IMPACT OF HEAT AND NUTRITIONAL STRESS ON ADAPTIVE CAPABILITY OF BUCKS

by

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(2010-20-106)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Impact of Heat and Nutritional Stress on Adaptive Capability of Bucks"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to

My Dear Parents

And

Loving Brother

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SYMBOLS AND ABBREVIATIONS

´	Minutes
°C	Degree Celsius
µL	Micro litre
A/G	Albumin/ Globulin
AAP	Aminoantipyrine
AHD	Animal Husbandry and Dairying
AIDS	Acquired Immunodeficiency Syndrome
ATP	Adenosine diphosphate
ATP	Adenosine triphosphate
B	Blank
BCG	Bromocresol green Green
BLAST	Basic Local Alignment Search Tool
BMR	Basal Metabolism Rate
BUN	Blood Urea Nitrogen
BW	Body Weight
C	Control
cDNA	complementary DNA
cDNA	complementary DNA
CER	Common Environment Specific Response
CHE	Cholesterol Esterase
CHOD	Cholesterol oxidase
cm	centimetre
CO_2	Carbon dioxide
CS	Combined Stresses
Cu^{++}	Copper(II) ion
DEPC	Diethylpyrocarbonate
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
E	East
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organisation

Fig.	Figure
g	gram
g/dL	gram/ decilitre
G-3-P	Glycerol-3-Phosphate
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
GDP	Gross Domestic Product
GK	Glycerol Kinase
GLM	General Linear Model
GOD-POD	Glucose oxidase- Peroxidase
GPO	Glycerol 3 Phosphate Oxidase
H and E	Haematoxylin and Eosin
H	hour
H_2O	Water
H_2O_2	Hydrogen Peroxide
Hb	Haemoglobin
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HPA	Hypothalamo Pituitary Adrenal
HR	Heart Rate
HRP	Horse Radish Peroxidase
Hrs	Hours
HS	Heat Stress
HSPs	Heat Shock Proteins
IU	International Unit
Kg	Kilogram
L	Litre
LN_2	Liquid Nitrogen
LPL	Lipoprotein lipase
Ltd.	Limited
M	Metre
mg/dL	Milli gram / decilitre
Min	Minutes
Ml	Millilitre
Mm	Milli meter

mRNA	messenger Ribonucleic Acid
N	North
NADH	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
Nm	nanometre
ns	Non-significant
NS	Nutritional Stress
º	degree
O_2	Oxygen
OD	Optical Density
PAP	Phenol + Aminophenazone
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
Pg	Picogram
PR	Pulse Rate
PRA	Pulse Rate Afternoon
PRM	Pulse Rate Morning
PUN	Plasma Urea Nitrogen
Pvt.	Private
qPCR	quantitative Polymerase Chain Reaction
RH	Relative Humidity
RNA	Ribonucleic Acid
Rpm	revolutions per minute
RR	Respiration Rate
RRA	Respiration Rate Afternoon
RRM	Respiration Rate Morning
r RNA	ribosomal RNA
RT	Rectal Temperature
RTA	Rectal Temperature Afternoon
RTM	Rectal Temperature Morning
RT qPCR	Real time quantitative Polymerase Chain Reaction
S	Standard

SEM	Standard Error Mean
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Sciences
SR	Sweating Rate
STF	Skin Temperature Flank
STFM	Skin Temperature Flank Morning
STH	Skin Temperature Head
STHA	Skin Temperature Head Afternoon
STHM	Skin Temperature Head Morning
STS	Skin Temperature Scrotum
STSA	Skin Temperature Flank Afternoon
STSA	Skin Temperature Scrotum Afternoon
STSM	Skin Temperature Scrotum Morning
T	Test
T_3	Triiodothyronine
T_4	Thyroxine
Tb	Body Temperature
T_{db}	Dry bulb temperature
TE	Tris Ethylenediaminetetraacetic Acid
THI	Temperature Humidity Index
TMB	Tetramethylbenzidine
TNZ	Thermo Neutral Zone
t RNA	transfer Ribonucleic Acid
T_{wb}	Wet bulb temperature
WAD	West African Dwarf
M	Overall Mean

INTRODUCTION

CHAPTER 1
INTRODUCTION

Livestock was considered as the oldest wealth resource of mankind and it also plays a vital role in providing nutritive value to humans all over the world. It is the major sector that accounts for 40 per cent of the world's agriculture Gross Domestic Product (GDP) (Sejian *et al*., 2015). India holds the largest livestock population in the world, and among agriculture, livestock is the major subsector that has a great significance to the Indian economy and particularly for the welfare of the rural farmers in India. India occupies the first and fourth positions in milk production and egg production respectively and also the meat and poultry products together accounted for 83 per cent of total export earning, while the export of milk and milk products contributed for about 17 per cent of the export of livestock products (AHD, 2007). In India among the livestock population, small ruminants play an important role in the rural economy since most of the farmers are mainly poor and marginal farmers, they cannot afford the huge maintenance expenses on large ruminants compared to the small ruminants.

India is the second largest in goat production. Developing countries accounts for 80 per cent of goat production. Goat rearing is considered one of the backbone of the Indian farming industry as it provides gainful employment to the farmers especially in the rural area. They can be reared both in intensive as well as semi-intensive systems of management. The shrinkage of land and water resources, due to the increasing population exacerbates the farmers to opt for goat rearing rather than the large ruminant production. High fertility rate, adaptability, high feed conversion ratio, low investment and managemental skills, small housing space, high disease resistance, well established local and sustainable markets, unpaid family labour, all add ups to the economic importance of goat farming in India (Devendra, 2007). Goats are multi-purpose animal that can produce meat, milk, hide, fiber and also manure. Goats give more production per unit of investment. The meat has less fat and is more in

demand. These animals are tolerant to hot climate and can well adapt to different agro-ecological zones where the climatic conditions can vary from dry to cold arid compared to other farm animals. These animals can survive in harsh hilly tracts as well as sandy zones. Unlike other farm animals, the goats can thrive well even by consuming shrubs, herbs and other poor quality roughages, because of the increased digestibility of crude fiber. Goats are also called as the foster mother of man, as their milk is considered better for human nutrition, easily digestible as well as cheap compared to any other livestock species. Goat milk has higher buffering qualities and is also used as ayurvedic medicine. Goat milk has higher phosphate content as well as B-complex vitamins, which is beneficial for vegetarian communities. Goat manure is 2.5 times richer in nitrogen and phosphoric acid than cow manure (Childs, 2011). In the changing climatic scenario, goat is the most admirable animal for physiological and biomedical research especially in the field of establishing the impact of climate change on livestock production.

Small ruminants such as sheep and goats are the major source of food as well as financial security to the small and marginal farmers especially in the rural areas of the different parts of the country. The farmers rely on these animals since goat farming require less initial investment, small housing space, less amount of feed because of their small body size as compared to large ruminants and also can thrive well on any kind of feed resources. The expenses that need to be diverted for meeting the different management strategies will be very less and that is the reason behind why goat is often called as “poor man’s cow”. Since these animals can be very well adapted to any type of climate they can be incorporated into different type of farming systems (Assan, 2014a). In the changing climatic scenario, the small ruminants are found to be more resistant to drought, less feed availability, poor quality feed and other impacts of climate change when compared to large ruminants such as cattle (Kosgey *et al* ., 2008).

There are many factors that influence the goat adaptation such as age, breed, sex, Temperature Humidity Index (THI) and other environmental stresses.

Among this, the most common and significant factors are climate, diseases, parasites, and nutrition (Lamy *et al*., 2012). Though the elder animals are vulnerable to environmental stress younger animals are more susceptible. Breed also influences the adaptation capabilities (Waziri *et al*., 2010). Animals that are poor in their nutritional status are prone to HS. Livestock productions are highly vulnerable to changing climatic factors since livestock are widely reliant on natural resources. Rise in temperature have adverse indirect impacts on pasture growth, water availability and spread of diseases (Thornton *et al*., 2009). Climate change and Carbon dioxide (CO_2) concentrations affect the function and distribution of plants and thus, alter the rangeland vegetation composition. Water scarcity, low quantity and quality of feed had led to negative impacts on the productivity and health status of livestock (Nardone *et al*., 2010). As animals suffer from nutritional stresses and energy deficits, they subsequently surrender themselves to extreme weather conditions.

According to Hanjra and Qureshi (2010), climate change shall immensely affect livestock production systems along with the existing factors such as rapid population and economic growth, increased demand for food and products, and increased conflicts over scarce resources (e.g. land tenure, water, and feed). Climate change can have both direct as well as indirect effect on goat production. Heat stress is the single detrimental factor which influences goat production. However, it's the indirect effects of climate change which causes worry. These effects include reduced feed and water availability and sudden outbreaks of diseases. All these can severely hamper goat production (Sejian, 2013). In the present changing climatic scenario, apart from HS there are other environmental stresses which hamper livestock productivity. These stresses do not occur in isolation rather they occur simultaneously. This results in multiple stresses simultaneously hampering the health, reproductive and productive capabilities of animal (Sejian, 2012).

High Ambient Temperatures (AT), direct and indirect solar radiation and Relative Humidity (RH) are the major environmental factors that impose HS on

livestock. Heat stress was observed to be common during the dry season, when the environmental temperature and RH are high with prolonged exposure to direct sunlight. Heat stress generally has significant influential role in the physiological responses (sweating, panting) and hormonal (cortisol, thyroid gland activity) of goat and also on their various systems (nervous and immune) have been implicated with specific responses and reciprocal regulatory influences (Castanheira *et al*., 2010). Heat stress reduces productivity, by causing reproductive problems such as reduced semen quality and lower birth weights, and compromise the immune system. Heat stress also reduces natural immunity making animals more susceptible to disease (Silanikove, 2000a). Factors such as water deprivation, nutritional imbalance and nutritional deficiency may aggravate the impact of HS. Under these circumstances the productivity may be severely affected which will lead to severe economic loss for the goat industry.

Environmental stress, nutritional Stress (NS), social stress, and prenatal stress can all have impact on innate and adaptive immunity of animals (Carroll and Forsberg, 2007). The production performance of an animal reflects its nutritional status. Factors such as water deficiency, nutritional imbalance, and nutritional deficiency aggravate the impact of HS on animals (Marai *et al*., 2007).Under climate change, extreme of temperatures further increase the demand of energy for sustenance and survival, however poor supply of forage and its quality in grazing lands restricts the energy intake leading to energy crisis, NS and loss of production. In addition, HS severely hampers pasture availability including both the quality and quantity of available feed resources. According to Craine *et al.* (2010), any future increases in precipitation would be unlikely to compensate for the declines in forage quality that accompany projected temperature increases. As a result, livestock species are likely to experience greater NS in the future. The NS increases the case of pregnancy toxaemia and neonatal death due to poor milk yield and immunity, prone to many infectious diseases (Sahoo, 2013). Hence it is the nutrition that hampers severely the productivity of livestock in the changing climate scenario.

Use of improved technologies in molecular analysis had permitted the improvement in the accuracy and intensity of use of genetic markers. Physiological responses like Respiration Rate (RR) and Rectal Temperature (RT) can also be indicated as stress markers. While the markers such as the Heat Shock Proteins (HSPs) are widely used to gauge stress tolerance, and it has become new approach to counter stress and also improve the stress tolerance in farm animals (Pawar *et al*., 2013). Many researchers found that the heat shock proteins especially HSP70 , HSP72 gene expression can be used as a marker for thermal adaptation in different species, and information generated will have significant implications in the future for the development of strategy to cope up with the challenges of climate change (Fehrenbach *et al*., 2000). Higher expression of HSP70 in caprine Peripheral Blood Mononuclear Cells (PBMCs) during HS, suggest a possible involvement of HSP70 in ameliorating the deleterious effect of thermal stress so as to maintain cellular integrity and homeostasis in goats (Mishra and Palai, 2014). There are also several other markers such as immunological markers, clinical markers, common environment-specific response genes (CER), oxidative stress markers and genomic and proteomic markers. The types of research might pave way for development of agro-ecological zone specific thermo-tolerant breeds.

Research efforts are needed to study the different environmental stress effect on goat production. In the changing climatic condition, these stresses occur simultaneously. Hence research agendas need to take into account this fact and try to study the cumulative impact of these environmental stresses on goat production. Such studies in goat are negligible. The proposed study will be the first to establish the cumulative impact of HS and NS in goat. It is very pertinent to conduct such study as under the changing climatic scenario these stresses do not occur in isolation rather they impact livestock production simultaneously. In addition, goat is the most preferred species for improving livelihood security in the changing climate scenario. From this study, it is possible to identify specific biological markers for both HS and NS for buck. Heat stress and NS are the

important factors which affect the health and productive performance of goats. Hence concerted efforts are needed to provide the base line information related to establishing the impact and adaptive mechanisms of goat to HS and NS simultaneously. This may pave way for development of goat breed that are genetically adapted to both HS and climate derived nutritional deprivation. With these backgrounds, the proposed study is the first of its kind to study the influence of two environmental stresses simultaneously on the adaptive capability of buck. Fig. 1 depicts the excepted results from this study through a concept figure.

The objectives of the study are

1. To observe the impact of HS and NS on physiological adaptability of bucks.
2. To determine the impact of HS and NS on blood biochemical and endocrine responses in bucks.
3. To observe the impact of HS and NS on HSP-70 expression in liver and Hypothalamo- Pituitary Adrenal axis (HPA axis) in bucks.

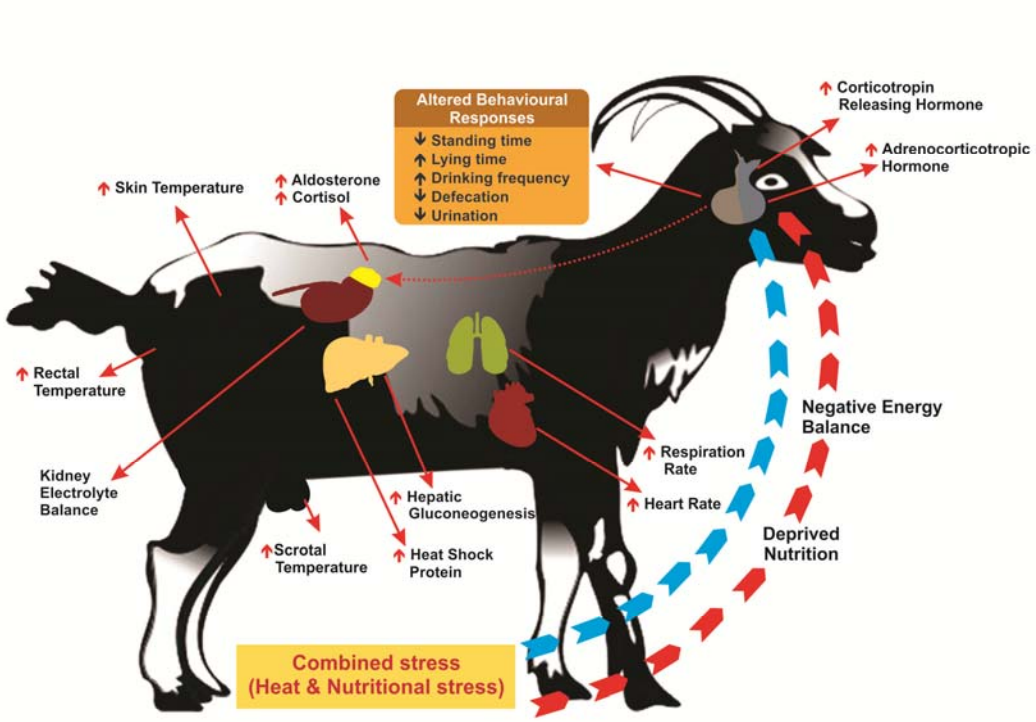


Fig. 1: Concept figure of the present study

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Livestock is considered as the oldest wealth resource of mankind and it also plays a vital role in providing nutritive value to humans all over the world. In the developing world, livestock constitutes 30 per cent of the agricultural GDP, and about 40 per cent of the global agricultural GDP (Steinfeld *et al.,* 2006). Small ruminants such as sheep and goats are the major source of food as well as financial security to the small and marginal farmers especially in the rural areas of the different parts of the country. The farmers rely on these animals since these animals require less amount of feed because of their small body size as compared to large ruminants and also can thrive well on any kind of feed resources. In the changing climatic scenario, the small ruminants are found to be more resistant to drought, less feed availability, poor quality feed and other impacts of climate change when compared to large ruminants such as cattle. Among the livestock sector, goat rearing is considered as the backbone of the Indian farming industry since it provides gainful employment to the farmers especially in the rural area. Also the high fertility rate, adaptability, high feed conversion ratio, low investment, managemental skills, small housing space, high disease resistance, well established local and sustainable markets, unpaid family labour, all add ups to the economic importance of goat farming in India. Goats are multi-purpose animal that can produce meat, milk, hide, fiber and also manure. Goats are considered as hardy animals that can thrive well in any extreme environmental conditions. They undergo changes in certain behavioural, physiological, blood biochemical, endocrine, cellular and molecular responses in order to adapt to the changing climatic scenario, which is being brought into perception through this review.

2.1 Importance of livestock to Indian economy

India has the largest livestock population of 485 million animals (Shinde, 2014). According to the 12th five year plan (2012-17), report of working group on Animal Husbandry and Dairying (AHD), India has 56.7 per cent of world's buffaloes, 12.5 per cent cattle, 20.4 per cent small ruminants, 2.4 per cent camel, 1.4 per cent equine, 1.5 per cent pigs and 3.1 per cent poultry. In 2010-11, livestock generated outputs worth Rs. 2075 billion (at 2004-05 prices) which comprised 4 per cent of the GDP and 26 per cent of the agricultural GDP and this total output worth was much higher than the value of food grains (AHD, 2012).

According to Kumar *et al.* (2008), livestock is the most crucial sector of agriculture that tremendously contributes to the foreign exchange, income, nutritional status,, employment, and poverty relief of the country. It is a continuous source for livelihood and it reduces the seasonality in income patterns particularly of the rural poor (Ali, 2007). 12 per cent of the world's population who relies on livestock for sustenance was mostly the poor farmers who consider livestock as a multifunctional asset (Randolph *et al.,* 2007). According to AHD (2007), livestock provides stability to family income of poor and marginal farmers and it has been the primary source that provides livelihood for one billion of the world's population living in poverty. Since the world population is increasing the global demands for agricultural products, especially the livestock products which includes the meat, milk, eggs, hides and skins and other by-products, such as manure, hides and skins, fat, offal, honey, transport services etc., were more or less expected to be doubled during the first half of this century (Naqvi and Sejian, 2011). Thornton *et al.* (2002) reported that the majority of the rural people depend on mixed as well as integrated farming systems where livestock will have a major role in contributing for the intensification and diversification of their income. This will recuperate and encounter the rising demand of millions of poor farmers and save them from being driven deeper into dearth (Iqubal, 2010). An anticipated rise in world population by 30 per cent will increase the demand for food by 70 per cent by 2050. These growing demand

further increases the real opportunities of livestock sector for economic growth as well as poverty alleviation of the country as a whole (Thornton, 2010). According to Iqubal (2010), the world's livestock sector is growing at an unprecedented rate by the contribution of developing countries and India being one among the developing countries that holds the largest number of livestock and has topmost position in milk production in the world, thereby improving the socio-economic conditions of people in general and especially rural people. In rural India, 15-20 per cent families are landless and about 80 per cent of farmers are landowners but belong to the category of small and marginal farmers whose livelihood is entirely relied on livestock sector (Thornton, 2010).

Milk production in India is growing at an annual rate of 4 per cent. The increase in milk prices along with rising domestic demand for a variety of milk products, supported by the growth of the Indian economy, are the primary factors driving increased production. By the end of 12^{th} Plan, demand for milk is expected to increase to 141 million tons and 15.8 million tons for meat, eggs and fish together (AHD, 2012). Global market for animal products is expanding fast, and is a prospect for India to expand its participation in global market. In addition, the growth of livestock sector is at the rate of 5.6 per cent, which is 3.3per cent higher than the agriculture sector over the last two decades. Many empirical studies illustrated that livestock rearing has substantial positive impact on equity in terms of income, employment and poverty alleviation, especially in rural areas (Thornton *et al.,* 2002; Ali, 2007). Moreover, livestock industry is mainly women oriented and growth in livestock production would help improve equity in terms of gender also.

2.2 Significance of rearing small ruminants

According to Oluwatayo and Oluwatayo (2012), small ruminants such as sheep and goat form an important economic and ecological role in agricultural systems and also play a significant part in the lives of households of the developing countries. The easiest and most readily available source of credit to

meet immediate social and financial obligations can be achieved through rearing small ruminants. In recent years, farmers have begun to strengthen the production of small ruminants owing to an increasing recognition by policy makers that they are a potential alternative source of farm income (Panin, 2000). The main reason for rearing small ruminants is that they are an easily liquidated resource that can be used for saving or raising cash. The small ruminants deliver their owners with a wide range of products and services. In rural areas there are no bank facilities, so the most appropriate way to store cash for future need is by purchasing the small ruminants and hence it is called as village bank in some areas (Mengesha and Tsega, 2012).

Oluwatayo and Oluwatayo (2012) reported that 35 per cent per cent of the total Nigerian meat supplies come from small ruminants. More than 50 per cent of milk produced for human consumption is from small ruminants reared in Niger and Somalia (Adedeji, 2012a). Small ruminant production, especially meat goat production, is one of the fastest growing agricultural production systems in the United States today. The population of sheep and goats in Pakistan are much higher than other livestock species indicating their economic importance and adaptation in the different agro-ecological zones of the country (Sarwar *et al*., 2010). Compared to large ruminants, small ruminants often produce about twice as much meat per animal unit in the tropical regions (Adams and Ohene, 2014). Sheep and goats are reared as a secondary occupation and way of life rather than a commercial enterprise in the dry land areas. Most small holder and pastoral/extensive farmers (on average 72 per cent) rear ruminants either for regular cash income or as an insurance against emergencies (Kosgey *et al.,* 2008).

Small ruminants have several advantages for being an integral part of the pastoral production system. Sheep and goats are a constant source of protein during and immediately following a period of drought, which makes them the most significant component of livestock in pastoral and agro-pastoral production system (Kumar *et al*., 2011a) Other than meat and milk, it also contributes to

fiber and skins to draught power in the highlands as well as food security in some extreme cases (Oluwatayo and Oluwatayo, 2012). Sheep *(Ovis aries)* and goat *(Capra hircus)* are more effective in converting non-grain feed into quality meat compared to other livestock species (Peacock, 2005).

Small ruminants are particularly relevant for subsistent agricultural systems, because of their unique biological attributes, including short gestation period, high prolificacy, rapid growth rate, high feed conversion efficiency from coarse roughage, and high tolerance to tannins and diseases, as well as easy marketability (Lebbie, 2004). Small ruminants (sheep and goat) have a vital role in contributing precious animal proteins such as meat and milk as well as food security in some cases (Oluwatayo and Oluwatayo, 2012).

Capital investment in housing and materials (such as iron sheets and wood) are lower in case of small ruminant production when compared with other livestock species (e.g. cattle) (Adams and Ohene, 2014). Sheep and goats are widely adapted to climatic conditions and are found in all production systems. They have higher survival rates under extreme weather conditions like drought etc., when compared to cattle and their short reproductive cycle permits them to quickly recover from rapid resumption of breeding following drought or devastating disease infestation (Aziz, 2010). Because of their small body size they have lower feed requirements compared to cattle. Furthermore, because of their reproductive rates, flock numbers can be restored more rapidly.

2.3 Importance of goat rearing

Comparing to other livestock species, goat is one of the sociable, versatile, and intelligent species, which has been used for its meat, milk, skin, and fiber from the time it was first domesticated ca. 10,000 years ago (Miranda-de la Lama and Mattiello, 2010). Out of the 674 million goats in the world, 94 per cent are found in the developing countries (Stroebel, 2004). Rymer (2006) reported that goats contribute approximately 30 per cent to the livelihood of resource poor livestock farmers. Kumar and Chander (2004) reported the

emerging importance of goat production globally with the evidence given by Von Veen (1999) in his study revealing that no other livestock species except pig, showing a rise in trend globally in population of goats, with more increase (56 per cent) in developing countries compared to developed countries (33 per cent) during 1975-95. According to Kosgey *et al*. (2008), at the household level, goats serve as a bank account which can be drawn at the time of economic crisis, whenever money is needed while the kids are the interest received. Moreover goats are small ruminants that need less initial investment, less labor, as well as low quality and quantity feed stuffs compared to large ruminants like cattle and it is also considered as an appropriate option for the poor farmers because of short gestation period, low capital investment and also because of the less maintenance charges (Gopala *et al.*, 2010; Rajkumar and Kavithaa, 2014). Devendra (2007) reported that goat rearing can be done in intensive as well as semi- intensive systems. According to Rohilla and Chand (2004), goat rearing is best suited for farmers with small land or community based free feeding resources. Seresinhe and Marapana (2011) in their study reported that the farmers even fed their goats with kitchen waste and refused coconut scrapings at the time of feed deficit, which is not at all possible in the case of other livestock species. According to Kumar *et al*., (2011a), goat is known as the poor man's cow as raising goat is ideal for poor resource farmers who cannot afford large ruminants especially in India. Further among the meat products, goat meat is the costlier which fetches huge profit in return to the poor farmers. Furthermore, there is no religious taboo for goat farming and chevon consumption. According to Ahuya *et al,* (2005), the goats are capable of producing twins or triplets annually because of their short reproductive cycle. According to Silanikove and Koluman (2015), goat will have significant role in harsh conditions, tropical, sub-tropical, desert and Mediterranean environments. Rural areas often faces environmental extremes like flood and droughts etc., and in such severe conditions, goat raising acts as an substantial source of survival for the rural landless farmers, who doesn't have any other means for their livelihood (Gopala *et al.*,2010; Rajkumar and Kavithaa, 2014). Ahuya *et al.* (2005) stated that goats recorded higher survival rates

compared to cattle under adverse drought conditions and hence they are resistant to a wide variety of environmental stress conditions due to their capability to survive even on drought resistant trees and shrubs without altering their production abilities. Assan (2014a) stated that goats can adapt easily to a wide variety of climatic conditions compared to other livestock species and survive on browse materials which are not at all used by other livestock species and in addition these animals are more capable to adjust to drought conditions during the dry, arid seasons and are also adaptable to harsh environments. Compared to other ruminants, efficient use of water and low quality feed resource utilization are the characteristic traits of goat and further their feeding behaviour ensures goats to be in less competition with other ruminant species (Ahuya *et al.,* 2005). Goats has low body mass, and low metabolic requirements, which minimize their maintenance and water requirements, in areas where food sources and water sources are scarce, further the ability to reduce metabolism allows goats to survive even after prolonged periods of severe limited food availability (Silanikove and Koluman, 2015).

According to Tanwar (2011), among various livestock species, goats play a significant role by providing milk as well as meat for nutrition and manure as agricultural input in case of integrated farming systems. Goat rearing is an important enterprise that not only provides livelihood for the weaker sections of the society at the meantime meets the nutritional requirement of families in arid and semi-arid areas. According to Thornton (2010), goat plays an important socio-cultural role, and they can alleviate the seasonal food variability and unavailability and can ensure the food security especially in semi-arid areas. According to Abad-ur-Rahman *et al*. (2012), chevon is considered as the quality meat since it has low quantity of fats and goat milk as the best substitute for mother's milk for infants and is often called as wet nurse in Europe.

2.4 Factors influencing goat adaptation

Each species, breed or animal category, has a comfort zone called Thermo Neutral Zone (TNZ), wherein the energy expenditure of an animal is minimal, constant and independent of environmental temperature. Outside this comfort zone, the animal experiences stress to maintain homeothermy and extra energy need to be diverted for thermoregulation, so that less energy is available for production processes. The animal modifies its behaviour and other physiological and metabolic functions as well as compromises the quantity and quality of its production when they are subjected to adverse environmental conditions (Nardone *et al*., 2006).

Animals develop various adaptive mechanisms in order to cope up with the harsh environment which causes stress in them (Helal *et al.,* 2010). Thus the adaptability of an animal can be defined as the ability to survive and breed within a defined environment or the degree to which an organism, population or species can remain/become adapted to a wide range of environments by physiological or genetic means (Lamy *et al.,* 2012).

According to Singh *et al.* (2012), climate change affects the animal health in four ways: heat-related diseases and stress, extreme weather events, adaptation of animal production systems to new environments, and emergence or re-emergence of infectious diseases, which are critically dependent on environmental and climatic conditions. However, distribution of goats all over the world depicts their ability to adapt to a variety of environments. The innate traits of goats such as capability to adjust to water scarcity and preference for browse and wide-range of feeds, allow them to survive in regions that receive less than 750 mm of rainfall (Sarwar *et al*., 2010).

According to Gupta *et al.* (2013), the adaptability of goats to thermal stress is due to water conservation capability, higher sweating rate (SR), lower Basal Metabolism Rate (BMR), higher RR, higher Skin Temperature (ST), constant Heart Rate (HR) and cardiac output. In addition, small body size, low

metabolic requirement, ability to reduce metabolism, efficiency of utilization of high forage diet i.e. high digestive efficiency, ability to economize nitrogen requirement, efficient use of water etc. are also important characteristics which help them to cope up with harsh environmental conditions (Silanikove, 2000a).

The different factors that affect the adaptability of goat are:

2.4.1 Breed

Hansen (2009) reported that there were differences in thermoregulatory abilities among the breeds. According to Silanikove (2000b), the indigenous breeds of the sub-tropical and tropical environments perform better than the exotic breeds in survival, reproduction and expression of their genetic potential for growth and milk production. However, the breeds in Pakistan like the dwarf goats and dairy goats in temperate zone are well adapted to thrive in humid tropics also (Sarwar *et al*., 2010).

da Silva *et al.* (2014) reported in their study that, Azul goat remained in the shade, longer than Grauna, and the latter was similar to Moxoto, which spent more time consuming food. Regarding the periods, the animals spent more time lying down during the afternoon. However, study conducted by Sharma *et al.* (1998) on Jamunapuri and Barbari goat breeds reported that there were no significant differences in the time spend on grazing during the summer periods and reduced feed intake due to high AT.Also Merino and Dorper sheep, Welsh Mountain and Scottish Blackface sheep (Fraser *et al.,* 2009 a,b) differed in their grazing/browsing behaviour.

Ocak and Guey (2010) reported that higher respiratory rate (RR) has been observed in Saanen goat breeds (54/min) which depicted that these breeds were more susceptible to stresses in comparison with others. However, in a study conducted in Balady and Damascus goats, by Helal *et al.* (2010) reported that the blood biochemical parameters such as total protein, globulin, albumin and cholesterol differed significantly in both breeds, when they were exposed to HS.

2.4.2 Sex

Adedeji (2012b) reported in West African Dwarf (WAD) goat that the sex influenced only the RR compared to other physiological responses and the males exhibited higher RR than females. This finding was in agreement with Butswat *et al*. (2000) and Sanusi (2008) among sheep of different breeds, where they reported that male sheep had higher values in all the physiological response parameters in comparison to females. According to Sharma and Puri (2013), one of the important factors that affect the concentration of blood constituents is sex. In their study conducted in Marwari goats they reported that males showed higher values of blood biochemical parameters in comparison to females in extreme climatic conditions. Further, Ocak and Guey (2010) observed lower cholesterol levels and added that these variations may be due to differences in sex. In contrast, the study conducted in Red Sokoto goats showed that sex had no significant influence on hematological parameters (Hassan *et al* ., 2013).

2.4.3 Age

Age and weather of the livestock can also affect their grazing behaviour, younger animals graze even less than older ones. Alam *et al.* (2011) reported that there were no changes in skin as well as RT in goats of different age groups, after being exposed under sun for a long time. This result was however in contrast with the findings of Marai *et al.*,(1997) and he further suggested that this variation in results may be due to different experimental conditions and/or breed and age of goats.

According to Njidda *et al.,* (2013), the blood composition of animal was also influenced by several factors, one important factor being age, which significantly influenced the blood values. The glucose levels were found to be higher in kids than adult goats. The observed tendency toward decrease in blood glucose in adult goat was in agreement with the findings of Toncho *et al*., (2007). The values for total protein, albumin and globulin showed significant differences

among breeds age and sex while cholesterol showed no significant differences in terms of age (Sharma and Puri, 2013). Further, they added that the animals of 0-1 year age group showed higher values in albumin, globulin. In addition, Hassan *et al*., (2013) reported that age influenced the values of hematological parameters in Red Sokoto goats.

2.4.4 Coat characteristics

Collier *et al.,* (2008) suggested that the characteristics of an animal that were found to affect the efficiency of evaporative heat loss from the skin surface are the sweat gland density, coat depth, skin colour, hair colour and hair length. According to Assan (2014a),water consumption was higher and feed intake was lower in the coloured Sirohi goats during the hotter part of the day and particularly the feed intake was observed lowest in black Sirohi goats.

The reflective properties of the hair coat is one of the factor that determines the heat exchange by radiation and it was reported by Hidalgo (2009) that the light-coloured sleek and shiny hair coats reflects more of the incident solar radiation in comparison to dark, dense and woolly coats. Further, Ocak and Guey (2010) reported that the body temperature (Tb) and the metabolic rate increased in black haired goats when they were exposed under sun. He further added that the hair colour also influences the RT, HR and also the RT. According to Scherbarth and Steinlechner (2010), the short haired goats had lowest tolerance as they exhibited more RR, HR compared to long haired goats and he added that coat colour and hair length had significant influence on the Tb and the metabolic rate. Bernabucci *et al.* (2010) concluded that skin colour is one of the factors that influence the adsorption of solar radiation. The study conducted by Al-Samawi *et al*., (2014) in black coloured Aardi goats showed significant increase in the coat temperature when they were exposed to sun. The other major characteristics of the hair coat involved in the thermoregulatory process of animals are hair density (Hidalgo, 2009) and coat depth (Turnpenny *et al*., 2000).

2.4.5 Environmental stressors

Among the physical environmental stressors, AT is ecologically the most important (Horowitz, 2002). High AT, high direct and indirect solar radiation and humidity are environmental stressing factors that impose stress and strain on animals (Silanikove, 2000a). Silva *et al*., (2014) suggested that the goats lying behaviour and greatest rest activity occurred during the afternoon hours when the AT is higher compared to morning. This statement was in baseline with the findings in feedlot cattle by Marques *et al*. (2006). The environmental stress activates the sweat secretion long before panting in goat and cow (Silanikove, 2000a). Further, Silanikove (2000b) stated that the most prominent response during the environmental stress conditions were the release of the stress relieving hormone plasma cortisol, by the activation of the HPA axis. Among the climatic factors the AT, humidity, air movement, radiation and photoperiod, temperature is the most important factor that influenced the animals (Ali *et al*., 2004). Sharma and Puri (2013) reported that the climatic factors, particularly hot weather conditions had substantial effect on globulin, A/G (Albumin/Globulin) ratio and total protein. Further, Sarwar *et al*. (2010) suggested that the goats thrive well in the drier tropics rather than in the wet humid tropics.

Temperature humidity index was used as an environmental factor to predict the production losses of an animal exposed to hot and humid climatic conditions (Karaman *et al*., 2007). Rising AT and RH as expressed in form of THI influenced the growth performance as well as the physiological responses of animals (Popoola *et al*., 2014). They further added that the body weight (BW) and average daily gain, RT and RR except pulse rate (PR) were significantly influenced by THI.

2.4.6 Nutrition

All livestock production depends on access to adequate amounts of good quality feed and water for proper growth and production. Animal adaptation includes not only heat tolerance but also to their ability to survive, grow and reproduce in the presence of poor seasonal nutrition as well as parasites and diseases (Scholtz *et al*., 2013). In the subtropics and tropics, majority of the livestock population undergoes an insufficient nutritional supply, with reduced productivity, mainly due to a combination of low pasture allowance, high fiber content and inadequate feeding management and poor nutrition being recognized as the major production constraint in smallholder systems (Thornton, 2010). Free-grazing animals always face reduction in the nutritional quality of forage especially during the hot and dry seasons (Aharoni *et al.,* 2004; Brosh *et al.,* 2004). Ruminants in pastoral and extensive mixed systems in many developing countries, suffer from permanent or seasonal NS (Ekou, 2014). Further, it was reported that feed intake and digestibility were significantly reduced by HS (Soren, 2012). In addition, reduced feed intake during HS impairs both the immune functions and reproductive performance in livestock (Ju, 2005).

2.5 Significance of understanding the impact of climate change on goat adaptation

Climate change has been proved to adversely affect the environment including the livestock, agriculture and other managemental practices which have been developed over the last 10,000 years (Silanikove and Koluman, 2015). Climate change results in causing multiple stress to animals which includes HS and NS affected the reproductive and productive capabilities of animal (Sejian *et al*., 2013a). The combined effect of temperature and RH induces HS in animals which can be quantified through the THI (Marai *et al*., 2001). High THI produced harmful effect on animals, particularly the livestock and dairy production were found to be more affected by HS. Warmer and humid conditions could also produced indirect effects on animal health and productivity through

diseases and parasitic infestations (Walthall *et al*., 2012). Assan (2014b) reported that changes in rainfall pattern also influence expansion of vectors during wetter years, leading to large outbreaks of diseases. Further, they stated that climate change will immensely affect fodder production and thereby affecting the nutritional security of livestock. Increased temperature will also enhance the lignification of plants, and thus reduces the digestibility. In the changing climatic scenario, drought conditions occur more frequently which results in water scarcity and thereby decline the production of feed and fodder.

According to Sejian (2013), climate change is likely to aggravate the HS in dairy animals, adversely affecting their reproductive performance. Climatic change will negatively affect the dairy industry and also the importance of goats to the dairy industry will increase in proportion to the severances of changes in environmental temperature (Silanikove and Koluman, 2015).

Ahuya *et al*. (2005), Gopala *et al*. (2010) and Rajkumar and Kavithaa (2014) reported that compared to other livestock species the goats were found to thrive in all extreme conditions initiated by the climate change such as drought and flood and thus they were said to be resistant to several stresses caused by the environment. Further, Alam *et al*., (2011) reported that the thermal environment is a major factor that can negatively affect goat performance and may result in hyperthermia and potentially have several physiological side effects. Further, Dossa *et al.* (2007) have reported that goats were less vulnerable to the impacts of drought, when compared to sheep and cattle, owing largely to their browsing capability. In addition, he reported that camels and goats recorded high adaptability scores with regard to feed shortage, indicating the ability of these animals to cope during conditions of feed resource shortage induced by unfavorable climatic conditions. However, goats that are grazing in the semi-arid conditions face problems in the availability of quality and quantity of feed resources (Martin *et al*., 2004; Sejian, 2013).

Scholtz *et al.* (2013) reported that majority of farmers (93 per cent) face shortages in the supply of sufficient feed for optimal goat production. This contradicts the common assumption that goats can survive exclusively on natural rangelands. However, goats may be able to forage more successfully than other large animals in wetter climates (Rust and Rust, 2013). It was concluded that goats continue to have an important role in harsh conditions, tropical, sub-tropical, desert and Mediterranean environments (Silanikove and Koluman, 2015).

Goats were considered as resilience than any other ruminants (Silanikove, 2000a). The nearing future water scarcity is considered to be the most vulnerable trauma all over the globe. This can lead to poor availability of fodder and feed resources for livestock. However, goats are ruminants with low body mass, and lower metabolic requirements, which therefore require minimum feed and water for their maintenance. This can make them survive in areas where water sources are widely distributed and food sources are limited by their quantity and quality (Silanikove and Koluman, 2015).

Goats have developed adaptive mechanisms that allow their survival at very high temperatures (45 to 50 °C) as well as cold temperatures (−20 to −40 °C). However, despite their extreme tolerance (Al-Tamimi, 2007), the productivity of these animals often declines due to thermal stress (Banergee *et al*., 2014). Although goats are considered as hardy animals that can survive in extreme weather conditions, they too had all type of stress responses as behavioural, physiological, blood biochemical, endocrine as well as cellular and molecular responses in order to cope up with the environmental stressors. Goat breeding as well as adaptation is gaining importance in changing climate scenario since it not only augment cash income, enhance food security, thus serving as an important component in household's livelihood strategies, but it also helps in empowerment of vulnerable groups such as women, HIV/AIDS (Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome), poor (Scholtz *et al.,* 2013).

2.6 Heat stress impact on goat adaptation

2.6.1 Behavioural adaptation

In tropical and sub-tropical regions, high AT were found to be the major limitation on animal production (Marai *et al*., 2007) and those animals which are exposed to the sun exhibited certain behavioural responses in their body posture and orientation, shelter-seeking, foraging pattern based on their thermoregulatory responses to the environment (Kadzere *et al*., 2002). According to Sejian *et al*. (2010a), the most significant behavioural changes observed during HS conditions were open-mouthed panting, decrease in feed intake, increased water intake, reduced movement, elevation in standing time, and reduced rumination activity. Alam *et al*. (2011) reported that goats that were subjected to HS showed an elevation in eating time. Increased intake of water is often observed in animals in order to compensate for the water lost through evaporation. The feeding behaviour also changes so as to ensure that the heat produced out of feed is lost through both conduction and radiation only during the cooler periods of the day (Al-Tamimi, 2007). Heat stressed goats depicts an elevation in standing and lying time. Alam *et al*. (2011) also reported that the number of urination and defecation were significantly decreased in heat treated goats. Reece (2015) reported that heat can be lost from an animal body to environment by means of evaporation through respiratory secretions, sweat or saliva. Loss of water through evaporation in sheep has been found to increase from 1L/24 hrs to 2.9L/24 hrs within the temperature ranging from 28ºC-38ºC. Therefore, number of urination is higher in non-exposure group than exposed groups (Alam *et al*., 2011). Some species of goats and cattle are able to reduce their internal Tb in the early morning as a preparatory mechanism to cope up with the increased amount of solar radiation they will be exposed during the warmer parts of the next day (Al-Tamimi, 2007). According to Marai *et al*. (2007), the first and foremost behavioural change exhibited by the animals exposed to HS were noticed as increased RR, body heat and salivation, seeking for shade, increased water intake, decreased feed intake and demanding to stand. It also been reported that under severe HS condition

animals will lick their body with their own saliva and nose secretions (Silanikove, 2000b). The frequency of urination as well as defecation also was found to be less in the heat stressed animals (Alam e*t al*., 2011).

2.6.2 Physiological adaptation

Several studies showed that the major parameters that are being used to elucidate the mechanism of physiological adaptation in small ruminants are RR, PR, RT and SR (Sevi *et al*., 2001; Srikandakumar *et al*., 2003; Maurya *et al*., 2004; Phulia *et al*., 2010, Sharma *et al*., 2013, Gupta *et al*., 2013). The studies conducted by Hooda and Upadhyay (2014) in goats and Sejian *et al*. (2014a) in sheep stated that the AT and THI values were more during the afternoon compared to the morning and thus the RT, RR and PR will peak during the afternoon hours (Habibu *et al*., 2014).

2.6.2.1 Respiration Rate

Increase in RR will be the first symptom exhibited by the animals when they are exposed out of their comfort zone (Maurya *et al*., 2007). Goats rely heavily on respiratory evaporative cooling mechanisms than cutaneous evaporation (Sivakumar *et al*., 2010). According to Alam *et al*. (2011), RR in goat was found to be increased in HS group compared to Control (C) group. Marai *et al*. (1997) stated that there was a further increase in the RR when a load of high RH was accompanied along with already existing high AT.

Sejian *et al*. (2010b) in their study done in Malpura ewes observed that RR did not differ significantly among the groups during morning. The study conducted by Indu *et al*. (2014a) in the same Malpura ewes reported that the RR was higher in the afternoon compared to morning. In goats Phulia *et al*. (2010) recorded aa increase in RR from 43.66 in morning to 77.33 in afternoon.

2.6.2.2 Pulse rate

According to Popoola *et al*. (2014), increase in AT increases the PR of animals. The increase in metabolism and muscle activity during the stress condition changes pulsation rates (Gupta *et al*., 2013). Alam *et al*. (2011) reported that panting and PR were increased in the HS group ($P<0.01$) compared to other groups. Further, Indu *et al*. (2014a) stated that PR recorded in afternoon was significantly higher in HS group as compared with C group during the afternoon. Sejian *et al.* (2010c) reported that the HS group showed significantly different PR in comparison with other groups both during morning and afternoon.

2.6.2.3 Rectal Temperature

Rectal temperature is another important indicator of thermal balance and might be used to evaluate the impact of HS (Darcan *et al*., 2008). According to Srikandakumar *et al*. (2003), the RT is indicated as one of the most significant indicator to identify the physiological status of an animal under stress. The increase in RT during HS is depicted by the fact that animals tries to accumulate its body heat (Sivakumar *et al*., 2010). Further, Marai *et al.* (2007) reported that the RT of goats was found to be increasing linearly with increase in environmental temperature. In the study conducted by Sejian *et al.* (2010c), it was proved that the RT of the C group did not differ with that of the HS group during morning hours, however during afternoon hours it increased significantly. The study conducted by Phulia *et al*. (2010) reported an increase in RT from 38.97°C to 39.35°C from morning to afternoon, when goats were subjected to HS for 6 hours. The RT was significantly ($P < 0.01$) high in animals exposed to heat as compared with the C group during afternoon (Indu *et al*., 2014a). However, Alam *et al*. (2011) reported that skin and RT did not differed significantly among the C and HS groups.

2.6.2.4 Blood biochemical changes

The elucidation of biochemical profiles is complex as various mechanisms control the blood level of various metabolites and to the large variation in levels of blood biochemical parameters with breed, age, physiological stage, diet, management of the animal, and the climate (Gomide *et al*., 2004). According to Sejian and Srivastava (2010), the mean plasma glucose level and the mean total plasma cholesterol increased significantly ($P \leq 0.05$) in goats after thermal exposure while the mean total plasma protein significantly ($P \leq 0$ .05) decreased. Al-Eissa *et al*. (2012) concluded in the study that the levels of glucose and total protein have higher concentration in the summer. Salama *et al*. (2013) reported that blood glucose levels did not change in goats that were subjected to HS. Ocak and Guey (2010) indicated that in a high-temperature environment, the levels of both blood glucose as well as blood cholesterol decreased in goats. According to Mundim *et al*. (2007), the decrease in cholesterol may be due to increased utilization of fatty acids for energy production as a consequence of the reduction of glucose in goats. Futher, Bahga *et al*. (2009) and Ocak and Guey (2010) reported that in goats blood glucose and total cholesterol level declines during summer while it increases during winter season in goats (Gupta *et al*., 2013). In addition, Indu*et al.* (2014b) reported that blood plasma glucose ($P < 0.01$) and total plasma cholesterol ($P < 0.05$) was lower in HS group.

Helal *et al.* (2010) in their study conducted in Balady goats exposed to HS, showed decreased total plasma protein, albumin and globulin, and this was purely in agreement with the results obtained by Sejian *et al*. (2010b) in Malpura ewes and the decrease in concentration was related to increased plasma volume during HS period. Indu *et al*. (2014b) reported that HS had no effect on total plasma protein, plasma albumin and plasma globulin. In contrast, goats that were exposed to sun for prolonged periods, depicted increased values of total protein, albumin and globulin (Helal *et al*., 2010). In addition, Okoruwa (2014) reported that the total protein, albumin and glucose were significantly highest in HS group

when compared to the C groups and they attributed this increase to favour hepatic gluconeogenesis to ensure regular energy supply to vital activities of body.

2.6.2.5 Endocrine responses

During stress conditions various endocrine responses are involved to recover the adaptive capability of the animals (Kannon *et al*., 2000). One such response is the activation of the HPA axis to induce the release of cortisol (Sivakumar *et al*., 2010). Blood hormonal profile, especially cortisol, Triiodothyronine (T_3), Thyroxine (T_4) and progesterone etc. are significantly influenced by the environmental stress along with the hematological and biochemical responses (Kour *et al*., 2014). Cortisol is a classic endocrine response to almost all types of stresses. According to Sivakumar *et al*. (2010), the estimation of concentrations of hormones, especially cortisol was considered as one of the important indicator for assessment of stress in animals. Indu *et al*. (2014b) reported that the plasma cortisol was significantly ($P < 0.01$) higher in HS group as compared with C group of sheep. Further, the results of Hooda and upadhyay (2014) depicted that the level of cortisol was found to be increased in heat stressed goats and this was in agreement with the findings of Sivakumar *et al*. (2010), and Sejian *et al*. (2010a). In addition, Sivakumar *et al*. (2010) reported that there was increase in plasma concentration of cortisol from 25.27 to 40.57 nmol/L in heat stressed goats and the cortisol level were significantly ($p<0.05$) higher when compared to the C groups.

Salem *et al*. (2011) reported that goats subjected to HS and water scarcity did not show any increase in plasma cortisol concentration. According to Mellado and Meza- Herrera (2002), the levels of cortisol was not altered significantly in Black Bengal (Kaushish *et al*., 1997) and in dairy goats (Olsson *et al*., 1995) subjected to extreme thermal stress (38–45 ºC, 55 per cent RH) in the climate chambers. Sivakumar *et al*., (2010) in his study reported that the level

of cortisol decreased in animals that were provided with antioxidant supplementation.

2.6.2.6 Cellular and molecular changes

Cellular responses were found to be an important mechanism by which animals are able to protect cells themselves from HS (Mishra and Palai, 2014). According to Gupta *et al*. (2013) thermal stress alters the gene expression, which is considered as the immediate cellular mechanism against the environmental stressors. Thermal stress generates a sequence of gene expressions and thereby produces biochemical adaptive mechanisms (Banergee *et al*., 2014). Heat stress alters the Tb which in turn effects the cellular function (Portner and Farrell, 2008; Indu *et al*., 2014b). Heat shock proteins are an evolutionary conserved family of proteins that are induced in both prokaryotes and eukaryotes which enables the cells to respond to elevated temperature or a variety of cellular stresses (Ross *et al*., 2003). Heat shock proteins are also considered to perform critical roles in environmental stress tolerance and adaptation mechanisms that the increase in HSP levels during the stress period plays a crucial role in cellular homeostatis (Banergee *et al*., 2014). According to Dangi *et al.* (2014), animals respond to HS in the cellular level by releasing HSPs which help to protect cells from heat induced damage. Expression of several HSPs including HSP32, HSP40, HSP60, HSP70, HSP90, HSP110 were found to be increasing during hyperthermic stress (Sharma *et al*., 2013). However, HSP70 is considered as the most abundant and temperature sensitive gene (Gade *et al.,* 2010). Xun *et al*. (2015) reported that HSPB9, HSPB10 were found to be upregulated and the expressions of HSP70 and HSP90 were also increased simultaneously due to HS in goats. Similar results have been reported in goat by Dangi *et al*. (2014) and Banergee *et al*. (2014).

2.7 Nutritional stress impact on goat adaptation

2.7.1 Behavioural adaptation

According to Tucker *et al.* (2009), the feed restricted cows exhibited behaviour like spending less time eating more time lying and vocalized more. These behavioural changes indicate that the level of feed restriction may cause hunger. Cândido *et al*., (2012) reported that the feed restricted cows of the breeds Guzerat and Sindhi spend less time on feeding and took more resting time compared to the *ad-libitum* fed animals. According to Sejian *et al*. (2010b), the study conducted in Malpura ewes subjected to NS did not exhibit any drastic change in their behaviour.

2.7.2 Physiological adaptation

According to Sejian *et al*. (2014a), the feed restricted study on sheep showed decrease in PR in the morning and also RR and PR in the afternoon. Similar finding was reported by Sejian *et al*. (2010a) in Malpura ewes and Samad *et al*. (2014) in goats where the NS group showed decrease in values of RR, PR, RT compared to the C group. The reduced feed intake causes reduction in thyroid activity which results in reduced RR (Sejian *et al*., 2014a). Reduced metabolic requirements and decreased oxygen consumption also resulted in decreased RR in goats (Hyder, 2012). However, they also reported that RT did not differ between the groups both during morning and afternoon. This could be due to the fact that the ruminants attempt to maintain internal temperature by means of certain behavioural and physiological mechanisms, when they are subjected under NS (Mohamed *et al*., 2010). Umesiobi *et al*. (2005) reported a decrease in RR in feed restricted WAD sheep while there were no significant changes in the RT in their entire study period.

2.7.3 Blood biochemical changes

Nutrition status of the animal reflects the level of blood cells and blood composition (Sejian *et al*., 2014b). Study conducted by Sejian *et al.,* (2014a) in

Malpura ewes, reported that the plasma glucose, total protein, albumin and total cholesterol decreased in NS group in comparison to the *ad libitum* fed group. Undernutrition leads to low levels of glucose circulating in the body of ewes, which was explained as one of the possible reason for the low level of glucose exhibited in the stress group. In order to combat the stress, glucose were synthesized by inducing hepatic gluconeogenesis which ultimately resulted in reduction of total plasma protein, while the total cholesterol reduced in blood due to restricted feed intake by the animals (Sejian *et al*., 2014a). These findings were purely in agreement with the reports of Sejian *et al*. (2010b) in Malpura ewes. Decrease in plasma protein was also reported in WAD sheep under feed restricted condition by Umesiobi *et al*. (2005). However, Rezapour and Taghinejad-Roudbane (2011) reported no significant differences for glucose, albumin and total protein among the groups on days 130 and 145 of pregnancy in Ghezel ewes which were subjected to NS, while there was decrease in the serum cholesterol concentration.

2.7.4 Endocrine changes

Nutritional state is closely associated with neuroendocrine and hormonal cues, and the connotation between different stresses and increased secretion of cortisol in small ruminants was well documented (Marai *et al*., 2007; Ali and Hayder, 2008). Sejian *et al*., (2014a) reported that the cortisol level decreased in the NS group in comparison with the *ad libitum* fed group. This was in agreement with their previous finding which proved that the level of cortisol declined in the nutrition deprived animals (Sejian *et al*., 2010b). They attributed this decreased production of cortisol in NS group to reduced blood cholesterol level. The decrease in cholesterol was reported due to the increased utilization of fatty acids for energy synthesis because of the lower levels of glucose concentrations in animals under restricted feeding (Sejian *et al*., 2014b). However, Hyder, (2012) reported increase in serum cortisol in feed restricted goat resulted in decreased leptin concentration, which in turn lead to increased cortisol, being a metabolic adaptation of undernutrition.

2.7.5 Cellular and molecular changes

HSPs are known to be highly conserved and ubiquitous proteins synthesized in response to several stimuli (Liu and Steinacker 2001; Kregel 2002a). According to Asea (2007) HSP inducers have been categorized as environmental, pathological and physiological. Caloric restriction, hypoglycaemia, or hyperlipidemia might also result in HSP gene expression in different parts of body (Eitam *et al*., 2009). Induction of HSP 70 in the cellular level was related with the development of tolerance to caloric stress (Yu and Chung 2001; Patel and Finch 2002; Kregel 2002b). It was reported that extended periods of low-energy diet also generated cell-specific HSPs where significant increase of HSP 90 was observed and unaltered levels of HSP 70 proteins were found in white blood cells of beef cows (Eitam *et al*., 2009).

2.8 Heat and nutritional stress markers for goat

Understanding the cellular dynamics behind the short and long term adaptation in the tropical food animals will be of use in developing mitigatory measures for improving the productivity. The rapid progress in understanding genomic tools now allows investigation of functional genomic regions with potential associations with adaptation. Improved tools in molecular analysis of gene expression upgrade the accuracy and strengthen the use of genetic markers. Understanding the molecular basis for the increased evaporative heat loss capability offers new opportunities for increasing thermal tolerance of animals especially in tropics (Collier *et al*., 2008). Sejian *et al*. (2010a) reported that the RR and RT have shown to be reliable HS markers in sheep. Maurya *et al*. (2004) in ewes also reported that RR and RT can be used as an effective indicator of environmental stress in ewes. Further, Sejian *et al*. (2011) reported RT as an indicator of walking stress in ewes. This proves that RT was one of the best indicators of any environmental stress in ewes. In addition, Daramola and Adeloye (2009) and Sejian *et al*. (2010c) reported that RR and RT have been shown to be good indicators of the stress condition in farm animals. McManus *et*

al. (2009) reported that Haemoglobin (Hb) and Packed Cell Volume (PCV) can be considered to be good blood biochemical markers during HS conditions in farm animals. Endocrine system gets activated during the stress conditions resulting in release of various hormones for relieving the stress impact on the organism. Kumar *et al.* (2012) and McEwen (2008) established cortisol to be the ideal endocrine marker in livestock. Further, according to Sejian *et al*. (2013b) plasma cortisol is considered as a good indicator of HS in Malpura ewes.

Given the complexity of the traits related to adaptation to tropical environments, the discovery of genes controlling these traits is a very difficult task. One obvious approach of identifying genes associated with acclimation to thermal stress is to utilize gene expression microarrays in models of thermal acclimation to identify changes in gene expression during acute and chronic thermal stress (Sejian *et al*., 2015). Another approach will be with single gene deletions exposed to a defined thermal environment. This permits the identification of those genes that are involved in key regulatory pathways for thermal resistance and thermal sensitivity. Finally, gene knockout models in single cells will also allow better delineation of the cellular metabolic machinery required to acclimate to thermal stress (Collier *et al*., 2012). Those genes identified as key to the process of thermal acclimation will then need to be mapped to their chromosomal location and the sequences of these genes will need to be determined in order to see if there are single nucleotide polymorphisms (SNPs) that are associated with changes in the coding for gene expression or protein function. Identification of SNP's that are associated with variation in animal resistance or sensitivity to thermal stress will permit screening of animal's presence or absence of desirable or undesirable alleles (Collier *et al*., 2008). However, further research is needed to quantify the genetic antagonism between adaptation and production traits to evaluate the potential selection response.

Studies evaluating genes identified as participating in the cellular acclimation response from microarray analyses or genome-wide association

studies have indicated that heat shock proteins are playing a major role in adaptation to thermal stress (Collier *et al*., 2012). In mammalian cells, non–lethal heat shock produces increased thermo tolerance through enhanced expression of heat shock genes. Additional genes of interest which two or more studies have identified are the genes for fibroblast growth factor, solute carrier proteins, interleukins and tick resistance genes. Genes which have only been identified by microarray analysis but not by genome-wide association studies include genes associated with cellular metabolism (phosphofructo kinase, isocitrate dehydrogenase, Nicotinamide adenine dinucleotide (NADH) dehydrogenase, glycosyltransferase, transcription factor and mitochondrial inositol protein). Other genes of importance were thyroid hormone receptor, insulin-like growth factor II and annexin (Collier *et al.,* 2008). Genes repressed in response to the environmental stress are mostly concerned with translation of genes for cytoplasmic ribosomal protein, Deoxyribonucleic Acid (DNA) polymerase I, II and III, transcription, t-RNA (transfer Ribonucleic Acid) synthetases, proteins required for processing ribosomal RNA (r-RNA) and a subset of translation initiation factors. The identification of the variety of CER genes involved in stress responses suggests that these responses are aimed at production of additional energy adenosine triphosphate (ATP), maintenance of environment as well as the repression of protein synthesis to ensure energy conservation and minimize unnecessary burden on the part of the cell (Sejian *et al*., 2015).

Heat shock proteins are group of protein family that can be used to assess the heat tolerance in animals (Lallawmkimi *et al*., 2013). According to Pawar *et al*. (2013), HSPs are used as potential markers to gauge the stress tolerance in farm animals. HSP ensures cytoprotection by the fact that its overexpression protects the cells from hyperthermia, circulatory shock, and cerebralischemia during HS (Lee *et al*., 2006). The most sensitive HSP being HSP 70 and it function as molecular chaperones in restoring cellular homeostasis and thereby promoting cell survival (Collier *et al.,* 2008). Xun *et al*. (2015) reported that the expressions of HSP 70 and HSP 90 were increased significantly in goats that

were subjected under HS. HSPs are the confirmatory biological marker for both HS and NS in livestock.

2.9 Concept of multiple stresses impacting livestock adaptation

Animals reared in tropical environments are generally subjected to more than one stress at a time. In the changing climatic scenario, the main stresses that affect the livestock adaptation are HS, NS and walking stress. And it is being a fact that none of these stresses occur in isolation, in real scenario all these stresses occurs simultaneously. These stresses severely affect the growth, production and adaptive capabilities of an animal. Climate change has both direct and indirect effect on the animals. The direct being HS and indirect effect being NS. During adverse environmental conditions, the animals cannot dissipate sufficient heat to maintain homeothermy, resulting in HS. Factors such as water deprivation, nutritional imbalance and nutritional deficiency may exacerbate the impact of HS (Silanikove, 2000a). The NS in animals occurs due to low pasture availability and water scarcity during summer season. The quantity as well as the quality of the pastures is hampered during the adverse environmental conditions resulting in aggravating the stress in animals. Further, the animals need to walk long distances for grazing in semi extensive or extensive production systems. In fact in the changing climatic scenario, the animals acquire only 30 per cent of the actual feed due to low pasture availability (Sejian *et al*., 2013a). Therefore the animals get induced to both walking stress, NS and HS when they go in search of feed under the scorching sun, especially in semi-arid tropics. The concept of the multiple stress originated from the fact that when an animal is subjected to only one stress at a time, it can counteract by efficiently using their stored body reserves, without altering any of the functions particularly the productive functions. However, if they are exposed to more than one stress simultaneously, the cumulative effects may alter all the functions of the animals. In this case, the animal's body reserves are not sufficient to cope up with the multiple stressors. As a result the animals are not able to maintain homeothermy and thus resulting in detrimental effects to their health, growth, reproductive and adaptive

capabilities. Moberg (2000) hypothesized that when animals are exposed to only one stress, they may not require the diversion of biological resources needed for other functions. However, if they are subjected to two stressors simultaneously, the total cost may have a severe impact on other biological functions. Thus, normal basal functions are drastically affected which jeopardizes production.

Sejian *et al*. (2013a) reported that the RR was found to be higher in the multiple stress group in comparison to the C group. Increase in the RR may be due to the efforts by the animals in order to maintain or re-establish the thermal balance (Al-Haidary, 2004; Kumar *et al*., 2011b). However, it was noticed that the RR was much lower in multiple stress group when compared with the Combined Stress (CS) group (HS and NS together) as well as single stress (Sejian *et al*., 2010b). This difference could be attributed to the additional walking stress in multiple stress group which leads to severe energy crisis.

It was observed that the PR in multiple stress group were much less in comparison to the C group both during morning and afternoon. The correlation between HR and metabolic heat production was found to be reason for low PR (Al-Haidary, 2004; Marai *et al*., 2007). Study conducted by Sejian *et al*., (2013a) reported that there was significant increase in RT during afternoon and this was because of the impaired or inefficient thermolytic activities under multiple stresses. Sejian *et al*., (2013a) reported that there was a significant increase in plasma cortisol in multiple stress group compared to normal range as described by Kramer (2000). This shows that the animals were under stress and tried to cope to this situation by increasing the cortisol production. However, the cortisol concentration obtained in this study was much lower than the CS group in sheep (Sejian *et al*., 2010c). This reduced cortisol concentration in multiple stresses group as compared to CS group could be to adjust the cortisol level to minimum possible increase to elicit the stress relieving effects as cortisol is thermogenic in nature which could contribute to additional heat load (Sejian *et al*., 2010c).

2.10 Concluding remarks

In the changing climatic scenario, HS and NS do not occur in isolation, and so concerted efforts are needed to provide the base line information related to establishing impact and adaptive mechanisms of goat to HS and NS simultaneously. Identification of specific biological stress markers will pave way for development of goat breed that are genetically adapted to both HS and climate derived nutritional deprivation.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

The experiment was carried out at the National Institute of Animal Nutrition and Physiology experimental livestock farm, Bengaluru, India which is located in southern Deccan plateau of the country at longitude 77º 38´E and the latitude of 12º 58´N and at altitude of 920 m above mean sea level. The average annual maximum and minimum ambient temperature ranges between 15 to 36ºC. The mean annual RH ranges between 20 and 85 per cent. The annual rainfall in this area ranges from 200 to 970 mm with an erratic distribution throughout the year. The average annual minimum and maximum temperature ranges between 15-22 and 27-34 ºC respectively. The average annual RH ranges between 40-85 per cent. The experiment was carried out during April-May. The temperature and RH variations during the study period (April-May) ranged between 24-38 and 30-38 per cent respectively under hot semi-arid environment. The average meteorological data for the entire study period both inside the shed as well as outside are given in table 3 and table 4 respectively. The THI values were calculated as per method described by McDowell (1972). Accordingly the formula used was THI = $0.72(T_{db} + T_{wb}) + 40.6$ where, T_{db} = Dry bulb temperature in ºC; T_{wb} = Wet bulb temperature in ºC. The THI values 72 and less are considered comfortable; THI values between75-78 are considered stressful and THI above 78 considered extreme distress.

3.2 Animals

Osmanabadi is a dual purpose (meat and milk) hardy goat breed, which originated in the semi-arid areas of central tropical India. The Osmanabadi breed derives its name from its habitat and distributed in Ahmednagar, Solapur and Osmanabad districts in Maharashtra (Motghare *et al.,* 2005; Deokar *et al.,* 2006). It has spread over a wide range of agro-climatic conditions in Maharashtra and

adjoining parts of Karnataka and Andhra Pradesh. The goats are large in size. Coat color varies, but mostly it is black (73 per cent) and the rest are white, brown or spotted. The average body weights of adult male and female animals are 34 kg and 30 kg respectively. The breed is considered useful both for meat and milk. Average daily yield varies from 0.5 to 1.5 kg for a lactation length of about 4 months. In favourable conditions the does will breed regularly twice a year and twinning is common in this breed.

The study was conducted in 24 (one year old) Osmanabadi bucks weighing between 15 to 20 kg. The animals were housed in well-ventilated sheds made up of asbestos roofing at the height 2.4 m and open from side and maintained under proper hygienic conditions. Prophylactic measures against goat diseases like goat pox, peste des petits ruminants, enterotoxaemia, endo and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

3.3 Technical program

The study was conducted for a period of 45 days. Twenty four adult bucks were used in the study. The bucks were randomly allocated into four groups of six animals each viz., C(n=6; Control), HS(n=6; Heat stress), NS(n=6; Nutritional) and CS(n=6; Combined stress). The animals were stall fed with a diet consisting of 60 per cent roughage (hybrid napier) and 40 per cent concentrate (maize 36 kg, wheat bran 37 kg, soya bean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5 kg/100kg of feed) as described in table 1. Control and NS bucks were maintained in the shed in thermo-neutral condition while HS and CS bucks were exposed outside to summer heat stress between 10:00 h to 16:00 h to expose them to heat stress. Control and HS bucks were provided with *ad libitum* feeding while NS and CS bucks were provided with restricted feed (30 per cent of intake of C and HS bucks) to induce nutritional stress. All four group animals were fed and watered individually throughout the

study period. All cardinal weather parameters were recorded both inside and outside the shed. Individual animal water intake was recorded on daily basis and the average was taken at fortnightly interval.Behavioural responses were recorded at fortnightly interval. Physiological responses were recorded twice daily (8:00 h and 14:00 h) at fortnightly interval. Blood samples were collected at fortnightly interval. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to both heat and nutritional stresses and to slaughter the animals for organ collection for gene expression and histopathological study. The entire technical program is represented through a flow chart depicted in Fig. 2.

Table 1: Ingredients and chemical composition of concentrate mixture and hybrid napier hay fed to goats

Attribute	**Concentrate mixture (kg/100 kg)**	**Napier hay** (*Pennisetum purpureum*)
Ingredients		
Maize	36	-
Wheat bran	37	-
Soybean meal	25	-
Mineral mixture	1.5	-
Salt	0.5	-
Chemical composition (per cent)		
Dry matter	92.9±0.079	94.0±0.289
Organic matter	95.9±0.190	95.4±0.298
Crude protein	19.6±0.176	6.21±0.098
Ether extract	1.82±0.183	1.49±0.026
Total ash	4.10±0.190	4.64±0.298
Fibre fractions (per cent)		
Neutral detergent fibre	40.4±1.400	82. 9±0.881
Acid detergent fibre	11.1±0.239	64.6±1.950
Acid detergent lignin	2.14±0.029	12.3±0.651
Nutritive value		
Total digestible nutrients per cent*	72.2	55.0
Digestible energy (kJ/kg) *	13.3	10.1
Metabolizable energy(kJ/kg) *	10.9	8.28

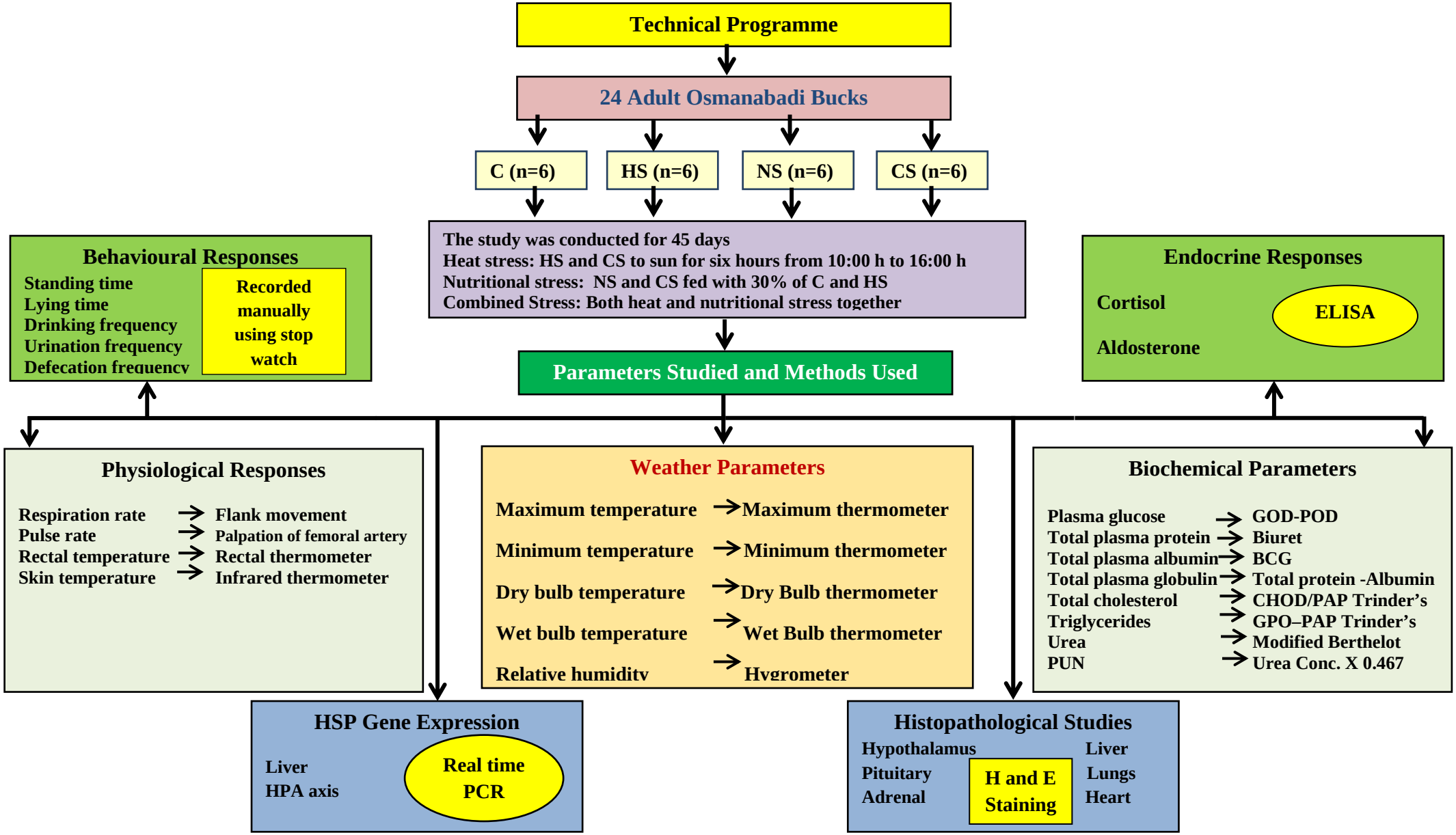


Fig. 2: Represents overall technical programme for the entire study. BCG-Bromocresol Green; C-Control; CHOD/PAP-Cholesterol oxidase/ Phenol + Aminophenazone; Conc.-Concentration;CS-Combined Stresses; ELISA- Enzyme Linked Immuno Sorbent Assay; GOD-POD-Glucose oxidase-Peroxidase; GPO-PAP- Glycerol phosphate oxidase- Phenol + Aminophenazone; h-Hour; H and E-Haemotoxylin and Eosin; HPA- Hypothalamo-Pituitary Adrenal;HS-Heat Stress; HSP-Heat Shock Protein; n- Number; NS-Nutritional Stress; PCR- Polymerase Chain Reaction;PUN- Plasma Urea Nitrogen.

3.4 Weather parameters recording

Weather parameters were recorded twice daily (8:00 h and 14:00 h) for the entire study period. Maximum temperature, minimum temperature, dry bulb temperature, wet bulb temperature were recorded using maximum thermometer, minimum thermometer, dry and wet bulb thermometers respectively. The RH was recorded by hygrometer.

3.5 Behavioural responses recording

Behavioural responses like standing time (min), lying time (min),drinking frequency (no. of times), defecation frequency (no. of times), and urination frequency (no. of times)were closely observed and recorded for all the four groups for 6 hours (10.00AM – 4.00PM), at fortnightly interval.

3.6 Water intake measuring

Water was measured and fed to the animals and the residue was recorded in every 24 hours interval and thus the water intake was calculated for the entire study period.

3.7 Physiological responses recording

3.7.1 Respiration Rate

The RR was recorded by counting flank movements/min with the help of a stop watch, from a distance of 4–5 m without disturbing the goats. The unit of measurement of RR was in breaths/min.

3.7.2 Pulse Rate

The PR was measured by palpating the femoral artery. For recording the pulse rate, goats were restrained gently. The unit of measurement of PR was in beats/min.

3.7.3 Rectal Temperature

RT was recorded using a clinical thermometer by inserting the thermometer by 6–7 cm inside the rectum inclined towards wall of the rectum. RT was recorded by gently restraining the goats. The unit of measurement of RT was in °C.

3.7.4 Skin Temperature

Skin temperature of an animal varies based on the quantum of sun rays to which the different body parts are exposed. Generally, in male animals the skin temperature is recorded on the head, scrotum and flank region. The skin temperatures were recorded using a non-contact infrared thermometer (B.S.K. Technologies, Hyderabad, India) by maintaining a distance of 5 to 15 cm. Aim the region where the temperature has to be taken, press the button of the device, the temperature is displayed immediately on the screen of the device. The unit of measurement of skin temperature was in °C.

3.8 Blood collection

Five ml of blood samples were collected at fortnightly interval from all the four groups simultaneously at 11:00 h using 20 gauge sterilized needles and plastic syringe from external jugular vein in tubes with heparin anticoagulant (Sisco Research Laboratories Pvt. Ltd, Bombay, India) @ 20 IU per ml of blood.

3.9 Plasma separation

Plasma was separated from blood by centrifugation at 3500 revolutions per minute (rpm) at room temperature for 20 minutes. The straw colored supernatant plasma was aspirated with the help of a sterile pasteur pipette and kept in sterilized vials. The plasma was preserved at -20° C until the estimation of various blood parameters.

Plate 1: Respiration rate recording

Plate 2: Pulse rate recording

Plate 3: Rectal temperature recording

Plate 4: Recording of skin temperature head

Plate 5: Recording of skin temperature flank

Plate 6: Recording of skin temperature scrotum

Plate 7: Blood Collection

3.10 Estimation of biochemical parameters

The biochemical parameters studied were plasma glucose, plasma total protein, plasma albumin, plasma globulin, plasma total cholesterol, plasma triglycerides, plasma urea and Plasma Urea Nitrogen (PUN).

3.10.1 Plasma Glucose

Plasma glucose was estimated by Glucose oxidase-Peroxidase (GOD-POD) method using microplate reader (Thermo Scientific Multiskan GO, Finland). The unit of measurement of glucose was mg/dL. Plasma glucose was estimated using kit method (Autospan, Gujarat, India).

Assay Principle:

GOD oxidises glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidases, released hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to form coloured quinoneimine dye. Absorbance of colored dye is measured at 505 nanometre (nm) and is directly proportional to glucose concentration in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

Where, O_2, H_2O, H_2O_2 represents oxygen, water and hydrogen peroxides respectively.

Reagents Composition:

Reagent No.	**Reagent**	**Composition**
1	Glucose reagent	Phosphate buffer, glucose oxidase, peroxidase, 4-AAP,stabilisers
2	Glucose diluent	Phenol preservative
3	Glucose standard	Dextrose preservative
4	Glucose standard	Dextrose preservative

Working Glucose Reagent Preparation:

Working glucose reagent was prepared by adding glucose diluent (100 ml) to glucose reagent (1 vial)**.**

Procedure:

Pipette into tube marked	**Blank (B)**	**Standard (S)**	**Test (T)**
Serum/Plasma	-	-	10µL
Glucose Standard	-	10µL	-
Working glucose reagent	1000µL	1000µL	1000µL

After mixing, incubation was done at 37ºC for 10 min or at room temperature (15-30ºC) for 30 min. The analyser was programmed as per assay parameters.

1. The analyser was made blank with reagent blank.
2. Absorbance of S as well as T was measured at 505 nm.

Calculation:

$$\text{Serum/Plasma glucose (mg/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 100$$

3.10.2 Total Plasma Protein

Total plasma protein was estimated by biuret method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement was g/dL. Total plasma Protein was estimated using kit method (Beacon Diagnostics Pvt. Ltd., Gujarat, India).

Assay Principle:

Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet colored complex. The intensity of the color formed is directly proportional to the amount of proteins present in the sample.

$$\text{Total Protein} + Cu^{++} \longrightarrow \text{Violet Complex}$$

Where, Cu^{++} represents copper (II) ion.

Procedure:

B, S and T were pipetted into the respective labeled test tubes as follows:

Pipette into tube marked	**Blank**	**Standard**	**Test**
Biuret Reagent	1000µL	1000µL	1000µL
Standard	-	10µL	-
Serum/Plasma			10µL

After mixing well, incubation was done for 5 min at room temperature. The absorbance of the S and T were measured against the reagent B, within 60 min.

The analyser was programmed as per assay parameters.

Calculation:

$$\text{Total Protein concentration (g/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 6$$

3.10.3 Plasma Albumin

Plasma albumin was estimated by bromocresol green (BCG) method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement was g/dL. Total plasma albumin was estimated using kit method (Proton Biologicals India Pvt. Ltd., Bangalore, India).

Assay Principle:

Albumin binds with BCG in a buffered medium to produce a green coloured complex. The absorbance of final colour is measured at 630 nm. The intensity of this colour is proportional to Albumin concentration in the sample.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 3.68}} \text{Green coloured complex}$$

Procedure:

B, S, T were pipetted into the respective labeled test tubes as follows:

Pipette into tube marked	**Blank**	**Standard**	**Test**
BCG Reagent	1000µL	1000µL	1000µL
Albumin Standard	-	10µL	-
Serum/Plasma	-	-	10µL

After mixing, absorbance of S and T were read against B at 578nm.

Calculation:

$$\text{Albumin (g/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 4$$

3.10.4 Plasma Globulin

Plasma globulin was calculated by subtracting the concentration of albumin from the total protein concentration. The unit of measurement was g/dL.

Plasma Globulin (g/dL) = Total Plasma Protein – Plasma Albumin

3.10.5 Total Plasma Cholesterol

Total plasma cholesterol was estimated by Cholesterol oxidase/Phenol + Aminophenazone(CHOD/PAP) trinder's Method using microplate reader (Thermo Scientific Multiskan GO, Finland). The unit of measurement of total plasma cholesterol was mg/dL. Total plasma cholesterol was estimated using kit method (Proton Biologicals India Pvt. Ltd., Bangalore, India).

Assay Principle:

Cholesterol esters are hydrolysed by Cholesterol Esterase (CHE) to give free cholesterol and fatty acids. In subsequent reaction, CHOD oxidises the 3-OH group of free cholesterol to liberate Cholest-4-en-3-one and H_2O_2. In presence of POD, Hydrogen Peroxide couples with 4-Aminoantipyrine (4-AAP) and phenol to produce Red Quinoneimine dye. Absorbance of coloured dye is measured at 505nm and is proportional to amount of total cholesterol concentration in the sample.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Free Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red Quinoneimine Complex} + H_2O$$

Procedure:

Pipette into tube marked	Blank	Standard	Test
Cholesterol Reagent	1000µL	1000µL	1000µL
Cholesterol Standard	-	10µL	-
Specimen	-	-	10µL

After mixing, incubation was done for 5 min at 37°C. Absorbance of S, T were read against B at 505 nm.

Calculation:

$$\text{Total Cholesterol (mg/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 200$$

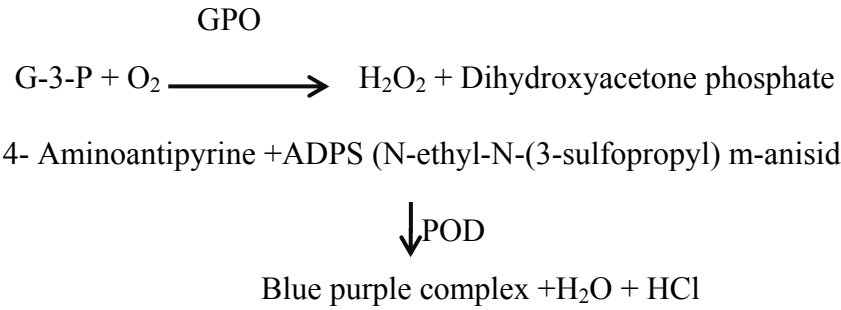
3.10.6 Plasma Triglycerides

Plasma triglyceride was estimated by Glycerol -3- Phosphate Oxidase - Phenol + Aminophenazone (GPO–PAP) trinder's method using microplate reader (Thermo Scientific Multiskan GO, Finland). The unit of measurement of triglycerides was mg/dL. Plasma triglycerides were estimated using kit method (Proton Biologicals India Pvt. Ltd., Bangalore, India).

Assay Principle:

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3- Phosphate (G-3-P)}$$



Where, LPL, ATP, GK, GPO and HCl represent Lipoprotein lipase, Adenosine Triphosphate, Glycerol Kinase, Glycerol -3- Phosphate Oxidase and hydrochloric acid respectively.

The triglycerides present in the Plasma/ Serum are catabolized into glycerol and free fatty acids by lipoprotein lipase. Liberated glycerol is converted to glycerol-3-phosphate in presence of glycerol kinase and ATP. Glycerol 3 phosphate is acted upon by glycerol-3-phosphate oxidase to form Hydrogen Peroxide. This together with phenolic compound ADPS and 4-Aminoantipyrine in presence of peroxidase gives the blue purple colour complex. The intensity of the colour is measured at 546 nm (540-546 nm) and is proportional to the triglycerides concentration in serum samples.

Procedure:

B, S and T were pipetted into the respective labeled test tubes as follows:

Pipette into tube marked	**Blank**	**Standard**	**Test**
BCG Reagent	1000µL	1000µL	1000µL
Triglycerides Standard	-	10µL	-
Serum/ Plasma	-	-	10µL

After mixing well, incubation was done for 10 min at 37°C.

Absorbance of S and T were read against B at 546 nm.

Calculation:

$$\text{Triglycerides (mg/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 200$$

3.10.7 Plasma Urea

Plasma urea was estimated by modified Berthelot method using microplate reader (Thermo Scientific Multiskan GO, Finland). The unit of measurement of urea was mg/dL. Plasma urea was estimated using kit method (Proton Biologicals India Pvt. Ltd., Bangalore, India).

Assay Principle:

Urease catalyzes the conversion of urea to Ammonia and carbon dioxide. The ammonia released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield a blue-green colored compound (Indophenol). The intensity of colour produced is proportional to the concentration of urea in the plasma and is measured photometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

$$\text{Ammonia} + \text{Salicylate} + \text{Hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2-2- DicarboxyIndophenol}$$

Procedure:

Pipette into tube marked	**Blank**	**Standard**	**Test**
Working Reagent	1000µL	1000µL	1000µL
Urea Standard (Conc. 50 mg/dl)	-	10µL	-
Serum/Plasma	-	-	10µL
Mix and incubate for 5 minutes at 37ºC			
Alkaline Reagent	1.0 ml	1.0 ml	1.0 ml
Mix and incubate for 5 minutes at 37ºC			

After mixing, absorbance of S and T were read against B at 578 nm.

Calculation:

$$\text{Urea concentration (mg/dL)} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 50$$

3.10.8 Plasma Urea Nitrogen

Plasma urea nitrogen (PUN) concentration was calculated from plasma urea concentration by the following formula. The unit of measurement was mg/dL.

$$\text{PUN (mg/dL)} = \text{Urea concentration (mg/dL)} \times 0.467$$

3.11 Estimation of endocrine parameters

The endocrine parameters included in the study were plasma cortisol and plasma aldosterone.

3.11.1 Cortisol

Principle:

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme – labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microplate reader (Thermo Scientific Multiskan GO, Finland). The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. A set of standards is used to plot a standard curve from which the amount of cortisol in patient samples and controls can be directly read.

Assay Procedure:

Plasma cortisol (analytical sensitivity, 0.4 µg/dl; intra-assay and inter-assay coefficient of variations were 9.4 % and 8.1 % respectively) was estimated using Enzyme Linked Immuno Sorbent Assay (ELISA) method (LDN kit, Nordhorn, Germany). All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption. The procedure was done as per the manufacturer's protocol as follows:

1. Working solutions of the cortisol- Horse Radish Peroxidase (HRP) conjugate and wash buffer were prepared.
2. 20µL of each calibrator, control and specimen sample were pipetted into the correspondingly labeled wells in duplicate.
3. Then 100µL of the conjugate working solution was pipetted into each well.
4. Incubation was done on a plate shaker (approximate 200 rpm) for 45 minutes at room temperature.

5. Wells were washed 3 times with prepared wash buffer (300µL/well for each wash) and the plate was firmly tapped against absorbent paper to ensure that it is dry (by hand 6 times).
6. 150µL of Tetramethylbenzidine (TMB) substrate was pipetted into each well at timed intervals.
7. The plate was incubated on a plate shaker at room temperature for 15-20 minutes.(or until Calibrator A attains dark blue color for desired OD)
8. 50µL of stopping solution was pipetted into each well at the same time intervals as in step 7.
9. The plate was read on a microwell plate reader at 450nm within 20 minutes after the stopping solution being added.

Calculations:

- The mean OD of calibrator was measured.
- OD of unknown samples was read against calibrator curve.
- 4–parameter calibrator curve was drawn using immunoassay software and analysed for results.

3.11.2 Aldosterone

Principle:

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme – labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microplate reader (Thermo Scientific Multiskan GO, Finland). The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. A set of standards is used to plot a standard curve from which the amount of cortisol in patient samples and controls can be directly read.

Procedure:

Plasma aldosterone (analytical sensitivity, 14 pg/ml; intra-assay and inter-assay coefficient of variations were 7.5 % and 9.4 % respectively) was estimated using ELISA method (LDN kit, Nordhorn, Germany). All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption. The procedure was done as per the manufacturer's protocol as follows:

1. Working solution of the wash buffer was prepared.
2. 50µL of each standard, control and specimen sample were pipetted into correspondingly labeled wells in duplicate.
3. Then 100µL of the aldosterone-HRP conjugate working solution was pipetted into each well.
4. Incubation was done on a plate shaker (approximate 200 rpm) for 1 h at room temperature.
5. The wells were washed 3 times with prepared wash buffer (300µL/well for each wash) and the plate was tapped firmly against absorbent paper to ensure that it is dry.
6. 150µL of TMB substrate was pipetted into each well at timed intervals.
7. The plate was incubated on a plate shaker at room temperature for 15-20 minutes (or until Calibrator A attains dark blue color for desired OD).
8. 50µL of stopping solution was pipetted into each well at the same timed intervals as in step 7.
9. The plate was read on a microwell plate reader at 450 nm within 20 minutes after the stopping solution being added.

Calculations:

- The mean OD of calibrator was measured.
- OD of unknown samples was read against calibrator curve.

- 4–parameter calibrator curve was drawn using immunoassay software and analysed for results.

3.12 Histopathology parameters

Histopathological observations in different groups of animals subjected to different kinds of stress: The tissues were collected immediately after sacrifice from same site in all the animals from C, HS, NS and CS in buffered 10 per cent formalin. The fixed tissues were processed routinely to get Haematoxylin and Eosin (H&E) stained sections (Luna, 1968). The organs collected for the study includes hypothalamus, pituitary, liver, lungs, heart and adrenal gland. The slides were interpreted in comparison with C group and representative lesions were photographed.

3.13 Expression of hsp-70 in liver and adrenal gland

Principle:

Samples are lysed and homogenized in lysis buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting Ribonucleic Acid (RNA) from endogeneous RNases. The lysate is then mixed with ethanol and loaded on a purification column. The chaotropic salt and ethanol cause RNA to bind to the silica membrane while the lysate is spun through the column. Subsequently, impurities are effectively removed from the membrane by washing the column with wash buffers. Pure RNA is then eluted under low ionic strength conditions with nuclease-free water.

Procedure:

3.13.1 Sample collection and storage

The adrenal and liver samples were collected from all the animals in each group immediately after slaughter. The samples were cut into small pieces, washed in Phosphate Buffered Saline (PBS) and immersed in RNA shield (Zymo Research, USA). All the samples were stored at -80°C till further use.

3.13.2 Sample preparation for RNA isolation

After thawing, the tissues were removed from RNA shield (Zymo Research, USA) and immediately processed for RNA isolation. The total RNA was isolated from tissues using GeneJET RNA Purification Kit (Thermo Scientific, Lithuania) and the procedure was done as per manufacturer's protocol with slight modifications as follows:

About 30 mg of tissue was homogenized by grinding in Liquid Nitrogen (LN_2) (-196°C) in RNAase ZAP (Ambion, USA) treated mortar and pestle. After homogenization, 300 µL of lysis buffer supplemented with β-mercaptoethanol (10 µL/ml) was added and the content was transferred to 1.5 ml microcentrifuge tube. The lysate was vortexed for 10 seconds. To the lysate, 10 µL of proteinase K in 590 µL of Tris Ethylenediaminetetraacetic Acid (TE) buffer was added, then vortexed and incubated at 15-25°C for 10 min. Then, the contents were centrifuged for 8 min at 12000 gravity (g) and the supernatant was transferred into a new RNase-free micro centrifuge tube. 450 µL of ethanol was added and mixed well by pipette. Then 700 µL of was transferred to a spin column with a 2 ml collection tube and centrifuged for 1 min at 12000 g. After discarding the flow through, 700 µL of wash buffer 1 was added and centrifuged for 1 min at 12000 g followed by two time washing with 600 µL and 250 µL of wash buffer 2 followed by centrifugation at 12000 g for 1 min and 2 min respectively. About 40 µL of warm nuclease free water was added to the membrane, and centrifuged at 10000 g for 1 min to elute RNA. The purified RNA samples were stored at -80°C until complementary DNA (cDNA) synthesis.

3.13.3 DNase treatment

Total RNA isolated from different tissues was treated with DNase (TURBO DNA-free, Ambion, USA) in order to eliminate the genomic DNA contamination in total RNA. During and after DNase treatment, 1µL of RNase inhibitor (20U/µL, Invitrogen, USA) was added. After DNase treatment quality and quantity of the isolated RNA was analyzed using Spectrophotometer (ND-1000, Thermo Scientific, USA).

3.13.4 cDNA Synthesis

The total RNA was reverse transcribed into cDNA using Maxima first strand cDNA synthesis kit for Real Time quantitative polymerase chain reaction (RT-qPCR) (Thermo Scientific, Lithuania). The procedure was performed as per manufacturer's protocol with modifications are as follows:

4µL of 5X Reaction Mix, 2 µL Maxima Enzyme Mix, 1µg of Template RNA for adrenal samples, while 1.5 µg of Template RNA was used for liver sample and 20 µL of nuclease free water were added into a sterile, RNAase-free tube. Then the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 minute at 25 ° C, followed by 20 minute at 50 ° C and the reaction was terminated by heating at 85 ° C for 5 minute). The product of the first strand cDNA synthesis was diluted to a final concentration of 25ng/µL with nuclease free water and 2 µL of diluted cDNA was used for each reaction in qPCR.

3.13.5 Primer design and synthesis

Gene specific primers were designed using online NCBI primer design software (Primer 3, http://bioinfo.ut.ee/primer3/) and specificity was checked using Primer3 and BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and sequence is depicted in table 2 given below. The preferences were given to the primers binding to the exon-exon junction. The primers were titrated with different concentrations (10, 5, 2.5 and 1 µM) for selecting optimum concentration to be used for qPCR experiments.

3.13.6 Quantitative RT-PCR analysis

The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20 µL reaction was carried out in duplicates using 50 ng of template and 0.5 µM primer concentrations. The real time qPCR reaction conditions were: enzyme activation at 95^0C for 10 min and amplification cycle (40 cycles; initial denaturation at 95^0C for 15 sec, annealing at 60°C for 30sec and extension at

72^0C for 30 sec). The melt curve analysis was performed to check the non-specific amplification. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control and the relative expression was analyzed using the formula, $2^{\Delta\Delta CT}$ (Tarif *et al*., 2012). The results were expressed in fold change as compared to untreated control (control=1fold).

Table 2: Primers used for HSP70 expression. GAPDH used as reference gene to normalize the gene expression of target genes.

Gene ID	Primers	Primer sequence (5”- 3”)	Primer Length (bp)	Product Size (bp)	Accession No.
HSP70	F	TGGCTTTCACCGATACCGAG	20	167	NM_001285703.1
	R	GTCGTTGATCACGCGGAAAG	20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG	20		

bp- base pair; HSP70 -Heat Shock Protein 70 ; F- Forward; R-Reverse; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase

3.14 Statistical analysis

The data was analysed by general linear model (GLM) repeated measurement analysis of variance (SPSS16.0). Effect of fixed factors namely group C, HS, NS and CS was taken as between subject factor and days (longitudinal time over which experiment was carried out; Day 0,Day 15, Day 30 and Day 45) were taken as within subject factor and also interaction between group and days was analysed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan’s multiple range tests as described by Kramer (1957).

RESULTS

CHAPTER 4

RESULTS

4.1 Weather parameters

The maximum, minimum, wet and dry bulb temperatures and RH were recorded and the THI was calculated on an average of fortnightly interval. The obtained THI inside during both morning and afternoon are described in table 3. The THI values inside the shed show that the animals were in comfort zone both during morning and afternoon. The THI outside the shed during both morning and afternoon are described in table 4. The THI values outside the shed shows that during the morning hours the animals were in comfort zone while during afternoon the animals were under extreme distress.

Table 3: Mean and SEM of climatological data during the experimental period inside the shed

Time of Recording	Minimum temperature (º C)	Maximum temperature (º C)	Dry bulb temperature (º C)	Wet bulb temperature (º C)	RH (%)	THI
Morning	21.50	32.37	23.80	20.10	78.67	72.21
(8:00 hrs)	±0.60	±0.20	±0.21	±0.17	±4.67	±0.25
Afternoon	24.4	35.60	26.47	20.93	45.67	74.73
(14:00 hrs)	±0.56	±0.72	±0.28	±0.09	±7.21	±0.22

SEM-Standard Error Mean; RH- Relative Humidity; THI- Temperature Humidity Index

Table 4: Mean and SEM of Climatological data during the experimental period in the outside environment

Time of Recording	Minimum temperature (º C)	Maximum temperature (º C)	Dry bulb temperature (º C)	Wet bulb temperature (º C)	RH (%)	THI
Morning	26.93	34.03	23.13	22.97	61.00	73.92
(8:00 hrs)	±2.87	±0.52	±0.20	±0.66	±7.77	±0.57
Afternoon	27.23	38.33	29.57	26.53	37.00	80.99
(14:00 hrs)	±3.46	±0.52	±0.38	±0.71	±4.16	±0.25

SEM-Standard Error Mean; RH- Relative Humidity; THI- Temperature Humidity Index

4.2 Behavioral responses in bucks

The effects of HS, NS and CS on behavioural responses are described in table 5. Standing time showed significant (P<0.01) variation between the groups for treatments. The standing time did not differ significantly between *ad libitum* fed groups (C and HS) and between restricted feeding groups (NS and CS). Further, the experimental days significantly (P<0.01) influenced standing time in the study. However, interaction between groups and experimental days did not influence significantly the standing time in the bucks. Lying time showed significant (P<0.01) variation between the groups for treatments. The highest lying time was recorded in CS group while the lowest in C and HS groups. Further, the experimental days significantly (P<0.01) influenced lying time in the study. However, interaction between groups and experimental days did not influence significantly the lying time in the bucks. Ruminating time showed significant (P<0.01) variation between the groups for treatments. The highest ruminating time was recorded in HS group while the lowest in CS group. However, both experimental days and interaction between groups and experimental days did not influence significantly the ruminating time in the

bucks. Drinking frequency showed significant ($P<0.01$) variation between the groups for treatments. The highest drinking frequency was recorded in CS group while the lowest in NS group. However, experimental days did not influence significantly the drinking frequency in the bucks. But interaction between groups and experimental days significantly ($P<0.05$) influenced drinking frequency in the bucks. Defecating frequency showed significant ($P<0.01$) variation between the groups for treatments. The highest defecating frequency was recorded in both C group while the lowest in CS group. Further, the experimental days significantly ($P<0.05$) influenced defecating frequency in the study. However, interaction between groups and experimental days did not influence significantly the defecating frequency in the bucks. Urinating frequency did not differ between the groups for the treatments. However, the experimental days significantly ($P<0.01$) influenced urinating frequency of the bucks in the study. Further, interaction between groups and experimental days also did not influence significantly the urinating frequency of the bucks.

Table 5: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the behavioral responses in goats

FACTOR	Standing Time (min)	Lying Time (min)	Drinking Frequency (no. of times)	Defecating Frequency (no. of times)	Urination Frequency (no. of times)
GROUP	**	**	**	**	ns
C	235.50^{a}±13.54	124.50^{b}±13.54	2.46^{bc}±0.22	03.13^{a}±0.21	03.04^{a}±0.32
HS	254.21^{a}±15.06	105.79^{b}±15.06	3.08^{ab}±0.32	2.00^{bc}±0.30	1.96^{ab}±0.23
NS	198.04^{ab}±13.34	161.96^{ab}±13.3	01.63^{c}±0.19	2.63^{ab}±0.17	2.25^{ab}±0.17
CS	145.33^{b}±13.10	214.67^{a}±13.10	04.00^{a}±0.43	1.25^{c}±0.26	01.58^{b}±0.25
DAY	**	**	ns	*	**
0	167.71±13.77	192.29±13.77	2.83±0.20	2.83±0.22	2.75±0.18
15	227.83±12.81	132.17±12.81	3.04±0.35	2.00±0.25	2.17±0.28
30	212.04±12.30	147.96±12.30	3.17±0.27	2.00±0.18	1.92±0.27
45	225.50±12.99	134.50±12.99	2.13±0.32	2.17±0.23	2.00±0.17
GROUP*DAY	ns	ns	*	ns	ns
C0	177.50±27.53	182.5±27.53	2.83±0.40	3.17±0.43	2.83±0.37
C15	265.00±25.61	95.00±25.61	3.00±0.71	3.33±0.51	3.33±0.56
C30	241.50±24.61	118.5±24.61	2.83±0.54	3.50±0.35	3.83±0.54
C45	258.00±25.98	102.0±25.98	1.17±0.64	2.50±0.46	2.17±0.35
HS0	184.17±27.53	175.8±27.53	3.00±0.40	2.67±0.43	2.67±0.37
HS15	271.50±25.61	88.50±25.61	3.00±0.71	1.00±0.51	1.33±0.56
HS30	274.00±24.61	86.00±24.61	3.17±0.54	1.67±0.35	1.50±0.54
HS45	287.17±25.98	72.83±25.98	3.17±0.64	2.67±0.46	2.33±0.35
NS0	161.67±27.53	198.3±27.53	2.67±0.40	2.83±0.43	2.67±0.37
NS15	239.67±25.61	120.3±25.61	1.33±0.71	2.67±0.51	2.50±0.56
NS30	206.50±24.61	153.5±24.61	1.83±0.54	2.67±0.35	1.67±0.54
NS45	184.33±25.98	175.7±25.98	0.67±0.64	2.33±0.46	2.17±0.35
CS0	147.50±27.53	212.50±27.5	2.83±0.40	2.67±0.43	2.83±0.37
CS15	135.17±25.61	224.8±25.61	4.83±0.71	1.00±0.51	1.50±0.56
CS30	126.17±24.61	233.8±24.61	4.83±0.54	0.17±0.35	0.67±0.54
CS45	172.50±25.98	187.5±25.98	3.50±0.64	1.17±0.46	1.33±0.35

C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other

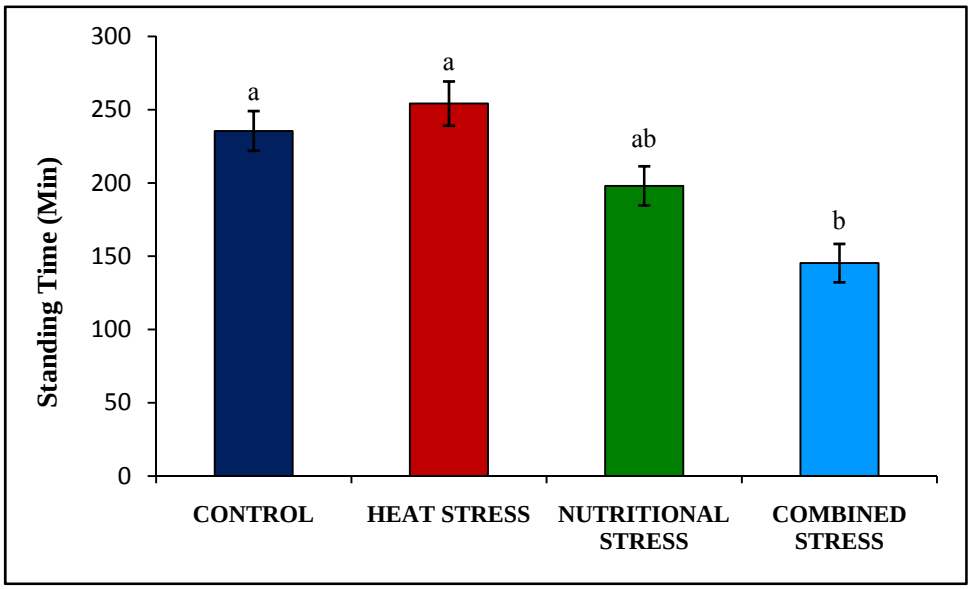


Fig. 3 (a) Standing time

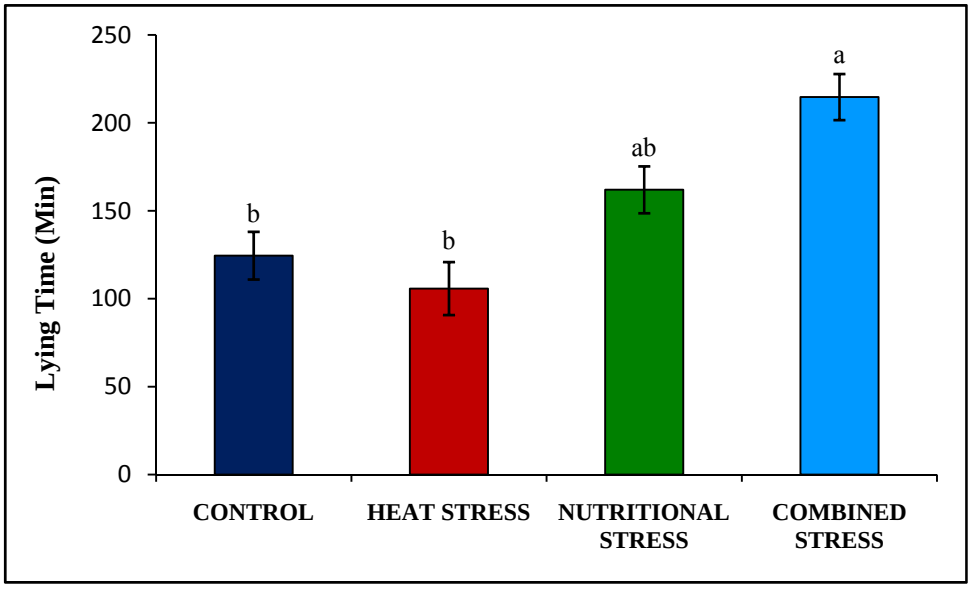


Fig. 3 (b) Lying time

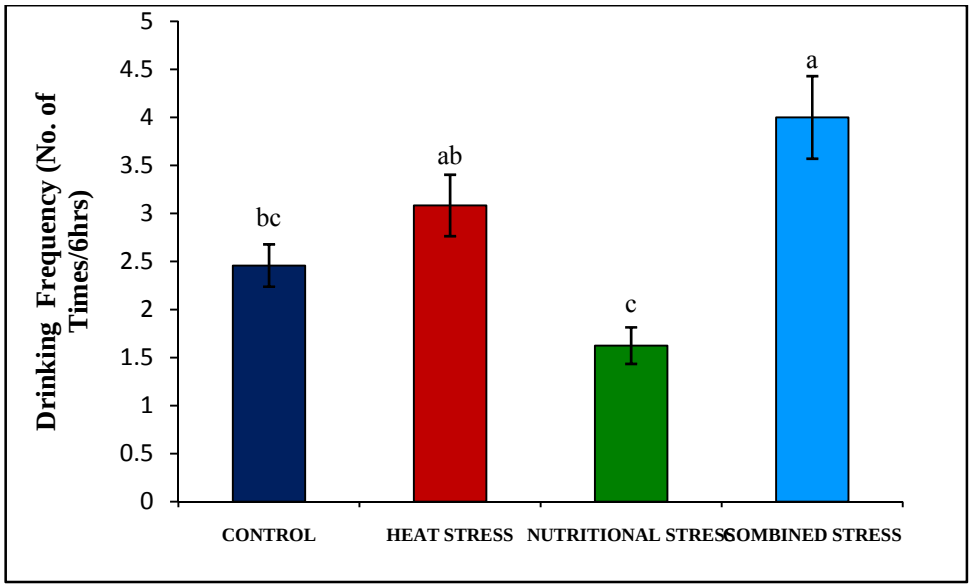


Fig. 3 (c) Drinking frequency

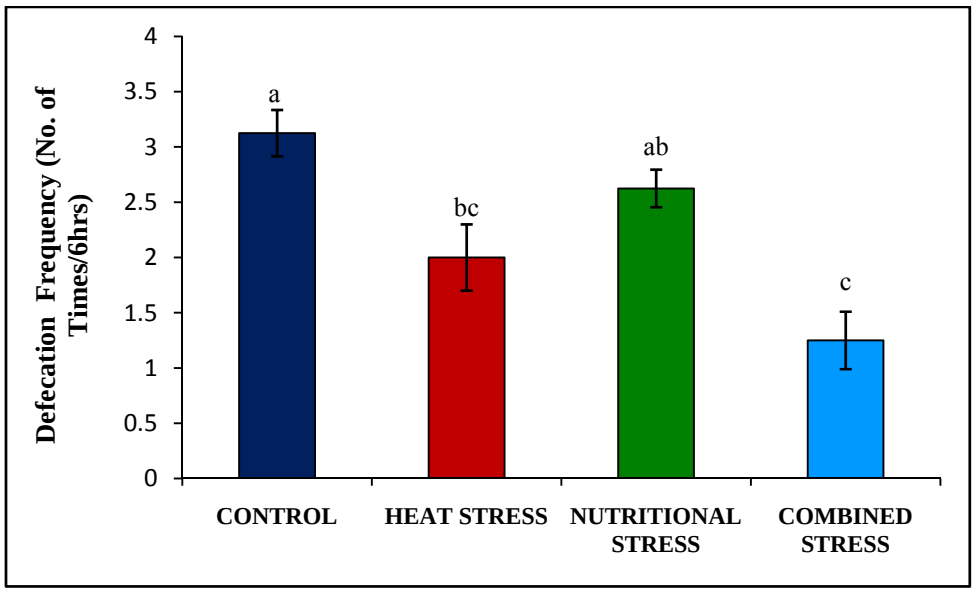


Fig. 3 (d) Defecation frequency

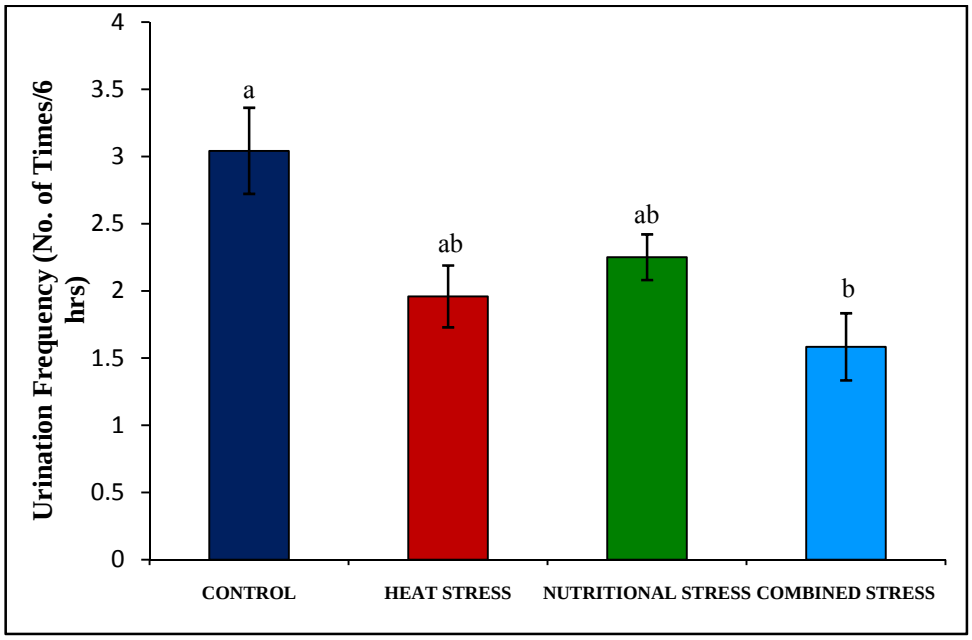


Fig. 3 (e) Urination frequency

4.3 Water intake changes in bucks

The effects of heat, nutritional and combined stress on water intake changes is described in table 6. Water intake showed significant (P<0.01) variation between the groups for treatments. The highest water intake was recorded both in HS and CS groups and the lowest in NS group. Further, the experimental days significantly (P<0.01) influenced water intake of the bucks in the study. In addition, interaction between groups and experimental days also significantly (P<0.01) influenced the water intake of the bucks.

Table 6: Effect of heat stress, nutritional stress and combined stress (heat and nutritional) on the water intake in goats.

FACTOR	**Water Intake**(L/Day)
GROUP	**
C	1.31^{b}±0.05
HS	1.60^{a}±0.06
NS	0.97^{c}±0.06
CS	1.50^{a}±0.06
DAY	**
0	1.35±0.03
15	1.45±0.05
30	1.38±0.05
45	1.21±0.04
GROUP*DAY	**
C0	1.32±0.07
C15	1.37±0.11
C30	1.48±0.09
C45	1.08±0.08
HS0	1.35±0.07
HS15	1.84±0.11
HS30	1.67±0.09
HS45	1.55±0.08
NS0	1.38±0.07
NS15	0.75±0.11
NS30	0.98±0.09
NS45	0.77±0.08
CS0	1.35±0.07
CS15	1.83±0.11
CS30	1.37±0.09
CS45	1.43±0.08

C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; Values bearing different superscripts within a column differ significantly with each other.

Fig. 4: Effect of heat stress, nutritional stress and combined stress (heat and nutritional) on the water intake in goats

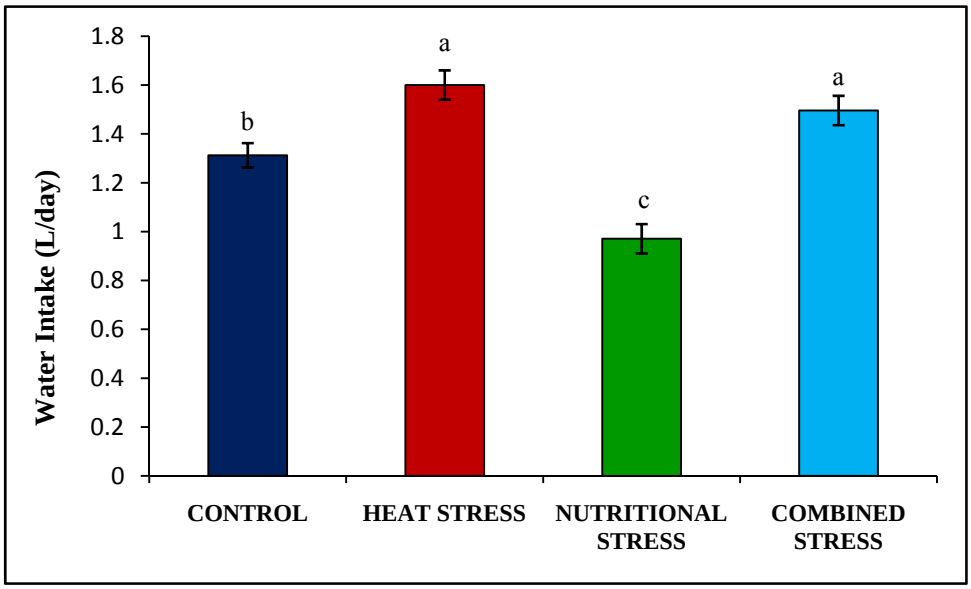


Fig. 4 Water intake

4.4 Physiological responses in bucks

The effects of heat, nutritional and combined stress on RR, PR, and RT both during morning and afternoon are described in table 7. Respiration rate morning showed significant ($P<0.01$) changes between the groups for the treatments. There were significant ($P<0.01$) differences in RRM between the *ad libitum* (C and HS) and restricted feeding (NS and CS) groups. However, experimental days did not influence significantly the RR during morning. But interaction between groups and experimental days significantly ($P<0.01$) influenced the RR during morning. Respiration rate afternoon showed significant ($P<0.01$) variation between the groups for treatments. The highest RR during afternoon was recorded both in HS and CS groups and the lowest both in C and NS groups. Further, the experimental days significantly ($P<0.01$) influenced RRA in the study. In addition, interaction between groups and experimental days significantly ($P<0.01$) influenced the RRA. Pulse rate morning showed significant ($P<0.05$) changes for the treatments. The highest PR recorded during morning was in C group while the lowest PR during morning was in CS group. However, both experimental days and interaction between groups and experimental days did not significantly influence PR during morning. PRA showed significant ($P<0.01$) changes between the groups for the treatments. The highest PRA was recorded in HS while the lowest in NS group. However, experimental days did not influence significantly the PR during afternoon. But interaction between groups and experimental days significantly ($P<0.01$) influenced the PR during afternoon. Rectal temperature morning showed significant ($P<0.01$) changes between the groups for the treatments. There were significant ($P<0.01$) differences in RTM between the *ad libitum* (C and HS) and restricted feeding (NS and CS) groups. However, experimental days did not influence significantly the RT during morning. But interaction between groups and experimental days significantly ($P<0.01$) influenced the RT during morning. Rectal temperature afternoon showed significant ($P<0.01$) variation between the groups for treatments. The highest RT during afternoon was recorded both in HS and CS groups and the lowest in NS group. Further, the experimental days

significantly (P<0.01) influenced RTA in the study. In addition, interaction between groups and experimental days also significantly (P<0.01) influenced the RT during afternoon.

Table 7: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the physiological responses in goats

FACTOR	RRM (breath/min)	RRA (breath/min)	PRM (beats/min)	PRA (beats/min)	RTM (ºC)	RTA (ºC)
GROUP	**	**	*	**	**	**
C	25.08^{a}±1.16	31.92^{b}± 1.38	61.58^{a}±1.94	69.58^{b}±1.82	37.73^{a}± 0.12	38.70^{b}± 0.08
HS	23.00^{a}±0.97	69.17^{a}± 5.33	59.17^{ab}± 1.44	79.17^{a}±2.39	37.61^{a}± 0.11	39.08^{a}± 0.08
NS	19.08^{b}±0.56	24.83^{b}± 1.08	56.46^{b}± 1.27	59.17^{c}±1.72	36.61^{b}± 0.18	38.40^{c}± 0.11
CS	19.75^{b}±0.87	62.75^{a}± 5.58	56.08^{b}± 1.56	71.67^{b}± 2.14	36.74^{b}± 0.21	39.23^{a}± 0.15
DAY	ns	**	ns	ns	ns	**
0	21.33 ± 0.84	30.33 ± 1.10	55.58 ± 1.35	67.83 ± 1.11	37.48 ± 0.08	38.55 ± 0.06
15	21.25 ± 0.89	47.50 ± 2.62	63.29 ± 1.39	73.17 ± 2.48	37.16 ± 0.10	39.05 ± 0.08
30	22.25 ± 0.74	52.42± 2.53	57.50 ± 1.63	67.08 ± 1.33	36.48 ± 0.13	38.81 ± 0.07
45	22.08 ± 0.58	58.42 ± 2.10	56.92 ± 1.26	71.50 ± 1.72	37.57 ± 0.14	39.01 ± 0.10
GROUP*DAY	**	**	ns	**	**	**
C0	19.33 ± 1.67	32.67 ± 2.20	53.67 ± 2.69	68.00 ± 2.22	37.40 ± 0.16	38.40 ± 0.13
C15	26.00 ± 1.77	32.67 ± 5.23	70.00 ± 2.78	76.00 ± 4.96	37.63 ± 0.20	38.95 ± 0.16
C30	26.00 ± 1.48	26.00 ± 5.05	61.33 ± 3.26	63.33 ± 2.66	37.73 ± 0.26	38.70 ± 0.14
C45	29.00 ± 1.16	36.33 ± 4.20	61.33 ± 2.53	71.00 ± 3.43	38.17 ± 0.28	38.77 ± 0.20
HS0	20.00 ± 1.67	30.67 ± 2.20	55.33 ± 2.69	68.00 ± 2.22	37.77 ± 0.16	38.63 ± 0.13
HS15	21.33 ± 1.77	67.33 ± 5.23	60.67 ± 2.78	82.67 ± 4.96	37.37 ± 0.20	39.32 ± 0.16
HS30	26.67 ± 1.48	86.33 ± 5.05	60.00 ± 3.26	80.33 ± 2.66	37.28 ± 0.26	39.22 ± 0.14
HS45	24.00 ± 1.16	92.33 ± 4.20	60.67 ± 2.53	85.67 ± 3.43	38.03 ± 0.28	39.17 ± 0.20
NS0	21.33 ± 1.67	30.67 ± 2.20	54.00 ± 2.69	67.33 ± 2.22	37.30 ± 0.16	38.88 ± 0.13
NS15	19.67 ± 1.77	24.00 ± 5.23	60.50 ± 2.78	56.00 ± 4.96	36.93 ± 0.20	38.35 ± 0.16
NS30	18.67 ± 1.48	23.33 ± 5.05	57.33 ± 3.26	61.33 ± 2.66	35.52 ± 0.26	37.78 ± 0.14
NS45	16.67 ± 1.16	21.33 ± 4.20	54.00 ± 2.53	52.00 ± 3.43	36.70 ± 0.28	38.60 ± 0.20
CS0	24.67 ± 1.67	27.33 ± 2.20	59.33 ± 2.69	68.00 ± 2.22	37.47 ± 0.16	38.28 ± 0.13
CS15	18.00 ± 1.77	66.00 ± 5.20	62.00 ± 2.78	78.00 ± 4.96	36.72 ± 0.20	39.58 ± 0.16
CS30	17.67 ± 1.48	74.00 ± 5.05	51.33 ± 3.26	63.33 ± 2.66	35.38 ± 0.26	39.53 ± 0.14
CS45	18.67 ± 1.16	83.67 ± 4.20	51.67 ± 2.53	77.33 ± 3.43	37.38 ± 0.28	39.52 ± 0.20

RRM-Respiration Rate Morning; RRA-Respiration Rate Afternoon; PRM- Pulse Rate Morning; PRA- Pulse Rate Afternoon; RTM- Rectal Temperature Morning; RTA-Rectal Temperature Afternoon; C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Fig. 5: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the physiological responses in goats (a) Respiration rate morning (b) Respiration rate afternoon (c) Pulse rate morning (d) Pulse rate afternoon (e) Rectal temperature morning (f) Rectal temperature afternoon

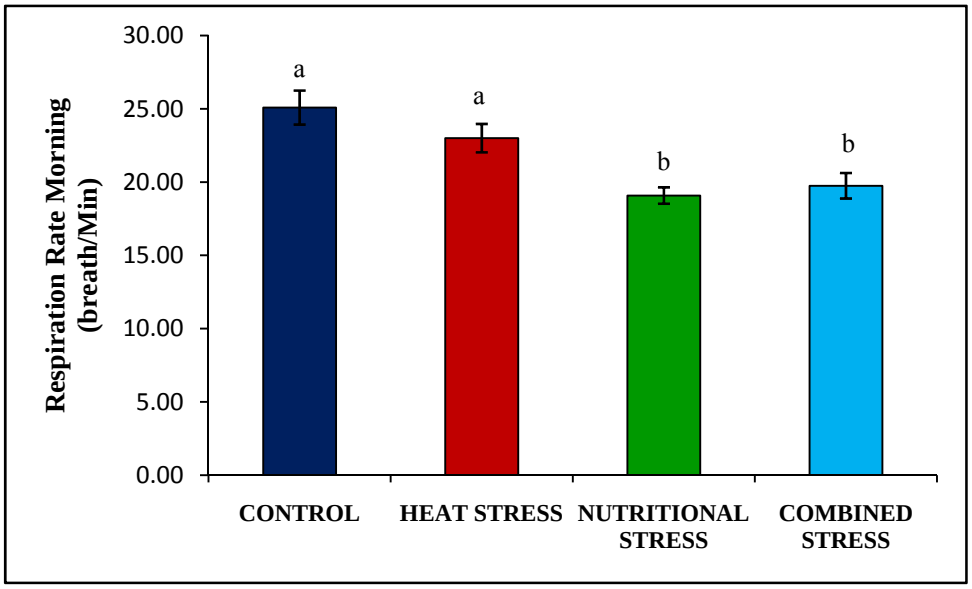


Fig. 5 (a) Respiration rate morning

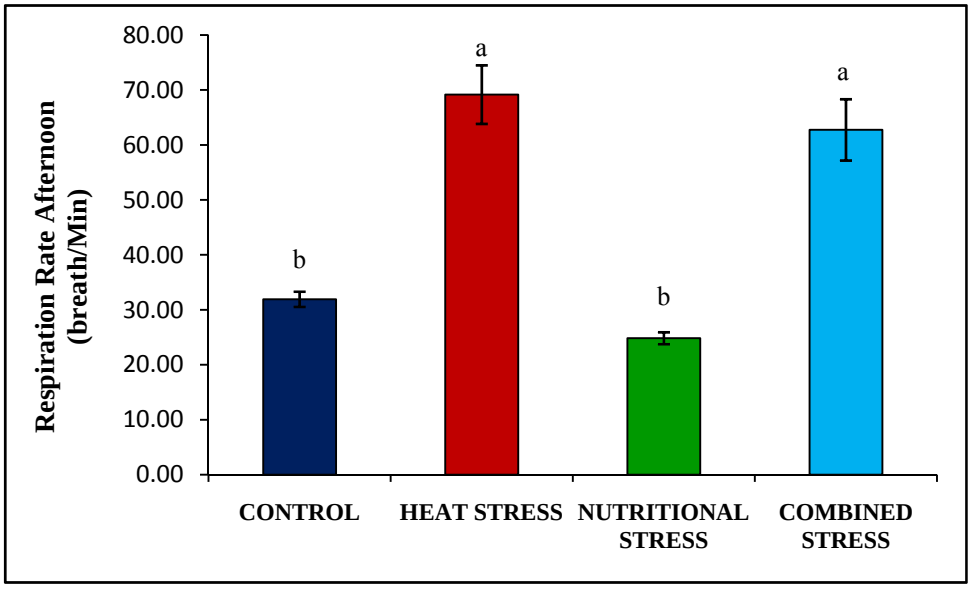


Fig. 5 (b) Respiration rate afternoon

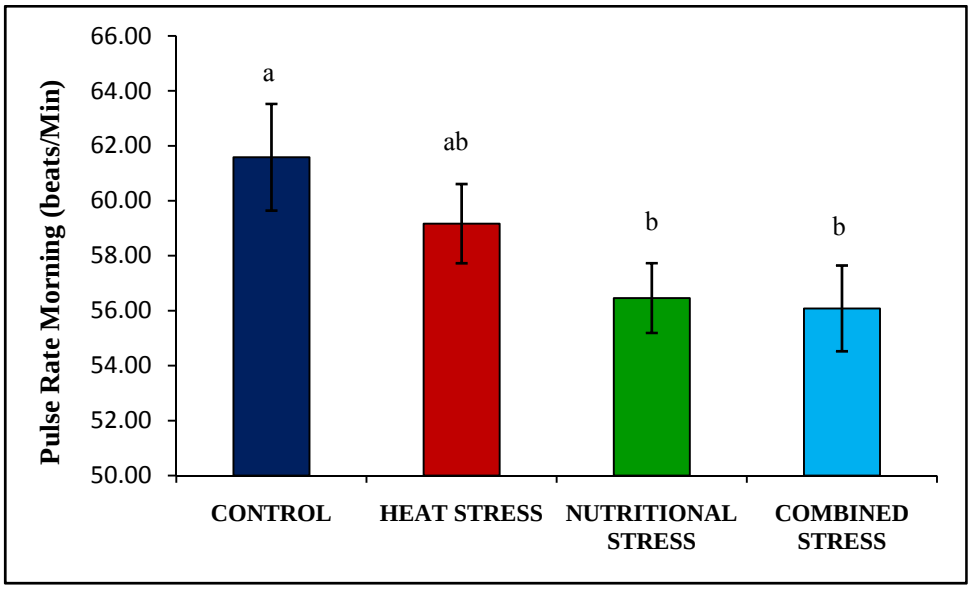


Fig. 5 (c) Pulse rate morning

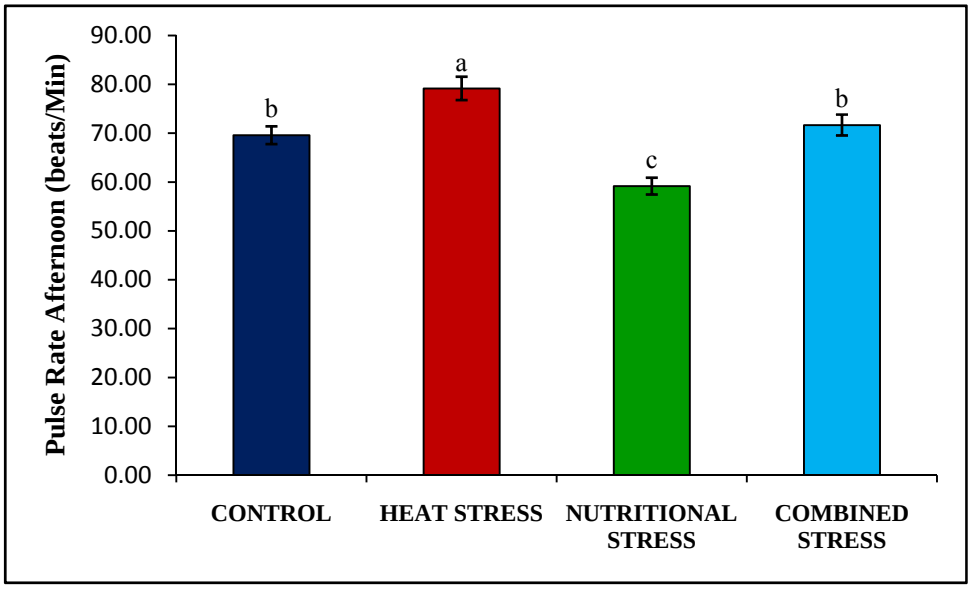


Fig. 5 (d) Pulse rate afternoon

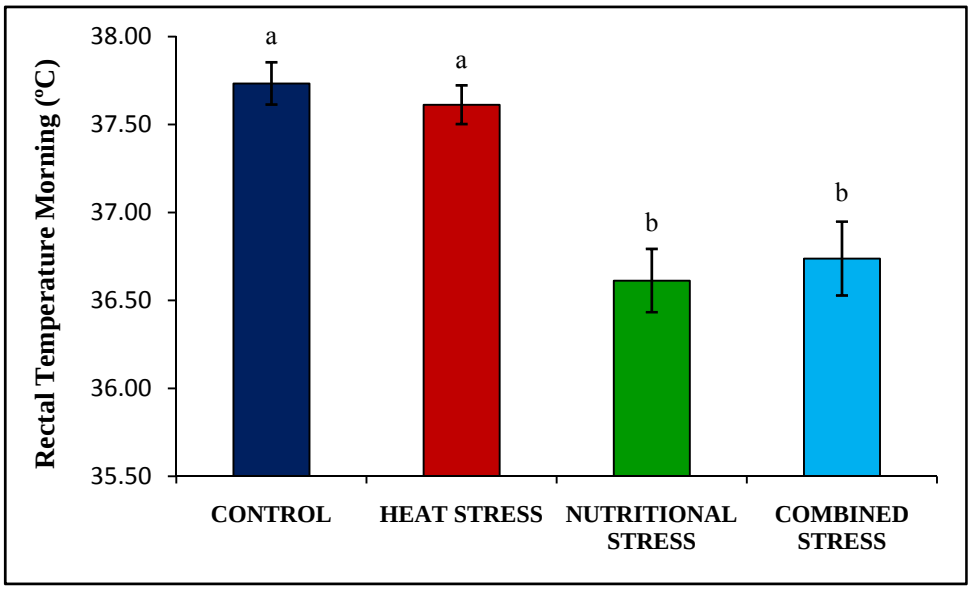


Fig. 5 (e) Rectal temperature morning

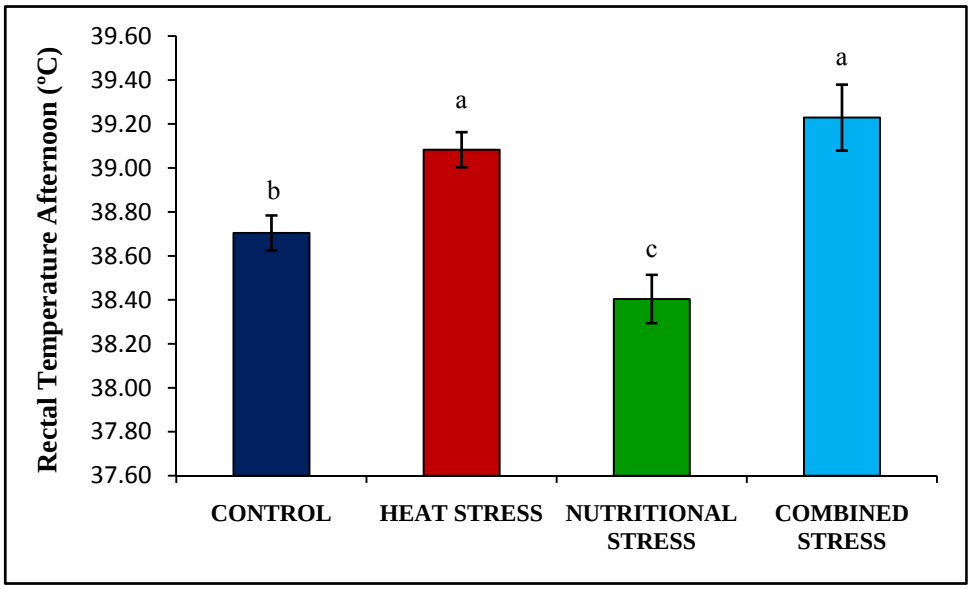


Fig. 5 (f) Rectal temperature afternoon

4.5 Skin temperature in bucks

The effects of heat, nutritional and combined stress on skin temperature at head, scrotum and flank both during morning and afternoon are described in table 8. Skin temperature head morning did not differ between the groups for the treatments. However, the experimental days significantly ($P<0.01$) influenced STHM. Further, interaction between groups and experimental days also did not influence significantly the STHM. Skin temperature head afternoon showed significant ($P<0.01$) changes between the groups for the treatments. The highest STHA was recorded in CS group while the lowest in both C and NS groups. Further, there were significant changes in STHA between the stress groups (HS, NS and CS). However, experimental days did not influence significantly the STH during afternoon. But interaction between groups and experimental days significantly ($P<0.01$) influenced the STH during afternoon. Skin temperature scrotum morning did not show significant changes for treatment, experimental days as well as for interaction between groups and experimental days in the study. Skin temperature scrotum afternoon showed significant ($P<0.01$) changes between the groups. The highest STSA was recorded in CS group while the lowest in C, HS and NS groups. Further, experimental days significantly ($P<0.05$) influenced STS during afternoon. In addition, interaction between groups and experimental days significantly ($P<0.01$) influenced the STS during afternoon. Skin temperature flank morning did not showed significant changes between the groups. However, experimental days significantly ($P<0.01$) influenced STF during morning. In addition, interaction between groups and experimental days significantly ($P<0.01$) influenced the STF during morning. Skin temperature flank afternoon showed significant ($P<0.01$) changes between the groups for the treatments. The highest STFA was recorded in CS group while the lowest in both C and NS groups. Further, there were significant changes in STFA between the stress groups (HS, NS and CS). However, experimental days did not influence significantly the STF during afternoon. But interaction between groups and experimental days significantly ($P<0.01$) influenced the STF during afternoon.

Table 8: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on skin temperature in goats

FACTOR	STHM (ºC)	STHA (ºC)	STSM (ºC)	STSA (ºC)	STFM (ºC)	STFA (ºC)
GROUP	ns	**	ns	**	ns	**
C	30.07^{a}±0.51	34.99^{c}±0.24	32.74^{a}±0.21	33.70^{b}±0.20	30.98^{a}±0.20	34.89^{c}±0.24
HS	29.79^{a}±0.21	37.69^{b}±0.78	32.11^{a}±0.24	34.51^{b}±0.24	30.25^{a}±0.19	36.51^{b}±0.38
NS	29.77^{a}±0.39	35.67^{c}±0.41	32.71^{a}±0.19	33.92^{b}±0.24	30.25^{a}±0.25	34.60^{c}±0.28
CS	29.10^{a}±0.26	40.32^{a}±0.76	32.30^{a}±0.26	35.87^{a}±0.42	30.37^{a}±0.25	37.93^{a}±0.64
DAY	**	ns	ns	**	**	ns
0	30.35±0.28	35.85±0.14	32.61±0.16	33.93±0.14	30.75±0.20	35.95±0.18
15	29.80±0.28	38.56±0.59	32.55±0.24	34.80±0.29	31.24±0.21	35.81±0.33
30	29.25±0.57	37.02±0.57	32.13±0.15	34.25±0.26	29.55±0.17	36.44±0.47
45	29.33±0.13	37.23±0.44	32.56±0.39	35.02±0.24	30.30±0.14	35.72±0.26
GROUP*DAY	ns	**	ns	**	**	**
C0	29.42±0.55	36.25±0.29	32.43±0.33	34.18±0.27	30.60±0.40	35.85±0.36
C15	30.53±0.56	35.40±1.17	32.57±0.48	33.82±0.58	31.77±0.43	35.57±0.66
C30	30.83±1.15	33.68±1.15	33.08±0.30	33.17±0.52	30.52±0.33	33.32±0.95
C45	29.48±0.26	34.63±0.88	32.87±0.78	33.65±0.48	31.05±0.27	34.82±0.51
HS0	30.40±0.55	35.48±0.29	33.13±0.31	33.70±0.27	30.65±0.40	35.97±0.36
HS15	30.22±0.56	38.67±1.17	31.77±0.48	35.98±0.58	30.97±0.43	35.73±0.66
HS30	28.90±1.15	39.67±1.15	31.28±0.30	34.22±0.52	29.17±0.33	38.13±0.95
HS45	29.65±0.26	36.95±0.88	32.25±0.78	34.13±0.48	30.23±0.27	36.20±0.51
NS0	31.58±0.55	36.18±0.29	32.52±0.31	33.95±0.27	31.37±0.40	36.32±0.36
NS15	29.52±0.56	38.35±1.17	32.78±0.48	33.73±0.58	30.75±0.43	34.17±0.66
NS30	28.62±1.15	33.22±1.15	32.37±0.30	33.22±0.52	29.25±0.33	33.15±0.95
NS45	29.35±0.26	34.93±0.88	33.17±0.78	34.78±0.48	29.62±0.27	34.75±0.51
CS0	29.98±0.55	35.47±0.29	32.37±0.31	33.90±0.27	30.40±0.40	35.68±0.36
CS15	28.95±0.56	41.88±1.17	33.07±0.48	35.67±0.58	31.48±0.43	37.78±0.66
CS30	28.63±1.15	41.50±1.15	31.80±0.30	36.40±0.52	29.27±0.33	41.15±0.95
CS45	28.83±0.26	42.42±0.88	31.97±0.78	37.50±0.48	30.32±0.27	37.12±0.51

STHM-Skin Temperature Head Morning, STHA- Skin Temperature Head Afternoon, STSM- Skin Temperature Scrotum Morning, STSA- Skin Temperature Scrotum Afternoon, STFM- Skin temperature Flank Morning, STFA- Skin Temperature Scrotum Afternoon; C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Fig. 6: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on skin temperature in goats (a)Skin temperature head morning (b)Skin temperature head afternoon (c) Skin temperature scrotum morning (d)Skin temperature scrotum afternoon (e) Skin temperature flank morning (f) Skin temperature flank afternoon

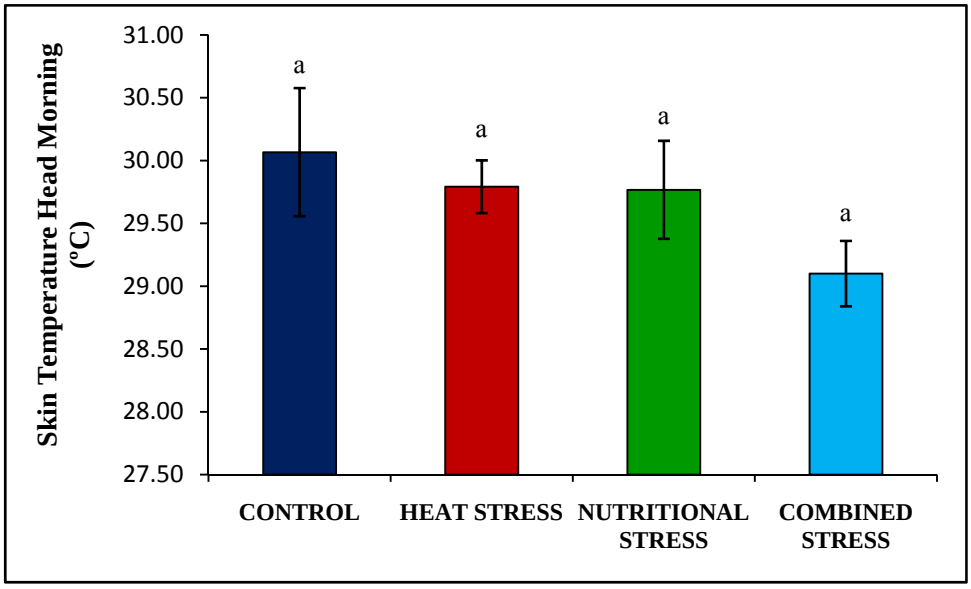


Fig. 6 (a) Skin temperature head morning

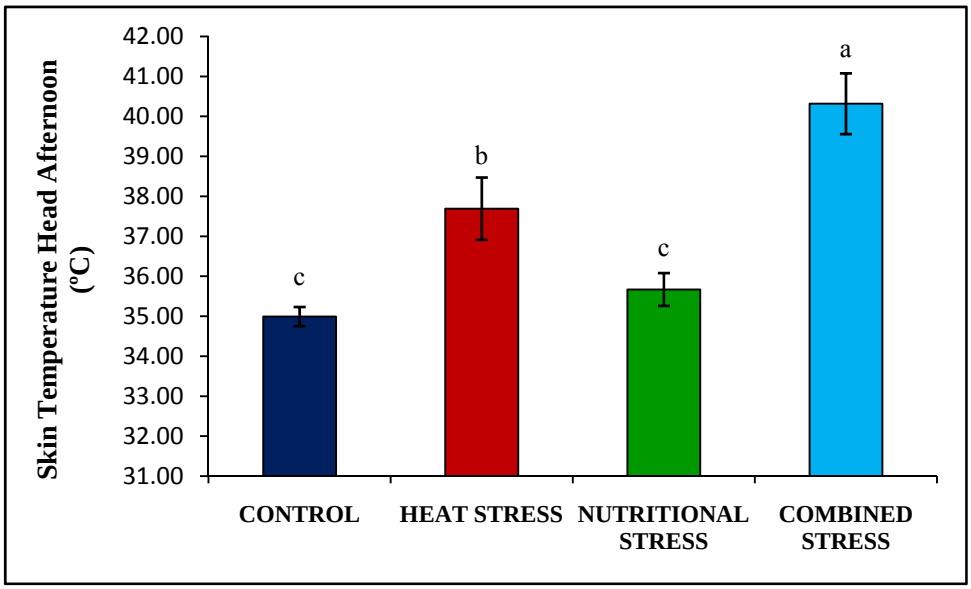


Fig. 6 (b) Skin temperature head morning

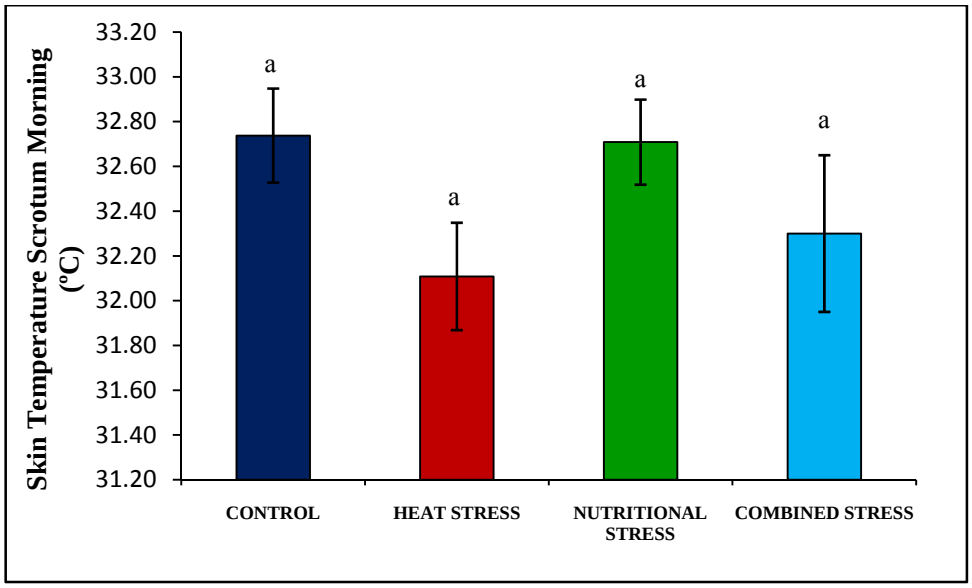


Fig. 6 (c) Skin temperature scrotum morning

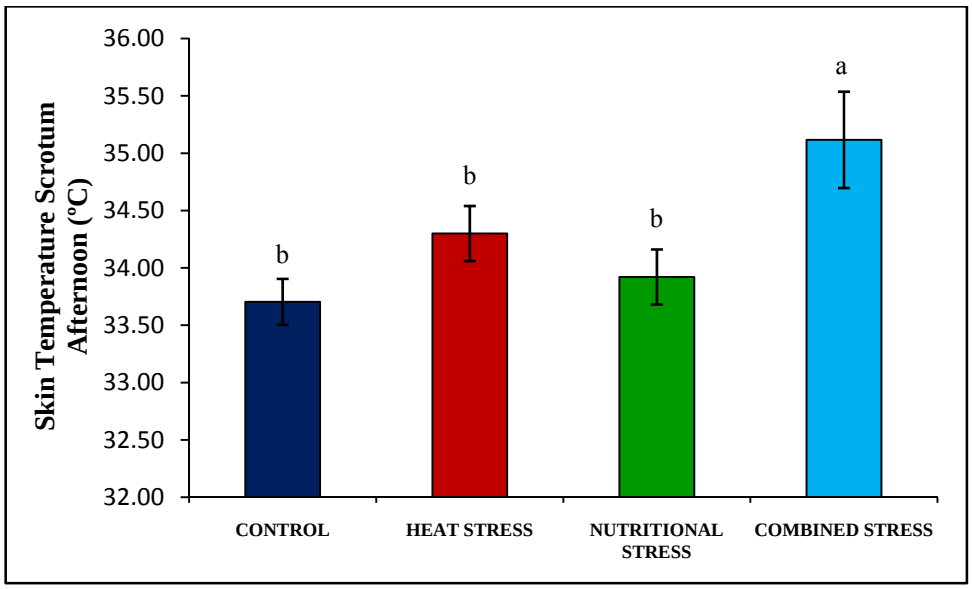


Fig. 6 (d) Skin temperature scrotum afternoon

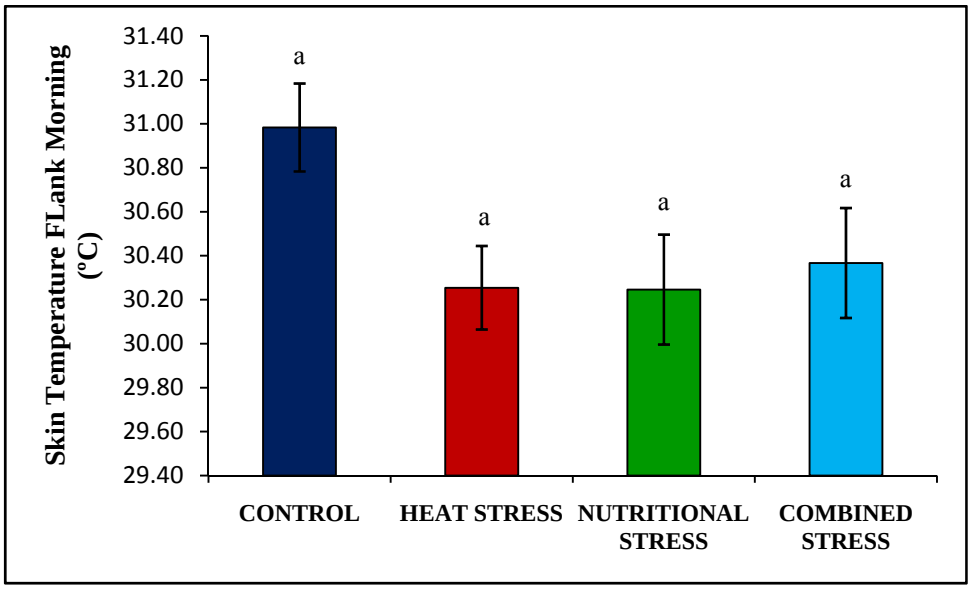


Fig. 6 (e) Skin temperature flank morning

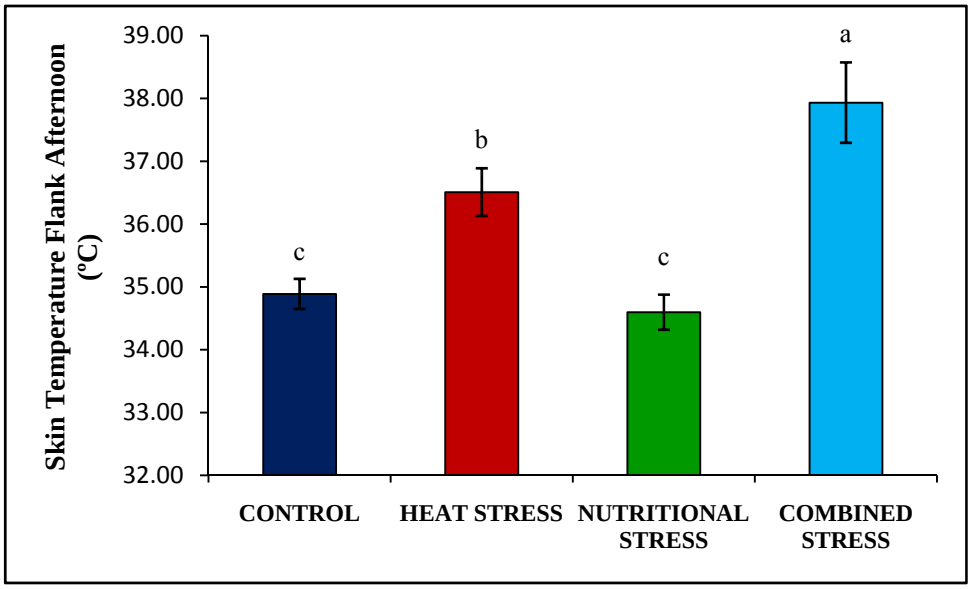


Fig. 6 (f) Skin temperature flank afternoon

4.6 Blood biochemical changes in bucks

The effects of heat, nutritional and combined stress on blood biochemical responses are described in table 9 and 10. Plasma glucose did not show significant changes for treatment, experimental days as well as for interaction between groups and experimental days in the study (table 9). Plasma total protein showed significant ($P<0.01$) changes between the groups for the treatments. The highest plasma total protein concentration was recorded in NS group while the lowest in C, HS and CS groups (table 9). However, experimental days did not influence significantly the plasma total protein. But interaction between groups and experimental days significantly ($P<0.01$) influenced the plasma total protein (table 9). Plasma albumin did not showed significant changes between the groups. However, experimental days significantly ($P<0.01$) influenced plasma albumin (table 9). In addition, interaction between groups and experimental days also significantly ($P<0.01$) influenced the plasma albumin concentration. Plasma globulin showed significant ($P<0.05$) changes between the groups for the treatments. The highest plasma globulin concentration was recorded in NS group while the lowest in C, HS and CS groups (table 9). Further, experimental days also significantly ($P<0.01$) influenced plasma globulin. However, interaction between groups and experimental days did not influence significantly the plasma globulin concentration (table 9). Plasma total cholesterol showed significant ($P<0.05$) changes for the treatments (table 9). The highest plasma total cholesterol recorded was in NS group while the lowest plasma total cholesterol was in C, HS and CS groups (table 9). However, both experimental days and interaction between groups and experimental days did not influence significantly the plasma total cholesterol concentration (table 9).

Plasma triglycerides showed significant ($P<0.01$) variation between the groups for treatments (table 10). The highest plasma triglycerides were recorded both in C and NS groups and the lowest in HS group. Further, the experimental days significantly ($P<0.01$) influenced plasma triglycerides concentration. In addition, interaction between groups and experimental days also significantly

($P<0.01$) influenced the plasma triglycerides concentration (table 10). Plasma urea showed significant ($P<0.01$) changes between the groups for the treatments. The highest plasma urea concentration was recorded in both C and HS groups while the lowest in CS group (table 10). Further, there were significant changes in plasma urea concentration between all stress groups (HS, NS and CS). However, experimental days did not influence significantly the plasma urea concentration. But interaction between groups and experimental days significantly ($P<0.01$) influenced the plasma urea concentration (table 10). Plasma urea nitrogen showed significant ($P<0.01$) changes between the groups for the treatments. The highest plasma urea nitrogen concentration was recorded in HS group while the lowest in CS group (table 10). However, experimental days did not influence significantly the blood urea nitrogen concentration. But interaction between groups and experimental days significantly ($P<0.01$) influenced the blood urea nitrogen concentration (table 10).

Table 9: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the blood biochemical responses in goats

FACTOR	Glucose (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Total Cholesterol (mg/dL)
GROUP	ns	**	ns	*	*
C	55.10^{a}± 2.07	6.09^{b}± 0.09	2.62^{a}± 0.07	3.46^{b} ± 0.12	61.26^{b}± 3.40
HS	56.18^{a}± 2.41	6.20^{b}± 0.10	2.62^{a}± 0.07	3.57^{ab}± 0.12	55.64^{b}± 3.83
NS	54.67^{a}± 3.58	6.77^{a}± 0.13	2.88^{a}± 0.06	3.90^{a}± 0.12	88.85^{a}± 9.44
CS	60.51^{a}± 5.61	5.98^{b}± 0.07	2.69^{a}± 0.05	3.29^{b}± 0.07	74.04^{ab}±4.01
DAY	ns	ns	**	**	ns
0	50.29± 3.06	6.10± 0.08	2.86± 0.05	3.24 ± 0.09	51.89± 2.63
15	58.69± 0.97	6.38± 0.10	2.71± 0.05	3.67± 0.09	96.25± 4.89
30	72.35± 4.51	6.21± 0.11	2.63± 0.07	3.58± 0.12	69.77± 4.38
45	45.12± 1.49	6.34± 0.09	2.62± 0.07	3.72± 0.11	61.87± 5.20
GROUP*DAY	ns	**	**	ns	ns
C0	50.27± 6.13	6.05± 0.16	3.00± 0.10	3.05± 0.18	54.18± 5.25
C15	61.12± 1.93	6.15± 0.20	2.57± 0.10	3.59± 0.18	77.22± 9.79
C30	61.89± 9.02	6.00± 0.22	2.46± 0.14	3.54± 0.25	57.41± 8.76
C45	47.12± 2.99	6.15± 0.19	2.47± 0.13	3.68± 0.23	56.23± 10.4
HS0	51.59± 6.13	5.93± 0.16	2.86± 0.10	3.07± 0.18	49.03± 5.25
HS15	61.29± 1.93	6.38± 0.20	2.63± 0.10	3.74± 0.18	71.95± 9.79
HS30	62.78± 9.02	6.11± 0.22	2.48± 0.14	3.64± 0.25	51.85± 8.76
HS45	49.06± 2.99	6.36± 0.19	2.53± 0.13	3.84± 0.23	49.74± 10.4
NS0	52.62± 6.13	6.21± 0.16	2.71± 0.10	3.50± 0.18	49.16± 5.25
NS15	54.29± 1.93	6.86± 0.20	2.93± 0.10	3.93± 0.18	138.7± 9.79
NS30	71.05± 9.02	6.91± 0.22	2.95± 0.14	3.96± 0.25	92.88± 8.76
NS45	40.71± 2.99	7.12± 0.19	2.93± 0.13	4.18± 0.23	74.61± 10.4
CS0	46.68± 6.13	6.21± 0.16	2.88± 0.10	3.33± 0.18	55.21± 5.25
CS15	58.08± 1.93	6.14± 0.20	2.71± 0.10	3.43± 0.18	97.10± 9.79
CS30	93.69± 9.02	5.82± 0.22	2.63± 0.14	3.19± 0.25	76.94± 8.76
CS45	43.58± 2.99	5.74± 0.19	2.55± 0.13	3.19± 0.23	66.90± 10.4

C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Table 10: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the blood biochemical responses in goats

FACTOR	**Triglycerides**(mg/dL)	**Urea**(mg/dL)	**PUN**(mg/dL)
GROUP	**	**	**
C	17.74^{a}± 0.85	61.30^{a}±2.23	27.40^{ab}±1.00
HS	08.46^{c}± 1.29	62.06^{a}±1.67	29.78^{a}±0.94
NS	14.99^{a}± 1.21	53.43^{b}±2.49	24.95^{bc}±1.23
CS	11.44^{b}± 1.40	45.90^{c}±2.10	22.49^{c}±1.04
DAY	**	ns	ns
0	16.55± 0.92	60.78±2.24	28.72±1.14
15	13.31±1.31	49.26±1.65	23.84± 0.83
30	12.63± 1.18	53.20±1.30	24.51± 0.63
45	10.15± 0.56	59.45±1.86	27.56±1.12
GROUP*DAY	**	**	**
C0	15.57±1.84	58.73±4.48	27.43±2.27
C15	17.51±2.63	52.10±3.31	26.22±1.65
C30	19.17±2.36	60.39±2.60	26.09±1.25
C45	18.72±1.13	73.96±3.72	29.87±2.24
HS0	15.23±1.84	63.28±4.48	29.61±2.27
HS15	09.26±2.63	56.50±3.31	26.38±1.65
HS30	07.47±2.36	61.15±2.60	28.56±1.25
HS45	01.86±1.13	67.33±3.72	34.56±2.24
NS0	16.13±1.84	62.96±4.48	29.40±2.27
NS15	16.51±2.63	48.88±3.31	22.83±1.65
NS30	15.22±2.36	50.67±2.60	23.66±1.25
NS45	12.11±1.13	51.20±3.72	23.91±2.24
CS0	19.26±1.84	58.14±4.48	28.43±2.27
CS15	09.94±2.63	39.54±3.31	19.91±1.65
CS30	08.65±2.36	40.59±2.60	19.73±1.25
CS45	07.91±1.13	45.32±3.72	21.90±2.24

PUN-Plasma Urea Nitrogen; C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Fig. 7: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the blood biochemical responses in goats (a) Plasma Glucose (b) Plasma Total Protein (c) Plasma albumin (d) Plasma globulin (e) Plasma total cholesterol (f) Plasma triglycerides (g) Plasma urea (h) Plasma urea nitrogen (PUN)

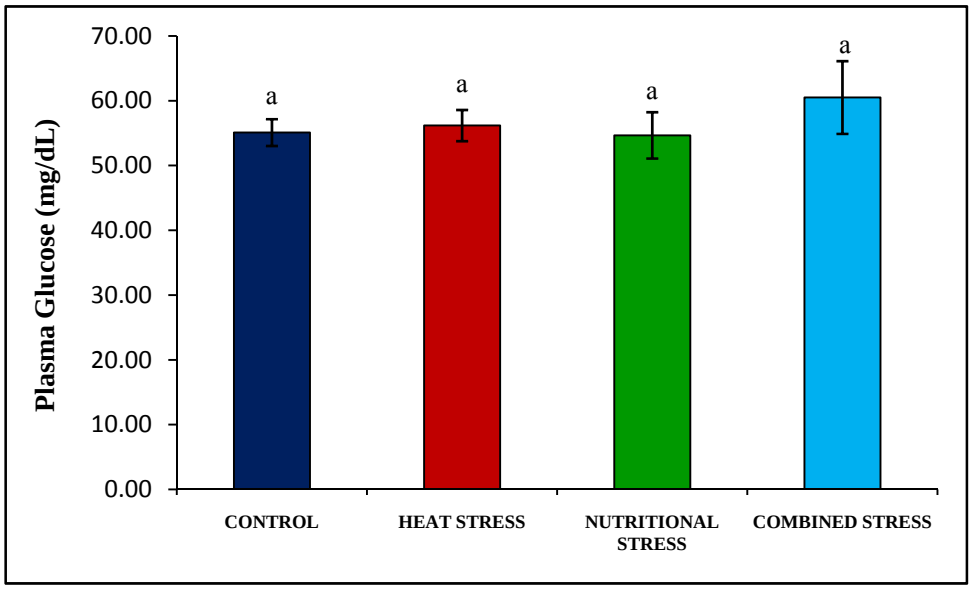


Fig. 7(a) Plasma glucose

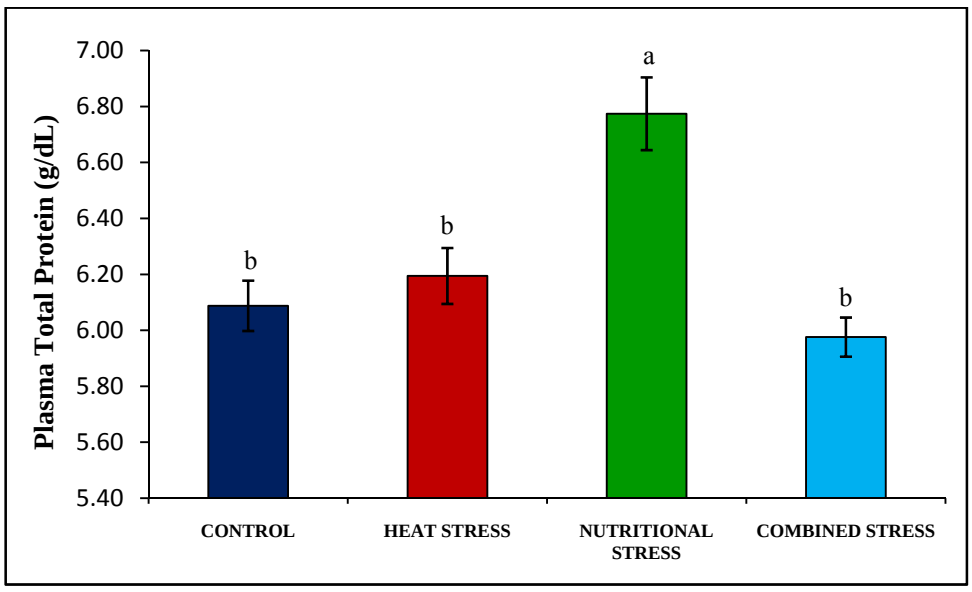


Fig. 7 (b) Plasma total protein

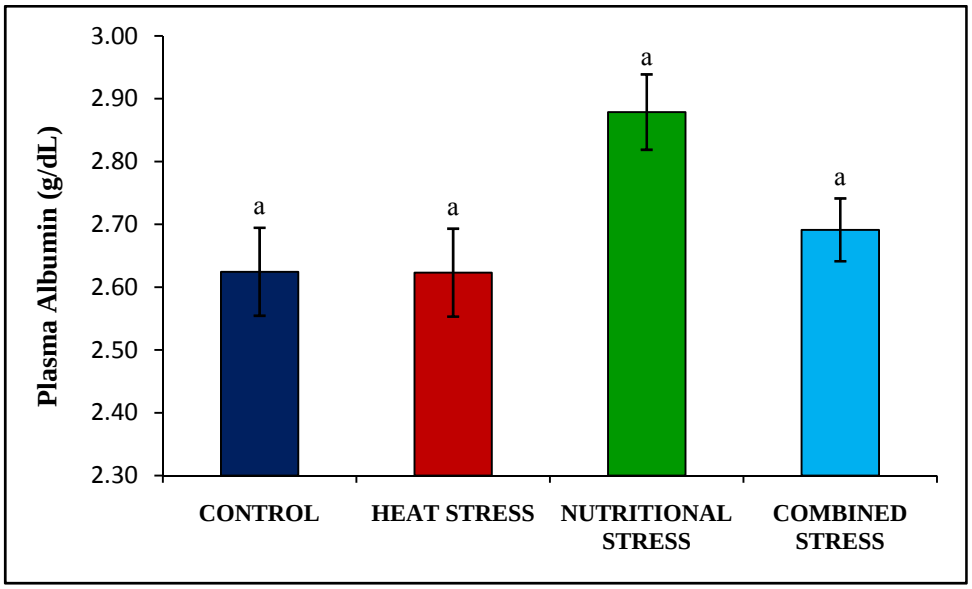


Fig. 7 (c) Plasma albumin

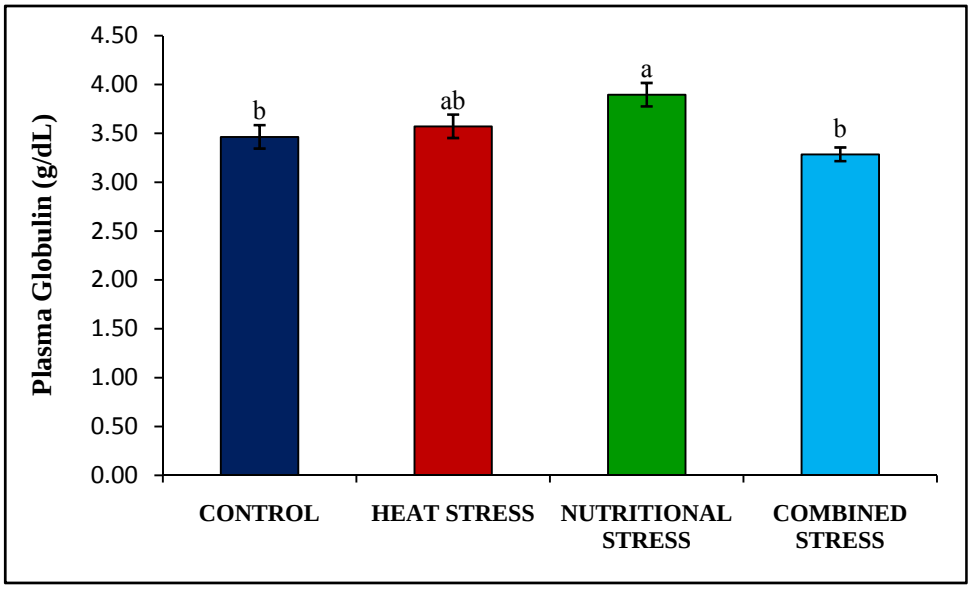


Fig. 7 (d) Plasma globulin

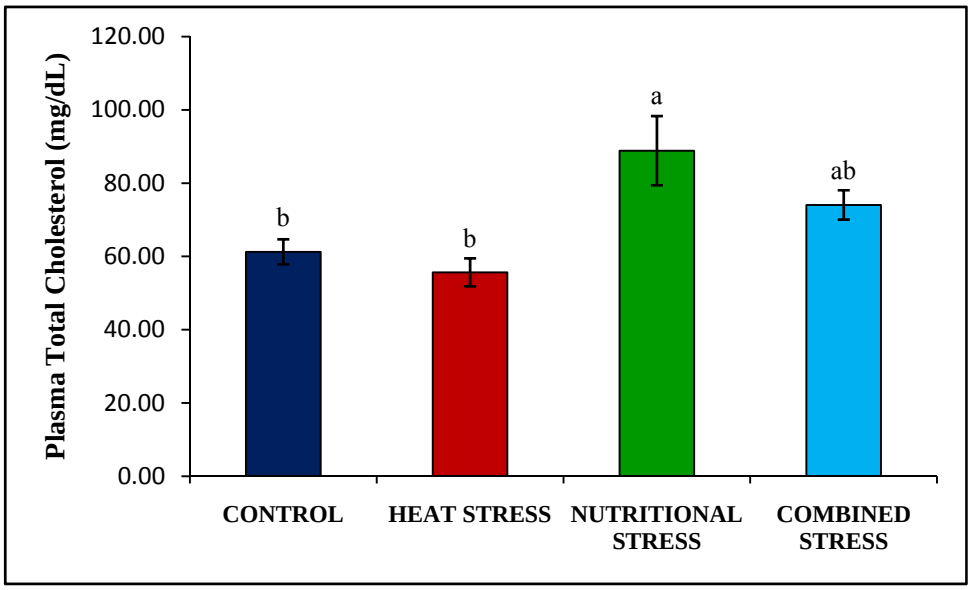


Fig. 7 (e) Plasma total cholesterol

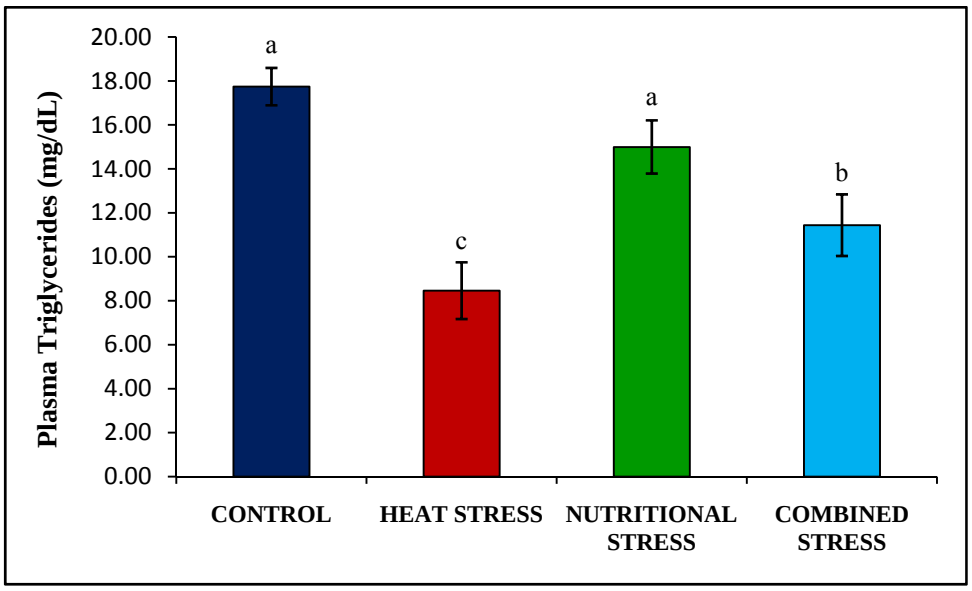


Fig. 7 (f) Plasma triglycerides

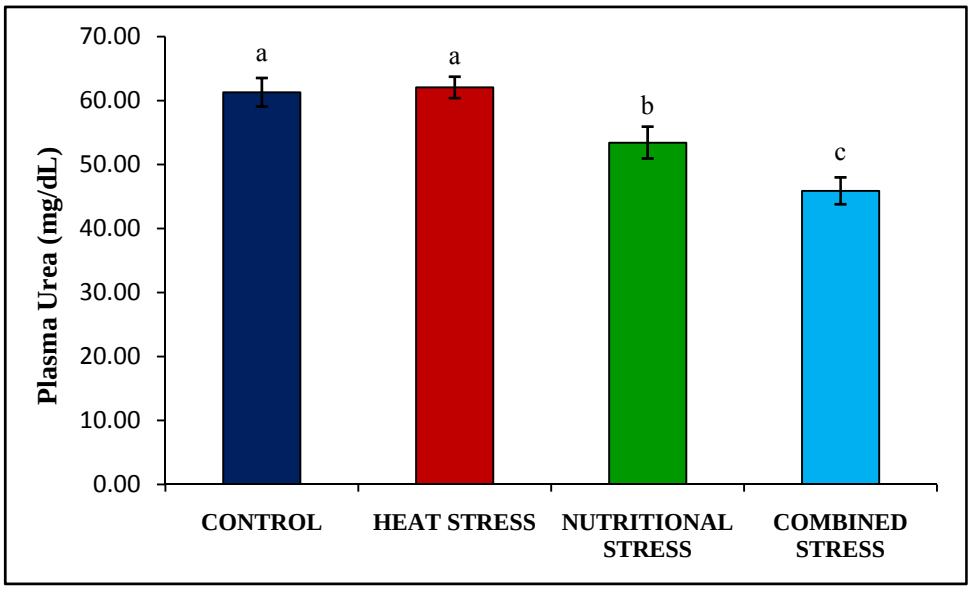


Fig. 7 (g) Plasma urea

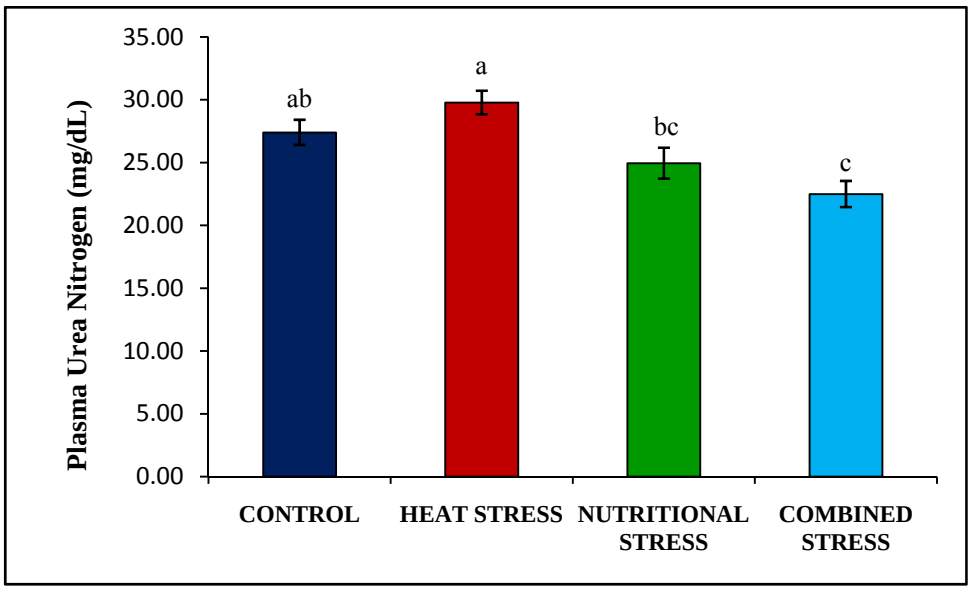


Fig. 7 (h) Plasma urea nitrogen

4.7 Endocrine responses in bucks

The effects of heat, nutritional and combined stresses on plasma endocrine profile are described in table 11. Plasma cortisol level showed significant ($P<0.01$) variation between the groups. The highest plasma cortisol was recorded in CS group and the lowest in rest of the groups (C, HS and NS). Among the stress groups, plasma cortisol concentration differed in CS group as compared to HS and NS group but did not differed between HS and NS groups. Further, the experimental days also significantly ($P<0.05$) influenced plasma cortisol concentration. However, interaction between treatment and experimental days did not influence plasma cortisol concentration. Plasma aldosterone level also showed significant ($P<0.01$) variation between the groups. The highest plasma aldosterone level was recorded in CS group and the lowest in C group bucks. Among the stress groups plasma aldosterone level did not differed between the groups. Further, the experimental days also significantly ($P<0.05$) influenced plasma aldosterone concentration. However, interaction between treatment and experimental days did not influence plasma aldosterone concentration.

Table 11: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the endocrine responses in goats

FACTOR	Cortisol (μg/dl)	Aldosterone (pg/mL)
GROUP	**	*
C	05.26^{b}±1.11	77.23^{b}±2.84
HS	08.67^{b}±1.29	103.30^{a}±7.81
NS	07.58^{b}±1.48	83.73^{ab}±4.09
CS	16.56^{a}±3.01	105.00^{a}±7.81
DAY	*	**
0	05.29±0.96	76.50±2.03
15	11.68±1.35	93.49±7.77
30	11.59±2.31	102.6±6.68
45	09.50±2.39	96.75±5.77
GROUP*DAY	ns	ns
C0	04.58±1.91	72.02±04.07
C15	05.83±2.71	76.28±15.54
C30	04.73±4.62	81.61±13.36
C45	05.90±4.78	79.01±11.54
HS0	05.03±1.91	77.60±04.07
HS15	12.74±2.71	105.8±15.54
HS30	10.54±4.62	119.8±13.36
HS45	06.36±4.78	110.1±11.54
NS0	05.51±1.91	76.84±04.07
NS15	07.99±2.71	84.73±15.54
NS30	09.70±4.62	93.22±13.36
NS45	07.12±4.78	80.14±11.54
CS0	06.02±1.91	79.53±04.07
CS15	20.18±2.71	107.1±15.54
CS30	21.41±4.92	115.6±13.36
CS45	18.63± 4.78	117.7±11.54

C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other

Fig. 8: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on the endocrine responses in goats (a) Plasma cortisol (b) Plasma aldosterone

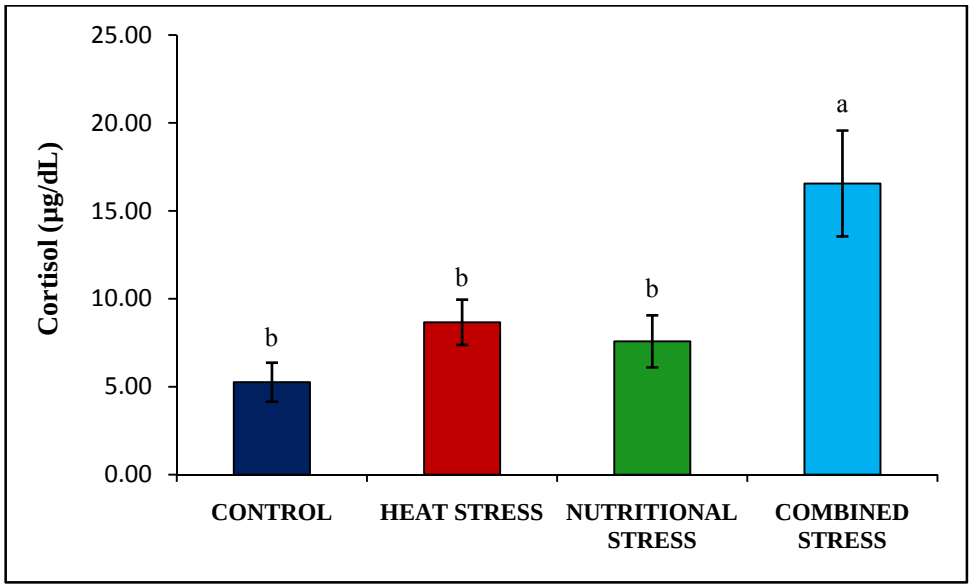


Fig. 8 (a) Plasma cortisol

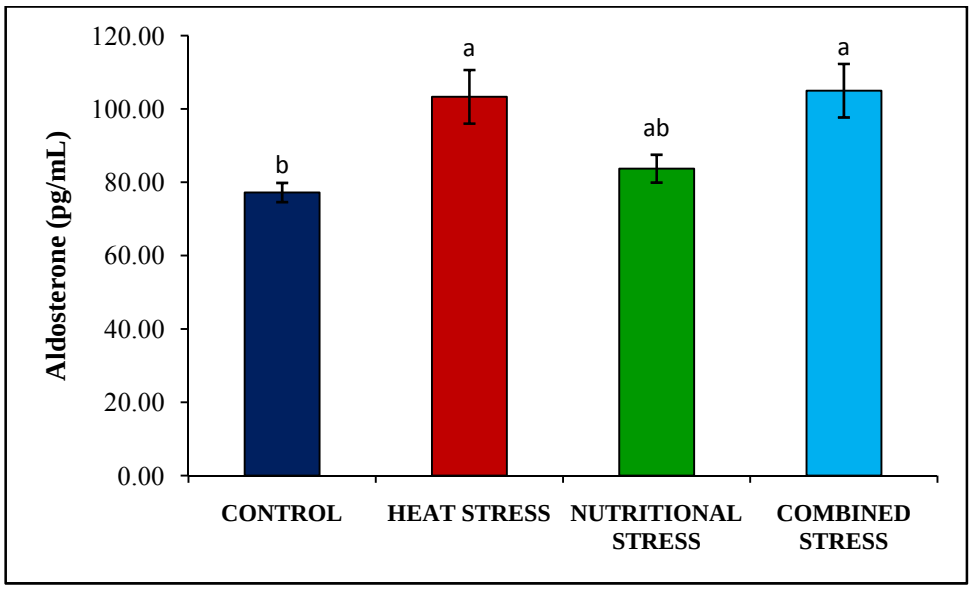


Fig. 8 (b) Plasma aldosterone

4.8 Histopathology

The H and E staining results of different organs studied revealed that only liver and adrenal gland sections showed significant changes between the groups. However, hypothalamus, pituitary, lung and heart did not showed significant changes for different treatments in the study. The histopathological changes in H and E stained sections from adrenal glands showed hypertrophic changes in adrenal endocrine cells and these changes were compared between different stress groups (HS, NS and CS) with the C group. The highest hyperactivity of endocrine cells was recorded in CS group followed by HS and NS as compared to C group as shown by the markings in the sections. Similarly the liver section showed significant changes for different stresses. These changes in different stress groups (HS, NS and CS) were compared with C group. The highest degree of degenerative changes was recorded in CS group followed by HS and NS groups as compared to C group.

4.9 Gene Expression

4.9.1 Adrenal HSP70 expression

Adrenal HSP70 mRNA transcript expression between the C, HS, NS and CS (heat & nutritional) groups of goats were described in Fig.8(a). The results revealed that adrenal HSP70 mRNA expression was evident in C (1 fold), HS (1.51 fold), NS (1.16 fold) and CS (2.03 fold). On comparative basis, the higher expression of adrenal HSP70 mRNA was recorded in CS goats (Fig. 8(a)). Within the stress groups, the highest adrenal HSP70 mRNA expression was recorded in CS group followed by HS and NS groups.

4.9.2 Hepatic HSP70 expression

Hepatic HSP70 mRNA transcript expression between the C, HS, NS and CS (heat & nutritional) groups of goats are described in Fig.8(b). The results revealed that hepatic HSP70 mRNA expression was evident in C (1 fold), HS (1.64 fold), NS (0.93 fold) and CS (0.86 fold). On comparative basis, the higher expression of hepatic HSP70 mRNA was recorded in HS goats (Fig. 8(b)). Within the stress groups, the highest hepatic HSP70 mRNA expression was recorded in HS group followed by NS and CS groups.

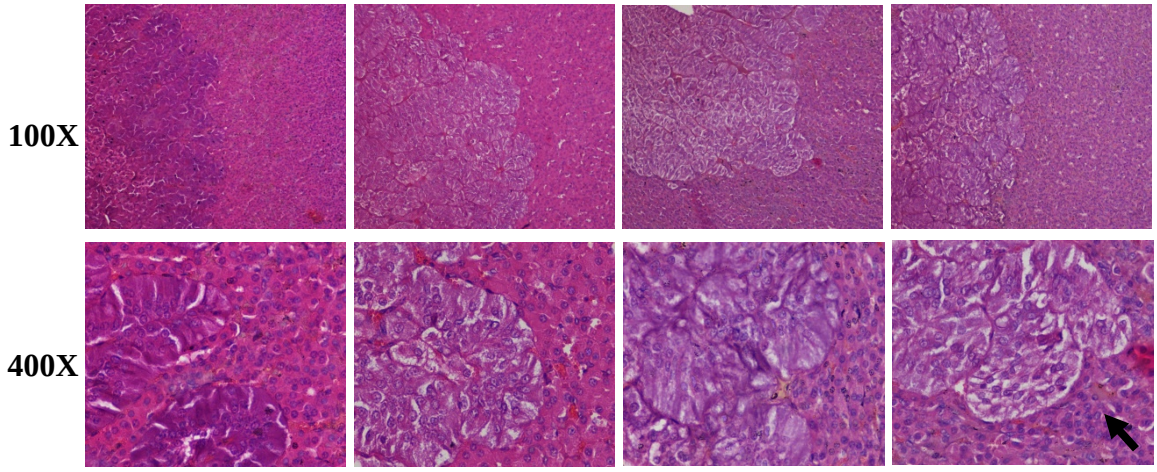


Plate 8: Histopathological changes in H&E stained sections from adrenal glands of C (Control; n=6), HS (Heat Stress; n=6), NS (Nutritional Stress; n=6) and CS (Combined Stresses; n=6) animals subjected to different kinds of stress. The increasing trend in the activity of adrenal endocrine cells was observed in NS, HS and CS compared to C, which is evidence by hypertrophy and increased activity of endocrine cells.

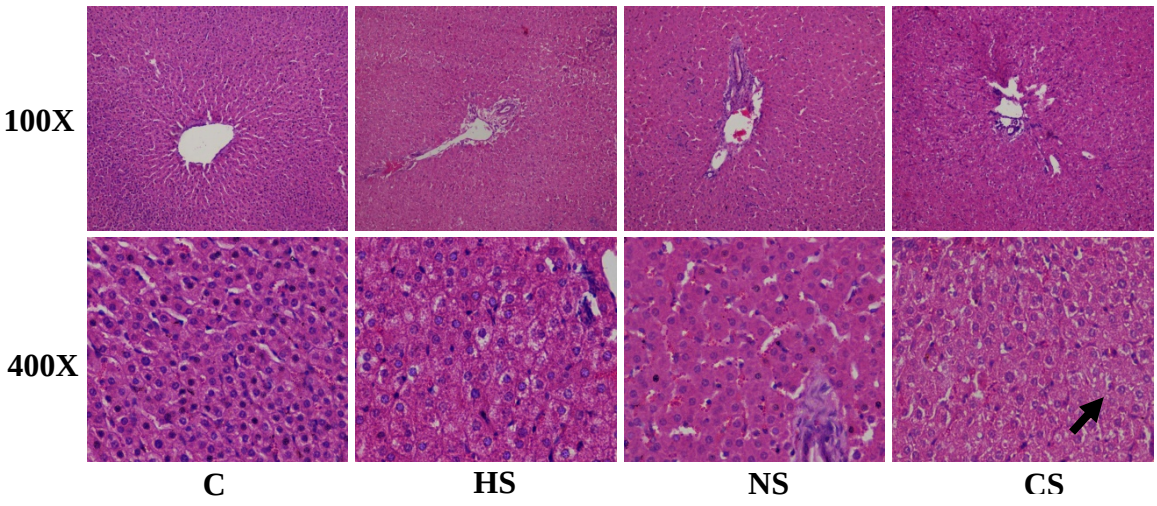


Plate 9: Histopathological changes in H&E stained sections from liver of C (Control; n=6), HS (Heat Stress; n=6), NS (Nutritional Stress; n=6) and CS (Combined Stresses; n=6) animals subjected to different kinds of stress. The hepatocytes revealed mild degenerative changes in CS followed by HS and NS compared to C.

Fig. 9: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on HSP 70 in (a) Adrenal (b) Liver

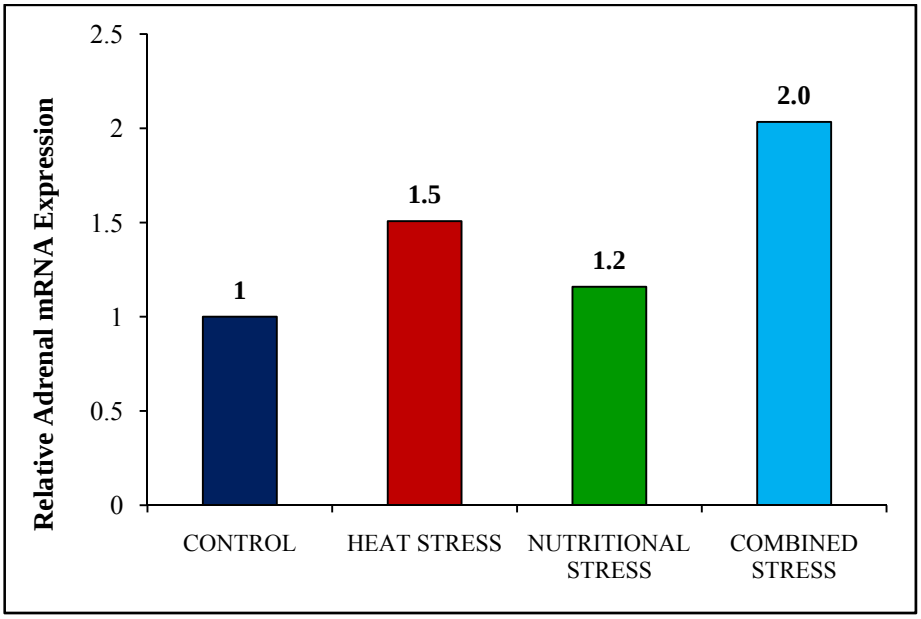


Fig. 9 (a) HSP70 expression in adrenal

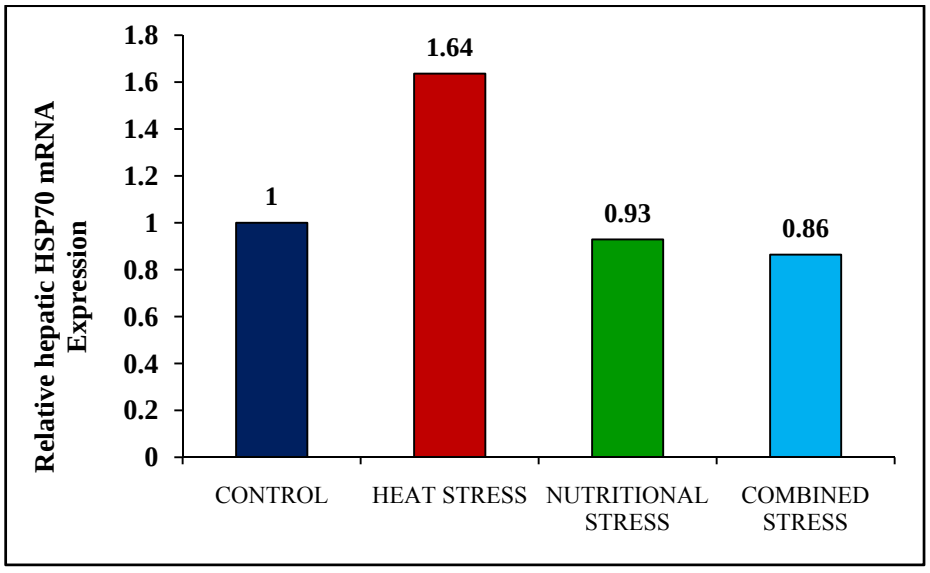


Fig. 9 (b) HSP70 expression in liver

DISCUSSION

CHAPTER 5

DISCUSSION

This experiment is an attempt to establish the cumulative impact of HS and NS in bucks. It is very pertinent to conduct such study as under the changing climatic scenario these stresses do not occur in isolation rather they impact livestock production simultaneously. HS and NS are the important factors which affect the productive and reproductive performance of livestock (Sejian *et al*., 2011). Hence concerted efforts are needed to provide the base line information related to establishing impact of HS and NS simultaneously in livestock. This may pave way for development of goat breed that are genetically adapted to both HS and climate derived nutritional deprivation.

5.1 Behavioural parameters

The reaction of the animal to stressors depends on the duration and intensity of the stressors, the animal's previous experience to the stressors, the physiological status and immediate environmental restraints. An animal may react either by physiological or a behavioural responses, but most often a combination of both (Stull, 1997). The treatments (HS, NS and CS) had significant influence on all behavioural parameter studied except urination frequency. Standing and lying time showed reverse trend between the groups. These behavioural adaptive mechanisms are to prevent additional heat load from ground as well as to facilitate effective heat dissemination. Both standing and lying time of CS group differed significantly with C and HS group. However, both NS and CS groups did not show significant difference between them. Standing can be characterized as a behavior that requires more energy than lying down. Further, standing diminishes the transmission of heat through direct contact with the ground and tends to decrease HS. The significantly lower level of standing time in CS as compared to HS group proved this in goat. This difference could be attributed to the difference in the level of feed intake between

these two groups. This could be the adaptive mechanisms exhibited by HS group to reduce surface contact with the ground and minimize conductive heat transmission to the body. Since CS group is also under severe nutritional deficiency they did not have sufficient energy to support the standing process for a long time. Similar finding was established in Saanen goats in the semi-arid northeast of Brazil (Paulo and Lopes, 2014). This shows the importance of optimum level of nutrition for adaptation in goat. The significant influence of experimental days on both standing and lying time showed that the recording time frequency also influenced these parameters. The highest drinking frequency was recorded in both CS and HS groups as compared to other groups. This shows the severity of HS in these animals. The lowest drinking frequency was recorded in NS group. This value in NS group was significantly lower than both CS and HS groups. This shows HS had more influence in these animals to consume water to get relieved from HS. The significant interaction between treatments and experimental days on drinking frequency shows that these animals are adapting to the existing stressful conditions by altering their drinking frequency. The defecating frequency showed reverse trend to drinking frequency with lowest frequency recorded in CS group. This could be the adaptive mechanism of these animals to conserve the body water. The significant influence of experimental days on defecating frequency indicates that the animals were responding in different way to conserve body water by reducing their defecating frequency throughout the study. The urinating frequency was significantly low in CS group compared to C group. The non-significant difference in urinating frequency of individual stress groups (HS and NS) with C group showed the severity of CS in maintaining the water balance. The reason for reduced urination frequency in CS goats could be due to increased respiratory and cutaneous cooling mechanism which might lead to severe dehydration thereby leading to reducing their urination frequency. Similar finding of reduced urination frequency due to HS in goat was reported by Alam *et al*. (2011). The non-significant influence of interaction between treatment and experimental days on all behavioural parameter except drinking frequency shows that the effect of treatment persisted

on behavioural parameter indicating their significance for adaptation to adverse environmental conditions in the changing climate scenario.

5.2 Water intake

The highest water intake was recorded in both CS and HS group. This showed the severity of HS in these two groups. The increase of water intake in both CS and HS groups might have occurred to compensate for the deficit of body water, which was caused by the increase of evaporation through the respiratory tract and skin surface. Similarly in a study conducted on sheep, Sejian *et al*. (2010a) reported significantly higher water intake when the sheep were subjected to HS and NS simultaneously. This shows that the high water intake, which is utilized to combat HS, may be indicating that restricted fed animals are under more stress (Minka and Ayo, 2009). This finding coincided with the findings of Darcan *et al*. (2008) who reported that the water requirement increased during HS which lead to more frequent drinking. Further, the highly significant influence of experimental days and also the significant interaction between treatments and experimental days on water intake shows that the treatments effect differed at some point while did not differed at some other time point indicating the ability of animals to adapt to different stressful conditions.

5.3 Physiological responses

The ability of an animal to withstand the rigors of climatic stress under warm conditions has been assessed physiologically by means of changes in Tb, RR and PR. Physiological adaptation is defined as a modification in an animal's behavioral or metabolic response, resulting from an experience that improves the ability of the animal to cope with a subsequent challenge (Al-Hidary *et al*., 2012). RR, PR and RT are the parameters which illustrate the mechanism of physiological adaptation. Several researchers studied physiological adaptation mechanisms such as RT, PR and RR in small ruminants (Otoikhian *et al*., 2009; Phulia *et al*., 2010, Sharma *et al*., 2013; Sejian *et al*., 2014a). However, there are no reports on how HS influences physiological responses when the animals were

on NS simultaneously in goats. This study is the first to report the influence of both HS and NS simultaneously on physiological response in goat. The treatments significantly influenced RR both during morning and afternoon. This showed that RR is one of the important physiological parameter to assess the severity of HS, NS and CS (heat and nutritional) in goat. The RR of CS group was significantly lower as compared to HS in morning while in afternoon there was no significant difference in RR between CS and HS group. This shows the adaptive capability of CS group goats as generally the animals which face extreme stress during day time tend to keep themselves cool during early morning hours by altering their metabolic activities (Al-Haidary, 2004; Marai *et al*., 2007). Further, it was observed from the study that RR significantly increased in both HS and CS groups after subjecting to HS as compared to C group. The increase in RR observed in CS goats in afternoon may have more homeostatic relevance for the dissipation of excessive heat and the maintenance of a lower body temperature (Rahardja *et al*., 2011). This shows RR is a very good indicator of measuring the severity of HS in goats. The highly significant increase in RR in afternoon in both HS and CS groups could be attributed to the efforts in maintaining or restoring thermal balance (Al-Haidary, 2004; Kumar *et al*., 2011b). In a similar study conducted on Malpura ewes, Sejian *et al*. (2010c) reported significantly reduced RR in CS group as compared to HS group. However, in the current experiment there were no significant differences in RR between CS and HS groups during afternoon. This showed the superior adaptive capability of goats for thermo-tolerance. In addition, interaction between treatment and experimental days also significantly influenced both RR in morning and afternoon. This shows that the relationship between the groups changed over time for RR indicating perhaps the groups differed in their responses at some time points but not others. This indicates the adaptive capability of Osmanabadi goats to the existing environmental conditions.

The PR reflects primarily the homeostasis of circulation along with the general metabolic status (Sejian *et al*., 2010c). The highest PR was recorded in

HS group while the lowest in NS group. The significantly higher PR in HS as compared to CS group shows the significance of appropriate nutrition for adaptation as HS group goats are under *ad libitum* feeding. This finding of higher PR in HS group coincides with the finding of Okoruwa (2014).This increase was attributable to the increased blood flow from the core to the surface to facilitate the heat loss by sensible and insensible means in goats (Gupta *et al*., 2013; Hooda and upadhyay, 2014). This finding supports the previous reports of McManus *et al*. (2009) and Sejian *et al*. (2010a) for sheep. Similar to RR, PR did not differ between HS and CS groups in morning while PR was significantly different between these groups in the afternoon. This also establishes the adaptive capability of CS group goats as they try to keep themselves cool by reducing their metabolic activity to cope up to increased temperature in the afternoon. The result of decreased PR in the CS group as compared to HS group in the afternoon might be due to a decrease in the metabolic rate as a result of restricted feeding in this group of animals. This view was supported by the findings of several investigators who have reported that there is a correlation between HR and metabolic heat production (Yamamoto and Ogura, 1985; Barkai*et al*., 2002; Popoola *et al*., 2014). Aharoni *et al*. (2003) have suggested that HR decreases during thermal stress as a general effort of the animal to decrease heat production. This reduction could be achieved by the animal either by intake reduction or by activity reduction or both (Al-Haidary, 2004; Rashid *et al*., 2013). Further, the significant interaction between treatment and experimental days on PR only in afternoon shows that the relationship between the groups changed over time for PR indicating perhaps the groups differed in their responses at some time points but not others. This again indicates the adaptive capability of Osmanabadi goats to the existing environmental conditions.

Rectal temperature also has been shown to be good indicators of the thermal stress and may be used to assess the adversity of the thermal environment (Marai *et al*., 2002; Daramola and Adeloye, 2009). Onset and degrees of thermal stress in an animal are best reflected by a rise in RT and rely

heavily on respiratory evaporative cooling mechanisms by rapid and shallow respiration (Marai *et al*., 2002; Daramola and Adeloye, 2009; Sejian *et al*., 2010a). Rectal temperature also showed significant changes between the groups for the treatments both during morning and afternoon. The significantly lower RT in CS group as compared to HS group in morning shows the adaptive capability of CS goats to keep them cool during morning hours to cope up to high environmental temperature during afternoon. This could be due to thermolability mechanism, which leads to passive lowering of RT (Slee *et al*., 1982). By this thermolability mechanism, these bucks were able to vary their core body temperature on a daily basis. This could be the adaptive mechanism exhibited by the bucks of NS and CS groups in order to cope with the water and feed scarcity (Wooden and Walsberg, 2002). This acts as the evidence for the ability of these bucks to adjust their energy expenditure through body temperature regulation. However, in the afternoon the RT increased significantly both in HS and CS groups. Further, the significant interaction between treatment and experimental days on RT both during morning and in afternoon shows that the relationship between the groups changed over time for RT indicating perhaps the groups differed in their responses at some time points but not others. This is again an indication of the adaptive capability of Osmanabadi goats to the existing environmental conditions. Since the interaction between treatment and experimental days influenced both RR and RT during morning and afternoon, it can be inferred that RR and RT can be good reliable indicators of environmental stress in goats.

The ST recorded at head, scrotum and flank are significantly different among the groups only during afternoon but non-significant during morning. The highest skin temperature was recorded in CS group as compared to C, HS and NS groups. This shows the severity of HS in these animals during exposure to solar radiation. In other words this reflects CS group inability to maintain homeostasis as their thermoregulatory mechanisms are compromised. There are few reports suggesting similar HS induced increase in skin temperature in goat (Al-Haidary

et al., 2012; Okoruwa, 2014; Hooda and upadhyay, 2014).They attributed this increase in ST to vasodilatation of skin capillary bed and consequently increase the blood flow to the skin surface to facilitate heat dissipation (McManus *et al*.,2009). ST could also be elevated due to solar radiation, as ST has been shown to be directly related to ambient solar radiation levels (Schutz *et al*., 2011).Although the ST did not differ between groups in morning, experimental days significantly influenced STHM, STSA and STFM. Further, interaction between treatment and experimental days significantly influenced STHA, STSA, STFM and STFA signifying that the relationship between the groups changed over time for these parameters indicating perhaps the groups differed in their responses at some time points but not others.

5.4 Blood biochemical parameters

While blood metabolic profile are helpful in assessing the nutritional status of the ewes (Sejian *et al*., 2010b), the references pertaining to impact of nutrition in combination with HS on blood biochemical parameters in goats are very limited. It is a surprising finding that the treatments or experimental days and interaction between treatment and experimental days did not influence plasma glucose concentration in the study. The reason for this could be the adaptive mechanisms of these native track goats to cope up to different environmental stress by initiating hepatic gluconeogenesis mechanisms to ensure regular glucose supply to maintain vital functions for their survival. However, in a similar study on influence of CS (heat and nutritional) on sheep Sejian *et al*. (2010b) reported significant reduction in plasma glucose level. This shows indigenous goats possess superior adaptive capability to alter their mechanisms to provide regular energy supply for vital body functions. Several researchers have studied the effect of thermal stress on blood glucose concentrations but conflicting results have been reported (More and Sahni 1980; Sejian and Srivastava, 2010).

Total plasma protein differed significantly between the groups. The highest total protein was recorded in NS while lowest being in C, HS and CS groups. The significantly higher level of plasma protein in NS group as compared to HS and CS shows the capacity for stimulating hepatic gluconeogenesis was more in NS group animals. This shows the severity of HS as compared to NS in inducing hepatic gluconeogenesis to cope up to stressful condition. The significantly lower level of plasma total protein in CS as compared to NS groups suggests the severity of CS on total plasma protein concentration. This shows that the additional HS in CS group require additional energy supply for heat dissipation which meets this requirement by favouring hepatic gluconeogenesis. Similar HS induced decrease in plasma protein concentration was reported in goat by other researchers (Helal *et al*., 2010; Gupta *et al*., 2013). In a similar study conducted on sheep, Sejian *et al*. (2010b) concluded that the total plasma protein concentration was significantly lower in CS (heat and nutritional) group. In the current study, this did not happen to strengthen the claim that goats are better adapted to CS than sheep. Further, in a study conducted in the same Malpura sheep, Sejian *et al*. (2014a) reported that NS significantly reduced plasma protein concentration. However, in the current study the level of plasma total protein was significantly higher in NS group. This shows the different capacities of both sheep and goat in inducing hepatic gluconeogenesis. The significant interaction between groups and experimental days on total plasma protein shows that the relationship between the groups changed over time for total protein indicating perhaps the groups differed in their responses at some time points but not others. Treatment did not influence plasma albumin concentration. However, experimental days and interaction between treatment and experimental days highly significantly influenced plasma albumin concentration. This shows that the relationship between the groups changed over time for albumin concentration indicating perhaps the groups differed in their responses at some time points but not others. Similarly NS group showed significantly higher plasma globulin in the study similar to that obtained for total plasma protein. The significantly lower level of globulin in CS as compared to

NS groups suggests the severity of CS on plasma globulin concentration. This shows that the additional HS in CS group require additional energy supply for heat dissipation which favours hepatic gluconeogenesis. Similar HS induced decrease in plasma globulin was reported in goat by other researchers (Helal *et al*., 2010; Gupta *et al*., 2013). Further, the non-significant influence of interaction between treatments and experimental days on plasma globulin concentration showed that the treatment effect persisted on globulin concentration throughout the study. Like plasma total protein, plasma total cholesterol also was significantly higher in NS group as compared to HS and CS groups. This gain shows the severity of HS and the lower capacity of HS and CS group bucks for hepatic gluconeogenesis as compared to NS group. Similar HS induced reduction in plasma cholesterol in goat was reported by Pandey *et al*. (2012).Further, the non-significant influence of interaction between treatments and experimental days on plasma total cholesterol concentration showed that the treatment effect persisted on total cholesterol concentration throughout the study.

Among the stress groups, the highest plasma triglyceride was observed in NS group followed by CS and HS groups. This again shows the severity of HS on plasma triglycerides concentration. Similar HS induced lower triglyceride level in small ruminants was reported by several researchers (Nazifi *et al*., 2003; Pandey *et al*., 2012).Further, significantly higher influence of both experimental days and interaction between treatment and experimental days on plasma triglycerides level shows that the effect of treatment varied between the groups throughout the study indicating the adaptive nature of these animals.

Plasma urea showed significant variation between the groups. Among the stress groups, the plasma urea level was significantly lower in CS groups. However, Srikandakumar *et al*. (2003) and Nazifi *et al*. (2003) reported significantly higher level of plasma urea in sheep during HS condition. The significantly lower urea concentration in CS and NS groups could be attributed to the feed restriction in these groups. Between C and HS groups the plasma urea

did not differed. Das *et al*. (2013) reported no significant effect on blood urea between control and HS groups in buffaloes. However, the significantly lower plasma urea in CS group as compared to NS group shows the animals of CS group exhibits different adaptive mechanisms for feed restriction during HS condition. PUN also showed similar trend to that of plasma urea. The lowest PUN level was recorded in CS group. This level was lower than the NS group level. The significantly lower PUN in CS as compared to NS may be due to HS in CS group. Similar HS induced decrease in PUN level was recorded in cows by Srikandakumar and Johnson (2004). Further, Habeeb *et al*. (1992) stated that during summer HS,PUN decreased significantly and they attributed this decline to the decrease in Dry Matter Intake (DMI), thereby lowering ruminal nitrogen recycling, and causing reabsorption of nitrogen into the rumen from the blood. In addition, Kreikemeier and Mader (2004) also reported changes in PUN concentrations and attributed this to increases in protein synthesis, decreased protein degradation, or a combination of both. The reduced PUN in CS group bucks could be both due to low energy level as well as heat stress in these animals. The reduced PUN in CS group could be due to higher deamination of amino acids. This indicates that the energy levels are low in CS group animals. Thus, when energy levels are low in these animals ultimately lead to high amino acid degradation by glutamate dehydrogenase facilitating energy production from the carbon skeletons derived from amino acids to support the life sustaining activities. Further, Thyroid hormones are the stimulation of protein synthesis and positive nitrogen balance (Nazifi *et al*., 1999). These authors established strong correlation between thyroid hormones and PUN and revealed that Triiodothyronine and Thyroxine had a significant effect on reduction of BUN in heat stress during the summer months in sheep (Nazifi *et al*., 2003). The reduced PUN in CS group bucks could be attributed to reduced plasma thyroid hormones in these animals in an effort to reduce metabolic heat production. Further, both plasma urea and PUN level showed significant variation for the interaction between treatment and experimental days. This shows that the relationship between the groups changed over time for urea and PUN indicating perhaps the

groups differed in their responses at some time points but not others. This again indicates the adaptive capability of Osmanabadi goats to the existing environmental conditions.

5.5 Endocrine parameters

The level of plasma cortisol differed significantly between the groups. The highest plasma cortisol concentration was recorded in CS group as compared to C, HS and NS group. This shows the severity of CS in CS group bucks. This indicates the much severity of physiological strain if bucks are subjected to two stresses simultaneously. The association between HS and increased secretion of cortisol, the principal glucocorticoid hormone in small ruminants is well documented (Ali and Hayder, 2008; Indu *et al*., 2014b). Further, cortisol is the principal stress reliever in ruminant species and hence its level is significantly higher in CS group in order to cope up to the both heat and NS condition. However, in a similar study conducted in Malpura ewes, Sejian *et al*. (2010b) reported significantly lower level of plasma cortisol in CS (heat and nutritional) group as compared to HS group. They identified that the *ad libitum* feeding in HS group ewes provided them the capacity to synthesize more cortisol. But in the current study the level of plasma cortisol was significantly higher in CS group as compared to HS group. This indicates that the CS group goats possessed the inherent ability to synthesize sufficient concentration of cortisol to relieve the HS condition although their nutritional status is compromised. This shows the superior adaptive capability of goats as compared to sheep when they are subjected to more than one stress simultaneously. In a recent study, Abdel-Fattah (2014) established that goat seemed to be more tolerable to higher heat storage and Tb than sheep. Hence plasma cortisol may be considered as an important biological marker for combined stresses in Osmanabadi bucks. Further, the non-significant interaction between treatment and experimental days for plasma cortisol concentration indicated that the treatment effect persisted for the entire

study period. This indicates the significance of cortisol concentration in goat to relieve environmental stresses in the changing climate scenario.

Plasma aldosterone concentration differed significantly only between C and CS groups. However there were no significant differences between the stress groups (HS, NS and CS). There is a general relationship between plasma aldosterone concentration and urine electrolyte excretion and this relationship may be used to assess the electrolyte homeostasis (El-Nouty *et al*., 1980). Aldosterone is produced by the adrenal gland and helps maintain the sodium/potassium balance within the body (Rabinowitz, 1996). Further, Phillips and Santurtun, (2013) reported simultaneous relationship among thermal stress, plasma aldosterone level and urine electrolyte concentration in bovines. Generally during HS, the blood concentration of aldosterone decreases as a result of dehydration leading to electrolyte imbalance and stimulates water intake (Collier *et al*., 1982; Asres and Amha, 2014). However, in our study in both HS and CS groups the level of plasma aldosterone was significantly higher. This difference could be attributed to the *ad libitum* water availability to these bucks indicating the adaptive capability of these bucks to counter HS. As aldosterone concentration increases, more sodium is retained and more potassium is excreted, thereby increasing water re-absorption (Farooq *et al*., 2010).

5.6 Histopathology

The H and E staining results of different organs studied revealed that only adrenal gland and liver sections showed significant changes between the groups but did not influence hypothalamus, pituitary, lung and heart. This shows the active involvement of both adrenal and liver in coping up to adverse conditions during environmental stresses in goat. The highest hyperactivity of endocrine cells was recorded in CS group. This indicates that the adrenal glands of CS group bucks are more active than other groups. This explanation was further justified by the higher level of cortisol in CS group as compared to other groups.

In addition, the higher HSP70 mRNA expression also suggests the hyperactivity of adrenal gland in CS group. Similarly the highest degree of degenerative changes was also recorded in liver of CS group as compared to other groups. This indicates the hepatic damage and burden on the CS group bucks liver to cope up to both heat and nutritional stress simultaneously.

5.7 Adrenal and hepatic HSP70 gene expression

HSP70 is one of the most abundant and best characterized HSP family that consists of highly conserved stress proteins, expressed in response to stress, and play crucial roles in environmental stress tolerance and adaptation in goat (Gupta *et al*., 2013; Banergee *et al*., 2014; Mohanarao *et al*., 2014). The higher expression of HSP70 in adrenal of CS group as compared to other groups could be the adaptive mechanism to counter both the HS and NS. Further, it is an established fact that HSP70 expression increases during HS in goat (Dangi *et al*., 2012; Banergee *et al*., 2014). The significantly higher expression of adrenal HSP70 in CS as compared to HS group could be attributed to additional NS in CS group. This shows that increased HSP70 expression in adrenal of CS group could be both due to restricted feeding as well as due to HS. The higher HSP70 expression in adrenal could also be attributed to the hyper activity of adrenal cortex to synthesize more cortisol as evident from this study.

In the current study, HSP70 messenger ribonucleic acid (mRNA) expression in liver was significantly higher in HS as compared to other groups. Further HSP70 was down regulated in NS and CS groups. There are reports which suggest key role for HSP70 during HS in goat (Banergee *et al*., 2014). Enhanced HSP70 expression in HS group may be a response to stressful environments and may improve cell survival by protecting proteins from degradation and facilitating their refolding (Dangi *et al*., 2014). Further, Dangi *et al*. (2014) reported HSP70, could play an important role during the initial phase of HS acclimation in goats. The hepatic HSP70 down regulation in both NS and

CS could be attributed to the nutrient deficiency in these bucks. The difference in HSP70 expression in CS of adrenal and HS of liver could be attributed to the hyperactivity of adrenal gland to relieve stress in CS group bucks as they are subjected to two stresses simultaneously. This shows that HSP70 might play an important role both in HS and NS tolerance in goats against harsh environmental conditions in the changing climate scenario.

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Heat stress and Nutritional stress are the important factors which affect the health and productive performance of goats. This experiment is an attempt to establish the cumulative impact of HS and NS on adaptive capability of bucks. It is very pertinent to conduct such study as under the changing climatic scenario these stresses do not occur in isolation rather they impact livestock production simultaneously. Hence concerted efforts are needed to provide the base line information related to establishing the impact and adaptive mechanisms of goat to HS and NS simultaneously. With these backgrounds, the proposed study is the first of its kind to study the influence of two environmental stresses simultaneously on the adaptive capability of buck.

Twenty four adult Osmanabadi bucks (average body weight (BW) 16.0 kg) were used in the present study. The bucks were divided into four groups viz., C (n=6; control), HS (n=6; heat stress), NS (n=6; nutritional stress) and CS (n=6; combined stress). The study was conducted for a period of 45 days. The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40 % concentrate (maize 36 kg, wheat bran 37 kg, soya bean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5kg/100 kg of feed). Control and HS bucks had *ad libitum* access to their feed while NS and CS bucks were under restricted feed (30% intake of C bucks) to induce NS. The HS and CS bucks were exposed to solar radiation for six hours a day between 10:00 h to 16:00 h to induce HS. Behavioural parameters, physiological responses, blood biochemical, endocrine responses were recorded at fortnightly interval. After slaughter different organs were collected for HSP70 gene expression and for histopathological observations. The data was analyzed using repeated measures analysis of variance.

The treatments (HS, NS and CS) had significant influence on all behavioural parameter studied except urination frequency. There were significantly ($P<0.01$) higher standing time in *ad libitum* (C and HS) fed groups as compared to restricted fed (NS and CS) groups. Standing and lying time showed reverse trend between the groups. These behavioural adaptive mechanisms are to prevent additional heat load from ground as well as to facilitate effective heat dissemination. The significantly lower level of standing time in CS and NS groups as compared to HS could be attributed to the difference in the level of feed intake between these two groups. However, the highest ($P<0.01$) lying time was recorded in CS group. The highest ($P<0.01$) drinking frequency was also recorded in CS group while the lowest in NS group. The highest ($P<0.01$) defecating frequency was recorded in C group while the lowest in CS group. The highest ($P<0.01$) water intake was recorded in both HS and CS groups. This shows HS had more influence in these animals to consume water to get relieved from HS. All these findings indicated that the CS groups are under severe stressful condition as compared to other groups. The non-significant influence of interaction between treatment and experimental days on all behavioural responses except drinking frequency indicates the stressful conditions persisted throughout the study period.

The animals exhibited different physiological adaptive behaviour in morning and afternoon. The RR, PR and RT were higher ($P<0.01$) in *ad libitum* fed groups in morning while in the afternoon it was higher ($P<0.01$) in both HS and CS groups. Both ST of head and scrotum in afternoon also are significantly ($P<0.01$) higher in CS groups. This shows the severity of CS on the physiological adaptability of Osmanabadi bucks. The significant influence of interaction between treatment and experimental days on majority of physiological responses indicated that the animals were trying to adapt to the different stressors. Since the interaction between treatment and experimental days influenced both RR and RT during morning and afternoon, it can be inferred that RR and RT can be good indicators of environmental stress in goats.

It is a surprising finding that the treatments or experimental days and interaction between treatment and experimental days did not influence plasma glucose concentration in the study. The reason for this could be the adaptive mechanisms of these native track goats to cope up to different environmental stress by initiating hepatic gluconeogenesis mechanisms to ensure regular glucose supply to maintain vital functions for their survival. The highest plasma total protein ($P<0.01$) and total cholesterol ($P<0.05$) was established in NS groups as compared to other groups. This shows the severity of HS as compared to NS in inducing hepatic gluconeogenesis to cope up to stressful condition. The significantly lower level of plasma total protein and plasma globulin in CS as compared to NS groups suggests the severity of CS. This shows that the additional HS in CS group require additional energy supply for heat dissipation which meets this requirement by favouring hepatic gluconeogenesis. Plasma triglycerides, plasma urea and PUN also differed significantly ($P<0.01$) between the groups. The significant interaction between treatment and experimental days on total protein, globulin, triglycerides, urea and PUN shows that the relationship between the groups changed over time for these parameters indicating the adaptive capability of Osmanabadi goats to the existing environmental conditions.

Both plasma cortisol and aldosterone also showed significant variations between the groups. The higher plasma cortisol ($P<0.01$) and aldosterone ($P<0.05$) was recorded in CS group as compared to other groups. This indicates the much severity of physiological strain if bucks are subjected to two stresses simultaneously. Further, the non-significant interaction between treatment and experimental days for plasma cortisol and aldosterone concentration indicated that the treatment effect persisted for the entire study period. This indicates the significance of cortisol concentration in goat to relieve environmental stresses in the changing climate scenario. Hence, plasma cortisol and aldosterone may be considered as an important biological marker for combined stresses in Osmanabadi bucks.

The higher expression of adrenal HSP70 mRNA was reported in CS goats. Within the stress groups, the highest adrenal HSP70 mRNA expression was reported in CS group followed by HS and NS groups. However, the higher expression of hepatic HSP70 mRNA was reported in HS goats. Within the stress groups, the highest hepatic HSP70 mRNA expression was reported in HS group followed by NS and CS groups. The H and E staining results of different organs studied revealed that only liver and adrenal gland sections showed significant changes between the groups. The highest degree of degenerative changes in liver was recorded in CS group followed by HS and NS groups as compared to C group. Similarly the highest hyperactivity of endocrine cells of adrenal was recorded in CS group followed by HS and NS as compared to C group.

The present study reveals that bucks subjected to HS and NS separately had less detrimental effects on buck's adaptive capability. Further, when compared to NS, HS had less significant effect on the adaptive capability in the bucks. This indicates that when nutrition is not a limiting factor then bucks were able to better cope up with HS. However, when both these stresses were coupled in CS group, it had serious consequences on adaptive capability of these bucks. This is evident from the significantly higher behavioural, physiological responses and endocrine responses in CS group. Hence, it is very pertinent to conclude that when two stressors occur simultaneously, the total impact on biological functions necessary to adapt to the stressful conditions may be severe. Further, the study indicated that RR, RT, plasma cortisol and adrenal HSP70 gene may act as ideal biological marker for assessing the impact of CS on adaptive capabilities in bucks. In addition, the study indicated that Osmanabadi bucks possess the capability to adapt to the detrimental effects of environmental stresses which is evident from the significant interaction of treatment and experimental days on majority of the parameters studied. Although NS was established from a quantitative point of view, further detailed study is required to understand the optimum nutritional requirements of bucks to adapt to HS condition.

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IMPACT OF HEAT AND NUTRITIONAL STRESS ON ADAPTIVE CAPABILITY OF BUCKS

by

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to assess the combined effect of heat stress and nutritional restriction on growth and reproductive performances in Osmanabadi Bucks. Twenty four adult Osmanabadi bucks (average body weight (BW) 16.0 kg) were used in the present study. The bucks were divided into four groups viz., C (n=6; control), HS (n=6; heat stress), NS (n=6; nutritional stress) and CS (n=6; combined stress). The study was conducted for a period of 45 days. C and HS bucks had *ad libitum* access to their feed while NS and CS bucks were under restricted feed (30% intake of C bucks) to induce nutritional stress. The HS and CS bucks were exposed to solar radiation for six hours a day between 10:00 h to 16:00 h to induce heat stress. The data was analyzed using repeated measures analysis of variance. There were significantly ($P<0.01$) higher standing time in *ad libitum* (C and HS) fed groups as compared to restricted fed (NS and CS) groups. However, the highest ($P<0.01$) lying time was recorded in CS group. The highest ($P<0.01$) drinking frequency was recorded in CS group while the lowest in NS group. The highest ($P<0.01$) defecating frequency was recorded in C group while the lowest in CS group. The highest ($P<0.01$) water intake was recorded in both HS and CS groups. The animals exhibited different physiological adaptive behaviour in morning and afternoon. The respiration rate (RR), pulse rate (PR) and rectal temperature (RT) were higher ($P<0.01$) in *ad libitum* fed groups in morning while in the afternoon it was higher ($P<0.01$) in both HS and CS groups. Both skin temperature of head and scrotum in afternoon differed significantly ($P<0.01$) between the groups. The highest plasma total protein ($P<0.01$) and total cholesterol ($P<0.05$) was established in NS groups as compared to other groups. Plasma triglycerides, plasma urea and plasma urea nitrogen also differed significantly ($P<0.01$) between the groups. Further, the higher plasma cortisol ($P<0.01$) and aldosterone ($P<0.05$) was recorded in CS group as compared to other

groups. The higher expression of adrenal Heat Shock Protein 70 (HSP70) messenger Ribonucleic acid (mRNA) was reported in CS goats. However, the higher expression of hepatic HSP70 mRNA was reported in HS goats. The highest degree of degenerative changes and hyperactivity of endocrine cells was recorded in CS group liver and adrenal gland respectively. It can be concluded from this study that when two stressors occur simultaneously, they may have severe impact on adaptive capabilities of Osmanabadi bucks as compared to that would occur individually. This is evident from the significantly higher behavioural, physiological responses and endocrine responses in CS group. Further, the study indicated that Osmanabadi bucks possess the capability to adapt to the detrimental effects of environmental stresses which is evident from the significant interaction of treatment and experimental days on majority of the parameters studied.