (2009) determined the diagnostic performance of an electronic β-hydroxybutyrate (BHBA) hand-held meter (Precision Xtra) for ketosis in dairy cattle. Specific objectives were to compare the electronic BHBA meter with serum BHBA concentrations determined photometrically and 2 commonly used chemical cow side tests (Ketostix, Ketolac) and to evaluate accuracy. The Precision Xtra test is a useful cowside ketone test for detection of subclinical ketosis in postpartum dairy cows. Using whole blood and a cut off value of ≥1,400 µmol of BHBA/L of blood, the Precision Xtra test achieved excellent test characteristics and a higher diagnostic performance than 2 chemical dipsticks.

Quiroz-Rocha *et al.* (2009), in a study to establish the reference limits of laboratory analytes in normal transition cows, revealed that all biochemical analytes (BHBA, fatty acids, glucose, cholesterol, urea, calcium and phosphorus) were statistically different between pre calving and post calving groups. However the hematological analytes were not significantly different except for eosinophils.

Oxidative stress resulting from increased production of free radicals and reactive oxygen species (ROS), and/or a decrease in antioxidant defence, leads in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which, in turn, may be related to various health disorders (Trevisan *et al.*, 2001; Abd Ellah, 2010).

Djokovic *et al.* (2010) reported significantly lower (P<0.05) blood levels of triglyceride, total protein, albumin and urea in the puerperal cows, which suggested the decreased synthetic capacity of liver cells. Blood bilirubin levels were significantly higher (P<0.01) in the puerperal cows than in the late pregnant cows clearly indicating the decreased excretory capacity of the liver. Blood calcium, phosphorus and magnesium levels in the postpartum cows were lower (P>0.05), suggesting a reduced supply of these minerals from alimentary sources and/or increased utilization by the mammary gland.

Serum cholesterol concentration was significantly decreased in cattle with moderate and severe fatty liver compared to the healthy cows and cows with mild fatty liver. The serum cholesterol concentration was inversely related to NEFA concentrations means decreased serum cholesterol, higher

NEFA, and higher NEFA/cholesterol ratio were recorded in cows with fatty liver (Holtenius, 1989; Kalaitzakis *et al*., 2010).

LeBlance (2010) observed high NEFA (> 0.4 mmol/l) in the last 7 to 10 days before expected calving is associated with increased risk of displaced abomasum (DA), retained placenta, culling before 60 days in milk, and less milk production in the first 4 months of lactation. Subclinical ketosis (Serum BHB > 1200 to 1400 μmol/l) in the first or second week after calving is associated with increased risk of DA, metritis, clinical ketosis, endometritis, prolonged postpartum anovulation, increased severity of mastitis, and lower milk production in early lactation. There are several validated and practical tools for cow-side measurement of ketosis.

Ospina *et al.* (2010) elevated concentrations of NEFA and BHBA in the transition period predicted clinical disease in dairy cow. The standard value of NEFA concentrations is ≥0.3 mEq/L for cattle, 14 to 2 day pre partum and NEFA concentrations ≥0.6 mEq/L and BHBA ≥10 mg/dL for cattle 3 to 14 day postpartum. Both pre- and postpartum NEFA concentrations and BHBA concentrations above these critical thresholds were associated with increased risk for subsequent disease (e.g., DA, CK, MET or RP).

Ketosis commonly results either from the lack of sufficient glucose precursors available for energy production or from a reduced gluconeogenic capacity by the liver and it is characterized by elevated concentrations of the ketone bodies acetoacetate, acetone and β-hydroxybutyrate in the blood, milk and urine. Serum β-hydroxybutyrate concentrations are typically dichotomized to distinguish between normal and hyper ketonemic cattle, with frequently recommended cut points of 1.000 to 1.400 μmol/L (Rollin *et al.*, 2010).

Stengarde (2010) studied the cows with displacement of abomasum displayed blood profiles indicating a severely altered energy metabolism (NEFA, BHB, insulin, cholesterol, RQUICKI), liver cell damage (AST, GD) and inflammatory responses (haptoglobin). At the herd level, energy markers (NEFA, insulin, glucose, cholesterol, RQUICKI) indicated altered metabolism in cows in high-incidence herds compared with cows in low-incidence herds. The markers of liver cell damage and inflammation were not different between high and low-incidence herds. Among high-incidence herds, BCS and change in BCS, and one metabolic marker (NEFA) were found most useful to pin point herd problems. Large herd size, high individual milk production level, keeping all dry cows in one group, and not cleaning the feeding platform daily, were found to be risk factors for a high incidence of DA or ketosis at the herd level.

In a study, Tripathi *et al.* (2010) observed that serum total cholesterol, HDL cholesterol, triglycerides, phospholipids increased from early to mid stage of lactation and then decreased from mid to late stage of lactation, while LDL cholesterol increased with advancing stage of lactation. Serum NEFA differs significantly with decreased level during mid lactation. The correlation between serum lipid profile with milk fat per cent was non-significant during all stages of lactation.

Zhang *et al.* (2010) found significantly high BHBA and NEFA levels and lowered glucose levels in cows affected with subclinical ketosis than healthy dairy cows. Likewise, significantly decreased serum concentrations of Zn was observed in dairy cows with subclinical ketosis and no significant difference was observed for serum Cu concentration between healthy and sub clinically ketotic dairy cows.

Asl *et al.* (2011), in their study to determine the cut off point for NEFA and glucose concentrations for diagnosis of sub clinical ketosis, found that the optimal cut off point of >0.26 mmol/L for NEFA, and <2.26 mmol/L for glucose with corresponding 82.54 per cent sensitivity and

91.89 per cent specificity for NEFA and 44.44 per cent sensitivity and 78.38 per cent specificity for glucose.

Cozzi *et al.* (2011) measured concentrations of a variety of blood-based markers in 740 Holstein cows in 33 dairy herds. They reported significant herd variance components for albumin as well as parity and season of production effects on total protein and globulin; however detailed study of diets or management practices was not conducted in their survey.

Davasaztabrizi (2011) revealed that NEFA, glucose and cholesterol serum value in anoestrous cows were 0.8±0.185 mmol/l,36.21±4.28 mg/dl and 167.13±15.42 mg/dl and in cyclic cows were 0.726±0.15 mmol/l, 38.43±4.47 mg/dl,162.53±33.02 mg/dl respectively. The T-test at the 95% confidence level demonstrated that there were non-significant difference between anoestrous and cyclic cows in NEFA, Glucose and Cholesterol serum values.

The values of AST were statistically higher ($P<0.05$) in early lactation in cows than in late pregnant cows and no significant difference ($P>0.05$) was observed between GGT activities in the two groups. Given that AST activity higher than 100 U/L is indicative of hepatic lesions, 2 early lactation cows (13.3%) in the study suffered from some degree of hepatic lesions, probably due to fat infiltration. These animals included 2 out of 7 cows considered to be ketotic and had blood NEFA values above 0.70 mmol/L. Meanwhile, none of the late pregnant cows had AST values higher than 100 U/L. A positive correlation between AST activity and lipomobilization (NEFA values) was observed by the significance coefficient ($r = 0.34$, $P<0.05$). In this study, the data regarding liver enzymes suggested that the process of lipomobilization was sufficient to cause liver lesions in 13.3% of the early lactating cows (Gonzalez *et al.*, 2011).

Piccione *et al.* (2011) measured serum protein fractions in cows during the late pre partum and early postpartum periods and determined that serum total protein concentrations decreased from the pre partum period to wk 1 postpartum, with decreased concentrations of globulins largely responsible for the decline in total protein concentrations.

Rezaeisaber *et al.* (2011) evaluated fatty liver syndrome in dairy cattle in Tabriz by measurement of NEFA and TG serum values. TAG aggregation in liver in last month of pregnancy had occupied more than 5% liver cells and amount of NEFA was more than 900 meq/lit, being nonspecific and some other reasons.The results showed that NEFA had a direct relationship with TG. Thus, with elevation of NEFA serum values, TG also increases.

Chapinal *et al.* (2012) sampled 45 herds and found that herds in which > 30 per cent of multiparous sampled animals had pre-partum NEFA concentrations > 0.5 mEq/L produced 3.0 kg less milk per day per cow and when > 50 per cent of multiparous cows had pre-partum NEFA concentrations >0.5 mEq/L, the odds of pregnancy to first insemination decreased by 50 per cent. In addition, when post-partum NEFA concentrations were > 1.0 mEq/L in > 30 per cent of cows, the odds of pregnancy to first insemination decreased by 40 per cent.

Kataria and Kataria (2012) reported that serum gamma glutamyl transferase enzyme as a biomarker of stress and metabolic dysfunctions in *Rathi* cattle of arid tract in India. The normal range in healthy animals was from 12 to 34 U/L. In affected group an average 23.69 times rise in the value was observed from that of healthy group.

Piccione *et al.* (2012) carried out experimental on five clinically healthy dairy cows (HF) on the basis of their pregnancy and lactation status. Blood samples were collected two days before the expected parturition (Late gestation), during the post partum, in early lactation, during the 2^{nd}, 5^{th} and 15^{th} week after parturition, at the end of lactation and at the dry period. On each serum sample urea,

creatinine, total proteins, albumin, total cholesterol, triglycerides, NEFA, β-hydroxybutyrate, total and indirect bilirubin, calcium, phosphorus and magnesium were determined. A significant effect of the physiological phase was observed on urea, creatinine, total proteins, total cholesterol, triglycerides, NEFA, β-hydroxybutyrate, calcium and phosphorus. It is concluded that the lactation period is the more sensible, by a metabolic point of view, for the high production dairy cow.

Ping Liu *et al.* (2012) reported the effects of post partum enzymes metabolic status in Holstein cows on 1 week pre partum (week 1), days delivery (week 0) and 1- 9 weeks postpartum (week 1-9). They were analyzed for Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT) and Alkaline Phosphatase (ALP) activities. The results showed a higher activity of AST in cows during the 1-3 weeks than others. ALT activity indicated a statistically significant increase from the 5-7 weeks of lactation and activity in the 7^{th} week postpartum periods significantly reached to the peak. GGT activity in the ante partum 1 week until delivery day was significantly lower in comparison to the first to reach the 9^{th} weeks postpartum. ALP activity in the delivery day and 6-8 weeks were significantly higher. Therefore, the activities of AST, ALT, GGT and ALP could be significantly change in the blood plasma of Holstein cows.

Roberts *et al.* (2012); study the elevated serum NEFA or BHBA concentrations and lower serum calcium concentrations from 1 wk before calving to 2 wk after calving were associated with an increased risk of being culled within the first 60 DIM (DAY IN MILK). When all metabolites were analyzed together, serum NEFA and calcium concentrations in wk −1 and serum NEFA concentration in wk +1 remained in the models. Measuring the concentration of selected metabolites around parturition may help to develop monitoring and intervention strategies to prevent early culling in transition dairy cows. Serum NEFA and calcium

concentrations in the week before calving in combination were associated with an increased risk of culling.

Saber *et al.* (2012) measured NEFA, APO-A, Ammoniac, TSH and Total Bilirubin serum values in fatty liver syndrome. The results showed that NEFA has a positive relationship with ammoniac and total Bilirubin serum values and reverse relationship with APO-A and TSH. Thus, with elevation of NEFA serum values, ammoniac and total Bilirubin also increased and on the contrary, TSH and APO-A diminished.

Ster *et al.* (2012) indicated that the increase in circulating NEFA impairs PBMC functions. The Peripheral blood mononuclear cells (PMNL) oxidative burst seemed less sensitive to NEFA, but may be affected by very high levels. Therefore, management approaches that decrease the negative energy balance and the increase in NEFA at the beginning of lactation, such as shortening the dry-off period, improving the diet during the transition period, or limiting milk production in the first days of lactation will likely improve resistance to infection. Several immune cell functions appear affected by the NEFA concentration. Therefore, strategies that prevent increases in blood NEFA during the transition period may limit post partum immunosuppression.

Zhang *et al.* (2012) observed that Ketosis is a common metabolic disorder frequently in dairy cows during the early lactation period. It is characterized by increased levels of ketone bodies in the blood, urine, and milk. Subclinical ketosis (SCK) in dairy cattle is an excess level of circulating ketone bodies in the absence of clinical signs of ketosis. Usually, detection of SCK is carried out by testing the ketone concentrations in blood, urine, and milk. The Ketolac BHBA test strip (with a cut-off threshold of 200 mM of BHBA in milk) is potentially useful tools for the routine monitoring of SCK in

early postpartum dairy cows.

Djokovic *et al.* (2013) reported that Cows in early lactation had significantly higher levels of serum BHB and NEFA, and lower glycaemia compared to the late pregnant cows. High lipomobilization (NEFA>0.4 mmol/L) was detected in 6 (40%) of early lactation cows but in none of the late pregnant cows, while subclinical ketosis (BHB>1.2 mmol/L) was detected in 14 (94.4%) of the early lactation cows and 4 (26.6%) of the late pregnant cows. AST activities above 100 U/L were detected in 2 early lactation cows and in none of the late pregnant cows. TG levels below 0.12 mmol/L and glucose below 2.5 mmol/L were found in 7 (44%) and 10 (66.6%) of the early lactation cows, respectively, and in none of the late pregnant cows. Early lactation cows were found to have lower blood serum levels of TG, albumin urea and GTT activities and higher concentrations of total bilirubin TP and AST activities compared to the late pregnant cows. The results of blood serum levels of glucose, TG, BHB, NEFA and AST in early lactation cows suggest metabolic disorders associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

Ilic *et al.* (2013) evaluated the metabolic status of early and mid-lactation in dairy cows through changes in blood biochemical indicators. Blood samples were collected to measure betahydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Early lactation cows had significantly higher ($P<0.05$) values of blood BHB and NEFA, and lower glycemia ($P<0.05$) and TG ($P>0.05$) values compared to mid lactation cows. High lipomobilization (NEFA > 0.4 mmol/l) and subclinical ketosis (BHB > 1.2 mmol/l) were detected in 6 (40%) and 14 (94.4%) early lactation cows, respectively, and in none of the mid lactation cows. AST activities above 100 IU/l were detected in 2 early lactation cows and none of the mid lactation cows. TG concentrations below 0.12 mmol/l were found in 7 (44%) early lactation cows and 2 (13.3%) mid lactation cows. Glucose levels were below 2.5 mmol/l in 10 (66.6%) early lactation cows and 5 (33.3%) mid lactation cows. Blood serum values for glucose, TG, BHB, NEFA and AST showed that early lactation cows suffered from metabolic disturbances, which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. They suggested that NEFA was useful indicators of the metabolic status of dairy cows during lactation.

Rezaeisaber *et al.* (2013) measured the NEFA, LDH and BHBA serum values in early lactation in hepatic lipidosis. In this inspection, age, body condition score and Pregnancy status of animals was investigated. At the time of testing, sera were defrosted and NEFA levels in serum by Randox kit and Auto analyzer were measured. In this study, levels of LDH and BHBA were measured by Pars test kits and by spectrophotometric method. Results showed that based on Pearson's Correlation index there is a direct correlation between NEFA and LDH and BHBA serum values. This index indicates a direct effect of NEFA on LDH and BHBA serum values. Thus, with elevating of NEFA serum values, the LDH and BHBA values also increased.

Wilson and Goodell (2013) measured Beta-hydroxybutyrate (BHB) in blood or milk of dairy cattle after calving for detection of ketosis. Results show that the BHB test methods agreed well for most non-ketotic cows, but tests did not agree well on classification of ketotic cows. Calibration improvements are a priority for improved Fossomatic testing of BHB in milk.

5. Summary and Conclusions

The present study was undertaken to assess the status of minerals, biomarkers of oxidative stress and energy status during transition period of dairy cows in Bikaner, Jodhpur and Pali-Marwar districts of western Rajasthan. A total 123 cows in transition period were sampled during the study period. Out of 123 dairy cows 94 were found healthy and 29 in subclinical ketotic. The energy status was assessed by estimating like NEFA, BHBA and glucose. Minerals viz. Ca, Pi, Mg, Na, K, Cu, Fe, Zn and oxidative stress in term of reduced glutathione, malondialdehyde and other biochemical changes were also estimated. A field therapeutic trial was conducted to determine the effect of propylene glycol (PG) in subclinical ketosis dairy cows.

- The overall prevalence of subclinical ketosis was 23.57 percent with a highest 26.08 percent in Pali-Marwar district followed by Bikaner and Jodhpur . Overall deficiency of Ca, Pi, Cu and Zn in cows were 34.15, 40.65, 33.33 and 2.43 per cent, respectively.
- The overall mean level of plasma glucose, BHBA, NEFA, total protein, BCS recorded were 56.293±0.611 and 42.768±0.937 mg/dl; 0.753±0.030 and 1.289±0.042 mmol/l; 0.262±0.010 and 0.683±0.016 mmol/l; 6.816±0.046 and 7.221±0.071 g/dl; 3.392±0.029 and 2.982±0.044 in healthy and subclinical ketotic cows, respectively.
- The overall mean plasma cholesterol, triglycerides levels were recorded as 95.543±1.313 and 4.241±2.013 mg/dl; 11.591±0.438 and 17.098±0.672 mg/dl in healthy and subclinical ketotic cows, respectively.
- The overall mean plasma AST and GGT levels were 53.393±1.062 and 60.185±1.628 IU/L; 26.480±0.809 and 31.232±1.240 IU/L in healthy and subclinical ketotic cows, respectively.
- The overall mean of GSH, MDA levels were recorded as 2.423±0.071 and 2.811±0.109 mg/dl per gm Hb and 38.813±0.729 and 49.753±1.118 nmol/ml per gm Hb in healthy and subclinical ketotic cows, respectively.
- The average milk yield among the healthy cows was 21.873±0.372 litre/day from the 3rd to 5th parity and 20.478±0.615 litre/day from above 5th parity; which is almost similar in both the parity in healthy groups. In subclinical ketosis cows average milk production were less to the extent of 15.50±0.51 and 16.818±0.79 litre/day in 3rd to 5th parity and more than 5th parity, respectively.
- The overall mineral plasma calcium and copper concentration were 10.095±0.095 and 8.876±0.146 mg/dl; 0.754±0.014 and 0.633±0.021 ppm in healthy and subclinical ketosis cows, respectively.
- A highly significant ($P \leq 0.01$) difference was observed in the overall mean glucose, BHBA, NEFA, cholesterol, triglyceride, total protein, AST, GGT, GSH, MDA, BCS, Ca, Cu and milk production and significant difference ($P \leq 0.05$) was observed in creatinine, globulin, potassium levels of healthy and SCK dairy cows .

- Circulating concentrations of energy-related metabolites (BHBA, NEFA and Glucose) were highly associated with postpartum outcomes related to disease and milk production in dairy cows. NEFA concentrations in plasma can provide additional insight into transition management opportunities.
- The results suggested that, a cut-off point of < 0.40 mmol/L for NEFA and BHBA > 0.6 mmol/L concentrations can be used during early lactation (pre partum) for diagnosis of subclinical ketosis and making management decisions for prevention and treatment. Glucose may not be a good

criterion for diagnosis of SCK. Reference ranges for NEFA and BHBA need to be established for Western Rajasthan conditions.

- Highly Significantly ($P \leq 0.01$) lower plasma glucose and total protein and higher BHBA, NEFA, AST and GGT levels were observed in cows suffering from SCK as compared to healthy cows.
- Marginal deficiency of Ca, P, Cu and Zn was recorded in dairy cows. However, no deficiency of Fe, Mg, Na and K was recorded in transition period.
- Significant correlations were observed among glucose, NEFA, BHBA, MDA, BCS, AST, GGT in subclinical ketotic cows.
- Our findings suggested that elevated concentrations of biomarkers related to stress (MDA) during the transition period were also associated with decreased milk yield in post partum transition period (early lactation).
- Propylene glycol @200 ml/ day given orally for 5 days was effective in decreasing plasma NEFA and BHBA levels, indicating its effectiveness for treatment of subclinical ketosis.

In general, BHBA was found to be a better predictor of negative downstream outcomes, easy and comparative inexpensive to measure accurately cow-side if the appropriate test is chosen. Therefore, BHBA should be used as the primary monitoring tool.

It is suggested that in conjunction with BHBA or NEFA monitoring, it is necessary to continuously evaluate other herd-level factors that increase the risk of excessive NEB, such as overcrowding, lack of heat abatement, excessive pen moves, excessive energy in dry cow diets, and unbalanced dietary protein.

Nevertheless the transition cow should be adapted to provide minimal risk of macro and micro mineral metabolism including Ca, Pi, Cu and Zn.

As the number of animals in the present study was statistically sufficient therefore the mean value of healthy group can be used as reference value for AST, ALT, GGT in crossbred dairy cows to interpret the variations of AST and GGT in subclinical ketosis. From the practical point of view AST and GGT activity were easy to measure which may be effectively used as a valuable diagnostic test for hepatic and metabolic disorders in the crossbred dairy cows.

It was concluded that dairy cows were in oxidative stress during transition period. This was based on the altered status of the free radical scavengers in the whole blood. The effect was more pronounced in post partum transition period in comparison to pre partum period. Extent of depletion was not pronounced in parity groups. The evaluation of the extent of oxidative stress in the form of values can be useful to redefine the role of oxidative stress in different physiological condition and can be used for health management in dairy cows. It can be recommended that dairy cows should be supplemented with high quality mineral mixture (anti-oxidant) during transition period. Although antioxidants an in erythrocytes have been reported to change under certain pathological condition, their use as early biomarkers of oxidative stress appear promising. Free radical scavengers are considered as non invasive peripheral markers of oxidative stress. As a part holistic approach, it is important to observe changes in the blood. For this purpose free radical scavengers were determined in the whole blood. The present investigation has tried to put various scavengers like reduced glutathione and malondialdehyde at one place for the purpose of production of physiological reference data to be used in transition period. The paucity of literature on this aspect in crossbred cows underlined the

importance of generation of appropriate physiological baseline values. This could help in realistic evaluation of the management practices including antioxidant supplementation and diagnosis of disease conditions. All the parameters investigated in this study belonged to the class whose level in the blood can be used diagnostically to determine the damage or dysfunction of the body at cellular level. This study elaborated signify the role of antioxidant supplementation prior to parturition for proper immunity development for the upcoming production diseases after parturition and proper growth of foetus. The data generated will be helpful in laying the foundation of physiological norms of enzymes for crossbred cows in future research in the field of veterinary clinical medicine.