PHYSIOBIOCHEMICAL CHANGES AND GROWTH PERFORMANCE IN YOUNG SURTI GOATS SUPPLEMENTED WITH RUMEN PROTECTED CHOLINE

BY

ROHITAS JAKHAR

B.V.Sc. & A.H.

Registration No. 2040419011

DEPARTMENT OF

VETERINARY PHYSIOLOGY & BIOCHEMISTRY

COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY

KAMDHENU UNIVERSITY, NAVSARI - 396 450

GUJARAT STATE

OCTOBER 2021

PHYSIOBIOCHEMICAL CHANGES AND GROWTH PERFORMANCE IN YOUNG SURTI GOATS SUPPLEMENTED WITH RUMEN PROTECTED CHOLINE

A THESIS SUBMITTED TO THE
KAMDHENU UNIVERSITY, GANDHINAGAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PHYSIOLOGY

BY

ROHITAS JAKHAR

B.V.Sc & A.H.

Registration No. 2040419011

DEPARTMENT OF
VETERINARY PHYSIOLOGY & BIOCHEMISTRY
COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY,
KAMDHENU UNIVERSITY, NAVSARI – 396 450
GUJARAT STATE

OCTOBER 2021

DEPARTMENT OF VETERINARY PHYSIOLOGY AND BIOCHEMISTRY
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY, KAMDHENU UNIVERSITY, NAVSARI – 396 450
GUJARAT STATE

Student	**Major Guide**
Rohitas Jakhar	**Dr. Virendra Kumar Singh**

PHYSIOBIOCHEMICAL CHANGES AND GROWTH PERFORMANCE IN YOUNG SURTI GOATS SUPPLEMENTED WITH RUMEN PROTECTED CHOLINE

ABSTRACT

Choline i.e. (beta-hydroxyethyl) trimethyl ammonium hydroxide is an essential nutrient that is required in high amounts. It is present naturally in feeds and also synthesized endogenously in ruminants. It acts as a lipotropic agent and prevents fatty liver. However its other effects are equally beneficial as it plays role in structural integrity and signalling function of cellular membranes, neurotransmission, methyl group donation, release of Ca^{2+} from intercellular storage, blood clotting, lipid transport, bile formation, one-carbon metabolism, osmoregulation, epigenetic as well as intestinal mucosa integrity, functionality and immunity. Even though roles of dietary choline have been mostly explored during transition and early lactation it is likely to benefit animals even in growth phase. To avoid ruminal degradation and increase bioavailability, choline is encapsulated and supplemented in diet as rumen protected choline. Such studies are very meagre especially in goats of Surti breed that are native of South Gujarat. Thus the present study was planned to study physiobiochemical changes and growth performance in young Surti goats supplemented with rumen protected choline.

Twelve young female Surti goats (9-11 months of age) were selected and divided into 2 groups: control (n=6) that was kept on basal diet and treatment (RPC, n=6) that was supplemented with rumen protected choline (RPC) in addition to basal diet. Duration of study was of 6 weeks but supplementation of RPC was done for only 4 weeks. Recording of meteorological conditions of goat shed was done throughout the study period. Recordings of physiological parameters at week 0, 2 and 4 and growth parameters week 0, 2, 4 and 6 were done. Blood was collected from all goats at week 0 i.e. beginning of study as well as RPC supplementation) and week 4 i.e. end of RPC supplementation. Blood was analyzed for hematological parameters, biochemical metabolites, hepatic enzymes, hormones, electrolytes and oxidative stress parameters.

Meteorological conditions of goat shed during study period viz. ambient temperature, relative humidity and temperature humidity index indicated thermal comfort for selected goats during the study period. Supplementation of RPC significantly ($P \leq 0.05$) lowered rectal temperature and heart rate at week 2 and 4. Hemoglobin, total erythrocyte concentration, hematocrit, lymphocytes were significantly ($P \leq 0.05$) higher whereas neutrophils and neutrophil: lymphocyte ratio

were significantly ($P \leq 0.05$) lower at week 4 in RPC supplemented group. RPC supplemented group at week 4 had significantly ($P \leq 0.05$) higher serum triglyceride, total cholesterol, HDL, total protein and globulin. Amongst the hepatic enzyme profile the mean concentrations of ALT, AST and GGT were significantly ($P \leq 0.05$) lower in the treatment group RPC at week 4. As compared to control group, hormones such as T3 and T4 were significantly ($P \leq 0.05$) increased and leptin was non-significantly elevated in RPC group at week 4. Between the groups levels of GSH and TAS were significantly ($P \leq 0.05$) higher whereas LPO was significantly ($P \leq 0.05$) lower in RPC supplemented group at week 4. Rest of the parameters did not show any significant difference due to supplementation of RPC.

Comparison between groups showed that body weights in RPC supplemented group were higher at all fortnights of study i.e. week 2, 4 and 6. Total gain and average daily gains in body weight during 1st, 2nd and 3rd fortnight as well as for whole study period were also higher due to RPC supplementation in treatment group. Non-significant higher values were also observed for body height and heart girth at week 6 in RPC group goats as compared to control. However none of the attributes related to body weight measurement were significantly different between the groups. It was interestingly observed that maximum gain in body weight due to supplementation of RPC occurred after week 4 i.e. after the supplementation was stopped.

Thus it was concluded that dietary supplementation of rumen protected choline in young female Surti goats is beneficial as it lowers rectal temperature and heart rate, improves hemogram and leukogram profile that indicates general health and immune status, increases nitrogen balance and reduces oxidative stress. It also has potential to improve growth performance.

KAMDHENU UNIVERSITY, GANDHINAGAR

COLLEGE OF VETERINARY SCIENCE & A.H., NAVSARI

CERTIFICATE – I

Date: /10/2021

This is to certify that the thesis entitled **PHYSIOBIOCHEMICAL CHANGES AND GROWTH PERFORMANCE IN YOUNG SURTI GOATS SUPPLEMENTED WITH RUMEN PROTECTED CHOLINE** submitted for the degree of M.V.Sc in the subject of **VETERINARY PHYSIOLOGY** embodies bonafide research work carried out by **ROHITAS JAKHAR** under my guidance and supervision and that no part of this thesis or research work has been submitted for any other degree. The assistance, guidance and help received during the course of investigation have been fully acknowledged.

Head of the Department

Major Advisor

Principal/Dean

iv

KAMDHENU UNIVERSITY, GANDHINAGAR

COLLEGE OF VETERINARY SCIENCE & A.H., NAVSARI

CERTIFICATE – II

Date: /10/2021

This is to certify that the thesis entitled **PHYSIOBIOCHEMICAL CHANGES AND GROWTH PERFORMANCE IN YOUNG SURTI GOATS SUPPLEMENTED WITH RUMEN PROTECTED CHOLINE** submitted by **ROHITAS JAKHAR** to the Kamdhenu University, Gandhinagar in partial fulfilment of the requirements for the award of the degree of **M.V.Sc** in the subject of **VETERINARY PHYSIOLOGY** after incorporating the suggestions and recommendations made by external examiner as discussed and defended by the candidate before the thesis Examination Committee, the performance of the candidate in the oral examination has been found satisfactory; we therefore, recommend the thesis be approved. All the corrections/modifications were made in the thesis as suggested during in the oral examination held on 22/10/2021.The corrected final copies of the thesis were submitted on **/10/2021.**

Major Guide

(Dr. Virendra Kumar Singh)

ACKNOWLEDGEMENT

It gives immense pleasure to express my deepest sense of gratitude and words of appreciation towards those, who helped me during the pursuit of my present study. It’s my proud privilege and pleasure to acknowledge all those who are directly or indirectly involved.

At this inexplicable moment, I express my heartfelt, sincere thanks to my Major advisor **Dr. Virendra Kumar Singh**, Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Navsari whose judicious supervision, keen interest, constructive criticism and expert guidance helped me traverse this phase of post-graduation and resulted in completion of this endeavor of research work.

I express deepest gratitude and appreciation to my Minor advisor **Dr. Sandhya S Chaudhary**, Professor & Head, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Navsari for her invaluable, judicious and constantly inspiring guidance with constructive criticism, moral support, persistent encouragement, active persuasion, diligent efforts and caring attitude throughout my post graduate program. She has not just supervised every aspect of my progress but also has been instrumental in creating a supportive atmosphere in all endeavors. I believe her blessings will always be with me.

I take this opportunity in expressing my heartfelt thanks to members of my advisory committee **Dr. Gopal Puri**, Associate Professor, Department of Veterinary Physiology and Biochemistry and **Dr R.R. Singh** Associate Professor, Department of Livestock Production and Management for their invaluable and critical suggestions and scholarly guidance, which served as a constant source of inspiration

I also take this opportunity to thank member of my advisory committee **Dr. N. B. Patel,** Research Scientist, LRS, and technical as well as non-technical staff at LRS, who helped directly or indirectly during the experiment and it was immense.

I express my respect and indebtedness to **Dr. S. B. Patel,** Assistant Professor and **Dr. Tanviben D. Manat** Assistant Professor, Department of Veterinary Physiology and Biochemistry and for their support and immense help during my Master degree.

I will be failing in my duties if I don’t recall the help offered by my senior **Dr. Sawan** for his motivational support during entire P.G. study and my research work.

I was fortunate to get not only unlimited help but also valuable suggestions from my senior **Dr. Sanjeev Kumar** and colleagues. I am indebted to them for their support and help during research work as they were with me all the time in the endeavors be it technical or non-technical work.

I am also thankful to the departmental staff especially **Dharmishthaben, Jigishaben, Binalben,** and **Parulben**. They work continuously in the department in order to facilitate the departmental activities and are always ready to share work load during the busy track of research work.

Words are short to express my deep sense of feelings, gratitude and regards to my beloved parents for their blessings, love, affection, sincere encouragement and

inspiration. They truly deserve more than a word of thanks, because without their psychological support nothing was possible.

I received numerous helping hands directly or indirectly. Since I could not mention them individually so I tender my apology to all those but I convey my thanks to all of them.

Eventually, I curtsy myself in front of the Almighty for showering upon me his divine providence throughout the journey of my life.

Date: /10/2021 (Rohitas Jakhar)

vii

CONTENTS

CHAPTER	TITLE	PAGE
	ABSTRACT	ii-iii
	ACKNOWLEDGEMENT	vi-vii
	LIST OF TABLES	ix
	LIST OF SYMBOLS AND ABBREVIATIONS	x-xi
1	**INTRODUCTION**	1-4
2	**REVIEW OF LITERATURE**	5-23
3	**MATERIALS AND METHODS**	24-44
4	**RESULTS AND DISCUSSION**	45-65
5	**SUMMARY AND CONCLUSIONS**	66-69
	REFERENCES	xii-xix

LIST OF TABLES

TABLE	TITLE	PAGE
4.1	Meteorological conditions of goats shed during the study period	45
4.2	Physiological parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	46
4.3.1	Hemogram profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	48
4.3.2	Leukogram profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	50
4.4.1	Blood biochemical metabolites (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	54-55
4.4.2	Hepatic enzyme profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	57
4.5	Hormonal parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	58
4.6	Oxidative stress parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	61
4.7	Blood electrolytes (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	62
4.8.1	Growth parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)	63
4.8.2	Average daily gain and cumulative weight gain (Mean±SE) in body weight of young Surti goats supplemented with rumen protected choline (RPC)	64

LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree celsius	MCV	:	Mean corpuscular volume
<	:	Less than	MCH	:	Mean corpuscular hemoglobin
>	:	Greater than	MCHC	:	Mean corpuscular hemoglobin concentration
%	:	Percent	MPV	:	Mean platelet volume
µl	:	Microlitre	LYM	:	Lymphocyte
µM	:	Micromole	TP	:	Total protein
µg	:	Microgram	TC	:	Total cholesterol
µg/ml	:	Microgram per millilitre	TG	:	Triglyceride
mm	:	Millimeter	NEFA	:	Non-esterified fatty acids
ml	:	Millilitre	NAD	:	Nicotinamide adenine dinucleotide
mm	:	Millimeter	ATP	:	Adenosine triphosphate
mg	:	Milligram	GOD	:	Glucose Oxidase
nM	:	Nanomole	°C	:	Degree Celsius
ng/g	:	Nano gram per gram	LPL	:	Lipoprotein Lipase
g/dl	:	Gram per deciliter	GK	:	Glycerol Kinase
g/l	:	Gram per litre	ATP	:	Adenosine Triphosphate
pH	:	Hydrogen ion concentration	ADP	:	Adenosine diphosphate
U/ml	:	Units per milliliter	GPO	:	Glycerol 3- phosphate oxidase
mEq/l	:	Mili equivalent per litre	DAP	:	Dihydroxyacetone Phosphate
nm	:	Nanometer	POD	:	Peroxidase
ng/dl	:	Nanogram per deciliter	AAP	:	Aminoantipyrine
mM	:	Milimole	NED	:	Naphthyl Ethylene Diamine
U	:	Units	ELISA	:	Enzyme Linked Immuno Sorbent Assay
S.E.	:	Standard error	TBARS	:	Thiobarbituric acid reactive substances
mg/dl	:	Milligram per deciliter	DTNB	:	5,5'-dithiobis nitro

					benzoic acid
mmol/l	:	Milimoles per litre	TCA	:	Trichloro acetic acid
µmoles/ min/ml	:	Micromoles per minute per milliliter	MTT	:	3-(4-5 Dimethyl Thiazol 2-xl) 2, 5 diphenyl tetrazolium bromide
µg/dl	:	Microgram per deciliter	P_4	:	Progesterone
ng/ml	:	Nanogram per milliliter	E_2	:	Estradiol
l	:	Litre	T_4	:	Thyroxine
mg/dl	:	Milligram/deciliter	T_3	:	Triiodothyroxine
%	:	Percent	SOD	:	Superoxide dismutase
et al.	:	et alibi	GPx	:	Glutathione peroxidase
ANOVA	:	Analysis of variance	GSH	:	Reduced Glutathione
TEC	:	Total erythrocyte count	MDA	:	Malondialdehyde
TLC	:	Total leucocyte count	GR	:	Glutathione Reductase
PCV	:	Packed cell volume	Hct	:	Hematocrit
Hb	:	Hemoglobin	RPC	:	Rumen protected choline
GGT	:	Gamma-glutamyl transferase	ALT	:	Alanine transaminase
AST	:	Aspartate aminotransferase	OD	:	Optical density
Na	:	Sodium	K	:	Potassium
Cl	:	Chloride	LPO	:	Lipid peroxide
i.e.		id est. (that is)	TP	:	Total protein

CHAPTER 1

INTRODUCTION

India ranks second in terms of goat population of the world. In India goats (135.17 million) comprise 26.40% of total livestock population as per 19th livestock census. In intensive system their rearing is scientifically managed and feeding is optimized. However generally they are reared in extensive rearing system without any extra care for dietary supplementation. Rearing in extensive system is successful because goats show better production and reproduction performance in spite of scarcity of feed resources thus requiring minimal investment. This makes them preferred species to be reared by marginal and poor livestock owners and so they are also referred as ‘poor man’s cow’. Surti goats are commonly reared in south Gujarat region which is their home tract.

Growth performance is important for any livestock species and it has economic implications throughout the life cycle of animal. An optimum as well as higher growth rate will result in higher body weight gain, early puberty and early onset of reproduction and production cycle. This is desired in both meat and milch purpose goats. Balanced nutrition is key for growth, production and reproduction. Intensive and more specifically extensive systems of rearing may sometimes face lack of essential nutrients. One such example is choline.

Choline i.e. (beta hydroxyethyl) trimethylammonium hydroxide is an essential nutrient. It is a water soluble vitamin like compound classified in vitamin B group but metabolized like an amino acid.

There are several compounds that contain choline. They are either water soluble or fat soluble. The examples of water soluble are choline, betaine, acetylcholine, cytidinediphosphate-choline, phosphocholine, and glycerophosphocholine. The examples of fat soluble biomolecules that contain choline are phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin. Zeisel and Niculescu (2006) have elucidated the role of choline in structural integrity and signaling function of cellular membranes, neurotransmission, methyl group donation, release of Ca^{2+} from intercellular storage, blood clotting, and lipid transport.

The physiological role of choline in the body are differently faceted and wide ranging viz. membrane integrity, bile formation, one-carbon metabolism, osmoregulation, epigenetic as well as intestinal mucosa integrity, functionality and immunity.

Choline is essential metabolically for building and maintaining cell structure. As a phospholipid component, choline is structural part of lecithin (phosphatidylcholine), certain plasmalogens and sphingomyelins. Lecithin is a part of animal cell membranes and lipid transport moieties in cell plasma membranes. Choline is required as a constituent of the phospholipids required for normal maturation of the cartilage matrix of the bone. Choline also plays an essential role in fat metabolism in the liver. It prevents fatty liver by preventing abnormal accumulation of fat in liver by promoting its transport as lecithin or by increasing the utilization of fatty acids in the liver. Choline is thus referred to as a "lipotropic" factor due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver.

The two principal methyl donors functioning in animal metabolism are choline and methionine, which contain "biologically labile methyl groups" that can be transferred within the body. This phenomenon is called transmethylation. Choline being a key source of labile methyl groups provides methyl groups and spares methionine from it. Choline furnishes labile methyl groups for formation of methionine from homocystine and creatine from guanidoacetic acid. Methyl groups function in the synthesis of purine and pyrimidine, which are used in the production of DNA. Methionine is converted to S-adenosylmethionine in a reaction catalyzed by methionine adenosyl transferase. S-adenosylmethionine is the active methylating agent for many enzymatic methylations. Studies in lactating dairy goats on rates of methyl group transfer revealed that only 6% of methionine methyl groups were derived from choline, while 28% of choline methyl groups were derived from methionine (Emmanual and Kennelly, 1984). This suggests that considerable dietary methionine is used for choline synthesis. Choline is not a major direct precursor of methionine, although it may spare methionine. Benoit *et al*. (2010) suggested that about 40% of methionine in the mammary gland underwent transmethylation with choline serving as the methyl donor.

Choline, unlike most vitamins, can be synthesized by most species. However in many cases its synthesis may not be either in sufficient amounts or rapid enough to meet the requirements. Even though choline can be synthesized by ruminants it may not be sufficient to meet the demands of growth and may become a reason for its retardation. The mechanism by which choline affects growth performance is possibly due to methionine sparing (Lobley *et al*., 1996) or changes in alterations in lipid metabolism or its transport (Bryant *et al*., 1999). Some studies for effect of choline supplementation on growth (Bryant *et al*., 1999; Bindel *et al*., 2005; Bindel *et al*., 2000) have been sporadically done.

Sources of choline for animal can be either exogenous from the diet or endogenous synthesis especially in ruminants. It is important for choline to be present post ruminally for absorption. Dietary choline is extensively degraded in rumen. Naturally occurring choline in feed is rapidly degraded by the rumen microbiota, where its N-methyl groups are converted into trimethylamine, and ultimately, methane. Post-ruminally most of the choline available for absorption is present in the form of translocated ruminal protozoa. To contribute to the body pool of choline dietary supplementation of choline that is rumen protected is essential. Studies done for observing effect of choline supplementation on growth are less, that are further lesser in Indian feeding conditions and almost nil for growing goats.

The roles of choline have been mostly explored during transition and early lactation. The study of choline supplementation has been mainly done in cattle and buffalo but rarely the study has been taken in goats that too during growing stage. Effects of supplementation of RPC in goats during growth phase are anticipated to range from physio-biochemical responses to growth performance.

Based on above considerations and the need to understand mechanism and effect of supplementing choline on growth performance in Surti goats, the present study entitled ‘Physiobiochemical changes and growth performance in young Surti goats supplemented with rumen protected choline’ was planned with the following objectives :

1. To study changes in physiological and hematological parameters in young Surti goats supplemented with rumen protected choline.
2. To study changes in biochemical, hormonal and oxidative stress parameters in young Surti goats supplemented with rumen protected choline.

3. To study changes in body weight and body measurement of young Surti goats supplemented with rumen protected choline.

CHAPTER 2

REVIEW OF LITERAURE

Choline is an essential nutrient that is naturally present in some foods and available as a dietary supplement. Choline i.e. (beta-hydroxyethyl) trimethyl ammonium hydroxide is a water soluble vitamin like compound rather quasi-vitamin and classified in vitamin B group but metabolized like an amino acid. Choline can donate 3 methyl groups present in its structure for several biochemical reactions in the animal's body. Daily requirement of choline is much more than expected and recommended for vitamins since vitamins are source of coenzymes and cofactors and are required in small quantities but choline has more roles as compared to vitamin. Predominant effects of choline supplementation are preventing fatty liver thus being referred to as a lipotropic agent.

Roles of methionine and choline are so intertwined that supplementation of either will spare the other for beneficial effects. So choline supplementation in diet may not only yield direct effects but also indirect effects that may improve livestock performance. However with ruminants as the mainstay of livestock rearing, choline degradation in rumen by microbes has been a cause of concern until some protection was found. The advent of rumen protected choline was a major breakthrough that made choline post-ruminally bioavailable in amounts adequate to benefit the livestock.

The roles of choline have been mostly explored during transition and early lactation and its supplementation has mainly been done in cattle and buffalo but rarely the study has been undertaken in goats that too during growing stage. Effects of supplementation of RPC in goats during growth phase are anticipated to range from physio-biochemical responses to growth performance.

2.1 EFFECTS OF RPC SUPPLEMENTATION

2.1.1 Physiological parameters

Deshpande *et al*. (2020) in their study studied physio-biochemical responses of buffalo heifers to betaine supplementation during hot humid season under field conditions. Fourteen buffalo heifers were selected and equally divided into two groups, i.e. control and treatment (supplemented betaine @25 g/animal/day over the farmers practice The physiological responses, *viz*. rectal temperature, skin

temperature, respiration rate and pulse rate were recorded using standard methods on day 0, 15, 30, 45, 60, 75 and 90. Due to supplementation of betaine the treatment group had lower rectal temperature, respiration rate, skin temperature and pulse rate generally in 6th and 7th fortnight. Betaine is a product that is formed by irreversible oxidation of choline in the body and the effects may be comparable between betaine supplementation in diet or its endogenous supply from choline.

Holdorf and White (2021) observed physiological responses as the effects of rumen-protected choline supplementation in Holstein dairy cows during electric heat blanket-induced heat stress. The primary objective of this experiment was to determine if supplementation of rumen-protected choline during, or before and during, an increased heat load would ameliorate the negative effects of HS. Heat stress was induced via an electric heat blanket model with a 3-d baseline period and 7-d HS period for all cows. Multiparous mid-lactation (208 ± 31 days in milk) Holstein cows were fed the same basal herd diet, blocked by pre-experiment milk yield, and randomly assigned to receive one of the following: (1) no rumen-protected (RP) choline (n = 7); (2) RP choline (60 g/d) via top-dress during the HS period (n = 8); or (3) RP choline (60 g/d) via top-dress during the baseline and HS periods (n = 8). Imposing HS via electric heat blanket raised respiration rate with all cows surpassing the HS threshold of 60 breaths/min. The increase in respiration rate tended to be ameliorated with either schedule of RP choline supplementation. Data showed that 2 g betaine/day can reduce the physiological measures of RR and TR relative to controls under both TN and to a lesser degree HE conditions. However, when the dose was doubled to 4 g betaine/day there appeared to be an increased heat load as indicated by elevated TR, TS, RR and HR. Betaine is a product that is formed by irreversible oxidation of choline in the body the effects may be comparable between betaine supplementation in diet or its endogenous supply from choline.

2.1.2 Hematological parameters

Nishiyama-Naruke and Curi (2000) studied phosphatidylcholine participation in the interaction between macrophages and lymphocytes. The role of phosphatidylcholine molecules as mediator for the control of lymphocyte proliferation by macrophages was investigated. Phosphatidylcholine added to the culture medium inhibited the concanavalin A-stimulated lymphocyte proliferation in a concentration-dependent manner. The potency of this effect was dependent on the presence of

arachidonic acid in the phosphatidylcholine molecules. The phosphatidylcholine transfer from macrophages to lymphocytes was then investigated. Macrophages incorporated phosphatidylcholine at a much higher rate than lymphocytes and exported phosphatidylcholine to the culture medium. When co-cultured, a significant amount of phosphatidylcholine incorporated by macrophages was transferred to lymphocytes. To examine the possible physiological importance of the transfer process, the lymphocyte proliferation was measured in co-culture conditions. Macrophages were treated with phosphatidylcholine and washed, and then these cells were co-cultured with concanavalin A-stimulated lymphocytes. The effect observed in co-culture was an inhibition of lymphocyte proliferation, which was also dependent on the molecular species of the phosphatidylcholine. Therefore it was concluded phosphatidylcholine may act as a mediator of the macrophage effect on lymphocyte proliferation.

Kawashima and Fujii (2008) in their review entitled ‘Basic and clinical aspects of non-neuronal acetylcholine: overview of non-neuronal cholinergic systems and their biological significance’ have highlighted that in lymphocytes the acetylcholine content correlates rather well with choline acetyltransferase activity. Acetylcholine (ACh) is a phylogenetically ancient molecule involved in cell-to-cell signaling in almost all life-forms on earth. Cholinergic components, including ACh, choline acetyltransferase, acetylcholinesterase, and muscarinic and nicotinic ACh receptors (mAChRs and nAChRs, respectively) have been identified in numerous non-neuronal cells and tissues, including keratinocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells, and reproductive organs, among many others. Stimulation of the mAChRs and nAChRs elicits cell-specific functional and biochemical effects. These findings support the notion that non-neuronal cholinergic systems are expressed in certain cells and tissues and are involved in the regulation of their function and that cholinergic dysfunction is related to the pathophysiology of certain diseases. They also provide clues for development of drugs with novel mechanisms of action.

Habeeb *et al.* (2017) studied evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. In his study twenty Zaraibi goat bucks were used which lasted 3 months during summer season of Egypt. The animals were divided randomly into two

equal groups. The first group was kept without treatment as control while in the second group, rumen-protected choline (RPC) at the level of 20 g/buck/day was added to the concentrate feed mixture at the morning feeding. RPC increased (P<0.05) red and white blood cell (RBC$\times 10^6$, WBC$\times 10^3$) counts and hemoglobin concentration and hematocrit percentage.

Garcia *et al.* (2018) did study entitled 'Choline Regulates the Function of Bovine Immune Cells and Alters the mRNA Abundance of Enzymes and Receptors Involved in Its Metabolism in vitro'. To determine whether increased choline concentrations (3.2, 8.2, 13.2 µM) in cell culture alter the function of bovine innate and adaptive immune cells, they isolated cells from dairy cows in early and mid-lactation as models of immuno-compromised and competent cells, respectively. Phagocytic and killing capacities of isolated neutrophils were linearly diminished with increasing doses of choline. In contrast, lymphocyte proliferation was linearly enhanced with increasing doses of choline. Furthermore, increasing doses of choline increased the mRNA abundance of genes involved in the synthesis of choline products (betaine, phosphatidylcholine, and acetylcholine) as well as muscarinic and nicotinic acetylcholine receptors in a quadratic and linear fashion for neutrophils and monocytes, respectively. Phagocytic and killing capacity of neutrophils and proliferation of lymphocytes were not affected by stage of lactation or its interaction with choline or LPS. In neutrophils from early lactation cows, choline linearly increased the mRNA abundance of muscarinic and nicotinic cholinergic receptors, whereas choline-supplemented monocytes from mid-lactation cows linearly increased the mRNA abundance of several genes coding for choline metabolism enzymes. Their results demonstrated that choline regulates the inflammatory response of immune cells and suggest that the mechanism may involve one or more of its metabolic products.

2.1.3 Blood biochemical metabolites

Bryant *et al.* (1999) studied effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on serum metabolites in lambs. Beef steers (n = 160; average initial BW = 350.9 kg) were fed a 90% concentrate diet with either 0, 0.25, 0.5, or 1.0% RPC (DM basis) for 112 to 140 d. Serum INS and NEFA concentrations increased linearly (P<0.05) and serum GH responded cubically (P<0.05) to RPC level on d 28, but no differences

were noted on d 56. Serum TG concentrations in weekly samples increased linearly ($P<0.10$) with RPC level, but, averaged over all weeks, serum CLSTRL concentrations did not differ ($P>0.10$) among treatments. They concluded that supplementing RPC in high-concentrate diets improved performance, but results were not consistent among RPC levels or between cattle and sheep. Potential effects of RPC might be mediated through alterations in fat metabolism and (or) metabolic hormones related to fat metabolism.

Bindel *et al.* (2000) conducted a 120 day study on 318 heifers (342 kg initial BW) to examine effects of ruminally protected choline (RPC) in diets containing graded concentrations of tallow. Heifers were given supplemental RPC (0, 20, 40, or 60 g of product daily, estimated to supply 0, 5, 10, or 15 g/d choline postruminally). Supplementation of RPC increased ($P<0.10$) ADG, with 20 g/d resulting in an 8.6% increase. Similarly, gain efficiency improved ($P<0.10$) by 7.6% with addition of 20 g/d RPC. Yield grade and kidney, pelvic, and heart fat both increased linearly ($P<0.10$) with fat supplementation. Plasma urea and serum insulin concentrations were not affected by RPC. Choline supplementation led to quadratic responses for total amino acids ($P<0.10$), with concentrations being greatest for intermediate levels of RPC. Moderate levels of supplemental RPC improved growth performance of finishing cattle without negatively affecting carcass characteristics. Optimum performance was achieved with 20 g of product daily.

Bindel *et al.* (2005) studied the effects of choline on blood metabolites associated with lipid metabolism and digestion by steers fed corn-based diets. In their study ruminally cannulated steers (281±18 kg) were used to evaluate effects of choline on digestion and metabolism. Four steers were implanted with 24 mg of estradiol and 120 mg of trenbolone acetate, and four steers were not implanted. Plasma urea was less ($P<0.05$) for implanted cattle, reflecting increased deposition of protein. Plasma cholesterol was greater ($P<0.05$) for steers fed 4% tallow. Changes in plasma triglycerides in response to an abomasal lipid dose were less ($P<0.05$) for steers fed 4% tallow, probably due to greater triglyceride concentrations at the time of lipid dosing. In summary, few responses to abomasally infused choline were observed in either digestion or plasma metabolites.

Guretzky *et al.* (2006) studied Lipid Metabolite Profiles and Milk Production for Holstein and Jersey Cows Fed Rumen-Protected Choline During the Periparturient

Period. Choline is important for assembly of very low density lipoproteins to export triglyceride from liver; however, studies to assess the effect of rumen-protected choline (RPC) supplementation on blood lipid metabolites in periparturient dairy cows have not been conducted. Thirty-two multiparous Holstein and 10 multiparous Jersey cows were randomly assigned to control or RPC treatments. A close-up diet was fed from approximately 3 wk before parturition through parturition, followed by a lactation diet from parturition through 49 d postpartum. For RPC, diets were top dressed once daily with 60 g of a RPC product (25% choline as choline chloride) from 21 d before expected parturition through 21 d postpartum. Jersey cows in the control group had lower concentrations of nonesterified fatty acids and β-hydroxybutyrate in plasma during d 1 to 10 postpartum than did other breed and treatment combinations. Cows fed RPC tended to have greater serum triglycerides prepartum (17.0 vs. 14.7 mg/dl) and lower plasma phospholipid at parturition (65.2 vs. 78.1 mg/dl) than control cows. Treatment did not affect cholesterol and phospholipid at other time points, but concentrations followed patterns of dry matter intake pre- and postpartum. Additionally, calculated Met balance was negative postpartum; supplemental RPC might not have spared enough Met to produce a physiological benefit. They concluded that more research is needed to determine how choline affects prevention or alleviation of fatty liver syndrome and to confirm potential differences between Holstein and Jersey cows.

Xu *et al.* (2006) conducted study on effect of rumen-protected choline addition on blood metabolic parameters in transition dairy cows. In Experiment 1, 14 Chinese Holstein dairy cows were supplemented with 0 or 20 g/d of RPC from 7 d before expected calving to 21 d post partum. Plasma concentrations of glucose tended to increase as cows consumed RPC, while plasma concentrations of triglycerides, very low density lipoproteins, cholesterol and nonesterified fatty acids were not significantly different between the two groups. In another experiment 36 Chinese Holstein dairy cows were supplemented with 0, 30, 60 or 90 g/d RPC from 15 d before expected calving to 15 d post partum. Plasma concentrations of glucose were remained at a higher level in 30 or 60 g/d RPC-supplemented groups, and nonesterified fatty acids were decreased in the 30 g/d group. Concentrations of triglycerides tended to reduce in 30 and 90 g/d RPC-supplemented animals, and cholesterol was reduced in 0 or 30 g/d group. Their results suggested that RPC

addition tended to improve blood metabolic parameters during transition dairy cows, and feeding 30 g/d of RPC may be the optimal.

Zom *et al.* (2011) studied effect of rumen-protected choline on performance, blood metabolites and hepatic triacylglycerols of periparturient dairy cattle. Thirty-eight multiparous cows were blocked into 19 pairs and then randomly allocated to either one of 2 treatments. The treatments were supplementation either with or without (control) rumen-protected choline. Treatments were applied from 3 wk before until 6 wk after calving. Both groups received the same basal diet, being a mixed feed of grass silage, corn silage, straw, and soybean meal, and a concentrate mixture delivered through transponder controlled feed dispensers. For all cows, the concentrate mixture was gradually increased from 0 kg/day (wk -3) to 0.9 kg of dry matter (DM)/d (day of calving) and up to 8.1 kg of DM/d on d 17 post-calving until the end of the experiment. Additionally, a mixture of 60 g of a rumen-protected choline supplement (providing 14.4 g of choline) and of 540 g of soybean meal or a (iso-energetic) mixture of 18 g of palm oil and 582 g of soybean meal (control) was offered individually in feed dispensers. Choline supplementation decreased the concentration of liver triacylglycerol during the first 4 wk after parturition. Results from this study suggest that hepatic fat export in periparturient dairy cows is improved by choline supplementation during the transition period and this may potentially decrease the risk for metabolic disorders in the periparturient dairy cow.

Pirestani *et al*. (2011) studied to determine beneficial effects of L-carnitine and choline in pre and postpartum for reproduction indices and milk somatic cell count (SCC) of Holstein cows. In their study 4 groups containing 15 cattle were selected with 2-4 parity and the same of milk production. Protected choline (60 gm/daily/cow) was used and L-carnitine (50 gm/daily/cow) like to top dress with diet ration from 1 week pre calving probably to 4 weeks after parturition in control group (without supplement), choline, L-carnitine, choline + L-carnitine treatments. Milk sample was collected as weekly from the calving time to fourth weeks of postpartum. Reproduction indices evaluated were open days, calving to first visible estrus, calving to first service and service per conception. The results were showed the choline + L-carnitine treatment group indicated significant decrease on rate of SCC and open days, calving to first visible estrus, calving to first service and service per conception compare to other groups. It was concluded that choline + Lcarnitine combination has

beneficial effect on improved reproduction indices and reduction of milk SCC than other treatment groups in Holstein Dairy cattle.

Sheikh (2012) conducted an experiment on 18 lactating crossbreed cows and reported that supplementation of 54 g RPC/animal/day led to significantly high total immunoglobulin concentration. The increased immunoglobulin concentration was attributed to increase folate in plasma and alpha-tocopherol in periparturient cows that might had positively affected immunoglobulin levels. They also reported that supplementation of 60 g RPC resulted in no significant effect on body weight and body weight change in lactating crossbred cows.

Pandurang (2012) conducted an experiment on 18 lactating crossbred cows and reported that supplementation of RPC @54 g/head had no significant effect on plasma NEFA (106.35 vs 106.80 mg/ml), glucose (55.61 vs 56.47 mg/ml), cholesterol (106.35 vs 106.80mg/ml) bun (18.13 vs 17.86 mg/ml) but resulted in high triglyceride concentration (13.40 vs 14.84 mg/L) and VLDL (2.68 vs 2.97 mg/L) in control and treatment group respectively (P<0.05). The high triglyceride and VLDL content in treatment groups was due to antilipolytic nature of choline.

Sheikh (2012) reported that supplementation of 60 g RPC had no significant effect on digestible intake of dry matter, organic matter, crude protein, ether extract, digestible neutral digestible fibre and acid digestible fibre in lactating crossbred cows. Digestibility of DM and EE was not influenced RPC supplementation whereas the digestibility of OM, CP, NDF and ADF were significantly higher in RPC group as compared to control. They deduced that increased protozoal number in rumen might be responsible for increased digestibility in treatment group. Nitrogen balance was also significantly high in treatment as compared to control. This was attributed to higher DCP value in the choline supplemented group. It was concluded that RPC supplementation caused higher digestibility and nitrogen balance.

Sheikh *et al.* (2015) investigated the effect of supplementation of rumen protected lysine plus methionine or choline on blood biochemical parameters in crossbred cows. In their study eighteen Karan Fries cows with most probable producing ability (MPPA) of around 4233 kg milk production were divided into 3 groups of 6 animals each in order to study the effect of rumen-protected lysine (RPL) plus methionine (RPM) and choline (RPC) on blood biochemical parameters. All the

animals were fed basal diet consisting of wheat (Triticum aestivum) straw (20%), chaffed green maize (*Zea mays*) fodder (40%) and concentrate mixture (40%) on DM basis to meet the requirements (NRC, 2001). The animals in group T1 (control) were given no supplement while those in groups T2 and T3 were given 7 g RPM + 6 g RPL and 60 g RPC per day, respectively. Supplementation of RPM+RPL and RPC resulted in higher ($P<0.05$) blood glucose and total plasma immunoglobulin concentration.

Sun *et al.* (2016) studied about regulation of nutritional metabolism in transition cows: energy homeostasis and health in response to feeding of rumen protected choline and methionine. Forty-eight healthy multiparous transition Chinese Holstein dairy cows were divided into four groups of twelve cows per group. Each group was assigned to one of the following four treatments: control (basal diet, T0), 15.0 gm/animal/day of RPC (TC), 15.0 gm/animal/day of RPM (TM), and 15.0+15.0 gm/animal/day of both (RPC+RPM) (TCM). Total duration of experiment was -21 to 21 day of parturition. During postpartum period, they observed that the plasma concentrations of non-esterified fatty acids (NEFA) (mmol/l) (RPC=0.97±0.021; RPM=1.06±0.021; RPM+RPC=0.90±0.021), β-hydroxybutyric acid (BHBA) (mmol/l) (RPC=0.92±0.009; RPM=0.94±0.009; RPM+RPC=0.86±0.009), total cholesterol (TC) (mmol/L) (RPC=3.22±0.024; RPM=3.23±0.024; RPM+RPC=3.12±0.024), urea (BUN) (mmol/l) (RPM=1.97±0.063; RPM+RPC= 1.90±0.063), low-density lipoprotein cholesterol (LDL-C) (mmol/l) (RPC= 0.84±0.012; RPM=0.85±0.012; RPM+RPC= 0.81±0.012) and HDL were significantly ($P<0.05$) lower in all the supplemented group than control group (NEFA= 1.21±0.021; BHBU=1.02±0.009; TC=3.36±0.024,; BUN=2.12±0.063 and LDL= 0.91±0.012) while, the level of glucose (mmol/l) (RPC=4.48±0.110; RPM= 4.38±0.110; RPM+RPC=4.94±0.110) and VLDL (mmol/l) (RPC=0.57±0.010; RPM= 0.56±0.010; RPM+RPC=0.59±0.010) were significantly ($P<0.05$) higher in all the supplemented group than control group (Glucose=4.10±0.110; VLDL=0.53±0.010). The plasma concentration of total protein, albumin, globulin, and triglyceride were not different between the groups during the whole study periods.

Habeeb *et al.* (2017) studied evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. In his study twenty Zaraibi goat bucks were used which lasted 3 months during summer season of Egypt. The animals were divided randomly into two equal groups. The first group was kept without treatment as control while in the

second group, rumen-protected choline (RPC) at the level of 20 g/buck/day was added to the concentrate feed mixture at the morning feeding. RPC increased total protein ($P<0.05$), globulin, and γ-globulin ($P<0.01$). RPC at level of 20 g/head/day increased significantly total protein, globulin, and γ-globulin by 17.8, 36.2, and 23.9%, respectively, while albumin value was not affected significantly by RPC supplementation. The improvement in protein fractions by rumen-protected choline additives may be due to improvement in DMI as well as nutrient feed digestibility. Glucose value increased significantly ($P < 0.01$) from 56.25 to 70.71 mg/dl (25.71%) due to RPC supplementation. Total lipids, total cholesterol, and triglyceride concentrations decreased significantly by 20.44, 17.05, and 14.09%, respectively, by RPC supplementation, while phospholipid value increased significantly by 24.54% due to RPC supplementation. Urea and creatinine concentrations were not affected significantly ($P > 0.05$) by RPC supplementation.

Rodriguez-Guerrero *et al.* (2018) studied the effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. Twenty-four lambs (Pelibuey x East Friesian), weighing 22.7±3.2 kg, were fed a basal diet of corn silage, oat hay, alfalfa hay, and concentrate (60% forage and 40% concentrate). Treatments consisted of oral doses of rumen protected methionine (RPM) (0 and 1.5 g/day) and herbal choline (biocholine) (0 and 4 g/day) in a completely random block design with factorial arrangement of treatments, where lambs were blocked by sex. The experiment was conducted for 60 days. Non-esterified fatty acids (NEFA) were increased by biocholine and unaffected by methionine (Met). Biocholine increased glucose and cholesterol, whereas methionine increased triglycerides, albumin and plasma protein. The dietary supplementation with biocholine and RPM did not improve lambs' growth; however, biocholine and Met showed a lipotropic effect by mobilizing NEFA and stimulating glucose and cholesterol synthesis.

2.1.4 Hepatic enzyme profile

Mohsen *et al*. (2011) conducted an experiment for 8 weeks in a switch over design on 12 lactating Friesian cows (2nd to 5th lactation) having body weight of 500±15 Kg and fed basal ration consisting of 40% concentrate mixture, 40% fresh berseem, 20% rice straw with supplementation of RPC (choline chloride) at levels of 0, 15 and 30 g/head/day. Results depicted that RPC supplementation significantly decreased concentrations of plasma cholesterol and triglycerides ($P<0.05$). However

the differences in plasma glucose, total protein, albumin, globulin and urea-N concentrations and activities of AST and ALT among the different experimental groups were not significant (P>0.05).

Rahmani *et al*. (2012) studied the effect of oral administration of choline on some liver function characterized blood plasma enzymes of early lactating dairy cows. Sixteen early lactating primiparous and multiparous Holstein cows were used for four weeks to investigate the effects of feeding choline on the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) in the plasma of the cows during early lactation period. Cows were randomly assigned to one of the following treatments: no supplement (control) and 90 g/d of rumen-protected choline (RPC) product. Treatments did not affect significantly ALT, AST and GGT during early lactation period, but for ALP there was a significant difference between the groups. The research results showed significant influence of feeding protected choline on ALP.

Sheikh *et al.* (2015) investigated the effect of supplementation of rumen protected lysine plus methionine or choline on blood biochemical parameters in crossbred cows. In their study eighteen Karan Fries cows with most probable producing ability (MPPA) of around 4233 kg milk production were divided into 3 groups of 6 animals each in order to study the effect of rumen-protected lysine (RPL) plus methionine (RPM) and choline (RPC) on blood biochemical parameters. All the animals were fed basal diet consisting of wheat (*Triticum aestivum*) straw (20%), chaffed green maize (*Zea mays*) fodder (40%) and concentrate mixture (40%) on DM basis to meet the requirements (NRC, 2001). The animals in group T1 (control) were given no supplement while those in groups T2 and T3 were given 7 g RPM + 6 g RPL and 60 g RPC per day, respectively. Supplementation of RPM + RPL and RPC resulted in lower (P<0.05) aspartate amino transferase activity compared to control group while there was no significant effect on alanine amino transferase activity due to supplementation, however, the values of all these parameters were within physiological limits.

Habeeb *et al*. (2017) studied evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. In his study Twenty Zaraibi goat bucks were used which lasted 3 months during summer season of Egypt. The animals were divided randomly into two

equal groups. The first group was kept without treatment as control while in the second group, rumen-protected choline (RPC) at the level of 20 g/buck/day was added to the concentrate feed mixture at the morning feeding. ALT and AST enzyme activities were not affected significantly (P>0.05) by RPC supplementation. Their results indicated that RPC addition to feed of bucks under heat stressful conditions of hot summer season do not affect liver function.

2.1.5 Serum hormones

Shahsavari (2012) studied the metabolic and reproductive responses of lactating dairy cows to supplementation with choline. In their first study, feeding 120 g/day rumen- protected choline (providing 30g choline chloride) to Holstein-Friesian cows during the transition period (3 weeks before to 6 weeks after calving) improved the metabolic state and postpartum reproductive function in dairy cows. Cows that received choline had greater plasma concentrations of leptin in early lactation and they showed a lesser decline in plasma insulin. Body condition was marginally better in cows that received choline.

Habeeb *et al.* (2017) studied evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. In his study Twenty Zaraibi goat bucks were used which lasted 3 months during summer season of Egypt. The animals were divided randomly into two equal groups. The first group was kept without treatment as control while in the second group, rumen-protected choline (RPC) at the level of 20 g/buck/day was added to the concentrate feed mixture at the morning feeding. The basal ration of bucks supplemented with RPC at a level of 20 g/head/day increased significantly T4 and T3 levels by 26.25 and 23.61 and 12.30%, respectively and decreased significantly the cortisol level by 17.82 compared with control bucks.

2.1.6 Oxidative stress parameters

Ossani *et al.* (2007) investigated oxidative damage lipid peroxidation in the kidney of choline-deficient rats. Phosphatidylcholine is the most abundant phospholipid constituent of cell membranes and choline is a quaternary amine required for phosphatidylcholine synthesis. The impairment of membrane functions is considered as an indication of oxidative damage. In order to kinetically analyze the time course of the pathogenesis of renal necrosis following to choline deficiency in weanling rats, we determined markers of membrane lipid peroxidation (thiobarbituric

acid reactive substances; TBARS and hydroperoxide-induced chemiluminescence (BOOH-CL) and studied the histophatological damage. Plasma TBARS ($t_{½}$=2.5 days) was an early indicator of systemic oxidative stress, likely involving liver and kidney. The levels of TBARS and BOOH-CL increased by 80 % and by 183 %, respectively, in kidney homogenates with $t_{½}$=1.5 days and 4 days, respectively. The levels of BOOH-CL were statistically higher in rats fed a choline-deficient diet at day 6, in a mixture of membranes (from plasmatic, smooth and rough endoplasmic reticulum and Golgi), in mitochondrial membranes and in lysosomal membranes. The results indicate that choline deficiency produces oxidative damage in kidney subcellular membranes. Necrosis involved mainly convoluted tubules and appered with a $t_{½}$=5.5 days. An increase in the production of reactive oxygen species, triggered by NADH overproduction in the mitochondrial dysfunction associated with choline deficiency appears as one of the pathogenic mechanism of mitochondrial and cellular oxidative damage in choline-deficiency.

Repetto *et al.* (2010) explained about oxidative damage and its the biochemical mechanism of cellular injury and necrosis in choline deficiency'. Oxidative stress and damage are characterized by decreased tissue antioxidant levels, consumption of tissue α-tocopherol, and increased lipid peroxidation. These processes occur earlier than necrosis in the liver, heart, kidney, and brain of weanling rats fed a choline deficient (CD) diet. In tissues, water-soluble antioxidants were analyzed as total reactive antioxidant potential (TRAP), α-tocopherol content was estimated from homogenate chemiluminescence (homogenate-CL), and lipid peroxidation was evaluated by thiobarbituric acid reactive substances (TBARS). Histopathology showed hepatic steatosis at days 1–7, tubular and glomerular necrosis in kidney at days 6 and 7 and inflammation and necrosis in heart at days 6 and 7. TRAP levels decreased by 18%, 48%, 56%, and 66% at day 7, with t1/2 (times for half maximal change) of 2.0, 1.8, 2.5, and 3.0 days in liver, kidney, heart, and brain, respectively. Homogenate-CL increased by 97%, 113%, 18%, and 297% at day 7, with t1/2 of 2.5, 2.6, 2.8, and 3.2 days in the four organs, respectively. TBARS contents increased by 98%, 157%, 104%, and 347% at day 7, with $t_{1/2}$ of 2.6, 2.8, 3.0, and 5.0 days in the four organs, respectively. Plasma showed a 33% decrease in TRAP and a 5-fold increase in TBARS at day 5. Oxidative stress and damage are processes occurring

earlier than necrosis in the kidney and heart. In case of steatosis prior to antioxidant consumption and increased lipid peroxidation, no necrosis is observed in the liver.

Elsawy *et al*. (2014) studied the effect of choline supplementation on rapid weight loss and biochemical variables among female taekwondo and judo athletes taekwondo and judo competitions are divided into weight categories. many athletes reduce their body mass a few days before competition in order to obtain a competitive advantage over lighter opponents. to achieve fast body mass reduction, athletes use a number of nutritional strategies, including choline supplementation. the goal of this study was to identify the effects of choline supplementation on body mass reduction and leptin levels among female taekwondo and judo athletes. twenty-two female athletes (15 taekwondo and 7 judo athletes) were selected from different weight categories and divided into two groups, according to weight. the players in the experimental group took choline tablets for one week before a competition. the results revealed significant differences between pre- and post-competition measurements of leptin, free plasma choline, urine choline and urine malondialdehyde levels; body mass was also reduced in the post-competition measurements. in conclusion, choline supplementation could rapidly reduce body mass without any side effects on biochemical levels or static strength.

Osorio *et al.* (2014a) studied Smartamine M and MetaSmart supplementation during the peripartal period alter hepatic expression of gene networks in 1-carbon metabolism, inflammation, oxidative stress, and the growth hormone-insulin-like growth factor 1 axis pathways. Thirty-nine Holstein cows were fed throughout the peripartal period (−21 d to 30 d in milk) a basal control (CON) diet (n = 14) with no Met supplementation, CON plus MetaSmart (MS; Adisseo Inc., Antony, France; n = 12), or CON plus Smartamine M (SM; Adisseo Inc.; n = 13). The Met supplements were adjusted daily and top-dressed over the total mixed ration at a rate of 0.19 or 0.07% (dry matter) of feed for MS or SM. Liver tissue was collected on −10, 7, and 21 d for transcriptome profiling of genes associated with oxidative stress pathways. The expression of glutathione synthase (GSS); glutamate-cysteine ligase, catalytic subunit (GCLC); and superoxide dismutase 1, cytosolic (SOD1) was lower in Met-supplemented cows than CON. A greater overall expression of nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) and greater upregulation of haptoglobin (HP) on d 7 occurred in Met-supplemented cows than CON. Despite

greater HP expression after calving, the lower expression of glutathione (GSS and GCLC) metabolism genes and SOD1 due to Met reflect a lower oxidative stress and mild inflammatory status. Increasing the supply of Met as SM or MS can affect expression of genes in the Met cycle to various extents and, hence, the supply of methyl donors such as S-adenosylmethionine and antioxidants such as glutathione. These compounds likely are in high demand during the peripartum period.

2.1.7 Growth parameters - body weight and body measurement

Bryant *et al.* (1999) studied the effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers. Ruminally protected choline (RPC) was evaluated in two experiments. Beef steers (n = 160; average initial BW = 350.9 kg) were fed a 90% concentrate diet with either 0, .25, .5, or 1.0% RPC (DM basis) for 112 to 140 d. Feeding .25% RPC increased ADG 11.6% compared with 0% RPC, but responses diminished with increasing RPC level (cubic response, $P < .10$). Daily DMI was not affected by RPC level, but feed: gain was improved 6.8% with .25% RPC compared with 0% RPC, and responses diminished with increasing RPC level (cubic response, $P<0.10$). Carcass yield grade increased linearly ($P<0.10$) as RPC level increased, but marbling score was lower for all three RPC-containing diets than for the 0% RPC diet (quadratic response, $P<0.05$).

Bryant *et al.* (1999) studied effects of dietary level of ruminally protected choline on growth in lambs. Suffolk lambs (initial BW=29.8 kg) were fed an 80% concentrate diet for 56 d with the same RPC levels. For the 56-d feeding period, ADG responded quadratically ($P<0.10$) to RPC level. They concluded that supplementing RPC in high-concentrate diets improved performance, but results were not consistent among RPC levels or between cattle and sheep.

Bindel *et al.* (2000) evaluated the effects of ruminally protected choline and dietary fat on performance of finishing heifers. A 120-d finishing study utilizing 318 heifers (342 kg initial BW) was conducted to examine effects of ruminally protected choline (RPC) in diets containing graded concentrations of tallow. Heifers were blocked according to previous nutrition (full-fed or limit-fed) and allotted to 24 pens containing 11 to 15 heifers. Two pens, one within each block, were assigned to each of 12 factorially arranged treatments including dietary tallow (0, 2, or 4%) and supplemental RPC (0, 20, 40, or 60 g of product daily, estimated to supply 0, 5, 10, or 15 g/d choline postruminally). Heifers were implanted with Revalor-H and fed a

finishing diet based on steam-flaked and dry-rolled corn (12.5% CP, 8% alfalfa on DM basis). Dry matter intake decreased (P<0.10) by 5.4% when tallow was increased from 0 to 4% but was not affected by RPC. Heifers receiving 4% tallow had 7.3% lower gains than those receiving none (P<0.10). Supplementation of RPC increased (P<0.10) ADG, with 20 g/d resulting in an 8.6% increase. Similarly, gain efficiency improved (P<0.10) by 7.6% with addition of 20 g/d RPC. Yield grade and kidney, pelvic, and heart fat both increased linearly (P<0.10) with fat supplementation. The percentage of carcasses grading USDA Choice was not affected by intermediate levels of RPC but decreased with the highest level (60 g/d). Dressing percentage, hot carcass weight, marbling, and 12th-rib fat thickness were not affected significantly by either tallow or RPC. On d 90, jugular blood was collected from all heifers at 2 h postfeeding. Moderate levels of supplemental RPC improved growth performance of finishing cattle without negatively affecting carcass characteristics.

Guretzky *et al*. (2006) studied lipid metabolite profiles and milk production for holstein and jersey cows fed rumen-protected choline during the periparturient period. Thirty-two multiparous Holstein and 10 multiparous Jersey cows were randomly assigned to control or RPC treatments. A close-up diet was fed from approximately 3 wk before parturition through parturition, followed by a lactation diet from parturition through 49 d postpartum. For RPC, diets were top dressed once daily with 60 g of a RPC product (25% choline as choline chloride) from 21 d before expected parturition through 21 d postpartum. Treatment did not affect dry matter intake either prepartum (12.0 vs. 12.1 kg/d for RPC and control, respectively) or during the first 3 wk postpartum (14.8 vs. 15.7 kg/d, respectively). Cows were in moderate body condition score (mean = 3.3) at the start of the study and did not lose excessive condition by 3 wk postpartum (mean body condition score loss = 0.5); therefore, cows might not have been at great risk for hepatic lipid accumulation. Additionally, calculated Met balance was negative postpartum; supplemental RPC might not have spared enough Met to produce a physiological benefit. They concluded that more research is needed to determine how choline affects prevention or alleviation of fatty liver syndrome and to confirm potential differences between Holstein and Jersey cows.

Sexson *et al*. (2010) studied the impact of short-term choline supplementation on performance and carcass characteristics of finishing steers. A total of 288 yearling

steers (315.2±3.7 kg) were used to determine the effects short-term choline supplementation on feedlot performance and carcass characteristics. At 29 d before slaughter, 4 pens of steers per trace mineral × water treatment combination were supplemented with choline at a rate of 20 g of rumen-protected choline/steer daily, and the remaining 4 pens served as the controls (no supplemental rumen protected choline). There were no effects of trace mineral source, water quality, or interactions with feeding period for BW, ADG, DMI, or G:F. Initial and final BW, ADG, DMI, and G:F were similar across trace mineral and water treatments. Choline supplementation for only the last 29 d on feed did not affect performance. Carcasses from steers fed supplemental choline were less likely (P<0.01) to qualify for the USDA YG 4 and 5 categories. Other carcass characteristics were not affected by water quality, trace mineral source, or choline supplementation. Results of this experiment indicated that short-term choline supplementation had no effect on animal performance.

Sheikh (2012) conducted an experiment on 18 lactating crossbreed cows and reported that supplementation of 60g RPC resulted in no significant effect on body weight and body weight change in lactating crossbred cows.

Rahmani *et al.* (2014a) evaluated the effects of feeding rumen protected choline and vitamin E on dry matter intake, body condition score and body weight in early lactating dairy cows. Twenty four primiparous and multiparous Holstein cows on early lactation, beginning five weeks postpartum, were used for four weeks to investigate the effects of supplementation of rumen-protected choline (RPC) or vitamin E on milk yield, milk composition, dry matter intake, body condition score and body weight. The cows were randomly assigned to one of the following treatments: I) no supplement (control), II) 90 g/d of RPC and III) 4400 IU/d of vitamin E. In this study, dry matter intake, body weight and body condition score were not affected by choline or vitamin E supplementation (P>0.05). The results showed that RPC or vitamin E supplementation into the diets of early lactating dairy cows did not affect dry matter intake, body condition score and body weight.

Budiarsana *et al.* (2016) conducted a study to observe effect of choline chloride supplementation on body weight characterstic of Etawah grade goats. The experiment was conducted in a completely randomized block design with three types of treatments and eight replications. The trial had two successive experimental

periods; the first, during the eight weeks of late pregnancy, and the second, during the first 12 weeks of lactation. Twenty-four Etawah Grade does in the second gestation period were divided into three treatment groups. Commercial choline chloride 60 % in corncobs-based powder was used as a source of choline chloride. The treatments were no supplementation (control) and supplemented with either 4 g or 8 g/2days of choline chloride. Choline chloride was given to the animals through a forced drinking technique, after dissolving it in 60 ml drinking water. The initial body weight of does was 38.81 ± 3.66 kg. The does were penned individually, and were given fresh chopped King Grass ad libitum and 700 g/day of concentrate diets containing Ca-fish oil, starting eight weeks prior to expecting kidding and continuing for 12 weeks of parturition. The supplementation with choline chloride did not affect ($p>0.05$) BW pre-parturition, ADG and FCR values during the last eight weeks of pregnancy. There were no different effects on BW, ADG and FCR during pre-partum period due to the same nutrients intake of goats among the treatments.

Habeeb *et al.* (2017) studied evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. Twenty Zaraibi goat bucks were used in this experiment which lasted 3 months during summer season of Egypt. The animals were divided randomly into two equal groups. The first group was kept without treatment as control while in the second group, rumen-protected choline (RPC) at the level of 20 g/buck/day was added to the concentrate feed mixture at the morning feeding. RPC additives to diet of Zaraibi goat bucks during the period of hot summer season increased ($P<0.01$) total gain and average daily gain compared to the control group.

Rodriguez-Guerrero *et al.* (2018) studied the effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. Twenty-four lambs (Pelibuey x East Friesian), weighing 22.7 ± 3.2 kg, were fed a basal diet of corn silage, oat hay, alfalfa hay, and concentrate (60% forage and 40% concentrate). Treatments consisted of oral doses of rumen protected methionine (RPM) (0 and 1.5 g/day) and herbal choline (biocholine) (0 and 4 g/day) in a completely random block design with factorial arrangement of treatments, where lambs were blocked by sex. The experiment was conducted for 60 days, and measurements of live weight and dry matter intake were obtained. No effects of the treatments were observed on performance variables (lamb growth, consumption and feed conversion).

Kawas *et al.* (2020) studied the effects of rumen-protected choline on growth performance, carcass characteristics and blood lipid metabolites of feedlot lambs. Their study was on 40 intact male Saint Croix lambs (average: 20.3 kg, 3-4 months of age) on a high grain-low roughage base feed were randomly assigned to four treatments (0, 0.1, 0.2, and 0.3% RPC on dry-matter basis; n=10 per group). RPC was offered for 90 days after 15 days of adaptation. RPC supplementation was not associated with significant differences in dry matter intake, weight gain, gain: feed ratio, carcass weights, and the dressing percentages. There was a linear decrease in height to the shoulder ($p=0.013$) and longissimus muscle area ($p=0.051$) with higher RPC levels, and a higher backfat thickness and yield grade with 0.3% RPC compared to 0.1% RPC ($p<0.05$).

CHAPTER 3

MATERIALS AND METHODS

The present study entitled 'Physiobiochemical changes and growth performance in young Surti goats supplemented with rumen protected choline' was conducted in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Navsari on twelve young apparently healthy Surti goats. The materials and methods used for conducting the study are mentioned below under following categories:

3.1 LOCATION AND CLIMATIC CONDITIONS

Navsari is geographically located at an altitude of 11.89 M above mean sea level, at latitude of 20°57'0" north and longitude of 72°54'0" east. The climate of the area forms the part of tropical and coastal region. Generally, winter remains cold and dry, summer is moderately hot and humid while monsoon is extremely hot and humid.

3.2 RESEARCH METHODOLOGY

3.2.1 Selection of animals and their management

Total 12 apparently healthy female young Surti goats of age 9-11 months were selected. Their body weight was recorded and based on their age as well as body weight they were randomly divided into 2 groups of six animals each viz. Control and Treatment (RPC). Average age (in months) of selected young Surti goats was 9.84±0.21 for Control group and 9.85±0.22 for RPC group. Average body weights (in Kg) were 12.08±0.60 for Control group and 12.07±0.38 for RPC group. They were housed in pucca shed with concrete floor and feeding was done as per the ICAR feeding standards, 2013. The experimental animals were maintained at Livestock Research Station, Navsari.

3.2.2 Dietary supplementation of rumen protected choline (RPC)

Both control and RPC group of goats were offered similar basal diet as per standard routine of LRS, NAU, Navsari during the study period. Goats in treatment group were individually fed rumen protected choline @ 4 grams/animal/day daily for 4 weeks. Supplementation of RPC was done from beginning of study upto 4 weeks i.e. 28 days.

Control group - 6 young Surti goats (Basal diet: Green fodder + dry fodder + concentrate)

RPC group - 6 young Surti goats (Basal diet + rumen protected choline @ 4 grams/animal/day daily for 4 weeks

3.3 METEOROLOGICAL CONDITIONS OF GOAT SHED

The meteorological variables like ambient temperature (AT) and relative humidity (RH) of goat shed were recorded with the help of datalogger continuously throughout the study period. The THI, a measure of thermal load on animals, was calculated from a formula (Tucker *et al.*, 2008):

$$THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)]$$

Where

AT = ambient temperature in °C and

RH = relative humidity in %.

Range of minimum, maximum and average values of ambient temperature (AT, °C), relative humidity (RH, %) and temperature humidity index (THI) was calculated and tabulated.

3.4 RECORDING OF PARAMETERS

3.4.1 Recording of body measurements

Body weight, body length, heart girth and height at withers of all the goats were recorded at the beginning of the study i.e. week 0 and thereafter at fortnight intervals i.e. at week 2, week 4 and week 6. Body measurements were done for 2 extra weeks after stopping of dietary RPC supplementation. Body weight was recorded using digital weight balance. Body length, heart girth and height at withers were determined using a measuring tape.

3.4.2 Recording of physiological parameters

Physiological parameters such as rectal temperature, respiration rate, heart rate and tympanic temperature were measured in all the goats at week 0, week 2 and week 4 of study. Rectal temperature was measured by placing a digital clinical thermometer in the rectum until the beep sound and reading was recorded. Respiration rate was measured by counting the exhalation from nostrils felt on the dorsal side of hand and corroborated with flank movements. Heart rate was measured by directly auscultating the heart using a stethoscope. Tympanic temperature was measured by infra red non-contact ear thermometer (BPL technologies).

3.5 SAMPLE COLLECTION

3.5.1 Collection of blood samples

Whole blood was collected at beginning of study/supplementation i.e. week 1 and week 4 i.e. at the end of supplementation. The samples collected at beginning were termed as samples of week 0. 5.0 ml of whole blood from each animal was collected from jugular vein and divided into vacutainers containing K_3EDTA and without anticoagulant (for serum separation). Whole blood with and without anticoagulant was centrifuged at 3000 rpm for 15 minutes and plasma and serum were separated in clean vials. They were stored at -20ºC in deep freeze for further analysis. Whole blood in K_3EDTA was also used to separate plasma by centrifuging it for 3000 and was used for glucose estimation

Some portion of whole blood collected in K_3EDTA was used for hematological examination as well as analysis of reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde assay (MDA) for Lipid peroxidation (LPO).

Serum was used for analysis of glucose, triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin, urea, creatinine, triiodothyronine (T_3), thyroxine (T_4), cortisol, leptin, sodium (Na^+), potassium (K^+) and chloride (Cl^-).

3.6 LABORATORY ANALYSIS

3.6.1 Analysis of hematological parameters (Hemogram and leukogram)

Hematological parameters such as hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume/hematocrit (PCV/Hct), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated using manual method. Indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and neutrophil: lymphocyte ratio (NLR) were calculated from their respective parameters. Following formulae were used to calculate hemalogical indices:

$$\text{Mean corpuscular volume, MCV (fL)} = \frac{\text{Hct or PCV (\%)}}{\text{TEC } ((\text{x}10^6/\mu\text{l}))}$$

$$\text{Mean corpuscular haemoglobin, MCH (pg)} = \frac{\text{Hb (g/dl)}}{\text{TEC } ((\text{x}10^6/\mu\text{l}))}$$

$$\text{Mean corpuscular hemoglobin concentration, MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{Hct or PCV (\%)}} \times 100$$

3.6.2 Analysis of biochemical parameters

Serum was used to analyze blood biochemical metabolites viz. glucose (Glu), triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein (TP), albumin (Alb), urea and creatinine (Creat) using Randox kits on semi-automated clinical chemistry analyzer (Merck, Vital Scientific N.V., Netherlands).

3.6.2.1 Glucose

Randox Glucose (GLUC-PAP) kit was used to estimate glucose concentration based on glucose oxidase (GOD-PAP) method. In this method, glucose is oxidized in the presence of enzyme glucose oxidase. The formed hydrogen peroxide catalysed by peroxidase enzyme further reacts with phenol and 4-aminophenazone to form red-violet quinoneimine dye as indicator. The intensity is directly proportional to the glucose concentration and is measured at 505 nm. The result is expressed as mg/dl.

10 µl of serum/standard was treated with 1000 µl glucose reagent and incubated at 37° C for 10 minutes. Absorbance of standard ($A_{standard}$) and the sample (A_{sample}) against the reagent blank was read at 505 nm. Concentration of glucose was calculated using the following formula:

$$\text{Glucose concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard concentration (mg/dl)}$$

3.6.2.2 Total Cholesterol (TC)

Estimation of total cholesterol was done as per enzymatic end point method. Total cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase. Absorbance is read at 546 nm. The results obtained were expressed as mg/dl.

10 µl of serum/standard was treated with 1000 µl cholesterol reagent. It was mixed and incubated at 37ºC for 5 minutes. Absorbance of the standard ($A_{standard}$) and

the sample (A_{sample}) against the reagent blank was read at 546 nm. Concentration of cholesterol was calculated using the following formula:

$$\text{Cholesterol concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard concentration (mg/dl)}$$

3.6.2.3 Triglyceride (TG)

Estimation of serum triglycerides was done as per GPO-PAP end point method in which triglycerides are determined after enzymatic hydrolysis with lipoprotein lipases. The indicator is a quinonemine formed from hydrogen peroxidase, 4-aminophenazone and 4- chorophenol under the catalytic influence of peroxidase. Absorbance of colored dye is measured at 505 nm and is proportional to triglycerides concentration in the sample. The results obtained were expressed as mg/dl.

10 µl of serum/standard was treated with 1000 µl triglyceride reagent. It was mixed and incubated at 37ºC for 5 minutes. Absorbance of the standard ($A_{standard}$) and the sample (A_{sample}) against the reagent blank was read at 505 nm. Concentration of triglyceride was calculated using the following formula:

$$\text{Triglycerides concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard concentration (mg/dl)}$$

3.6.2.4 High density lipoprotein (HDL)

Estimation of High density lipoprotein- cholesterol (HDL-C) was done by enzyme clearance assay using Randox kit. The assay principle consists of 2 distinct reaction steps : First step involves elimination of chylomicron, VLDL-Cholesterol and LDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase. Second step comprises of specific measurement of HDL-Cholesterol after release of HDL-Cholesterol by detergents in reagent 2 of kit. Finally the intensity of the quinone imine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm. The result was expressed in mg/dl.

15 µl of serum/standard was treated with 900 µl reagent I. It was mixed and incubated at 37°C for 5 min. Further 300 µl of reagent 2 was mixed in it and

incubated for 30 seconds followed by measuring initial absorbance (A1). Again after incubating for 150 seconds final absorbance (A2) was measured.

Following formulae was used for calculation:

$$A_2 - A_1 = \Delta A$$

$$\text{High density lipoprotein-cholesterol HDL-C (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{callibrator}} \times \text{Calibrator concentration (mg/dl)}$$

3.6.2.5 Low density lipoprotein (LDL)

Estimation of Low density lipoprotein- cholesterol (LDL-C) was done by enzyme clearance assay using Randox kit. The procedure is based on elimination method without any need of sample pre-treatment. The assay principle consists of 2 distinct reaction steps: First step involves elimination of chylomicron, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase. Second step comprises of specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by detergents in reagent 2 of kit. Finally the intensity of the quinoneimine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm. The result was expressed in mg/dl.

12 µl of serum/standard was treated with 900 µl reagent I. It was mixed and incubated at 37°C for 5 min. Further 300 µl of reagent 2 was mixed in it and incubated for 30 seconds followed by measuring initial absorbance (A1). Again after incubating for 150 seconds final absorbance (A2) was measured.

Following formulae was used for calculation:

$$A_2 - A_1 = \Delta A$$

$$\text{High density lipoprotein-cholesterol HDL-C (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{callibrator}} \times \text{Calibrator concentration (mg/dl)}$$

3.6.2.6 Total protein (TP)

Estimation of total protein was done by Biuret method in which cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex whose intensity was measured at 546 nm. The results obtained are expressed as g/dl.

20 µl of serum/standard was treated with 1000 µl biuret reagent. It was mixed and incubated for 10 min at 37ºC. Further the absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) against the reagent blank was measured at 546 nm. Following formula was used for calculation:

$$\text{Total protein concentration (g/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard concentration (g/dl)}$$

3.6.2.7 Albumin

Estimation of serum albumin was based on its quantitative binding to the indicator 3', 3'', 5', 5''-Tetrabromo-m-cresolsulfonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs light maximally at 600 nm, the absorbance being directly proportional to the concentration of albumin in the sample. The results obtained expressed as g/dl.

10 µl of serum/standard was treated with 1000 µl albumin reagent (BCG concentrate). It was mixed and incubated at 37ºC for 5 minutes. Absorbance of the standard ($A_{standard}$) and the sample (A_{sample}) against reagent blank was readat 600 nm. Concentration of albumin was calculated using the following formula:

$$\text{Albumin concentration (mg/dl)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard concentration (mg/dl)}$$

3.6.2.8 Globulin

Serum globulin was calculated using the following formula :

Serum globulin (g/dl) = serum total protein (g/dl) – albumin (g/dl)

3.6.2.9 Urea

Urea estimation was done by method based on L-glutamate dehydrogenase (GLDH) kinetic method. The procedure is based on hydrolysis of urea by urease enzyme to yield ammonium ion and carbonate ion. Ammonium ion combines with 2-oxoglutarate in the presence of glutamate dehydrogenase to form L-glutamate and

NAD^+ . The absorbance is then measured at 346 nm. The results obtained expressed as mg/dl.

10 µl sample was treated with 1000 µl test reagent. It was mixed thoroughly and absorbance was immediately and after 1 min at 346 nm. Following formula was used for calculation :

$$\Delta A_{\text{sample/standard}} = (A_2 - A_1) - \Delta A_{\text{blank}}$$

3.6.2.10 Creatinine

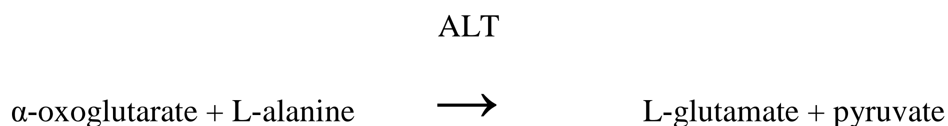
Randox creatinine kit was used for creatinine estimation. Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration. The result was obtained in mg/dl.

3.6.3 Analysis of hepatic enzymes

Serum was used to analyze hepatic enzymes using Randox kits on semi-automated clinical chemistry analyzer (Merck, Vital Scientific N.V., Netherlands).

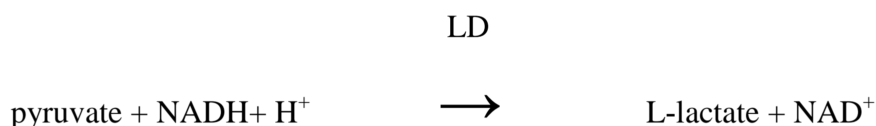
3.6.3.1 Alanine transaminase (ALT)

Randox ALT was used for ALT estimation from serum. In this method, α-oxoglutarate reacts with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The indicator reaction utilizes the pyruvate for a kinetic determination of NADH consumption. The intensity was measured in U/l.



ALT

α-oxoglutarate + L-alanine $\longrightarrow$ L-glutamate + pyruvate



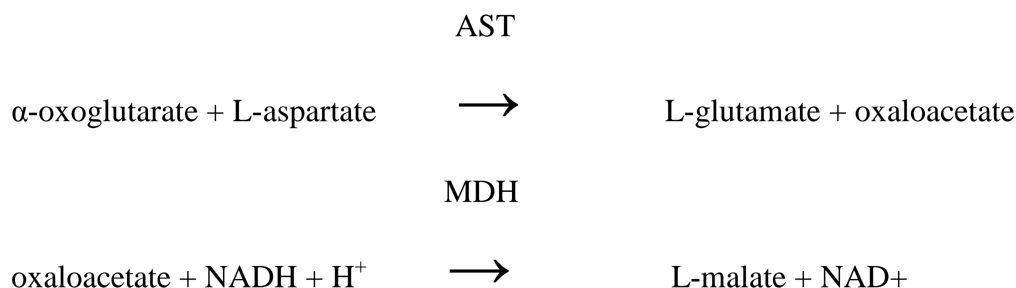
LD

pyruvate + NADH+ H^+ $\longrightarrow$ L-lactate + NAD^+

3.6.3.2 Aspartate aminotransferase (AST)

Randox AST was used for AST estimation from serum. In this method, α-oxoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus

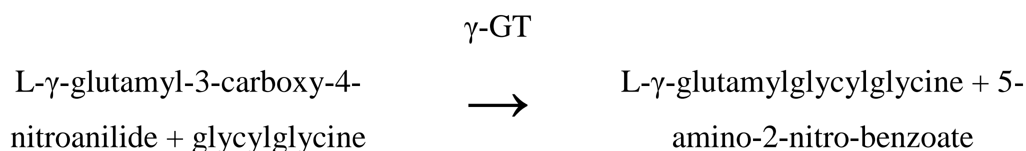
oxaloacetate. The indicator reaction utilizes the oxaloacetate for a kinetic determination of NADH consumption. The intensity was measured in U/l.



50 µl sample was mixed with 500 µl freshly prepared working reagent. It was mixed thoroughly and initial absorbance was recorded after 60 seconds followed by absorbance at 120 seconds at 340 nm wavelength.The mean absorbance change was determined.

3.6.3.3 Gamma-glutamyl transferase (GGT)

Randox GGT was used for GGT estimation from serum. The substrate L-γ-glutamyl-3-carboxy-4-nitronilide, in the presence of glycylgylcine is covered by γ-GT in the sample, to 5-amino-2-nitro-benzoate which absorbed at 405 nm. The result was obtained in U/L.



14 µl sample was mixed with 500 µl freshly prepared working reagent R1 reagent and incubated for 5 minutes at 37°C. It was mixed with 100 µl of R2 reagent. It was mixed thoroughly and incubated at 37°C for 1 minute and initial absorbance was at read at 410 nm. Further absorbance was read at 410 nm after incubating for 4 minutes at 37°C. The mean absorbance change was determined.

3.6.4 Analysis of serum hormones

Serum was used to analyze hormones T_3, T_4, cortisol and leptin using ELISA kits (Bioassay Technology Laboratory). Absorbance was measured using ELISA reader.

3.6.4.1 Triiodothyronine (T_3)

Estimation of Triiodothyronine (T_3) hormone was carried out using goat specific ELISA kit (Bioassay Technology Laboratory)

Principle

T3 present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Goat T3 Antibody is added and binds to T_3 in the sample. Further Streptavidin-HRP is added and binds to the Biotinylated T_3 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Goat T3. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

All reagents were brought to room temperature before use.

Standard preparation

120µl of the standard (64ng/ml) was reconstituted with 120µl of standard diluent to generate a 32ng/ml standard stock solution. Standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (32ng/ml) 1:2 with standard diluent to produce 16ng/ml, 8ng/ml, 4ng/ml and 2ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). Dilution of standard solutions mentioned above is as follows:

32ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
16ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
8ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
4ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer preparation

20ml of Wash Buffer Concentrate was diluted 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer and mixed gently avoiding formation of any crystals.

Assay Procedure

1. All reagents, standard solutions and samples were prepared as per instructions in the kit. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. 50 µl standards were added to standard well.
3. 40 µl samples were added to sample wells. Then 10µl anti-T3 antibody was added to sample wells, followed by addition of 50µl streptavidin-HRP to sample wells and standard wells.
4. Mixing of the well contents was done, plate was covered with a sealer and incubated for 60 minutes at 37°C.
5. Further sealer was removed and plate wells were washed 5 times with wash buffer. Wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blotting of the plate was done on absorbent material.
6. 50 µl substrate solution A was added to each well followed by addition of 50µl substrate solution B. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
7. 50 µl of stop solution was added to each well. Blue color changed into yellow immediately.
8. Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Computer-based curve-fitting software was used to plot average OD for each standard on the vertical (Y) axis against concentration on the horizontal (X) axis and a best fit curve through the points were drawn. Subsequently the concentration of sample was determined from their respective OD values.

3.6.4.2 Thyroxine (T_4)

Estimation of Thyroxine (T_4) hormone was carried out using goat specific ELISA kit (Bioassay Technology Laboratory)

Principle

T_4 present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Goat T_4 Antibody is added and binds to T_4 in the sample. Further Streptavidin-HRP is added and binds to the Biotinylated T_4 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution

is then added and color develops in proportion to the amount of Goat T_4. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

All reagents were brought to room temperature before use.

Standard preparation

120µl of the standard (960nmol/L) was reconstituted with 120µl of standard diluent to generate a 480nmol/L standard stock solution. Standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (480nmol/L) 1:2 with standard diluent to produce 240nmol/L, 120nmol/L, 60nmol/L and 30nmol/L solutions. Standard diluent served as the zero standard (0 nmol/L). Dilution of standard solutions mentioned above is as follows:

480nmol	Standard No.5	120µl Original Standard + 120µl Standard Diluent
240nmol	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
120nmol	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
60nmol/	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
30nmol/	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer preparation

20ml of Wash Buffer Concentrate was diluted 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer and mixed gently avoiding formation of any crystals.

Assay Procedure

1. All reagents, standard solutions and samples were prepared as per instructions in the kit. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. 50 µl standards were added to standard well.
3. 40 µl samples were added to sample wells. Then 10µl anti- T_4 antibody was added to sample wells, followed by addition of 50µl streptavidin-HRP to sample wells and standard wells.
4. Mixing of the well contents was done, plate was covered with a sealer and incubated for 60 minutes at 37°C.

5. Further sealer was removed and plate wells were washed 5 times with wash buffer. Wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blotting of the plate was done on absorbent material.
6. 50 µl substrate solution A was added to each well followed by addition of 50µl substrate solution B. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
7. 50 µl of stop solution was added to each well. Blue color changed into yellow immediately.
8. Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Computer-based curve-fitting software was used to plot average OD for each standard on the vertical (Y) axis against concentration on the horizontal (X) axis and a best fit curve through the points were drawn. Subsequently the concentration of sample was determined from their respective OD values.

3.6.4.3 Cortisol

Estimation of cortisol hormone was carried out using goat specific ELISA kit (Bioassay Technology Laboratory)

Principle

Cortisol present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Goat cortisol Antibody is added and binds to cortisol in the sample. Further Streptavidin-HRP is added and binds to the Biotinylated cortisol antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Goat cortisol. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

All reagents were brought to room temperature before use.

Standard preparation

120µl of the standard (64ng/ml) was reconstituted with 120µl of standard diluent to generate a 32ng/ml standard stock solution. Standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (32ng/ml) 1:2 with

standard diluent to produce 16ng/ml, 8ng/ml, 4ng/ml and 2ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). Dilution of standard solutions mentioned above is as follows:

32ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
16ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
8ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
4ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer preparation

20ml of Wash Buffer Concentrate was diluted 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer and mixed gently avoiding formation of any crystals.

Assay Procedure

1. All reagents, standard solutions and samples were prepared as per instructions in the kit. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. 50 µl standards were added to standard well.
3. 40 µl samples were added to sample wells. Then 10µl anti-cortisol antibody was added to sample wells, followed by addition of 50µl streptavidin-HRP to sample wells and standard wells.
4. Mixing of the well contents was done, plate was covered with a sealer and incubated for 60 minutes at 37°C.
5. Further sealer was removed and plate wells were washed 5 times with wash buffer. Wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blotting of the plate was done on absorbent material.
6. 50 µl substrate solution A was added to each well followed by addition of 50µl substrate solution B. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
7. 50 µl of stop solution was added to each well. Blue color changed into yellow immediately.
8. Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Computer-based curve-fitting software was used to plot average OD for each standard on the vertical (Y) axis against concentration on the horizontal (X) axis and a best fit curve through the points were drawn. Subsequently the concentration of sample was determined from their respective OD values.

3.6.4.4 Leptin

Estimation of leptin hormone was carried out using goat specific ELISA kit (Bioassay Technology Laboratory)

Principle

Leptin present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Goat leptin Antibody is added and binds to leptin in the sample. Further Streptavidin-HRP is added and binds to the Biotinylated leptin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Goat leptin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

All reagents were brought to room temperature before use.

Standard preparation

120µl of the standard (128ng/ml) was reconstituted with 120µl of standard diluent to generate a 64ng/ml standard stock solution. Standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (64ng/ml) 1:2 with standard diluent to produce 32ng/ml, 16ng/ml, 8ng/ml, 4ng/ml and 2ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). Dilution of standard solutions mentioned above is as follows:

64ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
32ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
16ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
8ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
4ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer preparation

20ml of Wash Buffer Concentrate was diluted 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer and mixed gently avoiding formation of any crystals.

Assay Procedure

1. All reagents, standard solutions and samples were prepared as per instructions in the kit. All reagents were brought to room temperature before use. The assay was performed at room temperature.
2. 50 µl standards were added to standard well.
3. 40 µl samples were added to sample wells. Then 10µl anti- leptin antibody was added to sample wells, followed by addition of 50µl streptavidin-HRP to sample wells and standard wells.
4. Mixing of the well contents was done, plate was covered with a sealer and incubated for 60 minutes at 37°C.
5. Further sealer was removed and plate wells were washed 5 times with wash buffer. Wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blotting of the plate was done on absorbent material.
6. 50 µl substrate solution A was added to each well followed by addition of 50µl substrate solution B. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
7. 50 µl of stop solution was added to each well. Blue color changed into yellow immediately.
8. Optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Computer-based curve-fitting software was used to plot average OD for each standard on the vertical (Y) axis against concentration on the horizontal (X) axis and a best fit curve through the points were drawn. Subsequently the concentration of sample was determined from their respective OD values.

3.6.5 Analysis of serum electrolytes

Serum was used to analyze electrolytes sodium (Na^+), potassium (K^+) and chloride (Cl^-).

3.6.5.1 Sodium and Potassium (Na and K):

Sodium and Potassium estimation was done by using flame photometer 128 (SYSTRONICS).

Principle:

Diluted samples (1:100) were introduced in the form of continuous spray into a non luminous gas flame. The emitted light of a wavelength characteristic for the ion being analysed focused on photoelectric cell. The electric response of the photoelectric cell was measured on suitable meter that converts the electrical impulse into ion concentration.

Procedure:

Serum dilution of 1:100 was used for either sodium or potassium analysis. Standard solutions were used along with unknown samples whenever analysis was performed.

Sodium standard solutions in the range of 100-160 mEq of sodium / l were used. Stock standard of sodium containing 100 mEq of sodium / l was prepared by dissolving 5.85 gm of NaCl in water and diluting it to one litre. Working standards were prepared from the stock standard by measuring out 10, 11, 12, 13, 14, 15 and 16 ml aliquots into one litre volumetric flask and diluting it to the mark with water. These standards represented 100,110,120,130,140,150 and 160 mEq of sodium / l respectively at a dilution rate of 1:100.

For potassium, standard solutions in the range of 3-7 mEq / l at a dilution of 1:100 were prepared. Stock standard of potassium containing 10 mEq of potassium / l was prepared by dissolving 0.746 gm of KCl in water and diluting it to one litremark. Working standards equivalent to 3,4,5,6 and 7 mEq of sodium / l at a dilution of 1:100 were prepared. Results obtained were expressed as mmol/l.

3.6.5.2 Chloride (Cl^-):

Randox Cl was used for serum chloride estimation. it is based on colorimetric method. In this method, Chloride ions react with mercurious thiocyanate to form mercury perchlorate and thiocyanate. The liberated thiocyanate forms a red complex with ferric chloride in the presence of nitric acid.

5 µl of serum sample was mixed with 500 µl reagent R1, mixed, incubated for 5 minutes at 37°C. Absorbance was measured at 456 nm.

3.6.6 Analysis of oxidative stress parameters

3.6.6.1 Processing of RBC for GSH, SOD and LPO

Whole blood was taken in tube and centrifuged at 2000 rpm for 15 minutes. Plasma and buffy coat were removed. Resulting RBC pellet was washed thrice with 0.15 M NaCl and centrifuged at 3000 rpm for 10 min. Supernatant was discarded. Dilution of 33% of packed RBC was made in PBS. Washed erythrocyte pellet was suspended in PBS (pH 7.4) and kept at 4 ºC until further analysis was done.

3.6.6.2 Lipid peroxides (LPO)

Membrane peroxidative damage in erythrocytes was determined in terms of Malondialdehyde (MDA) production by the method suggested by Rehman (1984).

Procedure

One ml of 33% packed erythrocyte was incubated at 37±0.5º C for 2 hours, and then one ml of 10% trichloro-acetic acid was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 minutes. To 1 ml of supernatant, 1 ml of 0.67% of thiobarbituric acid was added and kept in boiling water bath for 10 minutes, cooled and diluted with 1 ml of distilled water. Blank was prepared by adding all the reagents except the packed erythrocytes. The absorbance was read at 535 nm against the reagent blank and following formula was used for calculation

The amount of lipid peroxides was expressed as nanomole (nM) of MDA formed per ml of packed cells.

$$\frac{\text{O.D. of test} \times \text{Total volume of reaction mixture}}{\text{EC} \times \text{Volume of sample taken}} \times 10^{9} \times \text{DF} \times \text{Time of incubation}$$

Where,

Total volume of reaction mixture = 3 ml

Dilution Factor (DF) = 3

Time of incubation = 2 hr

Molar Extinction Co-efficient (EC) of MDA-TBA complex =

1.56×10^{8} /M/cm or 1.56×10^{5}

3.6.6.3 Reduced Glutathione (GSH)

The reduced glutathione levels in plasma were estimated as per the method described by Moron *et al*., (1979). Reduced glutathione on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) gives a yellow coloured complex and its absorbance was measured at 412 nm.

Reagents

1. Phosphate buffer (0.2M pH 8.0)
2. 5,5'- Dithiobis, 2- nitrobenzoic acid (DTNB, 0.6 Mm in 0.02 M phosphate buffer)
3. Tricholoacetic acid (TCA -20%)
4. Standard GSH (10 mg/100 ml)

Procedure

0.5 ml of plasma was precipitated with 2 ml of 20% TCA and centrifuged at 3000 rpm for 10 min. 2 millilitres of freshly prepared DTNB solution and 4.5 ml of 0.2 M sodium phosphate buffer were added to 1 ml supernatant. The intensity of yellow colour formed was read at 412 nm after 10 min in spectrophotometer against TCA as blank. The quantity of reduced glutathione was expressed as mg/dl of plasma.

3.6.6.4 Superoxide Dismutase (SOD)

Superoxide Dismutase was estimated as per method described by Madesh and Balasubramanian (1998). In this there is generation of superoxide by pyragallol auto-oxidation and the inhibition of superoxide dependent reduction of tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2, 5 diphenyl tetrazolium bromide] to its formazon, absorbance measured at 570 nm. The ongoing reaction is terminated by the addition of dimethylsulfoxide (DMSO), which helps to solubilize the formazon formed. It is expressed as SOD units (one unit of SOD is the µg of hemoglobin required to inhibit the MTT reduction by 50%).

Reagents

1. PBS (7 pH)
2. 1.25 mM MTT
3. 100 µM Pyragallol
4. DMSO

Procedure

Reagents were added in test, standard and blank as given in the order below.

Sr. no.	Reagents	Test	Standard	Blank
1	PBS (7 pH)	0.65 ml	0.65 ml	0.65 ml
2	1.25 mM MTT	30 µl	30 µl	30 µl
3	Hemolysate	10 µl	-	-
4	Pyragallol (100 µM)	75 µl	75 µl	75 µl
5	Incubated for 5 minutes at room temperature			
6	DMSO	0.75 ml	0.75 ml	0.75 ml
7	Hemolysate	-	10 µl	-

Absorbance was read at 570 nm against distilled water and following formula was used for calculation

$$Y\% = \frac{\text{O.D. of test}}{\text{O.D. of Standard}} \times 100$$

$$\text{SOD (U/mg of Hb)} = \frac{\text{mg of Hemoglobin in 0.01 ml}}{Y\%} \times 50 \times DF$$

Where, DF = Dilution Factor

Y% = % inhibition of MTT reduction by SOD protein

3.6.6.5 Total antioxidant status (TAS)

Total antioxidant status was measured in plasma by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

Principle

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

Reagents

1. FRAP Reagent

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water.

b) TPTZ (2, 4, 6-tripyridyl-s- triazine) (M.W. 312.34) 10 mM in 40mM HCl (M.W. 36.46)

c) $FeCl_3$. $6H_2O$ (M.W. 270.30) 20 mM

The working FRAP reagent was prepared by mixing a b & c in the ratio of 10:1:1 at the time of use.

Standard: Ascorbic Acid (M.W. 176.13) 1000 µM

Procedure

Solutions	Blank	Standard	Test
Sample (Plasma)	-	-	100 µl
Standard (Ascorbic acid)	-	100 µl	-
Working FRAP Solution	3000 µl	3000 µl	3000 µl

The components were mixed well.

1. Spectrophotometer was blanked with water.
2. OD of Standard and Test was measured at zero minute and again after four minutes at 593 nm.

Calculation:

Results were calculated as follows.

FRAP value of Sample (µM) =	Change in absorbance of sample from 0 to 4 minute / Change in absorbance of Standard from 0 to 4 minute)	X	FRAP value of standard (1000 µM)

Note: FRAP value of Ascorbic acid is 2.

3.6 STATISTICAL ANALYSIS

Statistical analysis of obtained data was carried out by student t test for independent samples for interpreting effect of different groups for the traits under study. Mean separation at 5% level of significance was considered for difference between means of different treatment groups (Snedecor and Cochran, 1994).

CHAPTER 4

RESULTS AND DISCUSSION

This chapter embodies the research results of the study conducted to observe physiobiochemical changes and growth performance in young Surti goats supplemented with rumen protected choline. The experimental animals were divided into 2 groups i.e. control and treatment that was supplemented with RPC. The results of the study of every aspect have been presented and described along with their statistical inference below.

4.1 METEOROLOGICAL CONDITIONS

The results of meteorological conditions of goat shed are mentioned in table 4.1.

The range of minimum, maximum and average values of ambient temperature (°C) were 19.8-23.5, 34.2-38.9 and 27.5-30; relative humidity (%) were 19.6-35.9, 76-90.2 and 54.5-61.9 and temperature humidity index (THI) were 62.38-65.41, 74.08-77.87 and 68.66-70.75 respectively during the study period.

Table 4.1: Meteorological conditions of goats shed during the study period

Groups	Minimum	Maximum	Average
	Ambient temperature (°C)		
Range	19.80-23.50	34.20-38.90	27.50-30.00
	Relative humidity (%)		
Range	19.60-35.90	76.00-90.20	54.50-61.90
	THI		
Range	62.38-65.41	74.08-77.87	68.66-70.75

The average values of ambient temperature, relative humidity and more importantly THI were near to those that are considered comfortable for livestock. In a study conducted by Srivastava *et al.* (2021) threshold THIs for PR, RR and RT in Jamunapari goats were 71.78, 75.14 and 85.94, respectively in India. Thus in the present study perusal of the meteorological variables revealed that the microclimate of the goat shed was comfortable and thus was least likely to cause any sort of thermal stress in young Surti goats.

4.2 PHYSIOLOGICAL PARAMETERS

The results (Mean±SE) for effect of supplementation of rumen protected choline on physiological parameters of Surti goats are mentioned in table 4.2

Table 4.2: Physiological parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)			
Groups	**Week 0**	**Week 2**	**Week 4**
	Rectal temperature (°F)		
Control (n=6)	101.07±0.18	101.38[a]±0.09	101.67±0.08
RPC (n=6)	101.05±0.14	101.08[b]±0.03	101.33±0.04
Overall (n=12)	101.06±0.11	101.23±0.07	101.50±0.07
	Tympanic temperature (°F)		
Control (n=6)	100.87±0.19	101.23±0.03	101.48±0.08
RPC (n=6)	100.95±0.16	101.18±0.04	101.32±0.08
Overall (n=12)	100.91±0.12	101.21±0.03	101.40±0.06
	Heart rate (beats/minute)		
Control (n=6)	102.00±1.86	113.00[a]±2.24	118.33±1.41
RPC (n=6)	100.67±2.04	104.33[b]±1.96	105.00±1.69
Overall (n=12)	101.33±1.33	108.67±1.93	111.67±2.27
	Respiration rate (breaths/minute)		
Control (n=6)	31.00±1.77	33.67±1.20	36.00±2.00
RPC (n=6)	32.67±1.12	34.33±1.58	37.67±0.61
Overall (n=12)	31.83±1.03	34.00±0.95	36.83±1.03
Means bearing different superscripts (a, b) differ significantly (P≤0.05) between groups			

Rectal temperature (°F) at week 0, week 2 and week 4 of study in control was 101.07±0.18, 101.38±0.09 and 101.67±0.08 and RPC supplemented group was 101.05±0.14, 101.08±0.03 and 101.33±0.04 respectively. Tympanic temperature (°F) at week 0, week 2 and week 4 in control was 100.87±0.19, 101.23±0.03 and 101.48±0.08 and RPC supplemented group was 100.95±0.16, 101.18±0.04 and 101.32±0.08 respectively. Heart rate (beats/minute) at week 0, week 2 and week 4 in control was 102.00±1.86, 113.00±2.24 and 118.33±1.41 and RPC supplemented group was 100.67±2.04, 104.33±1.96 and 105.00±1.69 respectively. Respiration rate (breaths/minute) at week 0, week 2 and week 4 in control was 31.00±1.77, 33.67±1.20 and 36.00±2.00 and RPC supplemented group was 32.67±1.12, 34.33±1.58 and 37.67±0.61 respectively.

Comparative analysis between groups showed that amongst physiological parameters rectal temperature and heart rate were significantly (P≤0.05) lower in RPC supplemented group at week 2 and 4. Tympanic temperature as well as rate of respiration were not affected by RPC supplementation whatsoever at any stage of study. Choline acts as a precursor of neurotransmitter acetylcholine and higher levels of acetylcholine are associated with lowering of body temperature and heart rate. Cholinergic signaling reduces heart rate, force of contraction of atria and conduction

velocity of both SA and AV nodes (Roy *et al.*, 2014). Shibasaki *et al.* (2002) have shown that acetylcholine released from cholinergic nerves is capable of modulating cutaneous vasodilation via NO-synthase mechanisms. This cutaneous vasodilation may cause increased heat loss and lowering of rectal temperature. Role of cholinergic sweating mediated by acetylcholine has been well established (Shibasaki and Crandall, 2010) that also seems to explain the heat loss and lowering of rectal temperature in the present study. In the present study significant lowering of body temperature as well as heart rate may also be attributed to reduced stress as choline aids in combating oxidative stress as discussed later in this chapter. Tympanic temperature and respiration rate generally accounts for acute and transient changes in relation to heat stress but as the microclimate of goat shed was comfortable they did not differed significantly between control and RPC supplemented group. However all the parameters were within normal range. The studies for observing the effect of RPC supplementation on the physiological parameters studied in the present study are lacking so direct resemblance with other studies is limited.

4.3 HEMATOLOGICAL PARAMETERS

Results for hematological parameters are presented and discussed under sub-headings hemogram profile and leukogram profile.

4.3.1 Hemogram profile

The results (Mean±SE) for effect of supplementation of rumen protected choline on hemogram profile of Surti goats are mentioned in table 4.3.1

Hemoglobin (g/dl) at week 0 and week 4 in control was 9.55±0.43 and 8.43±0.17 and in RPC supplemented group was 9.78±0.29 and 9.23±0.18 respectively. Total erythrocyte count (10^6cells/µl) at week 0 and week 4 in control was 15.73±0.80 and 12.81±0.12and in RPC supplemented group was 15.03±0.33 and 13.46±0.13 respectively. Hematocrit/Packed cell volume (%) at week 0 and week 4 in control was 26.28±1.08 and 24.12±0.39 and in RPC supplemented group was 27.23±0.81 and 26.88±0.27 respectively. Mean corpuscular volume (fL) at week 0 and week 4 in control was 16.81±0.67 and 17.92±0.27 and in RPC supplemented group was 18.13±0.33 and 21.00±0.27 respectively. Mean corpuscular hemoglobin (pg) at week 0 and week 4 in control was 6.12±0.32 and 6.27±0.13 and in RPC supplemented group was 6.52±0.17 and 7.21±0.16 respectively. Mean corpuscular hemoglobin concentration (g/dl) at week 0 and week 4 in control was 36.36±0.82 and

35.03±1.00 and in RPC supplemented group was 35.94±0.57 and 34.37±0.79 respectively.

Table 4.3.1: Hemogram profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)

Groups	Week 0	Week 4
	Haemoglobin, Hb (g/dl)	
Control (n=6)	9.55±0.43	8.43^{b}±0.17
RPC (n=6)	9.78±0.29	9.23^{a}±0.18
Overall (n=12)	9.67±0.25	8.83±0.17
	Total erythrocyte count - TEC (x10^{6}/µl)	
Control (n=6)	15.73±0.80	12.81^{b}±0.12
RPC (n=6)	15.03±0.33	13.46^{a}±0.13
Overall (n=12)	15.38±0.42	13.13±0.13
	Hematocrit/Packed cell volume, Hct/PCV (%)	
Control (n=6)	26.28±1.08	24.12^{b}±0.39
RPC (n=6)	27.23±0.81	26.88^{a}±0.27
Overall (n=12)	26.76±0.66	25.50±0.48
	Mean corpuscular volume, MCV (fL)	
Control (n=6)	16.81±0.67	17.92^{b}±0.27
RPC (n=6)	18.13±0.33	21.00^{a}±0.27
Overall (n=12)	17.47±0.41	19.46±0.50
	Mean corpuscular haemoglobin, MCH (pg)	
Control (n=6)	6.12±0.32	6.27^{b}±0.13
RPC (n=6)	6.52±0.17	7.21^{a}±0.16
Overall (n=12)	6.32±0.18	6.74±0.17
	Mean corpuscular hemoglobin concentration, MCHC (g/dl)	
Control (n=6)	36.36±0.82	35.03±1.00
RPC (n=6)	35.94±0.57	34.37±0.79
Overall (n=12)	36.15±0.48	34.70±0.61

Means bearing different superscripts (a, b) differ significantly (P≤0.05) between groups

Amongst the parameters for hemogram profile total erythrocyte concentration, hemoglobin and hematocrit (HCT or PCV) did not differ significantly initially but were significantly (P≤0.05) increased in the RPC supplemented group at the end of study i.e. week 4 as compared to control group. All the parameters of hemogram profile were in normal range.

Erythrocyte membrane contains phospholipids that are found as bilayer with phosphatidylcholine. Choline through CDP-choline pathway synthesizes phosphatidylcholine. Thus choline acts as a precursor as well as itself is a component of phosphatidylcholine. Consequently adequate availability of phosphatidylcholine can be one of the reasons for increased concentration of erythrocytes. Higher total

erythrocyte concentration is also normally accompanied with increased haemoglobin concentration as well as higher hematocrit. An increase in these parameters is also often associated with improved general health. As compared to control significantly ($P \leq 0.05$) higher PCV and hemoglobin resulted in significantly ($P \leq 0.05$) higher MCV and MCH at week 4 in RPC supplemented group.

Similar to the results of present study increased TEC, Hb and PCV have been reported by Habeeb *et al.* (2017) in male Zaraibi growing goats due to RPC supplementation. Due to peripartal RPC supplementation Zenobi (2018) has also reported such increase of erythrocyte, Hb and PCV in postpartum Holstein cows on high caloric intake and their preweaned female Holsteins that were receiving colostrum. Higher TEC, Hb and PCV in the present study are indicative of direct effect of RPC supplementation and indirect effect of better health status.

4.3.2 Leukogram profile

The results (Mean±SE) for effect of supplementation of rumen protected choline on leukogram profile of Surti goats are mentioned in table 4.3.2

Total leukocyte count (10^3cells /μl) at week 0 and week 4 in control was 13.31±0.94 and 10.47±0.68 and in RPC supplemented group was 13.89±1.61 and 11.36±1.19 respectively. Lymphocytes (%) at week 0 and week 4 in control were 53.83±2.15 and 51.67±1.15 and in RPC supplemented group was 51.33±1.56 and 57.00±1.41 respectively. Monocytes (%) at week 0 and week 4 in control were 5.50±0.62 and 5.17±0.70 and in RPC supplemented group was 5.00±0.58 and 5.33±0.33 respectively. Neutrophils (%) at week 0 and week 4 in control was37.50±2.38 and 39.67±1.41and in RPC supplemented group was 40.17±1.45 and 34.67±1.73 respectively. Eosinophils (%) at week 0 and week 4 in control was2.67±0.21 and 2.83±0.17 and in RPC supplemented group was 2.83±0.17 and 2.33±0.33 respectively. Basophils (%) at week 0 and week 4 in control were 0.50±0.22 and 0.67±0.21and in RPC supplemented group was 0.67±0.21 and 0.67±0.21 respectively. Neutrophils: Lymphocytes ratio at week 0 and week 4 in control was 0.71±0.07 and 0.77±0.04 and in RPC supplemented group was 0.68±0.07 and 0.61±0.05 respectively.

Table 4.3.2 : Leukogram profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)		
Groups	**Week 0**	**Week 4**
	Total leukocyte count (x10^3/µl)	
Control (n=6)	13.31±0.94	10.47±0.68
RPC (n=6)	13.89±1.61	11.36±1.19
Overall (n=12)	13.60±0.90	10.91±0.66
	Differential leukocyte count (DLC)	
	Lymphocytes (%)	
Control (n=6)	53.83±2.15	51.67^b±1.15
RPC (n=6)	51.33±1.56	57.00^a±1.41
Overall (n=12)	52.58±1.32	54.33±1.18
	Monocytes (%)	
Control (n=6)	5.50±0.62	5.17±0.70
RPC (n=6)	5.00±0.58	5.33±0.33
Overall (n=12)	5.25±0.41	5.25±0.37
	Neutrophils (%)	
Control (n=6)	37.50±2.38	39.67^a±1.41
RPC (n=6)	40.17±1.45	34.67^b±1.73
Overall (n=12)	38.83±1.39	37.17±1.30
	Eosinophils (%)	
Control (n=6)	2.67±0.21	2.83±0.17
RPC (n=6)	2.83±0.17	2.33±0.33
Overall (n=12)	2.75±0.13	2.58±0.19
	Basophils (%)	
Control (n=6)	0.50±0.22	0.67±0.21
RPC (n=6)	0.67±0.21	0.67±0.21
Overall (n=12)	0.58±0.15	0.67±0.14
	Neutrophils: Lymphocytes ratio	
Control (n=6)	0.71±0.07	0.77^a±0.04
RPC (n=6)	0.68±0.07	0.61^b±0.05
Overall (n=12)	0.70±0.05	0.69±0.04
Means bearing different superscripts (a, b) differ significantly (P≤0.05) between groups		

Leukogram profile showed similar concentration of all the parameters at the beginning of the study without any significant difference. However at week 4 between groups significant (P≤0.05) increase in lymphocytes and significant (P≤0.05) decrease in neutrophils as well as neutrophil: lymphocyte ratio in RPC supplemented group was observed. Rest of the parameters did not show any significant difference due to supplementation of RPC.

Leukogram profile indicates the potential of the body to fight infection and produce antibodies as and when required. Leukogram profile of an animal is

significantly impacted by stress. Presence of stress has been associated with higher neutrophil counts (Jain, 1993; Naik *et al.*, 2013; Koubková *et al.*, 2002; Mayengbam, 2009) and lower lymphocyte count in blood (Lacetera *et al.*, 2005; Lacetera *et al.*, 2006; Kamanga-Sollo *et al.*, 2011; Gomes *et al.*, 2013; Van Eerd, 2019; Devaraj and Upadhyay, 2007). This is mediated by stress induced glucocortocoid release that mobilizes the sequestered neutrophils apart from increasing its life span and suppresses the proliferation of lymphocytes. Lower cortisol levels in RPC supplemented group discussed later in this chapter indicated that control group might be in some sort of stress that was probably alleviated in treatment group due to RPC supplementation.

Increase in lymphocytes in the present study can also be directly attributed to choline mediated increased phosphatidylcholine. Higher biosynthesis of phosphatidyl choline may also result in its increased hydrolysis. Its hydrolytic products such as fatty acids, lysophosphatidylcholine, platelet-activating factor, choline, phosphatidic acid and diacylglycerol that participate in intra-and intercellular signaling have been reported to affect macrophage and lymphocyte function (Asaoka *et al.*, 1992; Exton, 1997; McIntyre *et al.*, 1994; Rotondo *et al.*, 1994; Sands *et al.*, 1994). It was concluded by Nishiyama-Naruke and Curi (2000) that phosphatidylcholine may act as a mediator of the macrophage effect on lymphocyte proliferation. It has been also reported that lymphocytes express choline acetyltransferase and acetylcholine receptors of muscarinic and nicotinic nature. So lymphocyte activation is associated with greater release of acetylcholine and upregulation of choline acetyltransferase (Kawashima and Fujii, 2008). Garcia *et al.* (2018) reported diminishing phagocytic and killing capacity of isolated neutrophils with increasing doses of choline in cell culture. They also reported that lymphocyte proliferation was enhanced with increasing dose of choline.

Neutrophil: lymphocyte ratio acts as inflammatory index and used to evaluate stress. Neutrophil: lymphocyte ratio is lower in the absence of stress. In the present study due to RPC supplementation and absence of stress in the treatment groups led to significantly ($P \leq 0.05$) lower neutrophil: lymphocyte ratio in treatment groups. Lower neutrophils, higher lymphocytes and lower neutrophil: lymphocyte ratio was also reported by Chaudhary *et al.* (2020) after mitigating heat stress in Surti buffaloes. As discussed later in this chapter absence of stress in treatment group was also indicated

by lower cortisol levels and lower oxidative stress in RPC supplemented group in the present study.

In the present study higher lymphocytes, lower neutrophils and neutrophil: lymphocyte ratio is suggestive of direct effect of RPC supplementation and indirect effect of improved general health along with lower stress.

4.4 BIOCHEMICAL PARAMETERS

Results for biochemical parameters are presented and discussed under sub-headings blood biochemical metabolites and hepatic enzyme profile.

4.4.1 Blood biochemical metabolites

The results (Mean±SE) for effect of supplementation of rumen protected choline on blood biochemical parameters of Surti goats are mentioned in table 4.4.1

Glucose (mg/dl) at week 0 and week 4 in control was 54.58±0.87 and 48.43±1.62 and in RPC supplemented group was 52.20±1.33 and 50.66±0.55 respectively. Total cholesterol (mg/dl) at week 0 and week 4 in control was 86.81±2.33 and 87.93±1.44 and in RPC supplemented group was 85.44±3.99 and 96.05±1.76 respectively. Triglyceride at week 0 and week 4 in control was 34.04±1.24 and 34.60±0.38 and in RPC supplemented group was 34.93±0.90 and 39.98±0.86 respectively. High density lipoprotein- cholesterol, HDL (mg/dl) at week 0 and week 4 in control was 35.90±1.22 and 34.06±0.81and in RPC supplemented group was 36.06±1.03 and 37.47±0.84 respectively. Low density lipoprotein-cholesterol, LDL (mg/dl) at week 0 and week 4 in control was 31.38±2.35 and 30.28±1.91 and in RPC supplemented group was 33.16±0.74 and 29.62±1.14respectively.

Glucose levels were marginally higher in RPC supplemented group at week 4 as compared to control but the difference was non-significant. Slightly higher blood glucose levels due to RPC supplementation can be attributed as choline leads to higher VLDL levels that get converted to glucose (Pinotti *et al.*, 2002) for energy purpose. Similar to present results non-significant change in glucose concentration due to RPC supplementation has also been reported by Chaurasia (2013) and Bindel *et al.* (2005). No effect on glucose levels of RPC supplementation in lactating cows have been reported (Pandurang, 2012; Mohsen *et al.*, 2011; Piepenbrink *et al.*, 2003). Choline supplementation can promote hepatic gluconeogenesis especially during increased energy demand stages such as postpartum. Therefore contrary to the present findings, Sheikh (2012) found significantly higher blood glucose in lactating cows

postpartum. Since in the present study the young Surti goats were not in any negative energy balance so the increase due to RPC supplementation was non-significant as the demand for hepatic gluconeogenesis was minimalistic.

Initial levels of serum triglyceride concentration were similar in both the groups. The concentration of serum triglyceride was significantly ($P \leq 0.05$) higher in RPC supplemented group as compared to control. It is known that VLDL is required to export triglyceride from the liver. Higher levels of choline are helpful in increasing the levels of phospholipids required for lipoprotein VLDL synthesis. Thus more choline causes more VLDL synthesis in liver which subsequently results in extra-hepatic transport of triglyceride thereby increasing serum triglyceride levels. Confirming the triglyceride clearance from liver, Piepenbrink and Overton (2000) have found that rumen protected choline prevents accumulation of liver triglycerides. Abeni *et al*. (2007) have also suggested that supplementation of RPC prevents liver triglyceride accumulation. Hepatic export of fat has been reported in transition cows due to RPC supplementation (Zom *et al*., 2011). Similar to the findings of present study there are reports of dietary RPC supplementation resulting in higher serum triglyceride levels in lambs (Bryant *et al*.,1999), finishing heifers (Bindel *et al*., 2000) and Holstein and Jersey cows (Guretzky *et al*., 2006).

Cholesterol levels were significantly ($P \leq 0.05$) higher in RPC supplemented group as compared to control after week 4 of supplementation. Since choline is essential for transport and metabolism of lipid cholesterol (Zeisel and DaCosta, 2009), the supplementation of rumen protected choline might have resulted in increasing the circulatory levels of cholesterol in RPC group of present study. The mechanism of cholesterol biosynthesis in ruminants yet remains to be deciphered completely. However the contribution of choline for phosphatidylcholine portion of VLDL may to some extent explain the rise in cholesterol levels in the present study due to RPC supplementation. Similar to the findings of the present study supplementation of herbal choline has been reported to increase blood cholesterol levels in lambs by Rodriguez-Guerrero *et al.* (2018) and in dairy cattle by Pinotti *et al*. (2004). An experiment conducted by Bárcena-Gama *et al.* (2020) showed that supplementation of RPC in feedlot lambs during their growth leads to higher cholesterol. Their findings are similar to the present study findings of higher cholesterol due to RPC supplementation in growing young surti goats. Contrary to increased cholesterol in the present study Li *et al.* (2015) have reported no effect on blood cholesterol

concentration in lambs. Variation in responses of cholesterol levels to RPC supplementation could be attributed to the differential fatty acid mobilization owing to variation in stages (growth or lactation) and age of animals as well as species

Due to supplementation of RPC in treatment group the levels of triglycerides, total cholesterol, HDL were significantly ($P \leq 0.05$) higher at week 4 whereas no significant effect was observed on glucose and LDL levels. HDL levels were significantly ($P \leq 0.05$) higher in treatment groups due to RPC supplementation at week 4 of study. The levels of LDL did not vary significantly between the groups at any stage of supplementation. Significantly higher HDL in RPC supplemented group at the end of supplementation might be due to synthesis of paroxonase in liver that protects HDL from oxidative damage (Turk *et al.,* 2004) and thus may spare HDL resulting in its higher concentration in serum. Increase in HDL in present study was similar in findings of Sun *et al.* (2016) and Rathwa (2020) however the latter study was done in postpartum Surti buffaloes and the effect was observed with supplementation of RPM along with RPC. Rathwa (2020) also reported no effect of RPM and RPC combined supplementation on LDL levels in postpartum Surti buffaloes that corroborates with the results obtained in the present study for LDL. Even though non-significant but lowering of LDL in treatment group is similar to the findings reported by Sun *et al.* (2016).

Table 4.4.1: Blood biochemical metabolites (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)

Groups	Week 0	Week 4
	Glucose (mg/dl)	
Control (n=6)	54.58±0.87	48.43±1.62
RPC (n=6)	52.20±1.33	50.66±0.55
Overall (n=12)	53.39±0.84	49.54±0.88
	Total cholesterol (mg/dl)	
Control (n=6)	86.81±2.33	87.93[b]±1.44
RPC (n=6)	85.44±3.99	96.05[a]±1.76
Overall (n=12)	86.12±2.21	91.99±1.64
	Triglyceride	
Control (n=6)	34.04±1.24	34.60[b]±0.38
RPC (n=6)	34.93±0.90	39.98[a]±0.86
Overall (n=12)	34.48±0.74	37.29±0.93
	High density lipoprotein, HDL (mg/dl)	
Control (n=6)	35.90±1.22	34.06[b]±0.81
RPC (n=6)	36.06±1.03	37.47[a]±0.84
Overall (n=12)	35.98±0.76	35.76±0.76

	Low density lipoprotein, LDL (mg/dl)	
Control (n=6)	31.38±2.35	30.28±1.91
RPC (n=6)	33.16±0.74	29.62±1.14
Overall (n=12)	32.27±1.20	29.95±1.06
	Total protein (g/dl)	
Control (n=6)	6.38±0.31	6.42 ^{b}±0.19
RPC (n=6)	6.65±0.16	7.11^{a} ±0.16
Overall (n=12)	6.52±0.17	6.76±0.16
	Albumin (g/dl)	
Control (n=6)	3.73±0.22	3.74±0.14
RPC (n=6)	3.80±0.16	3.77±0.09
Overall (n=12)	3.76±0.13	3.76±0.08
	Globulin (g/dl)	
Control (n=6)	2.65±0.12	2.68 ^{b}±0.21
RPC (n=6)	2.86±0.05	3.33^{a}±0.18
Overall (n=12)	2.76±0.07	3.01±0.17
	Urea (mg/dl)	
Control (n=6)	36.99±1.41	36.02±1.15
RPC (n=6)	37.49±0.79	33.47±0.80
Overall (n=12)	37.24±0.77	34.74±0.77
	Creatinine (mg/dl)	
Control (n=6)	0.76±0.03	0.77±0.03
RPC (n=6)	0.78±0.02	0.71±0.02
Overall (n=12)	0.77±0.02	0.74±0.02
Means bearing different superscripts (a, b,) differ significantly (P≤0.05) between groups		

Total protein (g/dl) at week 0 and week 4 in control was 6.38±0.31 and 6.42±0.19 and in RPC supplemented group was 6.65±0.16 and 7.11±0.16 respectively. Albumin (g/dl) at week 0 and week 4 in control was 3.73±0.22 and 3.74±0.14 and in RPC supplemented group was 3.80±0.16 and 3.77±0.09 respectively. Globulin (g/dl) at week 0 and week 4 in control was 2.65±0.12 and 2.68±0.21 and in RPC supplemented group was 2.86±0.05 and 3.33±0.18 respectively. Urea (mg/dl) at week 0 and week 4 in control was 36.99±1.41 and 36.02±1.15 and in RPC supplemented group was 37.49±0.79 and 33.47±0.80 respectively. Creatinine (mg/dl) at week 0 and week 4 in control was 0.76±0.03 and 0.77±0.03 and in RPC supplemented group was 0.78±0.02 and 0.71±0.02 respectively.

Comparison between the groups revealed that there was significant (P≤0.05) increase in total protein and globulin in the RPC supplemented group at week 4. Albumin levels were slightly higher and urea as well as creatinine levels were slightly lower but not significant due to RPC supplementation at week 4.

Significant (P≤0.05) increase in protein at week 4 can be explained partly by non-significant increase in albumin and significant increase in globulin in RPC supplemented group in the present study. Moderate increase in albumin fraction of protein might have occurred as adequate supply of choline leads to sparing of methionine for protein biosynthesis and albumin being a major fraction of plasma protein. Similarly Rahmani *et al.* (2014a) have reported an increase in albumin levels in Holstein cows. The reason for significantly (P≤0.05) increased globulin levels in RPC supplemented group can be viewed from two perspectives. The first reasons may be same as for albumin in the present study stated earlier. The other reason for rise of globulin levels might perhaps be the effect of antioxidant action of choline as dietary choline has been reported to decrease oxidant damage and regulate antioxidant system in immune organs of animal resulting in an increase in γ-globulin (Rahmani *et al.*, 2014b). The results of the present study were in concurrence with results reported by Habeeb *et al.* (2017) in male Zairabi growing goats in Egypt.

A non-significant decrease in urea and creatinine levels due to RPC supplementation in the present study could be due to anabolic shift favoring increased total protein as well as protein accretion and its reduced catabolism as explained earlier for higher albumin and globulin levels. Lower blood urea and creatinine levels indicate lower catabolism and higher anabolism of protein which is otherwise also a characteristic feature of growth phase that was present in selected young Surti goats. Likely anabolic effects in the treatment group of present study would have been boosted by RPC feeding and thus minimal protein breakdown might have occurred resulting in lower blood urea and creatinine. As serum urea and creatinine act as biomarker for renal function, no effect on these metabolites in treatment group of present study also indicates that RPC supplementation does not have any adverse effect on renal function.

Observations coherent to results of present study have also been reported in other studies. After RPC supplementation decreased levels of BUN have been reported both significantly (Chaurasia, 2013 and Bindel *et al.* 2005) as well as non-significantly (Pandurang, 2012 and Mohsen *et al.*, 2011). Habeeb *et al.* (2017) have also reported that RPC did not show any effect on urea and creatinine levels in male Zairabi goats.

4.4.2 Hepatic enzyme profile

The results (Mean±SE) for effect of supplementation of rumen protected choline on hepatic enzyme profile of Surti goats are mentioned in table 4.4.2

Alanine transaminase (U/l) at week 0 and week 4 in control was 24.59±1.46 and 24.26±0.81 and in RPC supplemented group was 25.17±0.80 and 20.06±0.74 respectively. Aspartate aminotransferase (U/l) at week 0 and week 4 in control was 50.36±2.25 and 47.39±1.75 and in RPC supplemented group was 49.45±2.37and 42.10±0.98 respectively. Gamma-glutamyl transferase (U/l) at week 0 and week 4 in control was 27.12±1.48 and 25.32±1.25 and in RPC supplemented group was 25.77±0.79 and 21.41±0.75 respectively.

Table 4.4.2 : Hepatic enzyme profile (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)

Groups	Week 0	Week 4
	Alanine transaminase, ALT (U/l)	
Control (n=6)	24.59±1.46	24.26^{a}±0.81
RPC (n=6)	25.17±0.80	20.06^{b}±0.74
Overall (n=12)	24.88±0.80	22.16±0.82
	Aspartate aminotransferase, AST(U/l)	
Control (n=6)	50.36±2.25	47.39^{a}±1.75
RPC (n=6)	49.45±2.37	42.10^{b}±0.98
Overall (n=12)	49.91±1.56	44.75±1.25
	Gamma-glutamyl transferase, GGT (U/l)	
Control (n=6)	27.12±1.48	25.32^{a}±1.25
RPC (n=6)	25.77±0.79	21.41^{b}±0.75
Overall (n=12)	26.44±0.82	23.37±0.91

Means bearing different superscripts (a, b,) differ significantly (P≤0.05) between groups

Amongst the hepatic enzyme profile the mean concentration of ALT, AST and GGT was significantly (P≤0.05) lower in the treatment group at week 4 i.e. at the end of supplementation. ALT, AST and GGT are indicators of liver health (Osorio *et al.*, 2014b) and are commonly referred as hepatic biomarkers. The levels of these enzymes are elevated during acute disease condition but generally are below normal if the disease persists to become chronic in nature. In the present study even though the values were in normal range for both the groups but significant lowering due to RPC supplementation indicates the possible hepatoprotective action of choline. Moreover being in normal range inspite of lowering of these enzyme levels shows that RPC supplementation does not have any adverse effect on liver. Similar to the results of present study lowering of ALT and AST enzymes has also been reported by

Chaurasia (2013). However many studies have also reported no effect of RPC supplementation on ALT and AST enzyme levels (Bindel *et al.*, 2005; Zahra *et al.*, 2006; Toghdory *et al.*, 2007; D'Ambrosio *et al.*, 2007; Habeeb *et al.,* 2017). No effect of RPC supplementation on AST, ALT, alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) levels have been reported by Rahmani *et al.* (2012) and Rahmani *et al.* (2014a) in Holstein cows during early lactation. Inability of hepatoprotective action of RPC supplementation to significantly alter liver enzymes in aforementioned studies as well as other studies can be because of the difference in physiological stage, species, dose and duration of RPC supplementation. During parturition, postpartum and early lactation existence of severe metabolic stress and derangement of liver enzymes might be overwhelming enough to reap any benefit from hepatoprotective action of choline. Therefore contrary to many other studies, RPC supplementation lowered hepatic enzymes in the present study possibly because there was no such stressful state in the selected young goats and thus they had conducive milieu favoring hepatoprotective effects of choline.

4.5 HORMONAL PARAMETERS

The results (Mean±SE) for effect of supplementation of rumen protected choline on serum hormones of Surti goats are mentioned in table 4.5.

Table 4.5: Hormonal parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)

Groups	Week 0	Week 4
	Triiodothyronine, T_3 (ng/ml)	
Control (n=6)	1.25±0.07	1.26^{b}±0.03
RPC (n=6)	1.26±0.02	1.41^{a}±0.01
Overall (n=12)	1.26±0.04	1.34±0.03
	Thyroxine, T_4 (ng/ml)	
Control (n=6)	60.21±2.54	61.15^{b}±1.40
RPC (n=6)	62.00±1.80	71.22^{a}±1.55
Overall (n=12)	61.10±1.51	66.19±1.81
	Cortisol (ng/ml)	
Control (n=6)	1.66±0.17	1.53±0.14
RPC (n=6)	1.58±0.15	1.32±0.07
Overall (n=12)	1.62±0.11	1.43±0.08
	Leptin (ng/ml)	
Control (n=6)	3.94±0.28	4.18±0.29
RPC (n=6)	3.89±0.26	4.50±0.22
Overall (n=12)	3.92±0.18	4.34±0.18

Means bearing different superscripts (a, b) differ significantly (P≤0.05) between groups

Triiodothyronine, T_3 (ng/ml) at week 0 and week 4 in control was 1.25±0.07 and 1.26±0.03 and in RPC supplemented group was 1.26±0.02 and 1.41±0.01 respectively. Thyroxine, T_4 (ng/ml) at week 0 and week 4 in control was 60.21±2.54 and 61.15±1.40 and in RPC supplemented group was 62.00±1.80 and 71.22±1.55 respectively. Cortisol (ng/ml) at week 0 and week 4 in control was 1.66±0.17 and 1.53±0.14 and in RPC supplemented group was 1.58±0.15 and 1.32±0.07 respectively. Leptin (ng/ml) at week 0 and week 4 in control was 3.94±0.28 and 4.18±0.29 and in RPC supplemented group was 3.89±0.26 and 5.39±0.11 respectively.

Between the groups the thyroid hormones i.e. triiodothyronine T_3 and thyroixine T_4 were significantly ($P \leq 0.05$) higher in the group supplemented with RPC at the end of supplementation i.e. week 4. The stress hormone cortisol was lower in RPC supplemented group at week 4 but it was not significant.

Thyroid hormones are released from thyroid glands under the influence of TSH secreted from pituitary gland. The levels of thyroid hormones are closely associated with metabolic rate that is generally optimal during phase of growth. Elevation of T_3 and T_4 in the present study might have occurred due to stimulation of TSH hormone secretion from pituitary under the influence of choline. Such increase in T_3 and T_4 has also been reported by Habeeb *et al.* (2017) due to RPC supplementation.

Cortisol level is indicative of stress in animal. Since the young Surti goats selected in present study were in growth stage and didn’t had any predisposing factor or reason of stress so any significant difference in cortisol level between groups was unlikely to be observed per se. However slightly lowered cortisol levels due to RPC supplementation may be due to antioxidant effect of choline that alleviates endogenous oxidative stress. Similar to the lowering of cortisol in present study, Habeeb *et al.* (2017) in male zairabi goats found that RPC supplementation lowered cortisol levels but in their study this effect was significant. Their study was done during heat stress and cortisol level was high in control group so the supplementation of RPC could produce effect that was significant for lowering of cortisol in treatment group.

Comparison between groups demonstrated that leptin hormone levels were elevated in RPC supplemented group at week 4 but the difference was non-significant as compared to control. Leptin hormone is an adipokine that is secreted from

adipocytes of subcutaneous fat. A positive energy balance state in animals is often associated with higher leptin levels. Choline has an effect on lipid metabolism and its transport. NEFA levels in circulation are increased due to lipolysis. Choline decreases lipolytic tendencies and thereby decreases NEFA levels. In the present study all the selected young Surti goats were in growing phase but the indicators for positive energy balance amongst lipid profile in present study were on the higher side in RPC supplemented group. So choline by virtue of its antilipolytic effect and maintenance of higher positive energy state may have caused an increase in leptin levels in RPC supplemented group of present study. Supplementation of RPC in transition cow has been reported to increase leptin levels in serum (Shahsavari, 2012). In the present study young Surti goats were in later phase of growth that is generally marked by increasing body weight. During this phase the normal physiology is programmed for increasing adiposity thereby causing concurrent increase in serum leptin levels. As discussed later in this chapter it is evident that RPC supplementation led to non-significantly higher body weight that might explain the increased serum leptin levels. Identical or similar studies for effect of RPC supplementation on leptin levels in young Surti goats is not much available to cite hence drawing comparisons with present study is difficult.

4.6 Oxidative stress parameters

The results (Mean±SE) for effect of supplementation of rumen protected choline on oxidative stress parameters of Surti goats are mentioned in table 4.6

LPO (nM of MDA produced/ml of packed cells) at week 0 and week 4 in control was 5.97±0.12 and 5.94±0.24 and in RPC supplemented group was 6.02±0.29 and 5.08±0.14 respectively. GSH (mg/dl) at week 0 and week 4 in control was 6.57±0.15 and 6.63±0.17 and in RPC supplemented group was 6.36±0.18 and 7.34±0.19 respectively. SOD (U/mg of Hb) at week 0 and week 4 in control was 2.61±0.23 and 2.65±0.14 and in RPC supplemented group was 2.75±0.11 and 2.88±0.08 respectively. TAS (µM) at week 0 and week 4 in control was 289.39±13.00 and 292.07±5.33 and in RPC supplemented group was 289.31±23.86 and 322.11±10.15 respectively.

Between the groups the levels of GSH and TAS were significantly (P≤0.05) higher whereas LPO was significantly (P≤0.05) lower in RPC supplemented group at week 4. There was no effect on SOD levels albeit the levels were slightly higher due to RPC supplementation.

Table 4.6: Oxidative stress parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)		
Groups	**Week 0**	**Week 4**
	LPO (nM of MDA produced/ml of packed cells)	
Control (n=6)	5.97±0.12	5.94[a]±0.24
RPC (n=6)	6.02±0.29	5.08[b]±0.14
Overall (n=12)	5.99±0.15	5.51±0.18
	Reduced glutathione, GSH (mg/dl)	
Control (n=6)	6.57±0.15	6.63[b]±0.17
RPC (n=6)	6.36±0.18	7.34[a]±0.19
Overall (n=12)	6.46±0.11	6.99±0.16
	Superoxide dismutase, SOD (U/mg of Hb)	
Control (n=6)	2.61±0.23	2.65±0.14
RPC (n=6)	2.75±0.11	2.88±0.08
Overall (n=12)	2.68±0.12	2.76±0.08
	Total antioxidant status, TAS (µM)	
Control (n=6)	289.39±13.00	292.07[b]±5.33
RPC (n=6)	289.31±23.86	322.11[a]±10.15
Overall (n=12)	289.35±12.95	307.09±7.10
Means bearing different superscripts (a, b) differ significantly ($P \leq 0.05$) between groups		

During normal metabolic processes in the body several free radicals and reactive oxygen species are generated that may cause oxidative stress. Reactive oxygen species (ROS) and free radicals (FR) may cause lipid peroxidation especially of cell membranes. SOD forms the line of cellular defense against ROS and catalyzes dismutation of superoxide radicals (O_2^{-}) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Glutathione acts as an antioxidant and prevents oxidative damage caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides etc. TAS signifies a pool of antioxidants that are present in the body to counter oxidative stress metabolites. Choline deficiency has been reported to produce oxidative damage in the liver, heart, kidney and brain with an increased lipid peroxidation of subcellular organelles and a decrease in tissue antioxidants (Ossani *et al.*, 2007). Repetto *et al.* (2010) observed that strong association between choline deficiency and decreased antioxidant content as well as increased lipid peroxidation exists. These studies indirectly implicate the findings of the present study wherein with supplementation of choline the levels of antioxidants increased and lipid peroxidation declined. Supportive evidence for present findings is also apparent from the work of Osorio *et al.* (2014b) wherein they demonstrated that in RPC supplemented cows hepatic gene

expression associated with antioxidant synthesis and DNA methylation status were improved. Role of choline as an antioxidant that protects cell and reduce the free radical production which damage liver cells are well established in dairy cows (Elsawy *et al.*, 2014).

4.7 Blood electrolytes

The results (Mean±SE) for effect of supplementation of rumen protected choline on blood electrolytes of Surti goats are mentioned in table 4.7

Sodium (mEq/l) at week 0 and week 4 in control was 141.42±2.82 and 140.37±2.62 and in RPC supplemented group was 143.08±2.33 and 145.30±2.66 respectively. Potassium (mEq/l) at week 0 and week 4 in control was 5.45±0.11 and5.58±0.17 and in RPC supplemented group was 5.47±0.17 and 5.88±0.13 respectively. Chloride (mEq/l) at week 0 and week 4 in control was 106.42±1.00 and 107.42±1.03 and in RPC supplemented group was 106.93±1.29 and 109.30±1.43 respectively.

Table 4.7 : Blood electrolytes (Mean±SE) of young Surti goats supplemented with Rumen protected choline (RPC)

	Week 0	**Week 4**
Groups	**Sodium , Na^+ (mEq/l)**	
Control (n=6)	141.42±2.82	140.37±2.62
RPC (n=6)	143.08±2.33	145.30±2.66
Overall (n=12)	142.25±1.76	142.83±1.93
	Potassium, K^+ (mEq/l)	
Control (n=6)	5.45±0.11	5.58±0.17
RPC (n=6)	5.47±0.17	5.88±0.13
Overall (n=12)	5.46±0.10	5.73±0.11
	Chloride, Cl^- (mEq/l)	
Control (n=6)	106.42±1.00	107.42±1.03
RPC (n=6)	106.93±1.29	109.30±1.43
Overall (n=12)	106.68±0.78	108.36±0.89

Serum levels of electrolytes sodium, potassium and chloride did not vary significantly between groups. The serum electrolytes measured in the present study were in normal range and similar in both the groups initially as well as after 4 weeks.

Sodium, potassium and chloride are vital serum electrolytes and are critically regulated homeostatically within narrow range. Osmolality in the body is affected by choline or its derivatives. Intracellular volumes are regulated by adjusting concentrations of intracellular inorganic (sodium, potassium and chloride ions) and

organic (betaine, glycerophosphocholine, myoinositol, sorbitol and amino acids such as taurine and glutamate) osmolytes (Wehner *et al.,* 2003). Endogenously choline can be oxidized to betaine. Betaine and glycerophosphocholine counteract the hyperosmotic environment of the renal medulla generated by high interstitial concentrations of sodium chloride and urea. Betaine and glycerophosphocholine protect natural protein structures and maintain normal cellular function by maintaining the tonicity within the renal medulla cells (Burg and Ferraris, 2008). The microclimate of shed in the present study was comfortable and there was no factor that might have caused any osmoregulatory derangement in both the groups. So the effect of RPC supplementation was not seen on normal levels of electrolytes sodium, potassium and chloride.

4.8 Growth parameters

4.8.1 Body weight and body measurement

The results (Mean±SE) for effect of supplementation of rumen protected choline on body weight, body height, body length and heart girth young Surti goats are mentioned in table 4.8.1.

Table 4.8.1: Growth parameters (Mean±SE) of young Surti goats supplemented with rumen protected choline (RPC)

	Week 0	**Week 2**	**Week 4**	**Week 6**
Groups	**Body weight(Kg)**			
Control (n=6)	12.08±0.60	12.45±0.75	13.22±0.69	13.95±0.68
RPC (n=6)	12.07±0.38	12.53±0.38	13.45±0.40	14.68±0.36
Overall (n=12)	12.08±0.34	12.49±0.40	13.33±0.38	14.32±0.38
	Body Height (cm)			
Control (n=6)	55.17±0.65	56.33±0.88	57.00±0.82	57.83±0.91
RPC (n=6)	54.50±1.09	55.83±1.05	57.33±1.09	58.17±0.87
Overall (n=12)	54.83±0.61	56.08±0.66	57.17±0.65	58.00±0.60
	Body Length (cm)			
Control (n=6)	50.67±0.76	52.17±0.95	53.33±0.92	54.50±0.96
RPC (n=6)	51.33±0.71	52.17±0.87	53.33±0.56	54.50±0.62
Overall (n=12)	51.00±0.51	52.17±0.61	53.33±0.51	54.50±0.54
	Heart Girth (cm)			
Control (n=6)	52.17±0.75	53.50±0.99	54.67±0.67	55.67±0.61
RPC (n=6)	51.83±0.48	53.67±0.80	55.00±0.58	55.83±0.54
Overall (n=12)	52.00±0.43	53.58±0.61	54.83±0.42	55.75±0.39

Body weight (Kg) at week 0, week 2, week 4 and week 6 in control was 12.08±0.60, 12.45±0.75, 13.22±0.69 and13.95±0.68and in RPC supplemented group

was 12.07±0.38, 12.53±0.38, 13.45±0.40 and 14.68±0.36respectively.Body Height (cm) at week 0, week 2, week 4 and week 6 in control was 55.17±0.65, 56.33±0.88, 57.00±0.82 and 57.83±0.91 and in RPC supplemented group was 54.50±1.09, 55.83±1.05, 57.33±1.09 and 58.17±0.87respectively. Body Length (cm) at week 0, week 2, week 4 and week 6 in control was 50.67±0.76, 52.17±0.95, 53.33±0.92 and 54.50±0.96 and in RPC supplemented group was 51.33±0.71, 52.17±0.87, 53.33±0.56 and 54.50±0.62 respectively. Heart Girth (cm) at week 0, week 2, week 4 and week 6 in control was 52.17±0.75, 53.50±0.99, 54.67±0.67 and 55.67±0.61and in RPC supplemented group was 51.83±0.48, 53.67±0.80, 55.00±0.58 and 55.83±0.54 respectively.

4.8.2 Average daily gain and cumulative weight gain

Results for average daily gain and cumulative weight gain (Mean±SE) in body weight of young Surti goats supplemented with Rumen protected choline (RPC) are mentioned in table 4.8.2.

Average daily gain (g/day) in body weight during 1st, 2nd and 3rd fortnight in control was 26.19±14.60, 54.76±18.23, 52.38±30.12 and RPC supplemented group was 33.33±20.01, 65.48±21.62 and 88.10±11.32 respectively. Average daily gain (g/day) in body weight for whole study period i.e. 6 weeks in control was 44.44±11.06 and 62.30±4.41 in RPC supplemented group. At the end of the study cumulative weight gain (Kg) for control group was 1.87±0.46 and for RPC supplemented group was 2.62±0.19.

Table 4.8.2: Average daily gain and cumulative weight gain (Mean±SE) in body weight of young Surti goats supplemented with rumen protected choline (RPC)

Groups	Average daily gain (g/day)				Cumulative weight gain (Kg)
	1st Fortnight	2nd Fortnight	3rd Fortnight	Throughout study period	
Control(n=6)	26.19±14.60	54.76±18.23	52.38±30.12	44.44±11.06	1.87±0.46
RPC (n=6)	33.33±20.01	65.48±21.62	88.10±11.32	62.30±4.41	2.62±0.19
Overall(n=12)	29.76±11.86	60.12±13.58	70.24±16.26	53.37±6.28	2.24±0.26

Comparison between groups showed that body weights in RPC supplemented group were non-significantly higher at all fortnights of study i.e. week 2, 4 and 6. Total gain and average daily gain in body weight during 1st, 2nd and 3rd fortnight as well as for whole study period was also non-significantly higher due to RPC supplementation in treatment group as compared to control group.

Choline supplementation has been associated with improved nutrient digestibility, nutritive values, feed conversion efficiency and altered lipid metabolism and transport. It acts as methyl donor and spares methionine that result in optimal nitrogen balance as well as protein accretion. These properties have been implicated in some studies to be reason for improved growth performance in terms of body weight and average daily gain in body weight. Few sporadic studies have also indicated increase in feed and dry matter intake as an effect of choline supplementation.

Similar to the findings of the present study non-significant increase in fortnightly body weight was observed in growing calves (Chaurasia, 2013) on RPC supplementation. Non-significant and numerically higher body weights after choline supplementation have also been reported by Sexson *et al.* (2010), Bindel *et al.* (2000) and Bryant *et al.* (1999). However Sexson *et al.* (2010) could not observe any effect on average daily gain in body weight due to choline supplementation.

In the present study RPC supplementation was done for only up to 4 weeks but growth performance was studied up to week 6. It was interestingly observed that between both groups difference in body weight, average daily gain, body height and heart girth was maximum and higher in RPC group during 3rd fortnight i.e. at week 6. This indicates that the effects of supplementation of RPC may be observed for a longer duration even after the supplementation is stopped.

The values of body measurements viz. body height, body length and heart girth did not vary significantly between control and RPC supplemented group either at beginning or at any of stage during study. However difference between body height and heart girth corroborated with the differences in body weights as well as average daily gain between both the groups during 3rd fortnight i.e. at week 6.

Choline causes a change in lipid metabolism as well as its transport and results in slight effect on subcutaneous depot of fat during growing phase. Therefore in the present study RPC supplementation could not elicit a significant change in body measurements. Kawas *et al.* (2020) reported no change in body measurement in RPC supplemented lambs but they observed a decrease in height with increase of RPC level in diet. Their results for body measurements were similar like the present study but difference in effect on height could be either due to younger age of lambs, longer duration of supplementation or variation in response in different species.

CHAPTER 5

SUMMARY AND CONCLUSIONS

5.1 SUMMARY

Choline i.e. (beta-hydroxyethyl) trimethyl ammonium hydroxide is an essential nutrient. Its daily requirement is much more than expected and recommended for vitamins since vitamins are source of coenzymes and cofactors and are required in small quantities but choline has more roles as compared to vitamin. Predominant effect of choline supplementation is prevention of fatty liver so it is also referred as a lipotropic agent.

Choline directly or indirectly has role in structural integrity and signalling function of cellular membranes, neurotransmission, methyl group donation, release of Ca^{2+} from intercellular storage, blood clotting, lipid transport, bile formation, one-carbon metabolism, osmoregulation, epigenetic as well as intestinal mucosa integrity, functionality and immunity. Roles of methionine and choline are so intertwined that supplementation of anyone of either will spare other for beneficial effects. So choline supplementation in diet may not only yield direct effects but also indirect effects that may improve livestock performance.

Choline is naturally present in some foods and also available as a dietary supplement. Sources of choline for animal can be either exogenous from the diet or endogenous synthesis especially in ruminants. Dietary choline is degradable in rumen. rumen protected choline evades ruminal degradation and makes choline more bioavailable.

Even though roles of choline have been mostly explored during transition and early lactation it is likely to benefit animals even in growth phase. Such studies are very meagre especially in goats of Surti breed that are native of South Gujarat. Thus the present study was planned with the objective to study physiobiochemical changes and growth performance in young Surti goats supplemented with rumen protected choline.

Twelve young female Surti goats of approximately 9-11 months of age were randomly selected and divided equally into 2 groups on the basis of age and body weight as control (n=6) and treatment (RPC, n=6). Average age (in months) of selected young Surti goats was 9.84±0.21 for Control group and 9.85±0.22 for RPC group. Average body weights (in Kg) were 12.08±0.60 for Control group and

12.07±0.38 for RPC group. Both control and RPC group of goats were offered similar basal diet as per standard routine of basal diet comprising greens, dry fodder and concentrate during the study period. In addition to basal diet goats in treatment group (RPC) were individually fed rumen protected choline @ 4 grams/animal/day. Supplementation of RPC was done from beginning of study up to 4 weeks i.e. 28 days but complete duration of study was 6 weeks. Recording of meteorological conditions (ambient temperature, AT and relative humidity, RH) of goat shed was done throughout study period. Recordings of physiological parameters (rectal temperature, tympanic temperature, heart rate and respiratory rate) at week 0, week 2 and week 4 and growth parameters (Body weight, body length, heart girth and height at withers) at beginning i.e. week 0 and subsequently at fortnightly intervals at week 2, week 4 and week 6 were done. Collection of blood samples from all goats were done at beginning i.e. week 0 and week 4 i.e. at the end of supplementation.

Blood samples were used for analysing hematological parameters (hemogram and leukogram profile) such as haemoglobin (Hb), total erythrocyte count (TEC), hematocrit/packed cell volume (HCT/PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC), differential leukocyte count (DLC) and neutrophils: lymphocytes ratio (NLR); biochemical metabolites such as glucose (Glu), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein (TP), albumin (Alb), globulin (Glo), urea and creatinine (Creat); hepatic enzymes viz alanine transaminase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT); serum hormones Triiodothyronine (T3), Thyroxine (T4), Cortisol and Leptin; serum electrolytes sodium (Na^+), potassium (K^+) and chloride (Cl^-) and oxidative stress parameters such as Lipid peroxidation (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and total antioxidant status (TAS).

The research results are summarized below with salient findings of the present study:

The range of minimum, maximum and average values of ambient temperature (AT) were 19.8-23.5, 34.2-38.9 and 27.5-30; relative humidity (RH) were 19.6-35.9, 76-90.2 and 54.5-61.9 and temperature humidity index (THI) were 62.38-65.41, 74.08-77.87 and 68.66-70.75 during the study period.

Amongst physiological parameters rectal temperature and heart rate were significantly lower in RPC supplemented group at week 2 and 4. Tympanic temperature as well as rate of respiration was not affected by RPC supplementation at any stage of study.

Amongst the parameters for hemogram profile total erythrocyte concentration, haemoglobin and hematocrit (HCT or PCV) did not differ significantly initially but were significantly ($P \leq 0.05$) increased in the RPC supplemented group at the end of study i.e. week 4 as compared to control group. As compared to control, MCV and MCH was significantly ($P \leq 0.05$) higher at week 4 in RPC supplemented group. All the parameters of hemogram profile were in normal range.

Leukogram profile showed similar concentration of all the parameters at the beginning of the study without any significant difference. However at week 4 between groups significant ($P \leq 0.05$) increase in lymphocytes and significant ($P \leq 0.05$) decrease in neutrophils as well as neutrophil: lymphocyte ratio in RPC supplemented group was observed. Rest of the parameters did not show any significant difference due to supplementation of RPC.

Due to supplementation of RPC at week 4 the levels of serum triglyceride, total cholesterol, HDL were significantly ($P \leq 0.05$) higher. Levels of glucose as well as LDL were lower but not significantly different in RPC supplemented group as compared to non-supplemented group i.e. control goats.

Comparison between the groups revealed significant ($P \leq 0.05$) increase in total protein and globulin in the RPC supplemented groups at the end of study i.e. at week 4. Albumin levels were slightly higher and urea as well as creatinine levels were slightly lower due to RPC supplementation at week 4 but their deviations from levels of control were non-significant.

Amongst the hepatic enzyme profile parameters the mean concentration of ALT, AST and GGT was significantly ($P \leq 0.05$) lower in the treatment group RPC at week 4.

Between groups thyroid hormones i.e. triiodityronine (T3) and thyroixine (T4) were significantly ($P \leq 0.05$) elevated whereas leptin was non-significantly increased in RPC group as compared to control at the end of supplementation i.e. week 4. Cortisol was non-significantly lower in RPC supplemented group at week 4.

Between the groups the levels of GSH and TAS were significantly ($P \leq 0.05$) higher whereas LPO was significantly ($P \leq 0.05$) lower in RPC supplemented group at

week 4. There was no effect on SOD levels due to RPC supplementation albeit the levels were slightly higher due to RPC supplementation.

Serum levels of electrolytes viz. sodium, potassium and chloride did not vary significantly between groups.

Comparison between groups showed that body weights in RPC supplemented group were higher at all fortnights of study i.e. week 2, 4 and 6. Total gain and average daily gain in body weight during 1st, 2ndand 3rd fortnight as well as for whole study period was also higher due to RPC supplementation in treatment group. Non-significant higher values were also observed for body height and heart girth at week 6 in RPC group goats as compared to control. However none of the attributes related to body weight measurement were significantly different between the groups. It was interestingly observed that between both groups difference in body weight, average daily gain, body height and heart girth was maximum and higher in RPC group during 3rd fortnight i.e. at week 6. This indicates that the effects of supplementation of RPC may be observed for a longer duration even after the supplementation is stopped.

5.2 CONCLUSIONS

Dietary supplementation of rumen protected choline in young female Surti goats

1. Reduced rectal temperature and heart rate.
2. Increased hemoglobin concentration, total erythrocyte concentration, hematocrit, lymphocyte and decreased neutrophils as well as neutrophil: lymphocyte ratio.
3. Increased serum triglyceride, total cholesterol, high density lipoprotein-cholesterol, total protein and globulin.
4. Increased reduced glutathione and total antioxidant status levels and decreased lipid peroxidation.
5. Resulted in non-significant increase in final body weight as well as its average daily gain.

Thus it was concluded that dietary supplementation of rumen protected choline in young female Surti goats is beneficial as it lowers rectal temperature and heart rate, improves hemogram and leukogram profile that indicates general health and immune status, increases nitrogen balance and reduces oxidative stress. It also has potential to improve growth performance.

REFERENCES

Abeni, F.; Speroni, M.; Terzano, M.G.; Migliorati, L.; Cavassini, P. and Pirlo, G. (2007). Effects of rumen protected choline on production responses in Italian Friesian dairy cows. *J. Dairy Sci*. **90** (Suppl.1): 354.

Asaoka, Y.; Oka, M.; Yoshida, K.; Sasaki, Y. and Nishizuka, Y. (1992). Role of lysophosphatidylcholine in T-lymphocyte activation: involvement of phospholipase A2 in signal transduction through protein kinase C. *Proc. Natl. Acad. Sci*., **89**(14): 6447-6451.

Bárcena-Gama, J.R.; Martínez-Aispuro, J.A.; Mendoza-Martínez, G.D.; Cordero-Mora, J.L.; Sánchez-Torres, M.T.; Figueroa-Velasco, J.L. and Ayala-Monter, M.A. (2020). Evaluation of polyherbal methionine and choline in feedlot rations for lambs. *S. Afr. J. Anim. Sci*., **50**(5): 731-737

Benoit, S.L.A.; Bequette, B.J. and Erdman, R. A. (2010), January). Rumen-protected choline affects methionine methyl group metabolism in lactating dairy cows. *J. Dairy Sci.* **93**: 437-437).

Benzie, I.F. and Strain, J.J. (1999). [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods. Enzymol.*, **299**: 15-27.

Bindel, D.J.; Drouillard, J.S.; Titgemeyer, E.C.; Wessels, R.H. and Löest, C.A. (2000). Effects of ruminally protected choline and dietary fat on performance and blood metabolites of finishing heifers. *J. Anim. Sci.*, **78**(10): 2497-2503.

Bindel, D.J.; Titgemeyer, E.C.; Drouillard, J.S. and Ives, S.E. (2005). Effects of choline on blood metabolites associated with lipid metabolism and digestion by steers fed corn-based diets. *J. Anim. Sci.*, **83**(7): 1625-1632.

Bryant, T. C., Rivera, J. D., Galyean, M. L., Duff, G. C., Hallford, D. M., and Montgomery, T. H. (1999). Effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on growth and serum metabolites in lambs. *J. Anim. Sci.*, **77**(11): 2893-2903.

Budiarsana, I.G.M.; Praharani, L.; Krisnan, R. and Sutama, I.K. (2016). Effect of choline chloride supplementation on milk production and milk composition of Etawah grade goats. *J. Anim. Sci. Technol*., **58**(1): 1-12.

Burg, M.B. and Ferraris, J.D. (2008). Intracellular organic osmolytes: function and regulation. *J. Biol. Chem*., **283**(12): 7309-7313.

Chaudhary, S.S.; Singh,V.K. and Manat, T.D. (2020). Effect of mitigating heat stress in dry period of Surti buffaloes on erythrogram, leukogram and neutrophil to lymphocyte ratio during subsequent lactation. *Int. J. Curr. Microbiol. Appl. Sci.,* **9**(11): 3244- 3251

Chaurasia, M. (2013). Effect of rumen protected choline (RPC) supplementation on growth, nutrient utilization and blood metabolites in calves. *Thesis Ph.D.*, National Dairy Research Institute, Karnal.

D'Ambrosio, F.; Campagnoli, A.; Susca, F.; Fusi, E.; Rebucci, R.; Agazzi, A.; Pinotti, L. and Baldi, A. (2007). Effects of rumen-protected choline supplementation in periparturient dairy goats. *Vet. Res. Commun*., **31**(1): 393-396.

Deshpande, A.; Singh, S.; Somagond, Y.M.; Sheoran, P.; Naskar, S. and Chahal, V.P. (2020). Physio-biochemical responses and growth performance of buffalo heifers to betaine supplementation during hot humid season under field conditions. *Indian J. Anim. Sci*., **90** (3): 416–423

Devaraj, C. and Upadhyay, R.C. (2007). Effect of catecholamines and thermal exposure on lymphocyte proliferation, IL–1α and β in buffaloes. *Ital. J. Anim. Sci*., **6**(2):1336-1339

Elsawy, G., Abdelrahman, O. and Hamza, A. (2014). Effect of choline supplementation on rapid weight loss and biochemical variables among female taekwondo and judo athletes. *J. Hum. Kinet*. **40**: 77-82

Emmanuel, B. and Kennelly, J.J. (1984). Kinetics of methionine and choline and their incorporation into plasma lipids and milk components in lactating goats. *J. Dairy Sci.*, **67**(9): 1912-1918.

Exton, J.H., 1997. Phospholipase D: enzymology, mechanisms of regulation, and function. *Physiol. Rev*.,**77**(2): 303-320.

Garcia, M.; Mamedova, L.K.; Barton, B. and Bradford, B.J. (2018). Choline regulates the function of bovine immune cells and alters the mRNA abundance of enzymes and receptors involved in its metabolism *in vitro*. *Front. Immunol.*, **9**: 2448.

Gomes, C.G.; Zuniga, J.E.; Karakaya, E.; Greco, L.F.; Sinedino, L.D.P.; Martinez, N., Bisinotto, R.S.; Ribeiro, E.S.; Junior, P.L.; Engstrom, M.A. and Driver, J.P. (2013). Effects of prepartum evaporative cooling and vitamin E

supplementation on immune function of Holstein cows during summer in Florida. *J. Dairy Sci.*, **97**(1): 725.

Guretzky, N.J.; Carlson, D.B.; Garrett, J.E. and Drackley, J.K. (2006). Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J. Dairy Sci*., **89**(1):188-200.

Habeeb, A. A. M.; Gad, A. E.; Atta, M. A. A. and Abdel-Hafez, M. A. M. (2017). Evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. *Trop. Anim. Health Prod.*, **49**(6): 1107-1115.

Holdorf, H.T. and White, H.M., (2021). Effects of rumen-protected choline supplementation in Holstein dairy cows during electric heat blanket-induced heat stress. *J Dairy Sci*.. DOI:https://doi.org/10.3168/jds.2020-19794

Jain, N.C. (1993). "*Essential of veterinary hematology*." Lea and Febiger Philadelphia.

Kamanga-Sollo, E.; Pampusch, M.S.; White, M.E.; Hathaway, M.R. and Dayton, W.R. (2011). Effects of heat stress on proliferation, protein turnover, and abundance of heat shock protein messenger ribonucleic acid in cultured porcine muscle satellite cells. *J. Anim. Sci.*, **89**(11): 3473-3480.

Kawas, J.R., Garcia-Mazcorro, J. F., Fimbres-Durazo, H., & Ortega-Cerrilla, M. E. (2020). Effects of Rumen-Protected Choline on Growth Performance, Carcass Characteristics and Blood Lipid Metabolites of Feedlot Lambs. *Animals*, **10**(9): 1580.

Kawashima, S.K. and Fujii, T., 2008. Basic and Clinical Aspects of Non-neuronal Acetylcholine: Overview of Non-neuronal Cholinergic Systems and Their *Biological. J. Pharmacol. Sci*., **106**(2): 167-173.

Koubkova, M.; Knizkova, L., Kunc, P.; Hartlova, H.; Flusser, J. and Dolezal, O. (2002). Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. *Czech. J. Anim. Sci.*, **47**(8): 309-318.

Lacetera, N.; Bernabucci, U.; Scalia, D.; Basiricò, L.; Morera, P. and Nardone, A. (2006). Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. *J. Dairy Sci.*, **89**(12): 4606-4612.

Lacetera, N.; Bernabucci, U.; Scalia, D.; Ronchi, B.; Kuzminsky, G. and Nardone, A., (2005). Lymphocyte functions in dairy cows in hot environment. *Int. J. Biometeorol*., **50**(2): 105-110.

Li, H.; Wang, H.; Yu, L.; Wang, M.; Liu, S.; Sun, L. and Chen, Q. (2015). Effects of supplementation of rumen-protected choline on growth performance, meat quality and gene expression in longissimus dorsi muscle of lambs. Arch. *Anim. Nutr*. **69**: 340-350.

Lobley, G.E.; Connell, A. and Revell, D. (1996). The importance of transmethylation reactions to methionine metabolism in sheep: effects of supplementation with creatine and choline. *Br. J. Nutr.*, **75**(1): 47-56.

Madesh, M. and Balasubramanian, K.A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian J. Biochem. Biophys*. **35**(3): 184-188.

Mayengbam, P. (2009). Heat shock protein 72 expression in relation to thermotolerance Sahiwal and Holstein-Friesian crossbred cattle. *Thesis Ph.D*., National Dairy Research Institute, Karnal.

McIntyre, T.; Patel, K.D.; Smiley, P.L. and Stafforini, D. (1994). Oxidized phospholipids with PAF-like bioactivity. *J. Lipid Mediat. Cell Signal*., **10**(1): 37-37.

Mohsen, M.K.; Gaafar, H.M.A.; Khalafalla, M.M.; Shitta, A.A. and Yousif, A.M. (2011). Effect of rumen protected choline supplementation on digestibility, rumen activity and milk yield in lactating Friesian cows. *Slovak J. Anim. Sci.*, **44**(1): 13-20.

Moron, M.S.; Depierre, J.W. and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim. Biophys. Acta. Gen. Subj.*, **582**(1): 67-78.

Naik, B.R.; Kumar, A.V.N.S.; Ravi, A.; Bramhaiah, K.V. and Chakravarthi, V.P. (2013). Effect of seasons on physiological and hematological values in Punganur cattle. *Int J Pharma Bio Sci*, **4**(4): 40-49

Nishiyama-Naruke, A. and Curi, R. (2000). Phosphatidylcholine participates in the interaction between macrophages and lymphocytes. *Am. J. Physiol. Cell Physiol.*, **278**(3): C554-C560.

Osorio, J.S.; Ji, P.; Drackley, J.K.; Luchini, D. and Loor, J. J. (2014a). Smartamine M and MetaSmart supplementation during the peripartal period alter hepatic

expression of gene networks in 1-carbon metabolism, inflammation, oxidative stress, and the growth hormone–insulin-like growth factor 1 axis pathways. *J. Dairy Sci*., **97**: 7451-7464.

Osorio, J.S.; Trevisi, E.; Ji, P.; Drackley, J.K.; Luchini, D.; Bertoni, G. and Loor, J. J. (2014b). Biomarkers of inflammation, metabolism, and oxidative stress in blood, liver, and milk reveal a better Immunometabolic status in peripartal cows supplemented with Smartamine M or MetaSmart. *J. Dairy Sci*., **97**:1-14

Ossani, G.; Dalghi, M. and Repetto, M. (2007). Oxidative damage and lipid peroxidation in the kidney of choline deficient rats. *Front. Biosci*. **12**, 1174-1183

Pandurang, P.S. (2012). Supplementing rumen protected choline to dairy during transition period: Effect on milk production and reproductive performance. *Thesis Ph.D*., National Dairy Research Institute, Karnal.

Piepenbrink, M.S. and Overton, T.R. (2000). Liver metabolism and production of periparturient dairy cattle fed rumen-protected choline. *J. Dairy Sci*, **83**(Suppl 1): 257.

Pinotti, L., Baldi, A. and Dell'Orto, V. (2002). Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. *Nutr. Res. Rev*., **15**(2): 315-332.

Pinotti, L.; Campagnoli, A.; Sangalli, L.; Rebucci, R.; Dell’Orto, V. and Baldi, A. (2004). Metabolism of periparturient dairy cows fed rumen-protected choline. *J. Anim. Feed. Sci*. **13**: 551-554.

Pinotti, L.; Paltanin, C.; Campagnoli, A.; Cavassini, P. and Dell’Orto, V. (2009). Rumen protected choline supplementation in beef cattle: effect on growth performance. *Ital. J. Anim. Sci.*, **8**(sup2): 322-324.

Pirestani, A.; Aghakhani, M.; Tabatabaei, S.N.; Ghalamkari, G. and Baharlo, F. (2011). Effects of dietary L-carnitine and choline chloride compound on reproduction indices and udder immune system in Holstein dairy cattle. In *Proc. International conference on life science and technology*, 59-61.

Rahmani, M.; Dehghan-banadaky, M. and Kamalyan, R. (2014a). Comparison between feeding rumen-protected choline and vitamin E on milk yield and blood metabolites in early lactation dairy cows. *Anim. Prod. Sci*., **55**(6): 752-757.

Rahmani, M.; Dehghan-banadaky, M.; Kamalyan, R.; Malekinejad, H.; Rahmani, F.; Tavatori, M.H.H. and Mohammadi, H. (2014b). Effects of feeding rumen-protected choline and vitamin E on serum protein fractions, total thiol molecules and total antioxidant capacity in early lactating dairy cows. *Global J. Animal Sci. Res*. **2**(4): 337-344.

Rahmani, M.G.; Kamalyan, R.G.; Dehghan-Banadrky M.J. and Marmaryan G.Y. (2012). The effect of oral administration of choline on some liver function characterized blood plasma enzymes of early lactating dairy cows. *Biol. J. Armenia*, **3** (64): 83-86.

Rathwa, S. (2020). Physiological and biochemical changes on feeding of rumen protected methionine and choline during transition period in Surti buffaloes. *Thesis Ph.D*., Navsari Agricultural University, Navsari.

Rehman, S.U. (1984). Lead induced regional lipid peroxidation in brain. *Toxicol. Lett*, **21**:333-337.

Repetto, M.G., Ossani, G., Monserrat, A.J. and Boveris, A., (2010). Oxidative damage: the biochemical mechanism of cellular injury and necrosis in choline deficiency. *Exp. Mol. Pathol*., **88**(1): 143-149.

Rodriguez-Guerrero, V.; Lizarazo, A.C.; Ferraro, S.; Miranda, L.A.; Mendoza, G.D. and Suárez, N. (2018). Effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. *S. Afr. J. Anim. Sci*, **48**(3): 427-434.

Rotondo, D., Earl, C.R.A., Laing, K.J. and Kaimakamis, D. (1994). Inhibition of cytokine-stimulated thymic lymphocyte proliferation by fatty acids: the role of eicosanoids. *Biochim. Biophys. Acta. Mol. Cell. Res.* **1223**(2): 185-194.

Roy, A., Guatimosim, S., Prado, V.F., Gros, R. and Prado, M.A., (2014). Cholinergic activity as a new target in diseases of the heart. *Mol. Med.* **20**(1): 527-537.

Sands, W.A., Clark, J.S. and Liew, F.Y. (1994). The role of a phosphatidylcholine-specific phospholipase C in the production of diacylglycerol for nitric oxide synthesis in macrophages activated by IFN-γ and LPS. *Biochem. Biophys. Res. Commun*, **199**(2): 461-466.

Sexson, J.L.; Wagner, J.J.; Schutz, J.S.; Seabrook, J. L.; de Veth, M.J., & Engle, T.E. (2010). The impact of trace mineral source, water quality, and short-term choline supplementation on performance and carcass characteristics of finishing steers. *Prof. Anim. Sci*., **26**(4): 380-386.

Shahsavari, A. (2012). The metabolic and reproductive responses of lactating dairy cows to supplementation with choline. *Thesis M.Phil.*, The University of Queensland, Gatton, Australia.

Sheikh, F. A. (2012). Nutrient utilization in lactating cows fed with rumen protected methionine, lysine and choline. *Thesis Ph.D.*, National Dairy Research Institute, Karnal.

Sheikh, F. A.; Neelam, K. and Thakur, S.S. (2015). Effect of supplementation of rumen protected lysine plus methionine or choline on blood biochemical parameters in crossbred cows. *Indian J. Anim. Nut.*, **32**(3): 344-347.

Shibasaki, M. and Crandall, C.G. (2010). Mechanisms and controllers of eccrine sweating in humans. *Front. Biosci.* (Scholar edition, **2**): 685-696.

Shibasaki, M., Wilson, T.E., Cui, J. and Crandall, C.G., (2002). Acetylcholine released from cholinergic nerves contributes to cutaneous vasodilation during heat stress. *J. Appl. Physiol*., **93**(6): 1947-1951.

Snedecor, G. W. and Cochran, W. G. (1994). *Statistical methods*. 8th edn. Iowa State University Press, United State of America.

Srivastava, A.; Yadav, P.; Mahajan, A.; Anand, M.; Yadav, S.; Madan, A.K. and Yadav, B. (2021). Appropriate THI model and its threshold for goats in semi-arid regions of India. *J. Therm. Biol*., **96**: 102845.

Sun, F.; Cao, Y.; Cai, C.; Li, S.; Yu, C. and Yao, J. (2016). Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. *PloS one*, **11**(8), p.e0160659.

Toghdory, A.; Emanuele, S.; Ghoorchi, T. and Naserian, A. (2007). Effect of choline and rumen protected choline on milk production, milk composition and blood metabolites and lactating dairy cows. *J. Dairy Sci.,* **90***:* 353-364

Tucker, C.B.; Rogers, A.R. and Schütz, K.E. (2008). Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Appl. Anim. Behav. Sci.***109**(2-4): 141-154.

Van Eerd, N.M. (2019). Evaluating heat stress response in lactating holstein cows with supplementation of a feed additive during mid lactation. *Thesis B.S. Honors*, The University Of Arizona

Wehner, F.; Olsen, H.; Tinel, H.; Kinne-Saffran, E. and Kinne, R.K. (2003). Cell volume regulation: osmolytes, osmolyte transport, and signal transduction. *Rev. Physiol. Biochem. Pharmacol.*, pp.1-80.

Xu, G.; Ye, J. A.; Liu, J. and Yu, Y. (2006). Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *Asian Australas. J. Anim. Sci.*, **19**(3): 390-395.

Zahra, L.C.; Duffield, T.F.; Leslie, K.E.; Overton, T.R.; Putnam, D. and LeBlanc, S.J. (2006). Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient dairy cows. *J. Dairy Sci.*, **89**(12): 4808-4818.

Zeisel, S.H. and DaCosta, K.A. (2009). Choline: an essential nutrient for public health. *Nutr. Rev.*, **67**(11): 615-623.

Zeisel, S.H. and Niculescu, M.D. (2006). Perinatal choline influences brain structure and function. *Nutr. Rev*. **64**: 197-203.

Zenobi M.G. (2018) Benefits of feeding ruminally-protected choline for lactating dairy cows and calves. *Thesis Ph.D.*, University of Florida.

Zom, R.L.; van Baal, G.J.; Goselink, R.M.A.; Bakker, J.A.; de Veth, M.J. and van Vuuren A.M. (2011). Effect of rumen-protected choline on performance, blood metabolites and hepatic triacylglycerols of periparturient dairy cattle. *J Dairy Sci*. **94**: 4016-4027.