COMPARATIVE ASSESSMENT OF THE ADAPTIVE CAPACITY OF DIFFERENT INDIGENOUS BREED GOATS TO SUMMER HEAT STRESS BASED ON CHANGES IN PHENOTYPIC TRAITS

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled "**Comparative assessment of the adaptive capacity of different indigenous breed goats to summer heat stress based on changes in phenotypic traits**" 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 beloved guide

and my family....

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SYMBOLS AND ABBREVIATIONS

µl- Micro Litre

A/G- Albumin/ Globulin

AAP- Aminoantipyrine

ACP- Acid Phosphatase

ACTH- Adrenocorticotropico Hormone

ALT- Alanine Aminotransferase

ANOVA- One-Way Analysis Of Variance

AST- Aspartate Aminotransferase

ATP- Adenosine Triphosphate

B- Blank

BCG- Bromocresol Green

BCS- Body Condition Score

CAT- Catalase

CDNA- Complementary DNA

CHE- Cholesterol Esterase

CHOD- Cholesterol Oxidase

Cm- Centimetre

CO_2- Carbon Dioxide

CRH- Corticotropin-Releasing Hormone

Cu^{++}- Copper(II) Ion

DeF- Defecating Frequency

DF- Drinking Frequency

DNA- Deoxyribonucleic Acid

ELISA- Enzyme Linked Immuno Sorbent Assay

ESR- Erythrocyte Sedimentation Rate

FAO- Food and Agriculture Organisation

FFA- Free Fatty Acid

Fig.- Figure

g- Gram

g/dl- Gram/ Decilitre

GAPDH- Glyceraldehyde 3-Phosphate Dehydrogenase

GDP- Gross Domestic Product

GOD-POD- Glucose Oxidase-Peroxidase

GPO- PAP- Glycerol Phosphate Oxidase- Phenol + Aminophenazone

h- Hour

H_2O- Water

H_2O_2- Hydrogen Peroxide

HR- Heart Rate

HRP- Horse Radish Peroxidase

HSF 1- Heat Shock Factor 1

HSP- Heat Shock Protein

IL- Interleukins

IPCC- Intergovernmental Panel on Climate Change

IU- International Unit

kg- Kilogram

km- Kilometre

LT- Lying Time

MAS- Marker Assisted Selection

MC1R- Melanocortin 1 Receptor

MCH- Mean Corpuscular Hemoglobin

MCON- Malabari Control

MCV- Mean Corpuscular Volume

MHS- Malabari Heat Stress

NEBL- Negative Energy Balance

NEFA- Non Estrified Fatty Acid

Nm- Nanometer

O_2- Oxygen

^{O}C – Degree Celsius

OCON- Osmanabadi Control

OHS- Osmanabadi Heat Stress

PBMC- Peripheral Blood Mononuclear Cell

PCR- Polymerase Chain Reaction

PCV- Packed Cell Volume

PMEL- Premelanosome

PR- Pulse Rate

PRA- Pulse Rate Afternoon

PRM- Pulse Rate Morning

PUN- Plasma Urea Nitrogen

RNA- Ribonucleic Acid

ROS- Reactive Oxygen Species

Rpm- Revolutions per Minute

RR- Respiration Rate

RRA- Respiration Rate Afternoon

RRM- Respiration Rate Morning

RT PCR- Real Time Polymerase Chain Reaction

RT- Rectal Temperature

RTA- Rectal Temperature Afternoon

RTM- Rectal Temperature Morning

RuT-Rumination Time

S- Standard

SCON- Salem Black Control

SHS- Salem Black Heat Stress

SNP- Single- Nucleotide Polymorphism

SOD- Superoxide Dismutase

ST- Skin Temperature

ST- Standing Time

STFA- Skin Temperature Flank Afternoon

STFM- Skin Temperature Flank Morning

STHA- Skin Temperature Head Afternoon

STHM- Skin Temperature Head Morning

STSA- Skin Temperature Shoulder Afternoon

STSM- Skin Temperature Shoulder Morning

T3- Tri-Ido Thyronine

T4- Thyroxine

TCZ- Thermal Comfort Zone

THI- Temperature-Humidity Index

TLR- Toll Like Receptor

TNZ- Thermal Neutral Zone

TRIP11- Thyroid Hormone Receptor Interacting Protein 11

UF- Urinating Frequency

UV – Ultraviolet

INTRODUCTION

CHAPTER 1

INTRODUCTION

Climate change is seen as a major threat to livestock production systems globally. The existing literatures clearly specify the foreseen effects of climate change on the stability of the world food security (Stocker, 2014). In fact, the climate change related effects would be mostly seen in countries already having severe hunger and malnutrition and are expected to increase over time (Wheeler and Von Braun, 2013). Potential impacts of climate change including high temperature, shift in rainfall patterns, increased intensity and occurrences of extreme weather events may reduce the global food production by about 10% by 2030 and by more than 20% in 2050 which may ultimately diminish the food availability (Wheeler and Von Braun, 2013).

Livestock rearing serve as a major economic activity in the lives and livelihoods of millions of poor and marginal farmers, particularly in the developing countries (FAO, 2015a). As per recent reports, livestock is considered as one of the fastest emerging dominant agricultural subsectors in the world (Thornton, 2010). However, the productivity of the animal is observed to be conserved in a narrow range of environmental conditions (Baumgard and Rhoads, 2012). Among the various climatic variables, ambient temperature fluctuations are found to be the most intriguing factor affecting livestock production potential (Sejian *et al.,* 2013a). Prolonged exposures of the animals to heat stress condition negatively affects their productive parameters such as growth, milk yield, meat production and reproduction, which in turn results in lower food production and higher economic loss to poor farmers (Rojas-Downing *et al.,* 2017). Considering the significance of livestock to the global food security, it becomes inevitable to improve their production in an effort to meet the global food demands of the future.

Climate change has been projected to be the major factor to negatively influencing the animal production in the coming decades (Rojas-Downing *et al.*, 2017). The livestock in developing countries are more vulnerable to the adverse

effects of heat stress as compared to developed countries primary due to the prevailing extensive rearing system in the former (Stocker, 2014). Approximately, at least 25% reduction in animal productivity is expected in these regions due to global warming (Seguin, 2008). This projected reduction in animal production as a result of heat stress may be attributed to the reduced growth, milk and meat production, feed utilization and performance (Rojas-Downing *et al.,* 2017).

Goat rearing acts as a critical source of income and nutrition for poor and marginal farmers in the rural areas (Mlambo and Mapiye, 2015). Recent statistics clearly established significantly increasing goat population throughout the world particularly in the poor countries (FAO, 2015). Further, goats are also proved to have high thermo-tolerance capacity compared to all other livestock species and are observed to be widely distributed throughout the world even in regions having extreme harsh climatic conditions (Silanikove and Koluman, 2015). Comparatively low body size and relatively low feed and water requirements enable them to thrive well during feed and water scarcity periods. Goats also possess better feed conversion ratio than other ruminants and are able to convert low quality feed into quality protein (Silanikove and Koluman, 2015). Therefore, these various unique characteristics of goat species specifically confirm their extreme potential to be considered as the ideal future animal to reduce the impacts of climate change in animal agriculture.

The vulnerability of the individual animal to the heat stress is clearly influenced by its genetic potential, and variations in efficiency of the adaptive responses determine the adaptability of the animal to the particular adverse environmental conditions (Silanikove and Koluman, 2015). Thermo-tolerant ability of the ruminants in particular established to be clearly varying between breeds evolved in diverged environmental region (da Silva *et al.*, 2017). Indigenous breeds which are produced and developed in the tropical and subtropical regions are proved to have higher adaptive capacity to stressful conditions than the exotic breeds (Habibu *et al.*, 2016). According to Valente *et al.* (2015) tropical breeds are comfortable even at a temperature of 38^0C while crossbreds perform better within

range of 5-25^{0}C. The well adapted breeds show increased tolerance to the climatic stressors by deviating less energy for the adaptation purposes compared to the susceptible breeds (Baumgard and Rhoads, 2013). Hence, genetic selection of adapted breeds is a promising strategy to reduce the impact of climate change in the livestock production.

Genetic merits of the indigenous goat breeds over exotic and crossbreds for adapting to heat stress challenges project them to be the future animals to sustain livestock production. Ample amount of information and research attempts are therefore necessary to select the best indigenous breed which apart from adapting to a particular agro-ecological zone may also produce optimally. Considering the importance of selecting highly adapted livestock breeds for the future, comparative studies on different indigenous breeds to assess their potential to survive and produce optimally may provide valuable information in identifying superior thermo-tolerant breeds specific to a tropical location. Further, such studies also assist in developing important biological markers which can be utilized in breeding programmes using marker assisted selection (MAS) to sustain livestock production in the hot climatic regions.

Performance of the livestock is influenced by several factors including type of production systems, breed, age, sex, nutritional level, hormonal status and environment (Habibu *et al.*, 2016). The agro-ecological zone as described by temperature, rainfall, topography and vegetation is found to be a significant source of variation for animal production (Mpofu *et al.*, 2017). Livestock having superior productive traits may produce poorly when the production environment is not favourable due to negative interaction between their genetic merit and environmental variables (Mpofu *et al.*, 2017). In most of the cases shifting of exotic breeds from their specific agro-ecological zone failed to become a sustainable strategy mainly due to their compromised production levels in the new region. Hence, the identification of agro-ecological zone specific breeds is considered an important strategy to sustain animal production level optimally in the changing climate scenario. Therefore, the present study was conducted to evaluate the effect

of shifting two extremely adapted indigenous breeds in their native tract to another agro-ecological zone and assess their adaptive capabilities in the new locality in comparison to the local breed. For this purpose, Malabari and Salem Black breeds, two breeds well known for their ability to survive in extremely hot and humid environment were shifted to a new locality where the heat stress was of much lower magnitude. Their thermo-tolerance ability was compared to the local Osmanabadi breed well known for its survival in the current experimental location. With this background, the study was conducted with the following objectives:

1. To assess the behavioural and physiological adaptability of different indigenous breed goats subjected to heat stress.
2. To compare differences in blood biochemical and endocrine responses between different indigenous breed goats exposed to heat stress.
3. To determine the differences in correlation between THI index and other phenotype traits between different indigenous breed of goats exposed to heat stress.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Significance of ruminant livestock production

Livestock sector is considered as one of the fastest emerging dominant agricultural subsectors in the world (Thornton, 2010) which supports and sustains livelihood security of 766 million rural poor farmers (FAO, 2015a). Being an economic activity, livestock sector contributes about 36% of the global agricultural gross domestic product (GDP), which is also predicted to have an augmenting trend (range of 50-60%) in the coming decades (Thornton, 2010). Further, the recent projections clearly show a rapid increase in human population from 6.5 billion in 2010 to 8.2 billion by 2020, which increases the demand for livestock and its products (FAO, 2012). Recent diet shifts towards the animal products also made livestock sector as a dominant source of world food economy (FAO, 2012). Apart from their socio-cultural and economic role, animal food products also act as a source of complete protein and help to maintain the balanced nutrition especially in poor rural areas (FAO, 2015a). Globally, 12.9% of the calorie consumption and 27.9% of the protein consumption is met through livestock products; however, the contribution varies largely between developing and developed countries (FAO, 2009).

2.2 Factors influencing livestock production

Livestock reared in a location is influenced by multitude of factors including both physical and physiological aspects of animal's surroundings. Farm animals are known to have range of thermal comfort zone where they are able to produce optimally and that are observed to be varied depending upon various factors such as species type, animal physiological status, relative humidity, air velocity and intensity of solar radiation (Atrian and Shahryar, 2012).

2.2.1 Climate

Geographic location and climate are established to be the most crucial factors significantly affecting both the animal productivity as well as their survival (Walthall *et al.,* 2016). The animals reared in arid and semi-arid regions are observed to be nutritionally deficient in almost all part of the year (Sejian *et al.,* 2010). Additionally, climate of the particular region indirectly influence the pest and disease cycle patterns thereby affects the quality of immune system in the animals making it more susceptible or resistant to disease and stress (Nardone *et al.,* 2010). Temperature, humidity and solar radiation are the important variables severely compromising the overall productive performance like milk, meat and egg production, creating multiple environmental stresses to the ruminants (Sejian *et al.,* 2013b).

2.2.2 Temperature

Within thermal comfort zone (TCZ) range animals try to maintain their body temperature through various physiological adjustments such as sensible heat exchanges, dilation or constriction of blood vessels and regulation of evaporation from lungs and skin etc. (Kingma *et al.,* 2014). Deviation from the TCZ may lead to enhanced physical as well as metabolic thermal regulations (Alameen and Abdelatif, 2012). Hence, variation of temperature either above or below TCZ range may lead to compromised production efficiency and profitability due to deviation of energy for thermoregulatory mechanisms (Baumgard and Rhoads, 2012). However, the net impact of the thermal radiation on an individual animal is also influenced by many other factors like shades, nearby structures and other animals, ground cover, clouds, surface characteristics of the animal, and insulation along with interior surfaces of housing (da Silva, 2017). In fact, ruminant species are observed to be highly vulnerable to hot environments than cold due to higher internal metabolic heat production through the microbial fermentative digestion (Philips, 2016; Garcia *et al.*, 2015).

In arid and semi-arid tropical regions, livestock are often exposed to higher ambient temperatures (Indu *et al.,* 2014). Studies showed a significant negative correlation between ambient temperature and production potential of the animal (Bernabucci *et al.,* 2014). Valente *et al.* (2015) defined thermal neutral zone (TNZ) for lactating dairy cattle ranges from −5◦ to 24◦C (23◦ to 75◦F). Direct effect of the heat stress reduces the production performances and lowers animal's reproduction efficiency (Key and Sneeringer, 2014). Further, reduced feed intake during heat stress condition can cause lower production (milk, meat and wool) as well as growth performances (Das *et al.,* 2016). Recent economic impact study of heat stress conducted in major US livestock industries estimated an annual cost between $1.69 and $2.36 billion, with approximately 40–60% costs arising from dairy sector alone (Key and Sneeringer, 2014). Moreover, projection models of livestock production also revealed highest vulnerability of dairy cattle against heat stress challenges in the coming decades. Mauger *et al.* (2015) projected heat stress to be the largest factor negatively influencing milk production and estimated approximately 6.3% milk production loss in US by 2080.

2.2.3 Relative humidity

The amount of water vapour content in the air influences an animal's thermal equilibrium, particularly in hot environments by determining the rate of evaporative cooling mechanism, thereby the heat stress level (Alejandro *et al.,* 2014). The greater ambient vapour pressure may lead to lower vapour pressure gradient between the skin/respiratory tract and the air resulting in lower rate of evaporative heat transfer (Caulfield *et al.,* 2014). In hot-dry weather evaporative cooling is rapid whereas in the hot-humid weather reduces the ability of the air to absorb moisture thereby enhances the level of heat stress to the animals (Kaliber *et al.,* 2016). Generally, high humid environments are observed to have greater impacts on the heat balance of panting species than sweating (Ward, 2013). In addition, high humidity also enhances the fungal infestations in animals thereby increases their susceptibility to disease (Fisher *et al.,* 2012). However, low humidity also affects the animal health by irritating the mucus membranes (Znamenskaya *et*

al., 2012). Therefore, optimum humidity is very much essential to maintain livestock productivity.

2.2.4 Solar radiation

In addition to elevated ambient temperature, high intensified direct solar radiation also acts as a source of heat to the animals (Da Silva *et al.*, 2017). Exposure of the animals to solar radiation during grazing will further increase the amount of heat load in them. However, the amount of heat generated in animal due to direct solar radiation is influenced by their coat colour and coat type (Da Silva *et al.*, 2017). Coat structures of the animal affects extend of penetration into, and location of absorption or reflection which in turn determines the amount of heat load in them (Sánchez *et al.*, 2016). The animals having white coat colour with hair coat type are observed to be highly resistant to direct solar radiation (Lee *et al.*, 2016).

2.2.5 Air movements

Air movements in the region assist the sensible as well as insensible means of heat transfer (Maia *et al.*, 2008). During higher ambient temperatures, rapid air movements are considered as comfortable for heat stressed animals. However, higher air movements during cold conditions can cause fast removal of heat to the surroundings and resulting in cold stress to the animals (de Melo Costa *et al.*, 2014). Further, increased air circulation is also essential to remove unwanted toxic gases to supply fresh air for breathing.

2.2.6 Genetic factors

Productivity of the animals in the prevailing geographical and climatic condition is determined by their genetic potential for adaptation as well as production (Singh *et al.*, 2016). Recent genome wide analysis studies clearly established the genetic influence on production and functional traits in dairy cattle (Walthall *et al.*, 2016). Production and reproduction efficiency difference between Holstein dairy cows and Brown Swiss cross-bred cows was reported by Endo *et al.*

(2017). The results from their experiment also showed higher conception rate and faster resumed ovarian cyclic activity in Brown Swiss cross-bred cattle while more milk production was observed in Holstein cows. Similarly, high influence of breed differences in cattle's milk quantity and quality was also demonstrated by Penasa *et al.* (2014). The findings from the study clearly proved the superiority of Holstein-Friesian for milk yield compared with both Brown Swiss and Simmental cows (Penasa *et al.*, 2014).

2.2.7 Nutrition factors

Sustained animal production demands optimum supply of nutrients and micronutrients throughout their growth period. Productive parameters like growth, milk and meat production and reproduction are greatly influenced by quantity and quality of the available feed (Xu *et al.*, 2010). Studies proved a strong correlation between the reproductive endocrine performances and nutritional status in various livestock species (Alejandro *et al.*, 2014). Optimum nutrition even assists the animals to cope to the adverse environmental stress condition effectively by ensuring adequate energy supply throughout the stress period (Shaji *et al.*, 2016). However, poor nutrition not only elevates the stress impacts but also compromises the productivity below their genetic potential. Extensively reared livestock solely depends on the rangeland and pasture land for their nutritional requirements (Nardone *et al.*, 2010). However, low feed and water availability during summer exacerbates the livestock production loss and thereby reduces their productive performances below their genetic potential, particularly in arid and semi-arid areas (Nardone *et al.*, 2010), also signifies the importance of nutritional supplementation during summer.

Animals try to cope within limits of the existing variations by changing their dietary intake, metabolism, and heat dissipation, which in turn cause deviation of energy from production to adaptation processes (Lamp *et al.*, 2015). Therefore, sustaining livestock production protecting from adverse climate change

consequences is very crucial to ensure livelihood securities of poor and marginal farmers.

2.3 Climate change and Livestock production

Climate change can have desolating affect on the livestock productivity by imposing greater thermal stress (IPCC, 2007), thereby compromising their growth, milk production and reproductive performances (Nardone *et al.,* 2010). In addition, climate change is also observed to have negative impacts on livestock's input resources- such as altering the feed as well as water availability and productivity and availability of grazing land. The most crucial constraints faced by livestock sector include limited resources such as land and water. Besides, a less recognized impact of the climate change is the varying animal products export demand and international prices in livestock output market (FAO, 2015a). All these factors in combination determine the livestock farmer's vulnerability to the climate change hazards.

Chronic exposures of the livestock to heat stress period hamper their production performances such as milk and meat production (Sejian *et al.,* 2013a). Susceptibility of the animal to heat stress is also depending upon the level of production and the higher producing animals are observed to be more vulnerable to heat stress condition (Garner *et al.,* 2017). A study estimated an annual economic loss of approximately $800 million dollars to the US dairy industry due to severe heat stress (Ziggers, 2012). In addition to heat stress, other factors associated to have negative impacts on livestock productivity are nutritional stress and walking stress (Sejian *et al.,* 2013b). Indirect impacts of heat stress can cause devastating effects on forage availability, quality and grazing land productivity and thereby reduces the feed availability for livestock (Sejian *et al.,* 2012). Scarce feed accessibility of the grazing animals during summer periods forces them to walk longer distances in search of feed. Enhanced energy requirements for the locomotion further demands more energy and cause negative energy balance in them (Sejian *et al.,* 2012).

Further, climate change is also expected to increase the frequency and intensity of extreme events such as heat waves, droughts, floods and which would eventually lead to loss of livestock assets (McKune *et al.,* 2015). Globally, floods and droughts respectively cause around 39% and 44% of crop and livestock production losses with the livestock sector especially influenced by droughts (86% of the impacts) (FAO, 2015a). An evaluation of post-disaster needs assessments in low and middle income countries revealed 36% of the total agriculture economic impact of USD 140 billion during 2003-2013 periods (FAO, 2015a).

Furthermore, the emergence, spread and distribution of livestock diseases are observed to be modified by recent climate change patterns and outcomes (Sejian *et al.,* 2013a). The change in weather parameters such as higher temperature and humidity resulted from climate change may lead to shift in disease distributions thereby increases the susceptibility of the animal populations to disease occurrences (Gale *et al.*, 2009). Changes in temperature and rainfall patterns may lead to incidents and spread of diseases and pests in new regions thereby compromises livestock productivity and which can also cause mortality in extreme cases (Bett *et al.,* 2017). Higher temperature and humidity events due to climate change favour the development and distribution of pathogens and also affect the abundance and distribution of disease vectors (Bett *et al.,* 2017).

2.4 Heat stress impact on livestock production

The global climate is progressing to change at the abnormal rates that are envisaged to be unfamiliar in the recent human history (IPCC, 2007). Livestock exhibits maximum production efficiency within narrow environmental conditions (Tripon *et al.,* 2014). Among various climatic variables, ambient temperature fluctuations are found to be the most intriguing factor impinging livestock production potential (Chauhan and Ghosh, 2014). In addition to ambient temperature, other factors like relative humidity and internal metabolic heat generation for meeting production and maintenance requirements also contributes substantially in determining degree of heat stress in them (Das *et al.,* 2016).

However, the impact of climate change on the livestock production varies depending on the genetic merit of the individual animal to cope up to the stressed condition. It is a widely believed fact that tropical indigenous breeds have better adaptability to the thermal environments. However, they do so by compromising their productive function.

2.5. Impact of heat stress on livestock adaptation

Heat stress critically alters almost all the adaptive mechanisms in the livestock. Behavioural adaptation is recognized as the first and foremost responses adopted by animals to reduce heat load (Shaji *et al.,* 2016). Behaviour of the animals represents their state of wellbeing and hypothalamus is the principal organ which controls the behavioural responses. Significantly increased standing time (ST), decreased lying time (LT), higher water intake (WI), increased shade seeking, decreased feed intake and rumination, lower urinating and defecating frequency are some of the behaviours that ruminants exhibit during heat stress condition (Valente *et al.,* 2015; Shaji *et al.,* 2016). Physiologically ruminants adapt to the heat stress conditions through enhanced respiratory and sweating activities to reduce the amount of heat load in them (Singh *et al.,* 2016; da Silva *et al.,* 2017). Further, significantly increased pulse rate and rectal temperature is also reported in farm animals during summer season (Al-Haidary *et al.,* 2012; Panda *et al.,* 2016). Endocrine alterations observed during heat stress condition include higher corticoids production, which ensures enhanced energy supply to the vital adaptive organs throughout the stress period (Baumgard and Rhoads, 2013). Additionally, lower thyroid hormone synthesis such as T3 and T4 reduces the internal metabolic heat production to cope with external heat load (Sejian *et al.,* 2010; Hooda and Upadhyay, 2014). Further, biochemical response plays a vital role in animals to cope up to the elevated temperature. In several experiments, a significant increased level of packed cell volume and haemoglobin were observed in various livestock species during heat stress condition (Sejian *et al.,* 2013b). In contrast, a decreased concentration of glucose (Rhoads *et al.,* 2009; Wheelock *et al.,* 2010), plasma protein (Hooda and Upadhyay, 2014), and cholesterol (Hooda and Upadhyay, 2014)

were recorded in livestock animals during elevated temperature condition. Further, there are reports which also established the decreased level of non estrified fatty acid (NEFA) (Rhoads *et al.,* 2009, Baumgard and Rhoads, 2013) and an increased concentration of free fatty acid (FFA) in livestock during heat stress condition (Chaiyabutr *et al.,* 2011). The animals also rely on the molecular and cellular responses to cope with heat stress challenges. Increased production of heat shock proteins (HSPs) is the cardinal cellular response of the livestock species for heat stress and these proteins function as an intra-cellular chaperone and prevent the protein and cell damages during stressed condition (Singh *et al.,* 2017).

2.6 Breed differences in livestock adaptation

Adaptive capacity variations between breeds are one of the widely discussed topics in the changing climate scenario. The investigation of the genetic traits for higher adaptive capability in extreme environmental conditions (High temperature, feed scarcity, water scarcity) is a promising strategy to mitigate the climate change impacts on livestock production. Adaptive nature of the animals is generally evaluated by their ability to produce and reproduce optimally in addition to possessing superior ability to survive in harsh climatic condition (Mcmanus *et al.,* 2009). Indigenous breeds evolved and developed in the tropical and subtropical regions are proved to have higher adaptive capacity to stressed conditions than the exotic breeds. The principles of higher adaptations to the extreme conditions of the indigenous breeds could be noticed from three sources of strains: (i) adaptation to high heat and radiation through efficient behavioural and physiological thermoregulation; (ii) adaptation to water constraints by low water requirements and high capacity to withstand severe dehydration, (iii) and adaptation to feed scarcity periods, supported by small body size, low and variable metabolic requirements, efficient digestive capacity and skilful grazing behaviour (Nyamushamba *et al.,* 2017). According to Valente *et al.* (2015) tropical Indian breeds are comfortable even at a temperature of 38^0C while crossbreds perform better within range of 5-25^0C. Tropical cattle breeds possess higher thermo-tolerance through their peculiar thermoregulation characteristics such as low

metabolic heat production and high heat dissipation (Nyamushamba *et al.,* 2017). In addition, indigenous tropical Zebu cattle also show higher resistance to ticks compared to European cattle (Ojong *et al.,* 2016). Low feed and water requirements due to small body size enable them to survive under extreme drought conditions (Nyamushamba *et al.,* 2017). Since these breeds have superior thermo-tolerance, the effects of heat stress on their production parameters (growth, milk and meat) are negligible. However, breeds developed in similar region also revealed alterations in adaptive capability. For example, Holstein cows are established to be more susceptible to heat stress than Jersey cows (Naskar *et al.,* 2014). The tropical sheep breeds are proved to be highly adapted to arid and semi-arid regions with efficient thermoregulatory mechanisms (Sejian *et al.,* 2010). Further, among various livestock species goats are considered as the most adaptive breeds due to their higher ability to survive, produce and reproduce in harsh climatic regions (Silanikove and Koluman, 2015). Goats can even tolerate severe heat stress and water and feed scarcity periods, making them more adaptable to harsh climatic regions, where other ruminants like cattle and sheep succumb to survive (Aziz, 2010).

2.6.1 Morphological adaptation

Morphological traits in livestock are highly important from the adaptation point of view as they directly influence the heat exchange mechanisms (Cutaneous convection, radiation and evaporation) between the animal and surrounding environment (McManus *et al.,* 2009). Morphological adaptive characteristic differences are noticed to be clearly varying between breeds evolved in diverged environmental region (McManus *et al.,* 2009). The light/ white coloured coats in animals are recognized as more advantageous for adapting to tropical regions as it reflects 50-60% of direct solar radiation compared to dark coloured animals (McManus *et al.,* 2009). In cattle, tropical breeds are reported to have more skin pigmentation compared to the exotic. Highly pigmented skin protects the deep tissues from direct short wave ultraviolet radiation by blocking its penetration (McManus *et al.,* 2009). Further, cattle breed with light coloured coat and highly

pigmented skin are characterised to be ideal for hot environments. Additionally, coat length, thickness and hair density also affects the adaptive nature of the animals in tropical region, where short hair, thin skin and less number of hair per unit area is directly linked to higher adaptability to hot conditions (Mahgoub *et al.,* 2010). Indigenous sheep breeds adapted to arid and semi-arid regions possess morphological characteristics such as carpet type wool, seems to provide better protection from direct solar radiation as well as allows effective cutaneous evaporative heat dissipation (Narula *et al.,* 2010; Mahgoub *et al.,* 2010). The fat tail observed in sheep also recognized as a morphological adaptation in indigenous breeds for better heat transfer by chest through external localization of body fat (Gootwine, 2011). Cutaneous evaporation is recognized as the most important mode of heat dissipation in cattle (Jian *et al.,* 2016). Similarly, Carvalho *et al.* (1995) also reported higher sweat gland diameter and volume in Bos indicus cattle than imported Simmental cattle. Moreover, lower sweat gland density was also reported in temperate adapted Sannen goats (Lallo *et al.,* 2012). Comparative study conducted between imported Bos taurus, native Bos taurus, and native Bos indicus cattle clearly established the difference in their morphological adaptation for coping to the tropical environment (Carvalho *et al.,* 1995). Sweat gland perimeter of the indigenous Bos indicus breed (540.5 +/- 19.1 mm) was found to be greater compared to both native (382.0 +/- 27.6 micrograms) as well as imported (497.2 +/- 17.4 micrograms) Simmental cattle, indicating the efficient heat dissipation mechanism in the former than later. Hence, the superior adaptive capability of the tropical indigenous breeds to extreme hot environments is clearly evident from their morphological characteristics like thin and short hair, high skin pigmentation and high number of sweat glands. In addition to all these characteristics indigenous breeds also possess efficient testicular thermoregulatory mechanisms during heat stress condition through higher ratios of testicular artery length and volume to the volume of testicular tissue compared to the crossbreds contributed high susceptibility of later to heat stress challenges (Brito *et al.*, 2004).

2.6.2 Behavioural adaptation

Behaviour of the animals clearly indicates the animal welfare and severity of the external environment. Grazing behaviour of the animals in the scorching sun can be directly interpreted as their extreme adaptive capability to heat stress condition. Tropical indigenous breeds are observed to be highly adapted to direct heat stress, spending more time for grazing than resting in shade. Comparative study conducted between Brahman, Senepol and Holstein heifers showed variations in amount of grazing time with highest value for Brahman followed by Senepol and Holstein respectively. Similar trend was also observed regarding the number of grazing cattle under heat stress condition with 75%, 61% and 59% in Brahman, Senepol and Holstein respectively. Similarly, in a study Carvalho *et al.* (1995) established the lower ability of imported Simmental cattle compared to indigenous cattle to walk long distances in the hot sun shine period during the mid-day. The exotic cattle were not able to complete a drive of 7 km at the temperature and humidity of 30^0C and 60-65% respectively while the indigenous cattle were able to withstand the heat stress conditions without having much effect on their grazing behaviour. The cattle Breed differences in water intake (WI) behaviour was also reported between indigenous Nellore and exotic Angus breeds with higher WI level in Angus breeds, which also indicated higher water requirement of these breeds for restoring their core body temperature in hot environments (Valente *et al.,* 2015). Goat breeds adapted to desert regions compensate higher water loss in heat stress condition through production of highly concentrated urine (Chedid *et al.,* 2014). Behavioural studies conducted in indigenous Osmanabadi bucks showed significantly increased WI and decreased defecating frequency as an adaptive mechanism to conserve body water (Shaji *et al.,* 2016). Additionally, indigenous bucks did not show any significant variation for standing time (ST), lying time (LT), drinking frequency (DF) and urinating frequency (UF), when they were exposed to summer heat stress. All these findings clearly indicate the inherent adaptive nature of the indigenous goat breeds to heat stress challenges as they were able to cope with the adverse conditions without relying on these behavioural variables.

2.6.3 Physiological adaptation

The higher adaptive capacities of the indigenous breeds are also evident from the differences in magnitude of physiological responses during stressed conditions. Generally, it is reported that genetic adaptation of the indigenous breeds allows them to maintain lower respiratory rate (RR) and rectal temperature (RT) compared to exotic breeds under similar environmental conditions (Valente *et al.,* 2015). Significantly increased respiration rate and eye temperature was reported in exotic Angus cattle (104 breaths/minute; 37.7 ^{0}C) compared to Nellore (45.2 breaths/minute; 36.6 ^{0}C) during heat stress exposures (Valente *et al.,* 2015). Similarly, Sahiwal breeds also showed significantly lower values for RR (26.70 beats/min) and RT (38.45 ^{0}C). However, the respiratory cooling mechanisms directly involve dissipation of the extra heat load by vaporizing more moisture to the surroundings (Kumar *et al.,* 2017). Hence, the rapid increase in the respiration frequency in exotic breeds also indicated their grater susceptibility to hot environments. Further, significantly higher heat tolerance coefficient and lower magnitude of thermal indicators (RR and RT) were also reported in Sahiwal breeds compared to Karan Fries in all seasons indicated that Sahiwal breed possess better thermoregulatory mechanisms and was less heat sensitive than crossbreds (Sailo *et al.,* 2017). Leite *et al.* (2017) established grater adaptability of the Morada Nova ewes (acclimatized to Brazilian conditions) to the arid regions. The ewes were able to maintain the normal RR even at an ambient temperature of 32^{0}C. In a comparative study, RT of the Holstein and Jersey cows were found to be higher than the Australian zebu cattle even during winter months and also act as a suggestive indicator for higher metabolic heat production in European breeds (Srikandakumar *et al.,* 2004). In addition to this the greater rate of increase for rectal temperatures in summer also confirms the least capability of the temperate breeds for maintaining normal body temperature reflecting the lower thermal adaptability of these breeds. Daily examination of the indigenous zebu breeds (Gir, Sindhi, Indubrasil) showed lower magnitude for physiological parameters such as RT, heart rate (HR) and RR during afternoon (35.9^{0}C) (Cardoso *et al.,* 2015). Similar to this,

the indigenous sheep breeds reared in the Indian semi-arid regions showed lower physiological variables (RR- 40.02 breaths/min, pulse rate (PR)- 81.8 beats/min, RT- 38.88 ^{0}C) compared to exotic Merino breeds (RR- 107.79 breaths/min, PR- 96.43 beats/min, RT- 39.36 ^{0}C), where the average temperature and humidity during the day was 33.72°C; 54.88% and 30.04°C; 48.55% respectively (Rathwa *et al.,* 2017; Wojtas *et al.,* 2013). The similar results of lower physiological response changes were also reported in Santa Inês and Morada Nova breeds during summer in semi-arid environments of Brazil (e Silva *et al.,* 2016). Likewise, Malpura ewes and Osmanabadi goats evolved in the Indian semi-arid regions showed extreme adaptability to the heat stress of higher magnitude, nutritional stress and combined stress (heat stress+ nutritional stress) conditions (temperature- 40°C; humidity- 55%) by altering their physiological variables (Sejian *et al.,* 2010; Shaji *et al.,* 2016). Both the ewes and goats demonstrated wide range of adaptive responses to overcome both the direct and indirect impacts of heat stress in the tropical regions. Further, the physiological examination of the Girolando cattle produced from the cross breeding between Holstein and Gir showed lower values for RR, heart rate and RT compared to the pure Holstein breed (Dalcin *et al.,* 2016). Similarly, comparative study conducted among crossbred Girolando cows also showed lower RT and RR values in cows with higher Gir grade which again signified the importance of selecting adapted indigenous breeds for cross breeding programmes to improve heat tolerance capacity of exotic breeds (da Costa *et al.,* 2015).

2.6.4 Genetic adaptation

Genetic adaptations infer genetic modifications that make an individual animal more suitable for existence under specific environmental conditions (Maibam *et al.,* 2014). The evidences from various researches suggest the genetic adaptation characteristics of the indigenous breeds to adapt to the hot environments. Characterization of genetic diversity of five African indigenous cattle by sequencing 48 genomes and further comparing with 53 commercial taurine breeds revealed highest genetic diversity among the African zebu and Sanga cattle (Kim *et al.,* 2017). In the same study authors also identified environmental adaptive traits

in zebu breeds including genes/pathways regulating the feeding behaviour, coat colour, horn development, heat tolerance and tick resistance across the African indigenous cattle. Cattle breeds having slick hair genes are observed as genetically more resistant to heat stress challenges and also exhibit higher productivity in hot environments (Olson *et al.,* 2003). Specific genome wide analysis of the Sahiwal cattle detected two SNPs in ATP1B2 gene in Intron 2 including g.2243G>A and g.2366T>C (Verma *et al.,* 2017). The g.2243G>A showed significantly lower values for the physiological variables such as respiration rate and rectal temperature and hence can be also used as a thermoregulatory indicator for selecting heat adapted breeds in hot environments. Likewise, genome wide analysis of the Sahiwal cattle for heat stress responses also quantified 140 and 77 up and down regulated transcripts in them (Mehla *et al.,* 2014). The gene expression alteration observed included activation of HSF-1 (heat shock factor 1), increased expression of HSPs, decreased expression of other proteins, improved immune responses through activation of immune genes also indicated higher genetic tolerance of these breeds for heat stress (Mehla *et al.,* 2014). In addition, Lamb *et al.* (2006) detected 8 SNPs in HSP gene of various cattle breeds and found 5 of them belongs to Brahman ancestary. However, a recent polymorphism study in indigenous sheep breeds (Chokla, Magra, Marwari, and Madras Red) for assessing the association of HSP90 and HSP70 with hemato-physio-biochemical parameters showed lower HSP gene expression in less adapted breed following the order Madras Red < Magra < Chokla < Marwari expression pattern (Singh *et al.,* 2017). A direct positive correlation between the quantity of gene expression and physiological parameters (rectal temperature, skin temperature and thyroid hormone production) indicated higher genetic adaptability of indigenous breeds to heat stress condition. Recent studies have proved approximately 13-17% of the variations in rectal temperature of the cows to genetic variability (Dikmen *et al.,* 2012) while the milk production accounts for 30% of the genetic merits (Pritchard *et al.,* 2013). Additionally, Karan Fries breeds also showed lower Melanocortin 1 receptor (MC1R) and premelanosome (PMEL) (melanogenic genes) expression in lymphocytes and lower plasma tyrosinase activity during heat stress, also signified the inability of

these breeds to protect themselves from UV radiation during summer season (Maibam *et al.*, 2014).

2.6.5 Cellular adaptation

High ambient temperatures activate excess production of free radicals and reactive oxygen species (ROS) in livestock, thereby imbalances their homeostasis (Jin *et al.*, 2016). Disproportion in the production of free radicals and ROS and inefficient antioxidant mechanism may lead to oxidative stress in heat stressed animals (Sheikh *et al.*, 2017). In response, body tissues stimulate excess production of antioxidant enzymes and heat shock proteins as a cellular adaptation to prevent the cellular damage by ROS (Sheikh *et al.*, 2017). Recent comparative studies clearly proved difference in level of cellular adaptation between indigenous and crossbred cattle (Sheikh *et al.*, 2017; Maibam *et al.*, 2017a, 2017b). In vitro cellular heat exposure (39, 41, and 43 °C) of Sahiwal and Holstein Friesian × Sahiwal crossbred showed lower cell count and viability in crossbreds indicating the higher susceptibility of crossbreds to heat stress challenges (Gill *et al.*, 2017). Similarly, in the same experiment, real time quantification of HSF 1 gene also showed an augmenting trend in crossbreds compared to the indigenous under extreme heat stress condition (Gill *et al.*, 2017). Likewise, significantly lower HSP70 expression was reported in indigenous Zebu cattle compared to Karan fries during summer (Maibam *et al.*, 2017a, 2017b). Similarly, lower increase of HSP level during heat stress in indigenous Sahiwal cattle was also observed by Sheikh *et al.* (2017). Lower HSP70 expression also indicates the less severity of heat stress, thereby reflects the higher thermo-tolerance of indigenous cattle breeds to heat stress condition (Sheikh *et al.*, 2017). However, HSP90 showed a reverse trend to that of HSP70 with lower gene expression and protein concentration found in the crossbred cattle (Deb *et al.*, 2014). The authors attributed the higher HSP90 expression to the superior cellular adaptive mechanisms in indigenous breeds to hot environments. In addition, the evaluation of differential expression nature of thermal stress associated microRNAs in crossbred Frieswal cattle revealed changes in expression pattern of approximately 65 miRNAs during peak summer periods (Sengar *et al.*, 2017).

Being greater in expression during heat stress period, the results also indicated higher severity level of hyperthermia in crossbred cattle. Further, the comparative gene expression analysis of Beetal and Assam Hill goats revealed higher HSP60 and HSP70 in Beetal goats during summer season (Hasin *et al.,* 2017). This may be due to the fact that exotic Beetal goats are highly vulnerable to direct solar radiation and thermal stress. Additionally, lower levels of ROS, and antioxidant enzymes superoxide dismutase (SOD), and catalase (CAT) were also seen in Tharparkar and Sahiwal cattle breeds than Karan Fries which also indicated lower status of oxidative stress in indigenous breeds during heat stress condition (Maibam *et al.,* 2017b; Sheikh *et al.,* 2017). Further, apoptosis enzymes such as CASP-3 and CASP-7 were significantly higher in indigenous breeds during summer. Higher abundance of caspases ensures the rapid removal of photo damaged cells indicating the superior skin defensive mechanism in indigenous breeds (Maibam *et al.,* 2017b). The higher expression of toll-like receptor (TLR) TLR2/4 and IL (Interleukins) 2/6 was reported in heat stressed Tharparkar cattle breeds during heat stress condition, which also implicated active immune functions in these breeds to counter heat stress effects (Bharati *et al.,* 2017). Similar results of increased TLR expression was also reported in indigenous Osmanabadi goats on exposure to different environmental stresses such as heat stress, nutritional stress and combined stress conditions. But in this study the magnitude of TLR expression was found to be significantly higher in combined stress groups as compared to the individual stress groups (Sophia *et al.,* 2016). The enhanced TLR expression clearly indicates the active immune system even during the nutritionally stressed condition establishing the extreme adaptive capability and disease resistance of indigenous Osmanabadi goats.

2.6.6 Endocrine adaptation

Hormones, specifically produced from the adrenal and thyroid glands, are recognized to have a significant role in thermoregulation and metabolic adjustments in animals particularly in hot environments. The activation of hypothalamo-pituitary adrenal axis (HPA axis) may lead to enhanced production of

glucocorticoids (cortisol), which act as the primary stress relieving hormone in heat stressed animals. Comparative studies clearly showed significant variation of endocrine responses in ruminants for breed differences (Kumar *et al.,* 2017). In comparison to their crossbreds, native Tharparkar cattle, Chokla sheep and Aardi goats showed lower rate of increase for cortisol production during summer season. Further, tropically adapted Sahiwal and Hariana cows showed lower increase of cortisol production during summer season (Kumar *et al.,* 2017). Relatively lower change in corticoid concentration also indicates reduced stress level in indigenous breeds. Similarly, breed variations was also reported for thyroid gland adaptation in summer. Crossbreds of Chokla sheep showed higher T3 concentration during summer compared to their pure breeds. The higher T3 level also proved the inefficient thyroid gland activity indicating the poor thermo-regulation of the exotic breeds to hot environments. Further, the wider adaptability of the indigenous sheep breeds to different temperature extremes was also proved by Sejian *et al.* (2013a). Malpura breeds, indigenous to the semi-arid tropical regions showed higher thermo-tolerance to the higher temperatures of 40°C and 42°C by altering the endocrine responses such as cortisol and thyroid hormone productions (Sejian *et al.,* 2013a). However, the severity of the stress was found to be higher in 42°C than 40°C, also signifying the vulnerability of even adapted breeds for each degree increase in upper critical temperature on livestock production and survivability.

2.6.7 Metabolic adaptation

The small size of the indigenous breeds is a natural synchronization of the genotype for adapting to the available feed resources in the tropical region (Nyamushamba *et al.,* 2017). The fewer amounts of nutrients required for the maintenance activities enable them to survive in low quality and quantity feed availability during summer in arid and semi-arid areas (Aharoni *et al.,* 2013). Additionally, indigenous ruminants also possess the characteristics of travelling long distances in search of feed and water during scarcity periods (Sejian *et al.,* 2012). Further, the percentage of feed intake reduction during hot conditions was found to be relatively lower in indigenous cattle compared to crossbreds (Valente

et al., 2015). Valente *et al.* (2015) found differences in amount of feed intake between Nellore and Angus breeds with significantly lower reduction in Nellore cattle (average intake -29.1, 29.8 and 30.2 g/kg) compared to Angus breed (average intake- 36.2, 35.4 and 31.6 g/kg) at 25, 29 and 33°C respectively. The relatively lower percentage of reduction in feed consumption of the indigenous breeds shows that the animals were able to maintain the core body temperature without significantly altering the feed intake reflecting their supreme thermo-tolerance capacity to hot environments. Efficient feed digestion and superior nutrient recycling ability and relatively lower locomotion costs helps them to survive in extreme feed scarcity periods compared to temperate breeds. Higher feed conversion efficiency of the indigenous breeds during extreme ambient temperatures is also evident from enhanced rumination time per kg of food consumed. Relatively small size also ensures limited metabolic requirements; thereby lower metabolic heat production; which is considered as one of the most important adaptation traits in the indigenous breeds for adapting to heat stress challenges by lowering the metabolic heat production (Aharoni *et al.,* 2013). Significantly lower metabolic heat production in Sahiwal cattle breeds compared to Karan Fries confers the superior adaptive capacity of indigenous breeds to hot humid environments (Kumar *et al.,* 2017). The results from the study further showed significantly lower methane emission in the Sahiwal breeds maintained in the same feeding regime. The less contribution of the indigenous breeds to methane emission also indicates their significance towards lesser global warming. In addition, the lower methane emission in Zebu cattle also shows their higher feed digestion efficiency deviating more availability of energy for the production processes per unit of dry matter intake. Further, the lower nutrient consumption and availability in the susceptible breeds may lead to negative energy balance in them (Rhoads *et al.,* 2009). The negative energy balance (NEBL) coupled with early post-partum period are reported to be highly vulnerable with the elevated risk of metabolic disorders and health problems in exotic breeds (Baumgard and Rhoads, 2013). Additionally, recent experiments also established heat stress disturbing adipose mobilization in NEBL cows (Baumgard and Rhoads, 2012). Although the

heat stressed Holstein cows were under NEBL, the unusual absence of non-esterified fatty acids (NEFA) clearly shows the altered metabolism in them. The significant reduced levels of NEFA in cows are probably in part explained by higher insulin circulating concentrations during heat stress condition (Wheelock *et al.,* 2010). However, indigenous Sahiwal and Hariana cattle breeds showed significantly higher NEFA concentration during summer season (Kumar *et al.,* 2017). This could be directly attributed as a metabolic adaptive alteration in these breeds to meet the additional energy requirements by initiating the mobilization of the body reserves during heat stress condition.

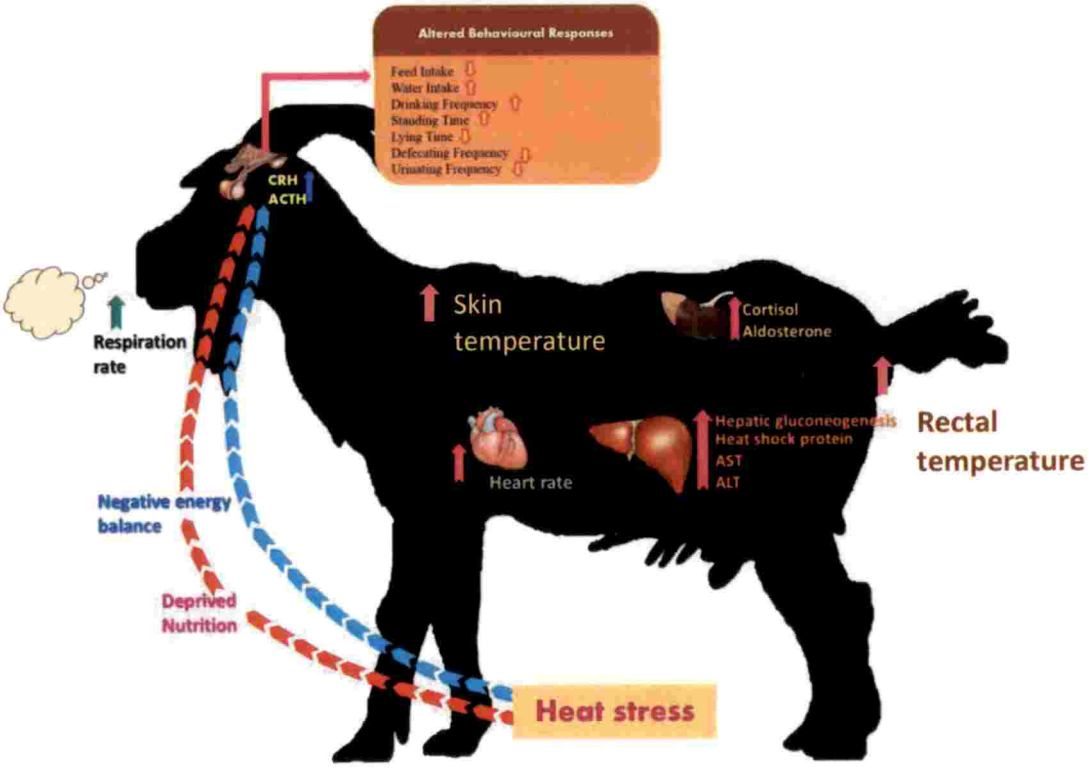
6.8 Blood biochemical adaptation

Generally, biochemical composition of the livestock indicates their health status and wellbeing. Heat stressed animals show variations in their biochemical profile corresponding to the level of the stress experienced. The low thermo-tolerance capacity of the exotic breeds was also reflected by their difference in biochemical adaptation. The PCV and Hb are recognized as good indicators of heat tolerance in sheep (Indu *et al.,* 2014). Significantly higher PCV level was also reported in Sahiwal crossbred compared to the pure breed (Sreedhar *et al.,* 2013). The elevated PCV and haemoglobin values clearly indicate severe dehydration during heat stress reflecting their lower thermo-tolerance. However, haematological profile analysis of Cholistani service bulls, native breeds of Pakistan did not show any seasonal change for PCV, MCV (Mean corpuscular volume) and MCH (Mean corpuscular haemoglobin). This could be directly attributed to higher adaptive capacity of these breeds to seasonal variations without being effected by heat stress during summer. Further, the crossbred cattle and goats also showed significantly lower glucose concentration than the indigenous breeds during summer for a long period of time (Ocak and Guey, 2010). Lower glucose level also represents increased energy demand of the crossbred breeds for the adaptation processes like enhanced respiratory muscular activity gain indicating high severity of heat stress in them (Sreedhar *et al.,* 2013; Indu *et al.,* 2014). However, Shaji *et al.* (2017) found non-significant influence of heat stress on glucose level in indigenous Osmanabadi

goats. This result clearly shows the efficient adaptive nature of the native track goat breeds to hot environments by maintaining regular glucose supply through hepatic gluconeogenesis. In contrast, Sreedhar *et al.* (2013) found significantly higher glucose level in Sahiwal crossbreds during summer. They attributed this to enhanced gluconeogenesis in the crossbred cattle for satisfying the elevated energy demands during stressed condition. Likewise, both creatinine and urea levels were also observed to be significantly higher in Sahiwal crossbreds exposed to heat stress condition (Sreedhar *et al.,* 2013). Higher creatinine levels clearly indicated the lower thermo-tolerance capacity of the crossbred cattle resulting in lower removal of creatinine during heat stress condition. The increased urea level in the crossbreds indicated reduced blood flow to the kidney as a result of redistributed blood flow to the periphery for enhanced heat dissipation to the surroundings. In addition, increased urea level could also have attributed to the compromised digestion process as the heat stress may cause inefficient conversion of rumen ammonia to microbial protein (Wheelock *et al.,* 2010). Further, lower concentration of total protein, cholesterol, albumin and globulin was also exhibited by cross bred goats exposed to heat stress, represented enhanced energy utilization for enhanced adaptation requirements (Ocak and Guey, 2010). The highly stressed goats were trying to meet the additional energy requirements for the adaptive mechanisms by initiating hepatic gluconeogenesis. However, no change in aspartate aminotransferase (AST) concentration was reported in tropical indigenous goats such as Marwari, Chokla and Sirohi breeds exposed to high ambient temperatures (Sharma and Kataria, 2011).

The above literature review clearly indicates the breed differences among the livestock species to cope with harsh environmental conditions and Fig.2.1 represents the overall concept figure of the study. Indigenous breeds specific to a location possess superior ability to adapt and produce optimally. Therefore, research efforts are needed to identify agro-ecological zone specific livestock breeds to sustain livestock production in the changing climate scenario. The differences among the breeds for thermo-tolerance should be established and the

underlying biological mechanisms and particularly the genetic traits those govern these adaptive principles must be identified and used in breeding programs to evolve a breed to with higher thermo-tolerant capability.



AST - Aspartate aminotransferase, ALT- Alanine aminotransferase, CRH- Corticotropin releasing hormone, ACTH- Adrenocorticotropic hormone

Fig. 2.1: Concept figure of the study

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, which is located between approximately 12° 58′N latitude and 77° 38′E, at an altitude of 920 m above mean sea level. The mean annual ambient temperature and relative humidity ranges from 15 to 36°C and 20 to 85 per cent respectively. The annual rainfall in this area ranges from 200 to 970 mm with an erratic distribution throughout the year. The experiment was conducted during the summer season (April-May). The annual minimum and maximum temperature ranges between 15-22 and 27-34 °C respectively. The annual RH ranges between 40-85 per cent. The temperature and RH variations during the study period (April-May) ranged between 26-40 and 28-59 per cent respectively under hot semi-arid environment.

3.2 Animals

Osmanabadi is a dual purpose (meat and milk) hardy goat breed in the semi-arid areas of central tropical India. The breed received its name from its habitat and is observed to be distributed in areas of 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 is predominantly black color (73 per cent) and few individuals are white, brown or spotted. The average body weights of adult male and female animals are 34 kg and 30 kg respectively.

The Salem Black goat breed is an important Indian goat breed originated from north-western part of Tamil Nadu, India. The breed owes its name from its place of origin (Salem) and colour (black). The Salem Black breeds are completely black in colour and found to be distributed in the Salem, Dharmapuri, Krishnagiri, Erode, Karur and Namakkal districts of Tamil Nadu (Jeyakumar *et al.,* 2014). Generally, these goats are tall with a lean body. The ears are observed to be medium-long, leaf

like and semi-pendulous. In adults the body weight ranges between 35-39 kg and 25-30 kg for males and females respectively (Thiruvenkadan *et al.,* 2014).

Malabari (Tellichery) is a native Kerala breed, well known for its high milk yield, excellent growth rate and adaptability to the hot humid conditions prevalent in the state (Alex and Raghavan, 2012). The Malabari breed derives its name from its place of origin, the Malabar region of Kerala state. They are observed to be widely distributed in Calicut, Kannur, Waynad and Malapuram districts of Kerala state in India (Verma *et al.,* 2009). They are medium sized dual purpose breeds reared for both milk and meat with coat colour ranging from white to admixtures and black (Alex *et al.,* 2013). The average body weight of adult males and female animals are 38.96 and 31.12 kg respectively.

The study was conducted in 36 female goats (8-10 months old) equally distributed into Osmanabadi, Malabari and Salem Black breeds weighing between 15-20kg, 10-15kg and 15-20kg respectively. 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 between April-May 2017. Thirty-six animals of 10 months to one-year-old were used in the study. The animals were randomly allocated into six groups of six animals each, OCON (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MCON (n=6; Malabari control), MHS (n=6; Malabari heat stress), SCON (n=6; Salem Black control) and SHS (n=6; Salem Black heat stress). The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40 % concentrate (Maize 36kg, wheat bran 37kg, soybean meal 25kg, mineral mixture 1.5kg, common salt 0.5 kg/ 100kg) as described in table 3.1. The GI, GIII and GV animals were maintained in the shed in thermo-neutral condition while GII, GIV and GVI animals were exposed

outside to summer heat stress between 10:00 h to 16:00 h to expose to heat stress during experimental period. The GI, GIII and GV animals were fed and watered inside the shed while GII, GIV and GVI animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily both inside and outside the shed. The behavioral responses were recorded twice both at the start as well as end of the experiment while the feed intake and WI 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 for estimation of enzymes, biochemical and endocrine parameters. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to heat stress.

3.4 Weather parameters recording

All the weather parameters of both inside as well as outside the shed were recorded twice daily (8:00 h and 14:00 h) for the entire study period. The maximum temperature, minimum temperature, dry bulb temperature, wet bulb temperature was recorded manually using maximum thermometer, minimum thermometer, dry and wet bulb thermometers respectively. Both ambient temperature and relative humidity were recorded by thermo-hygrometer. Photographs showing weather parameters recording are given in plate 3.1.

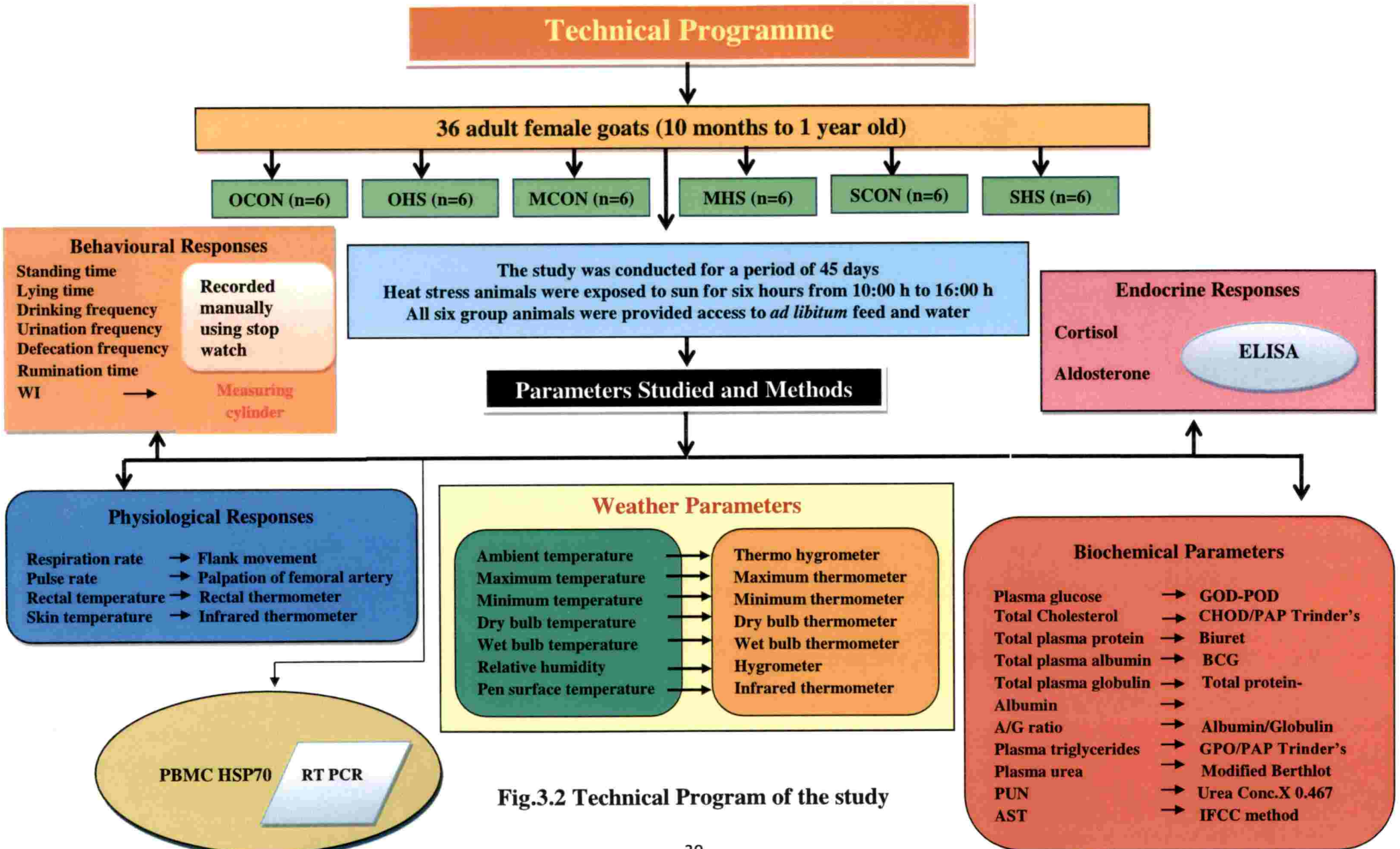


Fig.3.2 Technical Program of the study

ALT-Alanine aminotranferase; AST-Aspartate aminotranferase; BCG-*Bromocresol Green;* CHOD/PAP-Cholesterol oxidase/ Phenol + Aminophenazone; Conc.-Concentration; ELISA- Enzyme Linked Immuno Sorbent Assay; GOD-POD-Glucose oxidase-Peroxidase; GPO-PAP- Glycerol phosphate oxidase- Phenol + Aminophenazone; h-Hour; HSP-Heat Shock Protein; MCON- Malabari control; MHS-Malabari heat stress; n- Number; OCON-Osmanabadi control; OHS-Osmanabadi heat stress; PCR- Polymerase Chain Reaction; PUN- Plasma Urea Nitrogen; SCON- Salem Black control; SHS-Salem Black heat stress

Table 3.1: 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 (%)		
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 (%)		
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 (%)*	72.2	55.0
Digestible energy (kJ/kg) *	13.3	10.1
Metabolizable energy (kJ/kg) *	10.9	8.28

*Calculated values

Plate 3.1: Weather parameters recording (a) Ambient temperature and Relative humidity recording (b) Wet and Dry bulb temperature recording

Plate 3.1 (a) Ambient temperature and Relative humidity recording

Plate 3.1 (b): Wet and Dry bulb temperature recording

3.5 Behavioural responses recording

All the behavioural responses like ST (min), LT (min), drinking frequency (no. of times), defecation frequency (DeF; no. of times), urination frequency (no. of times) and rumination time (RuT; min) were closely observed and recorded for all the six groups for 6 hours (10.00 AM – 4.00 PM), twice both at the start as well as end of the experiment. Photographs showing behavioural responses recording in both inside as well as outside the shed are given in plate 3.2.

3.6 Water intake measuring

Water was measured and offered to the animals and the residues were recorded in every fortnightly interval and using that the WI was calculated for the entire study period. Photograph showing WI recording is given in plate 3.3.

3.7 Physiological responses recording

3.7.1 Respiration Rate

From a distance of 4–5 m, stop watch was used to record the RR by counting flank movements/min, without disturbing the goats. The unit of measurement of RR was in breaths/min. Photograph showing RR recording is given in plate 3.4 (a).

3.7.2 Pulse Rate

The goats were gently restrained for measuring the PR. The PR was recorded by palpating the femoral artery. The unit of measurement of PR was in beats/min. Photograph showing PR recording is given in plate 3.4 (b).

3.7.3 Rectal Temperature

Using a clinical thermometer, the RT was measured by inserting the thermometer inside the rectum for 6–7 cm, inclined towards wall of the rectum. The RT was recorded by gently restraining the goats. The unit of measurement of RT was in °C. Photograph showing RR recording is given in plate 3.4 (c).

Plate 3.2: Behavioural responses recording (a) Inside the shed (b) Outside the shed

Plate 3.2(a): Inside the shed

Plate 3.2(b): Outside the shed

Plate 3.3: WI recording

Plate 3.4: Physiological responses recording (a) Respiration rate (b) Pulse rate (c) Rectal temperature

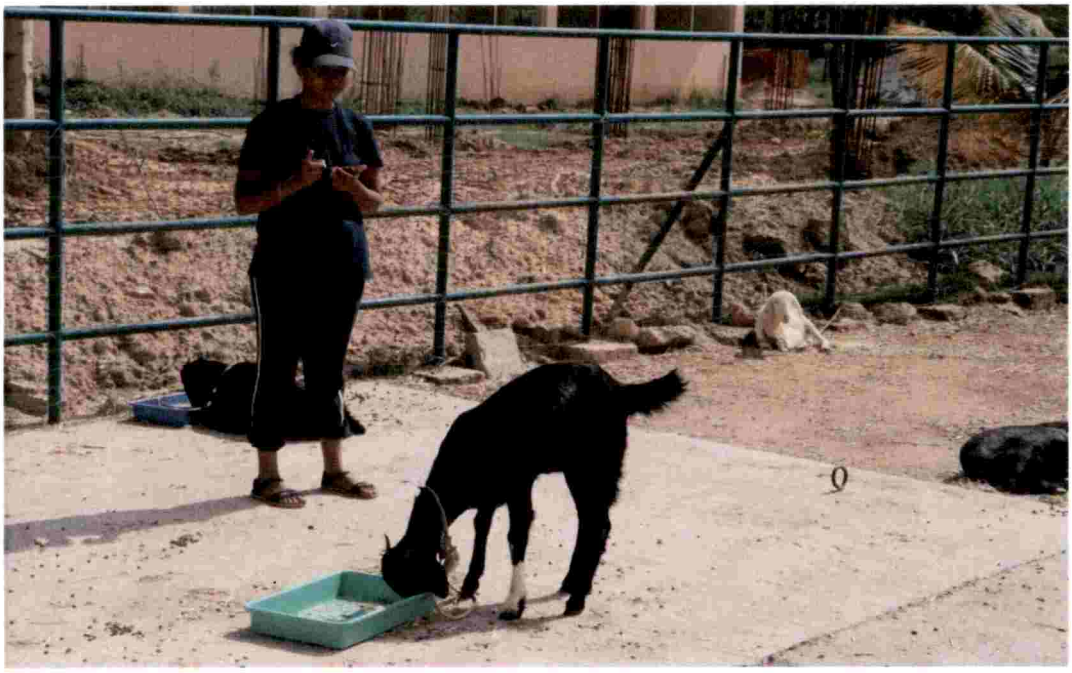


Plate 3.4(a): Respiration rate recording

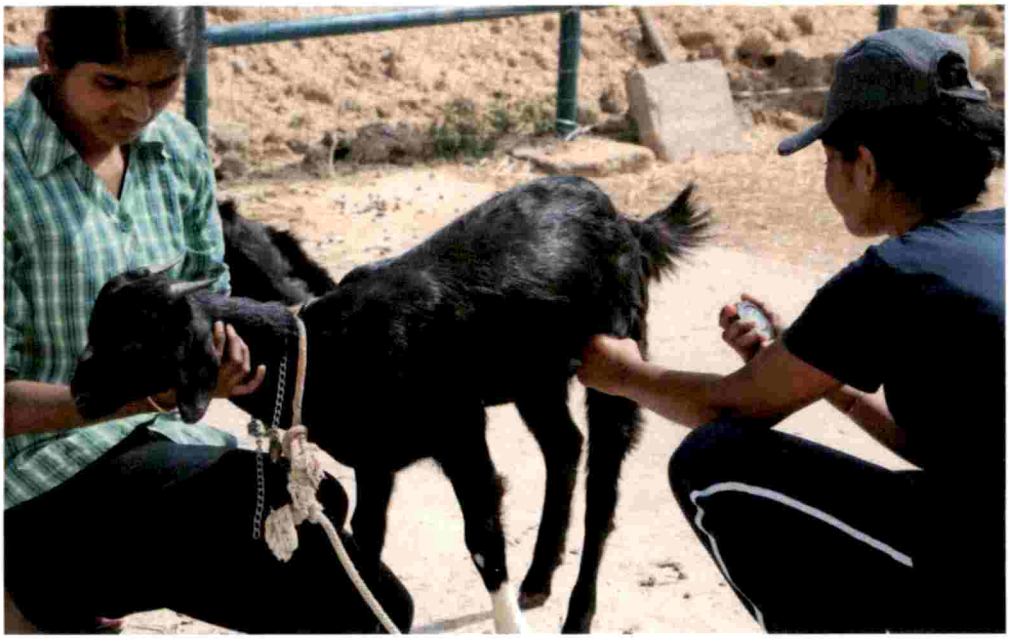


Plate 3.4(b): Pulse rate recording

Plate 3.4(c): Rectal temperature recording

3.7.4 Skin Temperature

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The ST at the head, shoulder and flank were measured using a non- contact infrared thermometer (B.S.K. Technologies, Hyderabad, India). The measurements were taken by pointing the infrared laser at the target site from a distance of 5 to 15 cm. The unit of measurement of skin temperature was in °C. Photographs showing ST recording in various regions are given in plates 3.5.

Plate 3.5: Skin temperature recording (a) Head temperature (b) Shoulder temperature (c) Flank temperature

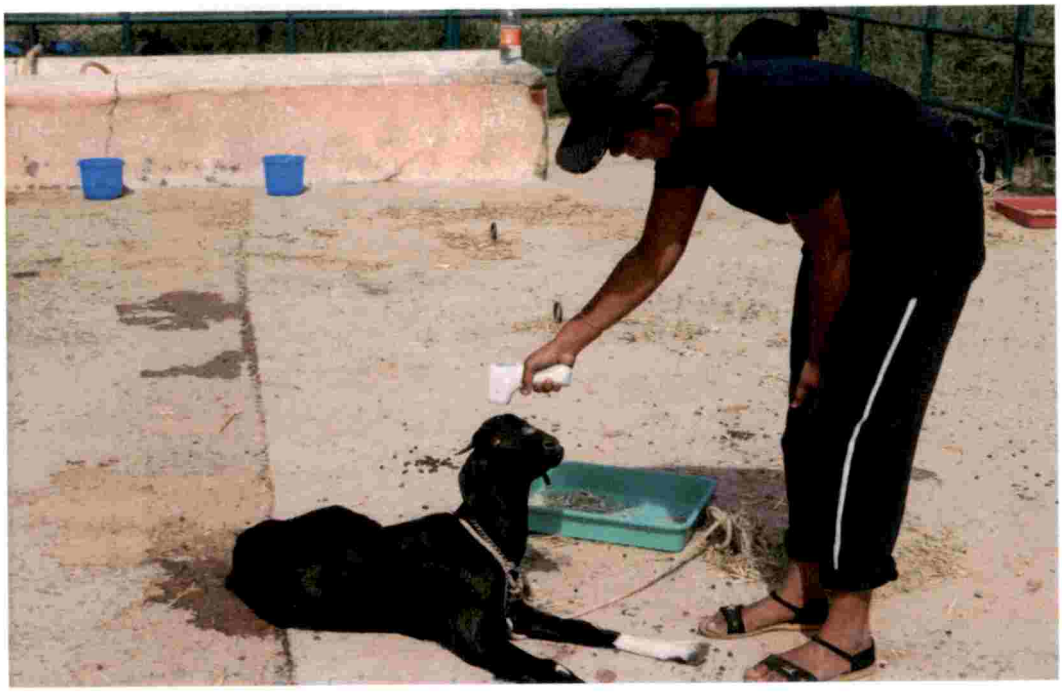


Plate 3.5(a): Head temperature

Plate 3.5(b): Shoulder temperature

Plate 3.5(c): Flank temperature

3.8 Blood collection

Blood samples of 8 ml were collected from the external jugular vein using 20 gauge sterilized needles and a plastic syringe in tubes with heparin anticoagulant (20 IU per ml of blood) (Sisco Research Laboratories pvt. Ltd, Bombay, India) at 11:00 h simultaneously from all the six groups at fortnightly interval.

3.9 Plasma separation

Plasma was separated from the blood using centrifugation technique, involving centrifugation at 3500 revolutions per minute (rpm) at room temperature for 20 minutes. The supernatant straw color plasma was separated using a sterile pasteur pipette and stored in sterilized vials. The plasma was then preserved frozen at −20°C till further analysis. The separated plasma samples were used to estimate all biochemical and endocrine parameters.

3.10 Estimation of biochemical parameters

The biochemical parameters studied were plasma glucose, plasma total protein, plasma albumin, plasma globulin, A/G ratio, plasma total cholesterol, plasma triglycerids, plasma urea, plasma urea nitrogen (PUN), (AST), alanine aminotransferase (ALT).

3.10.1 Plasma glucose

Plasma glucose was estimated by Glucose oxidase-Peroxidase (GOD-POD) method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement of glucose was mg/dL. Plasma glucose was estimated using kit method (Autospan, Surat, 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 colored 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.

Procedure

Blank (B), Standard (S) and Test (T) were pipetted to the labelled test tubes as shown below:

Addition Sequence	Blank	Standard	Test
Serum/ Plasma	-	-	10 µl
Reagent 2	-	10 µl	-
Reagent 1	1000 µl	1000 µl	1000 µl

The solutions were mixed well and incubated at 37 ^{0}C for 10 minutes.

Calculations

$$\text{Serum/ Plasma Glucose (mg/ dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

3.10.2 Total plasma protein

Total protein was estimated by Biuret method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement was g/dL. Total protein was measured using kit method (Asritha Diatech India Private (Pvt.) Limited (Ltd.) Telangana, India).

Assay principle

Cupric ions combine with peptide bonds of proteins and polypeptides containing at least two peptide bonds in the presence of an alkaline medium to produce a blue-violet coloured complex. The absorbance of the complex at 555 nm is directly proportional to the amount of total protein in the sample.

$$\text{Protein} + Cu^{++} \longrightarrow \text{Blue violet coloured complex}$$

Procedure

Blank (B), Standard (S) and Test (T) were pipetted to the labelled test tubes as shown below:

Addition Sequence	Blank	Standard	Test
Biuret Reagent (A1)	1000 µl	1000 µl	1000 µl
Standard (S)	-	10 µl	-
Plasma	-	-	10 µl

The solutions were mixed well and incubated at room temperature for 10 minutes. The absorbance of S and T were measured against B at 555 nm.

Calculations

$$\text{Total protein in g/dL} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 6$$

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 protein was measured using kit method (Asritha Diatech India Private (Pvt.) Limited (Ltd.) Telangana, India).

Assay principle

Albumin specifically binds with BCG in a buffered medium to produce a green coloured complex. The absorbance of the complex at 630 nm is directly proportional to the albumin concentration in the sample.

Albumin + Bromocresol Green ⟶ Green Albumin BCG complex

Procedure

Blank (B), Standard (S) and Test (T) were pipetted to the labelled test tubes as shown below:

Addition Sequence	Blank	Standard	Test
BCG Reagent (A1)	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard (S)	-	10 µl	-
Sample	-	-	10 µl

After mixing, incubation was done for 5 minutes at room temperature. The absorbance of S and T were recorded against B at 630 nm.

Calculations

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

3.10.4 Plasma globulin

The estimated total protein and albumin concentrations were used to calculate the plasma globulin by the following formula:

Globulin in g/ dL = Total plasma protein (in g/ dL) – Plasma albumin (in g/ dL)

3.10.5 Albumin/Globulin Ratio

The Albumin/ Globulin (A/G) ratio was calculated by dividing the concentration of albumin with globulin.

$$\text{A/G Ratio} = \frac{\text{Albumin (in g/ dL)}}{\text{Globulin (in g/ dL)}}$$

3.10.6 Total plasma cholesterol

Plasma cholesterol was estimated by cholesterol oxidase/phenol+ Aminophenazone (CHOD/PAP) trinder's method using bio spectrophotometer

basic (Eppendorf, Hamburg, Germany). The unit of measurement was mg/dL. The total cholesterol was estimated using kit method (Asritha Diatech India Private (Pvt.) Limited (Ltd.) Telangana, India).

Assay principle

Cholesterol esters are hydrolysed to cholesterol by cholesterol esterase. The free cholesterol is oxidised to form cholestenone and hydrogen peroxide (H_2O_2) in the presence of cholesterol oxidase (CHOD). The liberated H_2O_2 further combines with 4 Aminoantipyrine and Phenol by catalytic action of peroxidase (POD) to form red coloured Quenonimine dye complex. The intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterone} + H_2O_2$$

$$H_2O_2 + \text{4 Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Procedure

Blank (B), Standard (S) and Test (T) were pipetted to the labelled test tubes as shown below:

Addition Sequence	**Blank**	**Standard**	**Test**
Cholesterol Reagent (A1)	1000 µl	1000 µl	1000 µl
Standard (S)	-	10 µl	-
Serum/ Plasma	-	-	10 µl

After mixing, incubation was done for 10 minutes at room temperature. The absorbance of S and T were recorded against B at 505 nm.

Calculations

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

3.10.6 Plasma triglycerides

Plasma triglyceride was estimated by Glycerol -3- Phosphate Oxidase - Phenol + Aminophenazone (GPO–PAP) method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement of triglycerides was mg/dL. Plasma triglycerides were estimated using kit method (Asritha Diatech India Pvt. Ltd. Telangana, India).

Principle

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which inturn is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of 4-aminoantipyrine in the presence of Peroxidase (POD) and hydrogen peroxide produces a purple coloured dye. The intensity of the colour formed is directly proportional to the triglycerides concentration in the sample.

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol + Free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol 3 Phosphate + ADP}$$

$$\text{Glycerol 3 Phosphate} \xrightarrow{\text{Glycerol-3-PO}} \text{Dihydroxyacetone Phosphate + } H_2O_2 + O_2$$

Peroxidase

H_2O_2 + 4 Aminoantipyrine ⟶ Purple Quinoneimine dye + H_2O + Phenol

Procedure

Blank (B), Standard (S) and Test (T) were pipetted to the labelled test tubes as shown below:

Addition Sequence	Blank	Standard	Test
Triglyceride Reagent	1000 µl	1000 µl	1000 µl
Triglyceride Standard	-	10 µl	-
Sample	-	-	10 µl

The solutions were mixed well and incubated at room temperature for 15 minutes. The absorbance of the S and T against B was recorded at 546 nm within 60 minutes.

Calculations

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

3.10.7 Plasma Urea

Plasma urea was estimated by Urease-Berthlot method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement of urea was mg/dL. Plasma urea was estimated using kit method (Asritha Diatech India Pvt. Ltd. Telangana, India).

Principle

Urea in plasma is hydrolysed to ammonia in the presence of urease. The produced ammonia reacts with hypochlorite phenolic chromogen to form green cloured complex. The intensity of the colour formed is directly proportional to the amount of urea present in the sample.

Urea + H_2O ⟶ Ammonia + CO_2

Ammonia+ Phenolic chromogen+ Hypochlorite ⟶ Green coloured complex

Procedure

The solutions were pipetted into clean and dry test tubes labelled as B, S and T

Addition Sequence	Blank	Standard	Test
Working enzyme reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Mixed and incubated for 5 minutes at room temperature			
Chromogen Reagent	1000 µl	1000 µl	1000 µl

After mixing, incubation was done for 10 minutes at room temperature. The absorbance of S and T were recorded against B, at 570 nm.

Calculations

$$\text{Urea in mg/ dL} = \frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times 40$$

3.10.7 Plasma urea nitrogen

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

PUN (mg/dL) = Urea concentration (mg/dL) X 0.467

3.10.8 Aspartate aminotransferase

The enzyme AST was estimated using IFCC method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement of AST was IU/L. The AST was estimated using kit method (Asritha Diatech India Pvt. Ltd. Telangana, India).

Principle

The AST catalyzes the transfer of amino group between L-Aspartate and α-Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts

with NADH in the presence of Malate Dehydrogenase (MAD) to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the AST activity in the sample.

$$\text{L-Aspartate acid} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-glutamate}$$

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

Procedure

The solution and sample were pipetted into a clean dry test tube labelled as T

Addition Sequence	**Test**
Working Reagent	1000 µl
Sample	100 µl

The solutions were mixed well and the initial absorbance was recorded at 340 nm exactly after 1 minute (A_0) and the absorbance reading was taken repeatedly after 1, 2 and 3 minutes. The mean absorbance change per minute was calculated (ΔA/min).

Calculations

ALT activity in IU/L at 37°C = ΔA min. x 1746 X tF

Where, tF is the Desired reporting temperature.

3.10.9 Alanine aminotransferase

The enzyme ALT was estimated using Mod. IFCC Method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement of ALT was IU/L. The ALT was estimated using kit method (Asritha Diatech India Pvt. Ltd. Telangana, India).

Principle

The ALT catalyzes the transfer of amino group between LAlanine and a-Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of

oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the ALT activity in the sample.

$$\text{L-Alanine + Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L—Glutamate}$$

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{\text{LDH}} \text{Lactate + NAD}^{+}$$

Procedure

The solution was pipetted into a clean dry test tube labeled as T :

Addition Sequence	**Test (T) 37^{0}C**
Working Reagent	1.0 ml
The solution was incubated at the assay temperature for 1 minute	
Sample	0.1 ml

The solutions were mixed well and the initial absorbance was recorded at 340 nm exactly after 1 minute (A_0) and the absorbance reading was taken repeatedly after 1, 2 and 3 minutes. The mean absorbance change per minute was calculated (ΔA/min).

Calculations

ALT activity in IU/L at 37°C = ΔA min. x 1746

3.11 Estimation of endocrine parameters

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

3.11.1 Plasma Cortisol

Principle

The Cortisol Quantitative Test is based on a widely used immunoassay technique, based on the principle typical competitive binding. A sample (serum/ plasma/urine) containing an unknown amount of Cortisol to be assayed (unlabelled antigen) is added to a standard amount of a Cortisol-horseradish peroxidise conjugate (labelled antigen). The labelled and unlabelled antigens are then allowed

to compete for high affinity binding sites on a limited number of antibodies coated on to the plate. After incubation the unbound conjugate is washed off. The amount of bound peroxidise conjugate is inversely proportional to the concentration of the cortisol in the sample. After washing away, substrate solution is added and the enzyme allowed reacting for a fixed time. 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 colour formed is inversely proportional to the concentration of cortisol in the sample.

Assay Procedure:

Plasma cortisol was estimated using Enzyme Linked Immuno Sorbent Assay (ELISA) method (LDN kit, Nordhorn, Germany). All reagents and specimens must be allowed to reach room temperature before use. All reagents must be mixed without foaming. 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 200µL of the enzyme conjugate was pipetted into each well.
4. Incubation was done on a plate shaker (approximate 200 rpm) for 60 minutes at room temperature.
5. Wells were washed 3 times with prepared wash buffer (400µL/well for each wash) and the plate was struck sharply on absorbent paper to remove residual droplets (by hand 6 times).
6. 100µL of substrate solution was pipetted into each well at timed intervals.
7. The plate was incubated on a plate shaker at room temperature for 15 minutes
8. 100µL of stopping solution was pipetted into each well at the same timed intervals as in step 7.

9. The plate was read on a micro well plate reader at 450 nm within 10 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 Plasma Aldosterone

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme – labelled 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 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 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 Expression of HSP70 mRNA in peripheral blood mononuclear cells

The total RNA was isolated from the PMBC pellet using GeneJET Whole Blood RNA Purification Mini Kit (Thermo Scientific, Lithuania). The total RNA was reverse transcribed into complementary DNA (cDNA) using Maxima first strand cDNA synthesis kit for real-time quantitative polymerase chain reaction (RTqPCR) (Thermo Scientific, Lithuania). Sequences of the primer used for amplifying the target regions of HSP70 and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) have been published by Shaji *et al.* (2016). The primer sequences are described in table 3.2. The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The PBMC HSP 70 mRNA expression was quantified as per the published procedure (Shaji *et al.*, 2016) The GAPDH gene was used as an internal

control, and the relative expression was analyzed using the formula, $2^{-\Delta\Delta CT}$ (Shaji *et al.,* 2016).

3.13 Statistical analysis

The data was analyzed using general linear model (GLM) repeated measurement analysis of variance (SPSS 18.0). Effect of fixed factors namely breeds (Osmanabadi, Malabari, Salem Black) and treatment (OCON, OHS, MCON, MHS, SCON and SHS) 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 breed, treatment and experimental days was analyzed 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). The changes in relative expression of different genes in relation to the reference gene were analysed using SPSS (18.0) software using one-way analysis of variance (ANOVA) with Tukey's post-hoc analysis to compare the means between the groups. The changes in relative expression of PBMC HSP70 mRNA in relation to GAPDH as the house keeping gene were analyzed by ANOVA with Tukey's post hoc analysis to compare the means between the groups. Further, the correlation coefficient between the THI and all phenotypic traits were established by Pearson's correlation coefficient test using SPSS (version 18.0) software. The R^2 values were used to establish the correlation association between THI and various phenotypic traits. Results are shown as mean ± standard error (SE) and the significance level was set at $P<0.05$.

Table 3.2: 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	NM001285703.1
	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

RESULTS

CHAPTER 4

RESULTS

4.1 Weather parameters

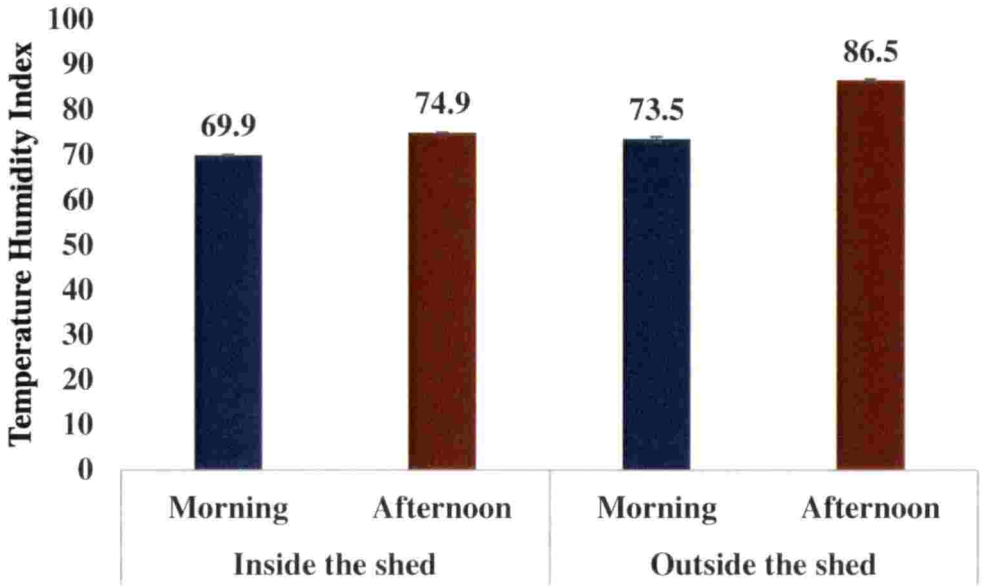
The average meteorological data for the entire study period both inside the shed as well as outside are given in table 4.1. The severity of the heat stress was estimated using both temperature and humidity, termed as THI. The THI outside the shed during both morning and afternoon are described in Fig 4.1. 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.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 °C; T_{wb} = Wet bulb temperature in °C. The obtained THI values indicated 72 and less as comfortable; THI values within the range of 75-78 as stressful and THI above 78 as extreme distress.

Table 4.1: Mean and SEM of weather parameters for the entire study period for both inside and outside the shed

	Time of Recording	DBT (°C)	WBT (°C)	MaxT (°C)	MinT (°C)	RH (%)	AT (°C)	PST (°C)
Inside	Morning (8:00 h)	23.2± 0.11	17.5±0.17	41.5±1.40	22.5±0.92	56.7± .76	26.6±0 .31	25.5±0 .20
	Afternoon (14:00 h)	26.0± 0.16	21.6±0.15	44.6±0.94	24.1±1.02	37.1± 1.62	34.2±0.22	30.6±0.46
Outside	Morning (8:00 h)	24.5± 0.55	21.2±0.30	44.0±1.27	23.0±0.93	58.6± 2.54	28.8±0.61	29.5±0.61
	Afternoon (14:00 h)	34.6± 0.37	29.1±0.43	44.9±0.81	24.4±1.27	29.1± 1.75	39.9±0.63	47.4±0.76

DBT- dry bulb temperature; WBT- wet bulb temperature; MinT- minimum temperature; MaxT- maximum Temperature; RH-relative humidity; AT-Ambient Temperature; PST-Pen Surface Temperature

Fig. 4.1: Average temperature humidity index (THI) for the study period both inside and outside the shed



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 °C; T_{wb} = Wet bulb temperature in °C. The THI values 72 and less are considered comfortable; THI values between 75-78 are considered stressful and THI above 78 considered extreme distress

4.2 Behavioural responses

The effects of heat stress on behavioural responses in Osmanabadi, Malabari and Salem Black goat breeds with the respective graphical representations are presented in table 4.2 and Fig. 4.2 respectively. Both ST and LT did not show any significant variation for both breed and treatment. However, the experimental days significantly influenced both ST ($P<0.01$) and LT ($P<0.05$). Further, interaction between breed, treatment and experimental days for ST and LT were non-significant. The DF also did not show any significant difference for breed variation.

However, DF showed significant (P<0.01) variation for the treatment. Heat stress groups of all the three breeds (OHS, MHS and SHS) showed significantly (P<0.01) higher DF than their control groups (OCON, MCON and SCON). In addition, experimental days significantly (P<0.01) influenced the DF in goats. However, the interaction between breed, treatment and experimental days did not influence the DF. The DeF showed completely different trend than the other behavioural parameters in that it did not differ for breed, treatment, experimental days and their interaction. The UF showed significant (P<0.05) variation for both the breed and treatment effect. But the UF did not differ between the control groups whereas within heat stress groups, SHS showed significantly lower UF than MHS while OHS did not show any significant variation with other breeds. Further, the experimental days also significantly (P<0.01) influenced UF in goats. However, the interaction between breed, treatment and experimental days did not influence the UF. The RuT showed similar trend to UF. The breed (P<0.01), treatment (P<0.01) and experimental days (P<0.01) significantly influenced the RuT in the study. However, these independent factors interaction did not significantly influence the RuT in goats. The effects of heat stress on WI in indigenous goat breeds are given in table 4.3.The WI did not show significant variation for the breed factor. However, WI showed variation for the treatment with significantly higher (P<0.01) value in heat stress groups as compared to their respective control groups. Further, the experimental days significantly (P<0.01) influenced WI in goats, but, the interaction between breed, treatment and experimental days did not influence the WI.

Table 4.2: Effect of heat stress on behavioural responses in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
ST (minutes)	0	140.50	148.33	132.33	132.83	196.00	198.33	NS	NS	**	NS
	45	153.33	244.00	204.33	227.00	191.33	142.83				
	Mean	**146.9^{a}**	**196.2^{a}**	**168.3^{a}**	**179.9^{a}**	**193.7^{a}**	**170.6^{a}**				
	Pooled SE	±16.55	±16.55	±16.55	±16.55	±16.55	±16.55				
LT (minutes)	0	219.50	211.67	227.67	227.17	164.00	161.67	NS	NS	*	NS
	45	206.67	116.00	178.00	133.00	168.67	217.17				
	Mean	**213.1^{a}**	**163.83^{a}**	**202.83^{a}**	**180.08^{a}**	**166.33^{a}**	**189.42^{a}**				
	Pooled SE	±16.85	±16.85	±16.85	±16.85	±16.85	±16.85				
DF (no. of times)	0	2.67	3.17	3.17	3.50	2.83	2.83	NS	**	**	NS
	45	8.67	22.00	9.33	21.00	8.33	18.67				
	Mean	**5.67^{b}**	**12.58^{a}**	**6.25^{b}**	**12.25^{a}**	**5.58^{b}**	**10.75^{a}**				
	Pooled SE	±1.08	±1.08	±1.08	±1.08	±1.08	±1.08				
DeF (no. of times)	0	3.33	3.67	3.17	3.00	3.33	3.33	NS	NS	NS	NS
	45	4.33	1.33	3.33	2.33	4.00	0.67				
	Mean	**3.83^{a}**	**2.50^{a}**	**3.25^{a}**	**2.67^{a}**	**3.67^{a}**	**2.00^{a}**				
	Pooled SE	±0.65	±0.65	±0.65	±0.65	±0.65	±0.65				
UF (no. of times)	0	2.00	2.50	3.33	3.17	1.50	1.67	*	*	**	NS
	45	1.67	1.33	1.00	2.67	0.67	1.33				
	Mean	**1.83^{ab}**	**1.92^{ab}**	**2.17^{ab}**	**2.92^{a}**	**1.08^{b}**	**1.50^{b}**				
	Pooled SE	±0.40	±0.40	±0.40	±0.40	±0.40	±0.40				
RuT (minutes)	0	49.67	41.83	70.67	67.00	42.50	47.33	**	**	**	NS
	45	24.83	0	60.33	29.00	39.17	0				
	Mean	**37.25^{b}**	**20.91^{c}**	**65.50^{a}**	**48.00^{ab}**	**40.83^{b}**	**23.67^{c}**				
	Pooled SE	±8.52	±8.52	±8.52	±8.52	±8.52	±8.52				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; ST- standing time; LT- lying time; DF- drinking frequency; DeF- defecating frequency; UF- urinating frequency; RuT- rumination time, SE- standard error

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

Further among behavioural responses, strong positive (P<0.01) and correlations were established between THI and DF and WI (R^2-0.82**) while a strong negative correlation was established only between THI and DeF (Table 6).

Table 4.3: Effect of heat stress on water intake in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
WI (L/day)	0	1.08	5.00	0.73	3.37	0.77	4.67	NS	**	**	NS
	15	0.87	2.08	0.61	2.27	0.71	2.53				
	30	0.77	3.25	0.78	3.05	0.53	2.53				
	45	0.88	2.47	0.97	2.22	0.87	2.15				
	Mean	**0.90^{c}**	**3.2^{a}**	**0.77^{c}**	**2.73^{b}**	**0.72^{c}**	**2.97^{ab}**				
	Pooled SE	±0.14	±0.14	±0.14	±0.14	±0.14	±0.14				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; WI- WI; SE- standard error

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

Table 4.4: Correlation association between THI and Behavioural responses

	THI	ST	LT	DF	DeF	UF	RuT
THI	1						
ST	0.16	1					
LT	-0.21	-0.95**	1				
DF	0.76**	0.28	-0.31	1			
DeF	-0.50**	-.50**	0.05	-0.35	1		
UF	0.26	0.53**	-0.50**	0.39*	0.17	1	
RuT	-0.44	-0.17	0.17	-0.33	0.22	-0.17	1

THI- Temperature humidity index; ST- standing time; LT- lying time; DF- drinking frequency; DeF- defecating frequency; UF- urinating frequency; RuT- rumination time **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

Figure 4.2: Effect of heat stress on behavioural responses in Osmanabadi, Malabari and Salem Black goat breeds (a) Standing time (b) Lying time (c) Drinking frequency (d) Defecating frequency (e) Urinating frequency (f) Rumination time (g) WI

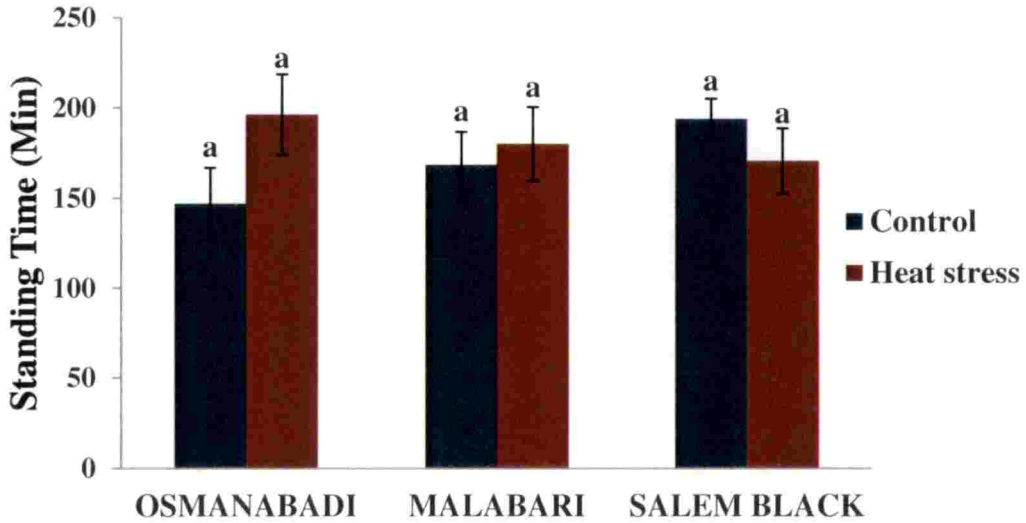


Fig. 4.2(a): Standing time

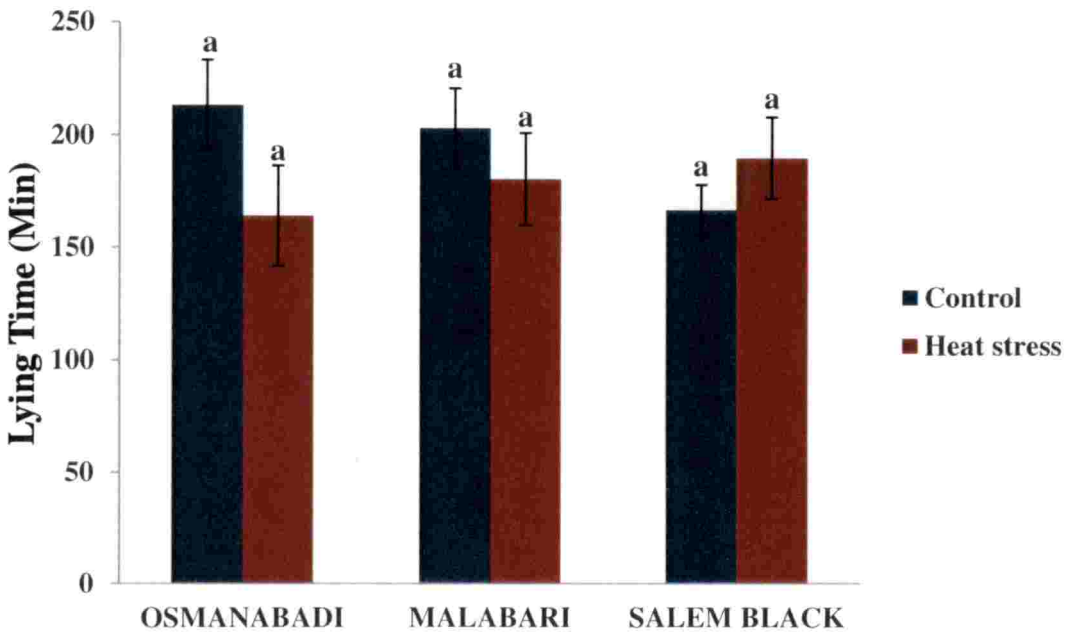


Fig. 4.2(b): Lying time

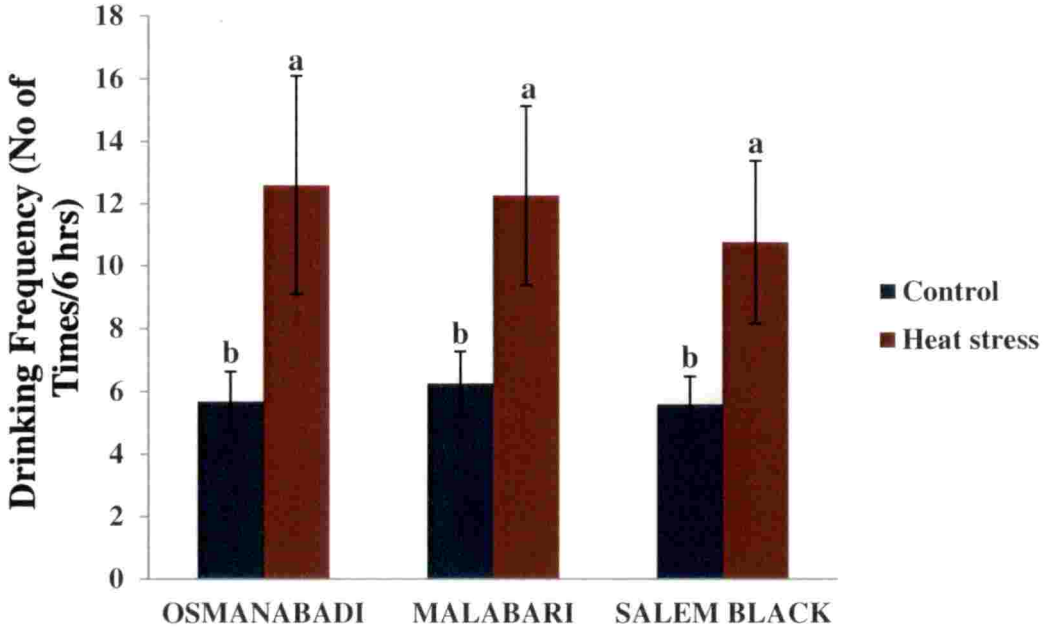


Fig. 4.2(c): Drinking frequency

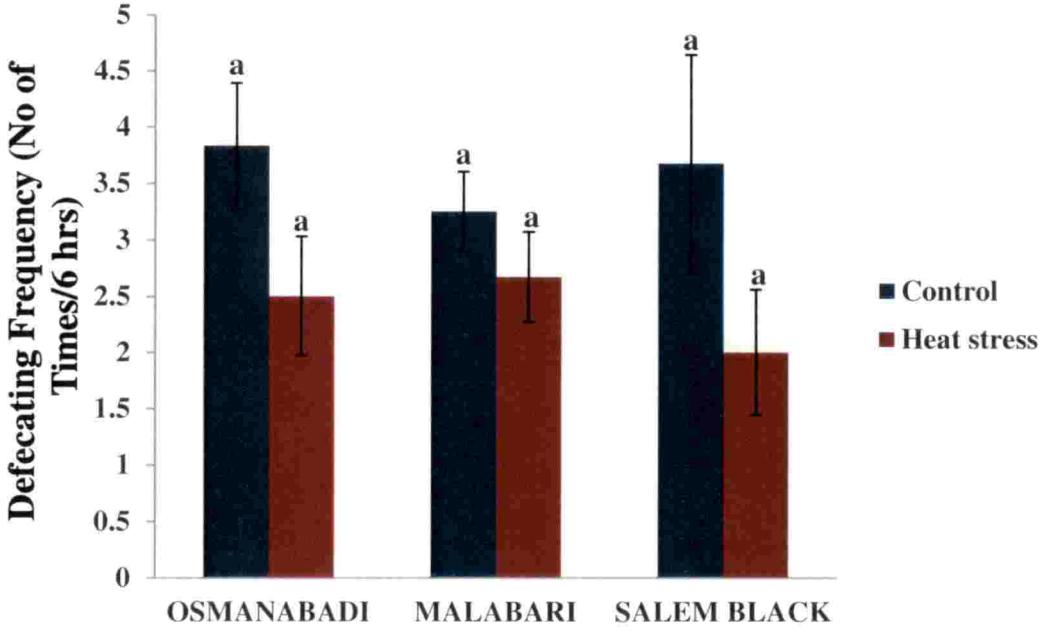


Fig. 4.2(d): Defecating frequency

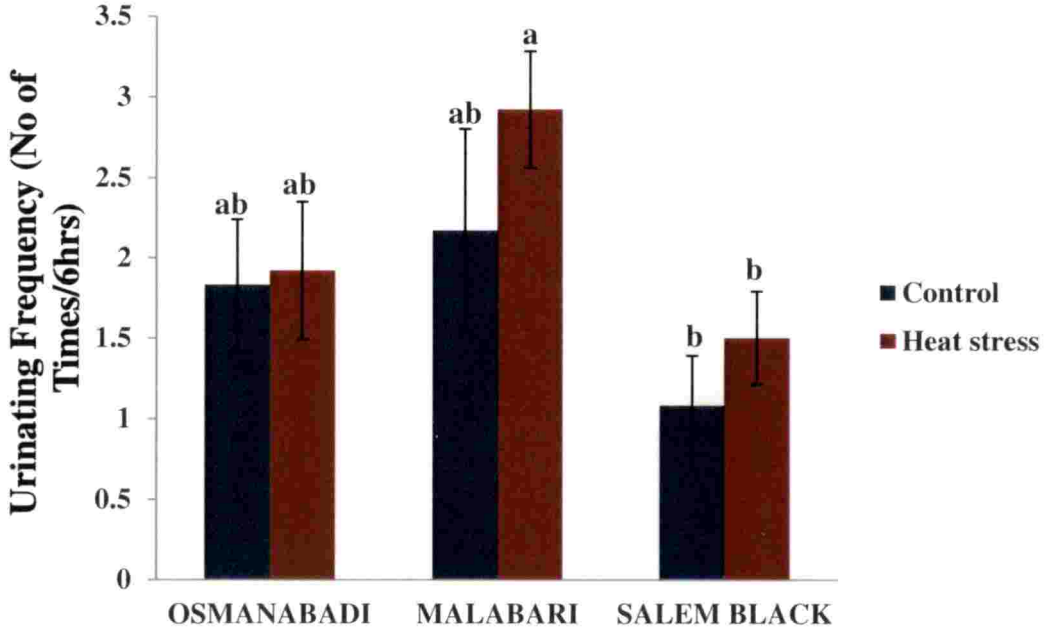


Fig. 4.2(e): Urinating frequency

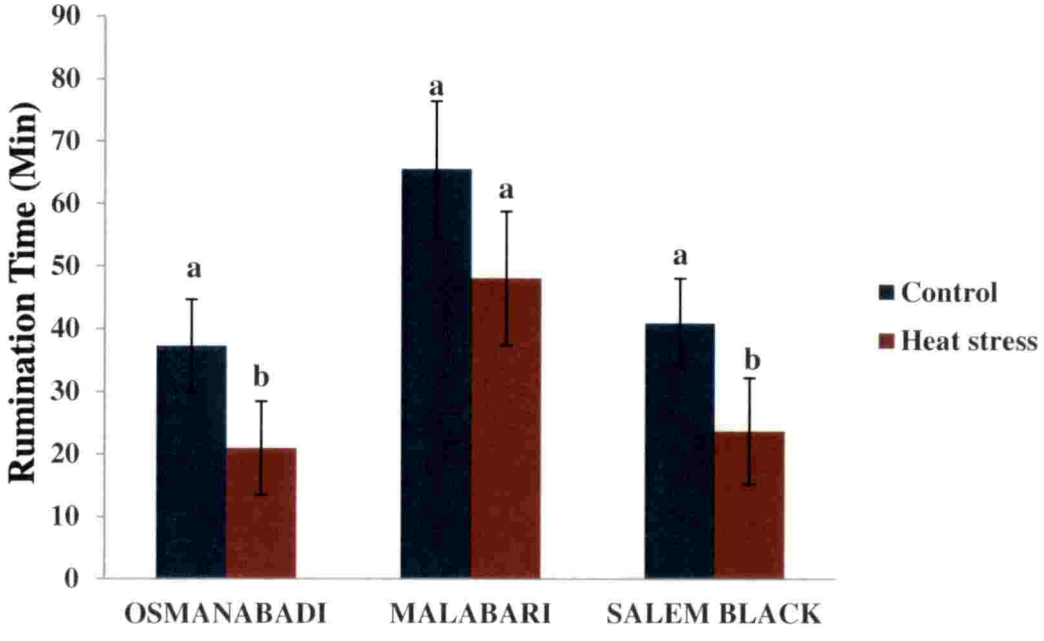


Fig. 4.2(f): Rumination time

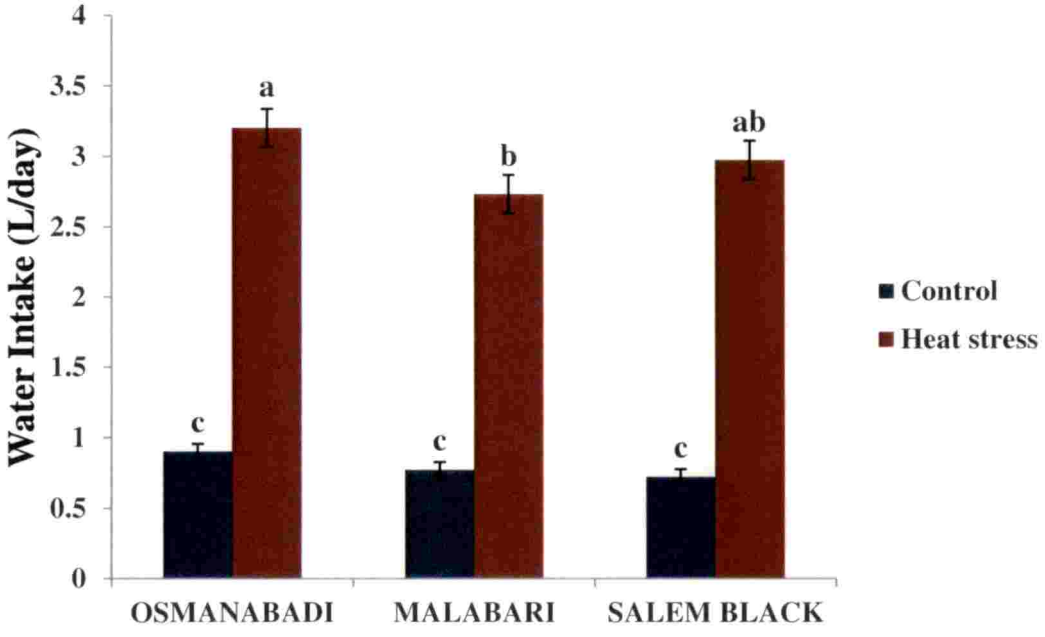


Fig. 4.2(g): Water intake

4.3 Physiological responses in goats

The effects of heat stress on respiration rate, pulse rate and rectal temperature both during morning and afternoon in Osmanabadi, Malabari and Salem Black goat breeds with the respective graphical representations are presented in table 4.5, table 4.6 and Fig. 4.3 respectively. The RRM did not show any significant difference for the breed factor. However, the RRM significantly ($P<0.01$) reduced in heat stress groups compared to their respective control groups for the treatment. The RRM values showed significant variations within control groups with significantly ($P<0.01$) higher value in OCON compared to SCON while within heat stress groups non-significant difference were recorded. In addition, experimental days ($P<0.01$) and interaction ($P<0.05$) between breed, treatment and experimental days significantly influenced RR in goats during morning. The RRA showed significant ($P<0.01$) variation between groups for the breed as well as treatment. The RR during afternoon did not show any significant variation between control groups of all the three breeds. However, RRA values between heat stress groups showed treatment variations. The SHS group showed significantly ($P<0.01$) lower RR compared to both OHS and MHS during afternoon. Further, experimental

days and interaction between breed, treatment and experimental days also had significant ($P<0.01$) influence on RRA. The PRM significantly ($P<0.01$) differed for the breed factor. However, the PRM did not show any significant variation between the groups for the treatment. Additionally, experimental days significantly ($P<0.01$) influenced PRM in all goat breeds. However, the interaction between treatment and experimental days showed non-significant influence on PRM. The PRA showed significant variations for both the breed and treatment. Additionally, experimental days also showed significant ($P<0.01$) influence on PRA value in the study. However, the interaction between breed, treatment and experimental days did not influence the PRA. The RTM also showed similar trend to that of PRA in that the breed ($P<0.01$), treatment ($P<0.05$) and experimental days ($P<0.01$) having significantly influenced the RTM. However, the interaction between breed, treatment and experimental days did not significantly influence the RTM in the study. Rectal temperature afternoon (RTA) showed significant variation for both the breed ($P<0.05$) and treatment ($P<0.01$). Further, both experimental days and interaction between breed, treatment and experimental days did not significantly influence the RTA values in goats.

Table 4.5: Effect of heat stress on physiological responses in Osmanabadi, Malabari and Salem Black goat breeds during morning

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
RRM (breaths/minute)	0	35.75	35.48	35.62	35.98	35.73	35.72	NS	**	**	*
	15	22.33	18.00	23.00	25.00	19.67	21.33				
	30	21.33	17.33	21.00	18.67	18.67	19.50				
	45	24.67	17.33	22.00	19.33	19.67	20.67				
	Mean	**26.02^{a}**	**22.04^{c}**	**25.4^{ab}**	**24.75^{ab}**	**23.43^{bc}**	**24.30^{ab}**				
	Pooled SE	±.671	±.671	±.671	±.671	±.671	±.671				
PRM (beats/minute)	0	51.50	49.33	56.33	59.33	57.67	54.00	**	NS	**	NS
	15	51.50	49.33	56.33	59.33	57.67	54.00				
	30	60.67	54.00	58.00	80.67	60.00	59.67				
	45	55.33	54.00	57.50	70.67	55.67	56.00				
	Mean	**54.75^{bc}**	**51.67^{c}**	**57.04^{bc}**	**67.50^{a}**	**57.75^{b}**	**55.92^{bc}**				
	Pooled SE	±1.84	±1.84	±1.84	±1.84	±1.84	±1.84				
RTM (°C)	0	38.70	38.62	38.63	38.97	38.78	38.43	**	*	**	NS
	15	38.30	38.25	38.58	38.88	38.32	37.78				
	30	37.90	37.62	38.45	38.37	37.97	37.38				
	45	37.97	37.32	38.50	37.92	37.78	37.12				
	Mean	**38.22^{ab}**	**37.95^{bc}**	**38.54^{a}**	**38.53^{a}**	**38.21^{ab}**	**37.68^{c}**				
	Pooled SE	±0.14	±0.14	±0.14	±0.14	±0.14	±0.14				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT, treatment; Breed*TRT* Day, breed treatment and day interaction; RRM, respiration rate morning; PRM, pulse rate morning; RTM, rectal temperature morning, SE, standard error, **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns- Indicates non-significant; Values bearing different superscripts within a row differ significantly with each other

Table 4.6: Effect of heat stress on physiological responses in Osmanabadi, Malabari and Salem Black goat breeds during afternoon

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
RRA (breaths/minute)	0	36.02	35.92	36.32	36.08	36.45	36.33	**	**	**	**
	15	36.50	143.33	34.00	141.33	32.00	106.00				
	30	25.50	144.00	25.50	131.33	23.50	100.00				
	45	28.67	142.00	24.50	131.33	23.33	107.33				
	Mean	**31.67^{c}**	**116.31^{a}**	**30.08^{c}**	**110.02^{a}**	**28.82^{c}**	**87.42^{b}**				
	Pooled SE	±2.31	±2.31	±2.31	±2.31	±2.31	±2.31				
	Pooled SE	±1.84	±1.84	±1.84	±1.84	±1.84	±1.84				
PRA (beats/minute)	0	69.33	67.33	76.00	73.00	69.33	65.33	**	**	**	NS
	15	69.33	67.33	76.00	73.00	69.33	65.33				
	30	72.67	80.00	73.83	96.00	68.67	77.33				
	45	65.67	84.00	67.50	95.33	65.83	72.67				
	Mean	**69.25^{bc}**	**74.67^{b}**	**73.33^{bc}**	**84.33^{a}**	**68.29^{c}**	**70.17^{bc}**				
	Pooled SE	±1.88	±1.88	±1.88	±1.88	±1.88	±1.88				
RTA (°C)	0	39.10	39.43	39.47	39.98	39.53	39.27	*	**	NS	NS
	15	39.33	40.43	39.58	40.68	39.67	40.12				
	30	39.32	40.33	39.40	40.50	39.02	40.18				
	45	39.15	40.02	39.18	40.25	39.10	39.95				
	Mean	**39.23^{c}**	**40.05^{ab}**	**39.41^{c}**	**40.35^{a}**	**39.33^{c}**	**39.88^{b}**				
	Pooled SE	±0.11	±0.11	±0.11	±0.11	±0.11	±0.11				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT, treatment; Breed*TRT* Day, breed treatment and day interaction; RRA, respiration rate afternoon; PRA- pulse rate afternoon; RTA- rectal temperature afternoon; SE- standard error, **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns- Indicates non-significant; Values bearing different superscripts within a row differ significantly with each other

Figure 4.3: Effect of heat stress on physiological responses in Osmanabadi, Malabari and Salem Black goat breeds (a) Respiration rate morning (b) Respiration rate afternoon (c) Pulse rate morning (d) Pulse rate afternoon (e) Rectal temperature morning (f) Rectal temperature afternoon

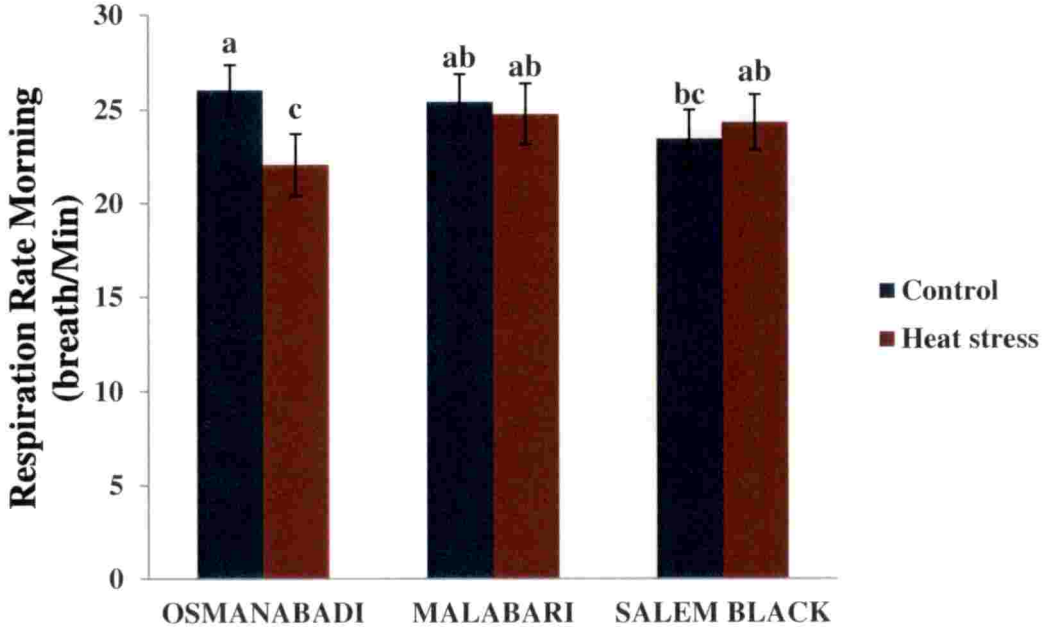


Fig. 4.3(a): Respiration rate morning

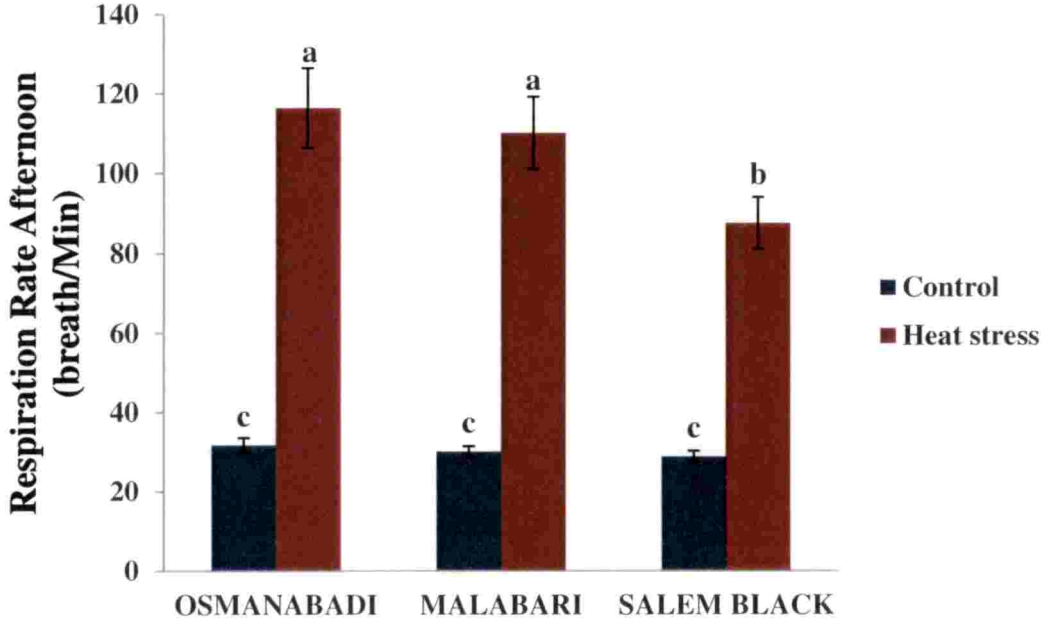


Fig. 4.3(b): Respiration rate afternoon

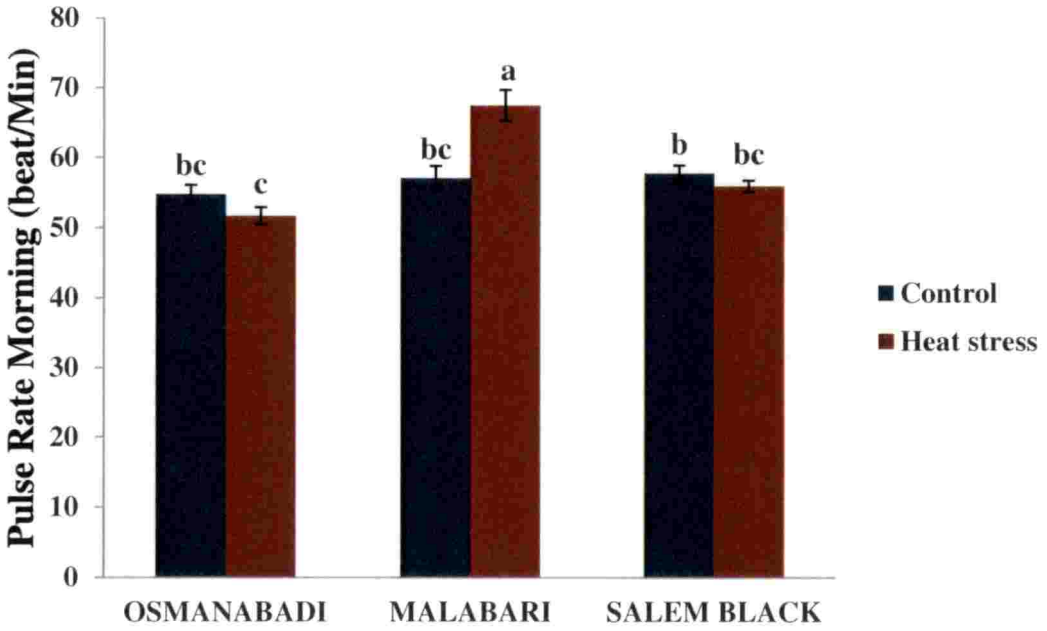


Fig. 4.3(c): Pulse rate morning

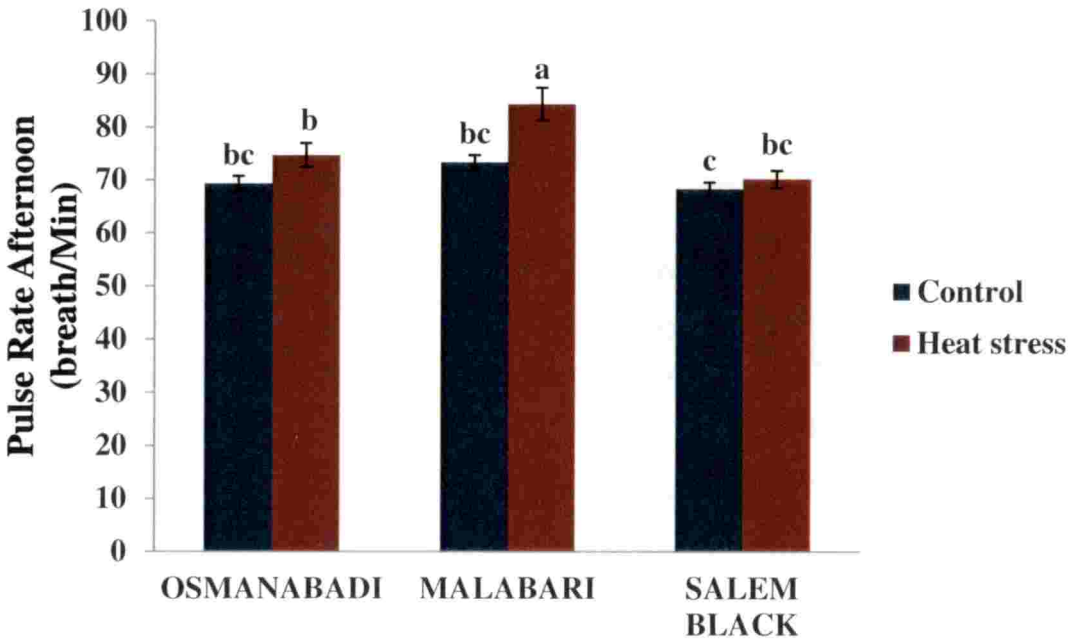


Fig. 4.3(d): Pulse rate afternoon

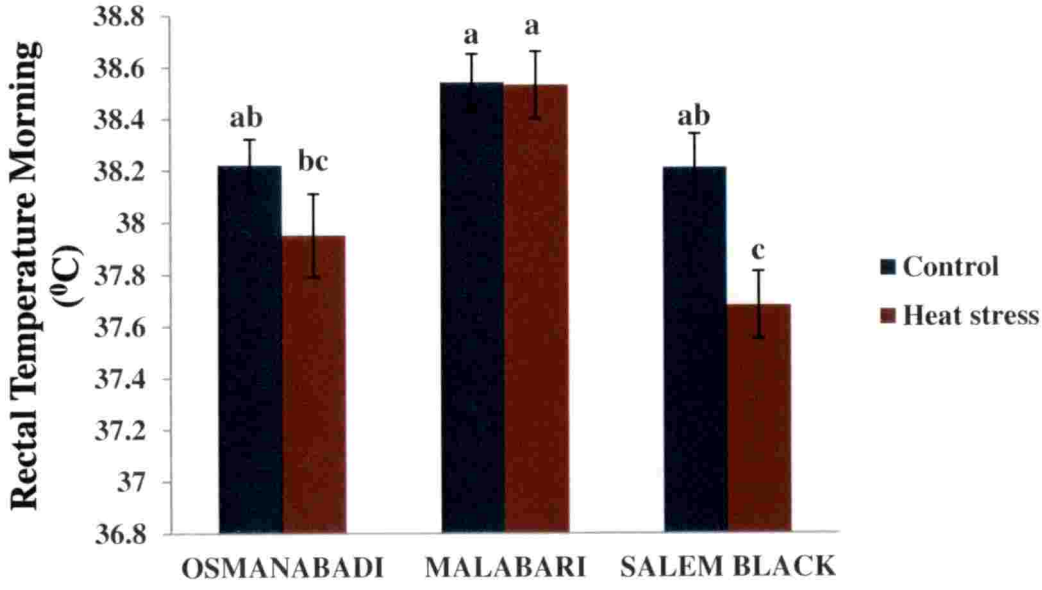


Fig. 4.3(e): Rectal temperature morning

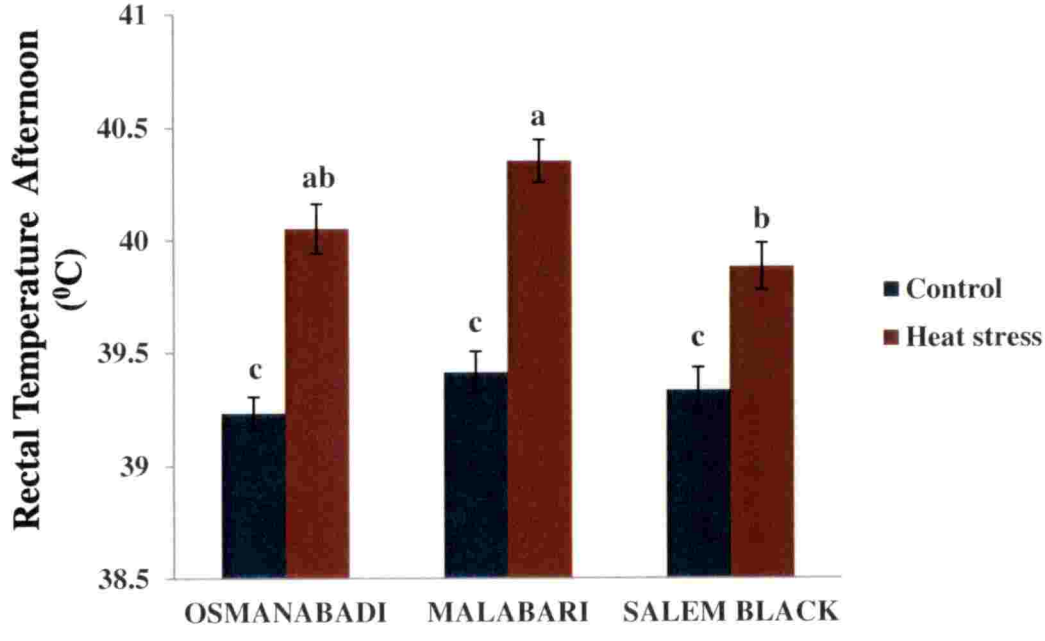


Fig. 4.3(f): Rectal temperature afternoon

4.4 Skin temperature in goats

The effects of heat stress on skin surface temperature at head, shoulder and flank in Osmanabadi, Malabari and Salem Black goat breeds both during morning and afternoon with respective graphical representations are presented in table 4.7, 4.8, Fig. 4.4 respectively. The STHM showed significant ($P<0.05$) variation for the breed as well as treatment. Further, experimental days significantly ($P<0.01$) influenced STHM in goats. However, interaction between breed, treatment and experimental days did not significantly influence the STHM. The STHA showed significant variation for the breed. Additionally, STHA showed significant ($P<0.01$) variation for the treatment and the effect was significantly higher in the heat stress groups than the control groups. Further, experimental days ($P<0.01$) and breed, treatment and experimental days interaction ($P<0.05$) significantly influenced the STH during afternoon. The STSM did not show significant variation for breed factor. However, STSM showed significant ($P<0.05$) variation for the treatment. Further, experimental days significantly ($p<0.01$) influenced STSM but interaction between breed, treatment and experimental days did not influence STSM significantly. The STSA showed significant ($P<0.01$) variation for both breed and treatment. Further, experimental days significantly ($P<0.01$) influenced STSA, while interaction between breed, treatment and experimental days did not influence STSA. The STFM showed highly significant variation ($P<0.01$) for breed and treatment. Experimental days significantly ($P<0.01$) influenced STFM values in goats while the breed, treatment and experimental days interaction did not show significant influence on STFM. The STFA showed significant ($P < 0.01$) variation for breed and treatment. In addition, both experimental days ($P<0.01$) and interaction ($P<0.05$) between breed, treatment and experimental days significantly influenced the STF during the afternoon. In addition, among the physiological responses in the morning, negative correlation was established only between THI and both RRM ($P<0.05$) and RTM ($P<0.01$) (Table 4.9). Furthermore, among the physiological responses in the afternoon, strong positive ($P<0.01$) correlations were established between THI and all physiological variables during afternoon (Table 4.10).

Table 4.7: Effect of heat stress on skin temperature in Osmanabadi, Malabari and Salem Black goat breeds during morning

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
STHM (°C)	0	34.78	34.95	34.68	34.82	34.92	34.60	*	*	**	NS
	15	35.38	35.20	35.68	35.33	35.22	35.12				
	30	29.68	29.67	29.78	30.72	29.98	28.95				
	45	28.12	29.18	29.52	29.88	29.30	28.68				
	Mean	**31.99^{bc}**	**32.25^{abc}**	**32.42^{ab}**	**32.69^{a}**	**32.35^{abc}**	**31.84^{a}**				
	Pooled SE	±0.17	±0.17	±0.17	±0.17	±0.17	±0.17				
STSM (°C)	0	35.53	34.93	35.02	35.47	35.33	35.15	NS	*	**	NS
	15	35.48	35.43	35.68	35.57	35.42	35.35				
	30	31.18	30.98	31.00	30.55	30.43	29.92				
	45	30.58	29.52	30.40	29.63	30.30	29.50				
	Mean	**33.20^{a}**	**32.72^{ab}**	**33.03^{ab}**	**32.80^{ab}**	**32.87^{ab}**	**32.48^{b}**				
	Pooled SE	±0.18	±0.18	±0.18	±0.18	±0.18	±0.18				
STFM (°C)	0	35.75	35.48	35.62	35.98	35.73	35.72	**	**	**	NS
	15	35.63	35.52	35.72	35.75	35.63	35.42				
	30	31.37	30.38	31.63	31.20	30.26	29.73				
	45	30.92	29.65	30.53	30.57	30.03	29.52				
	Mean	**33.42^{a}**	**32.76^{c}**	**33.38^{ab}**	**33.38^{ab}**	**32.92^{bc}**	**32.60^{c}**				
	Pooled SE	±0.15	±0.15	±0.15	±0.15	±0.15	±0.15				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; STHM- skin temperature head morning; STSM- skin temperature shoulder morning; STFM- skin temperature flank morning; SE, standard error

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

Table 4.8: Effect of heat stress on skin temperature in Osmanabadi, Malabari and Salem Black goat breeds during afternoon

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
STHA (°C)	0	35.63	35.73	35.65	35.62	35.33	35.82	**	**	**	*
	15	35.80	46.50	35.97	39.35	35.67	46.93				
	30	32.43	41.18	32.72	34.63	32.45	42.22				
	45	30.82	37.32	32.08	35.88	34.25	36.93				
	Mean	**33.67^{c}**	**40.18^{a}**	**34.10^{c}**	**36.37^{b}**	**34.42^{c}**	**40.48^{a}**				
	Pooled SE	±0.38	±0.38	±0.38	±0.38	±0.38	±0.38				
STSA (°C)	0	36.03	35.77	35.78	35.62	36.02	36.07	**	**	**	NS
	15	35.23	47.43	34.87	38.58	34.87	47.30				
	30	33.98	40.93	34.02	35.40	32.90	39.52				
	45	32.70	38.33	33.20	35.23	33.50	37.25				
	Mean	**34.49^{c}**	**40.62^{a}**	**34.47^{c}**	**36.21^{b}**	**34.32^{c}**	**40.03^{a}**				
	Pooled SE	±0.42	±0.42	±0.42	±0.42	±0.42	±0.42				
STFA (°C)	0	36.02	35.92	36.32	36.08	36.45	36.33	**	**	**	*
	15	36.02	48.32	35.57	39.03	35.13	51.58				
	30	33.93	42.57	33.90	35.90	33.03	45.20				
	45	33.42	40.07	33.85	35.57	34.18	38.18				
	Mean	**34.85^{d}**	**41.72^{b}**	**34.91^{d}**	**36.65^{c}**	**34.70^{d}**	**42.83^{a}**				
	Pooled SE	±0.37	±0.37	±0.37	±0.37	±0.37	±0.37				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; STHA- skin temperature head afternoon; STSA- skin temperature shoulder afternoon, STFA- skin temperature flank afternoon; SE- standard error

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

Table 4.9: Correlation association between THI and physiological responses during morning

	THI	RRM	PRM	RTM	STHM	STSM	STFM
THI	1						
RRM	-0.22*	1					
PRM	0.15	-0.13	1				
RTM	-0.26**	0.34**	0.19	1			
STHM	0.001	0.15	-0.16	0.43**	1		
STSM	-0.09	0.21*	-0.22*	0.45**	0.93**	1	
STFM	-0.09	0.20*	-0.17	0.46**	0.93**	0.95**	1

THI-Temperature Humidity Index; RRM- respiration rate morning; PRA- pulse rate morning; RTA- rectal temperature morning, STHA- skin temperature head afternoon; STSA- skin temperature shoulder afternoon, STFA- skin temperature flank afternoon

**Indicates statistical significance at $P < 0.01$

Table 4.10: Correlation association between THI and physiological responses during afternoon

	THI	RRA	PRA	RTA	STHA	STSA	STFA
THI	1						
RRA	0.95**	1					
PRA	0.40**	0.42 **	1				
RTA	0.76**	0.79**	0.32**	1			
STHA	0.66**	0.62**	-0.44	0.53**	1		
STSA	0.62**	0.58**	-0.08	0.52**	0.87**	1	
STFA	0.64**	0.58**	-0.10	0.49**	0.86**	0.86**	1

THI-Temperature Humidity Index; RRA- respiration rate afternoon; PRA- pulse rate afternoon; RTA- rectal temperature afternoon, STHA- skin temperature head afternoon; STSA- skin temperature shoulder afternoon, STFA- skin temperature flank afternoon

**Indicates statistical significance at $P < 0.01$

Figure 4.4: Effect of heat stress on skin temperature in Osmanabadi, Malabari and Salem Black goat breeds (a) Skin temperature head morning (b) Skin temperature head afternoon (c) Skin temperature shoulder morning (d) Skin temperature shoulder afternoon (e) Skin temperature flank morning (f) Skin temperature flank afternoon

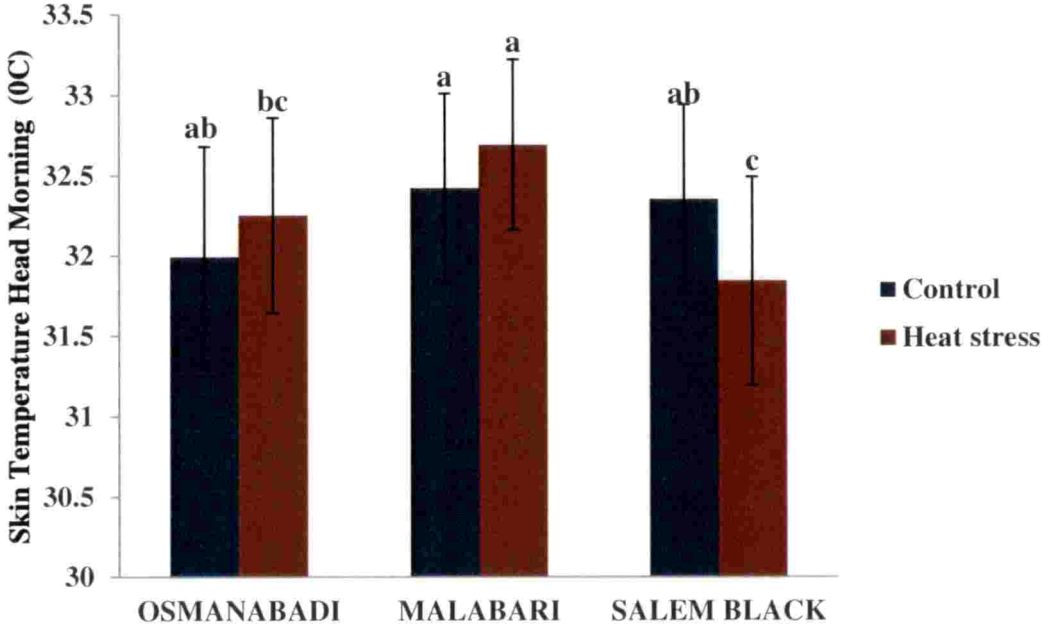


Fig. 4.4(a): Skin temperature head morning

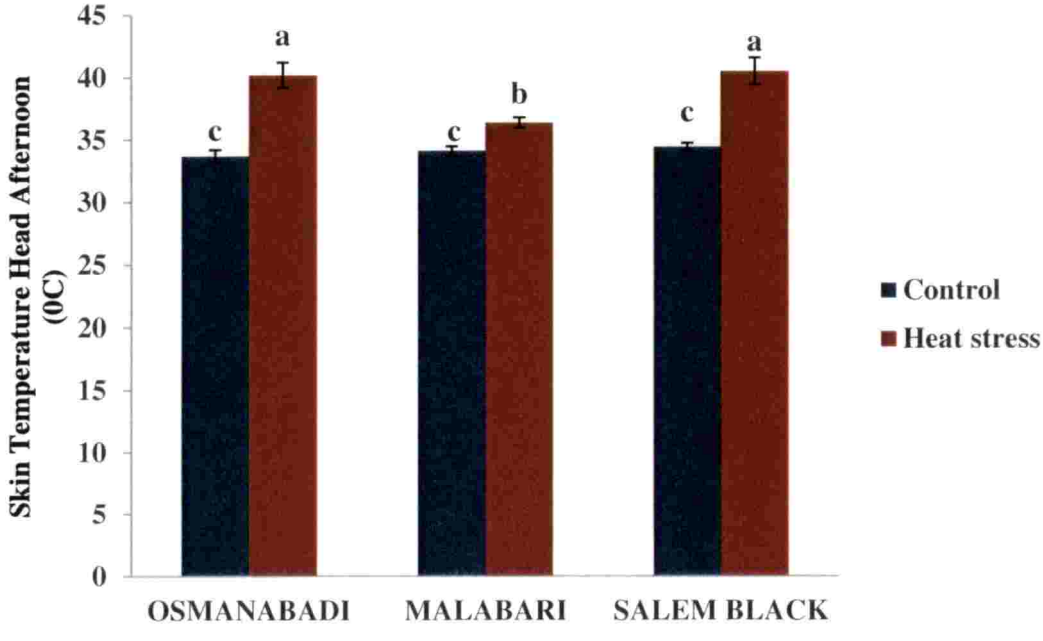


Fig. 4.4(b): Skin temperature head afternoon

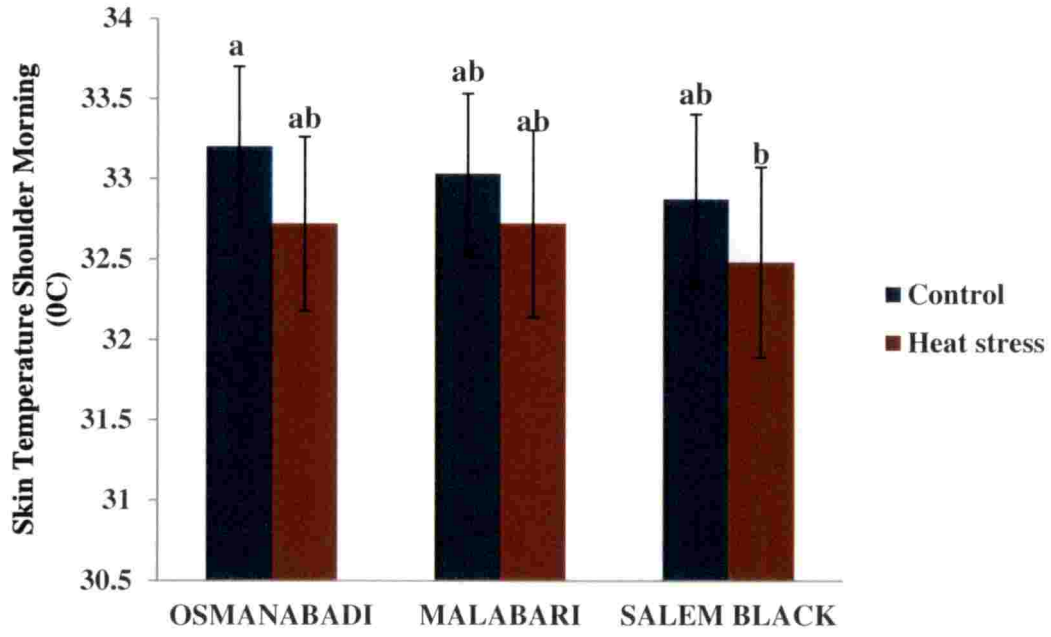


Fig. 4.4(c): Skin temperature shoulder morning

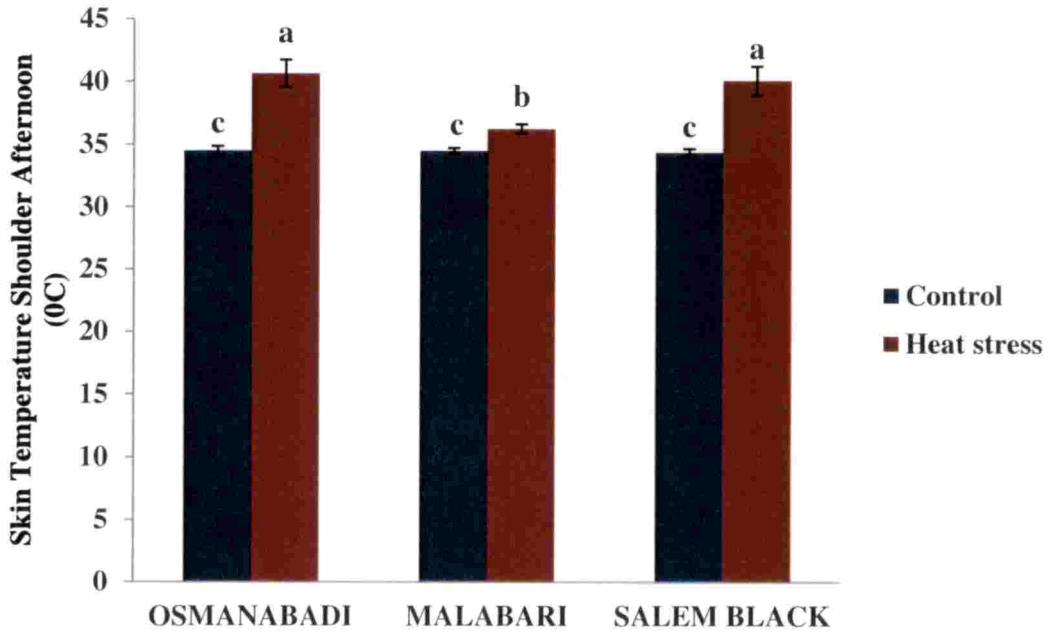


Fig. 4.4(d): Skin temperature shoulder afternoon

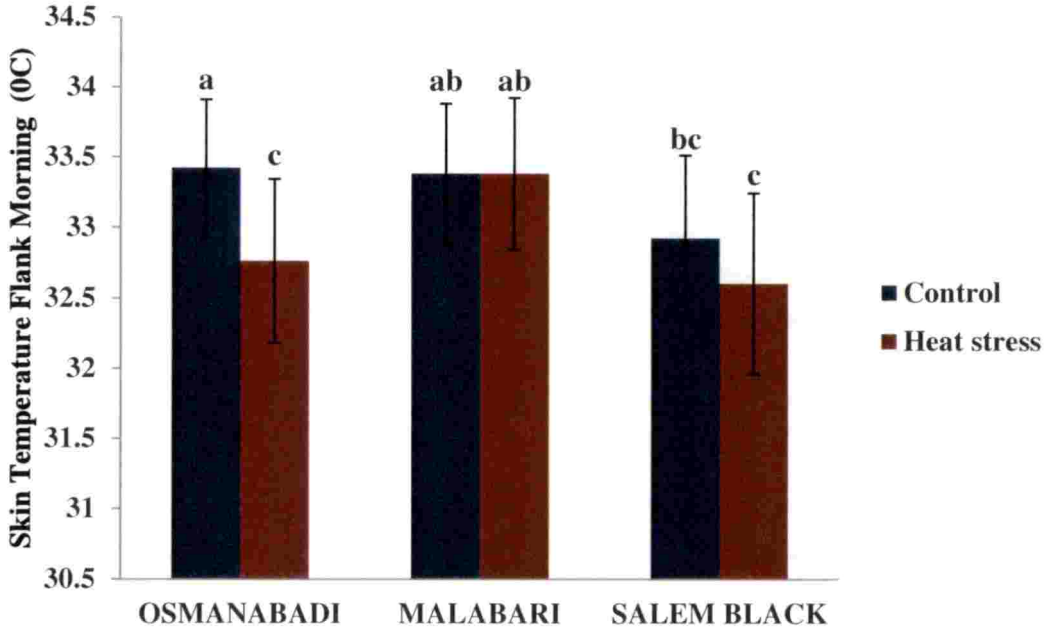


Fig. 4.4(e): Skin temperature flank morning

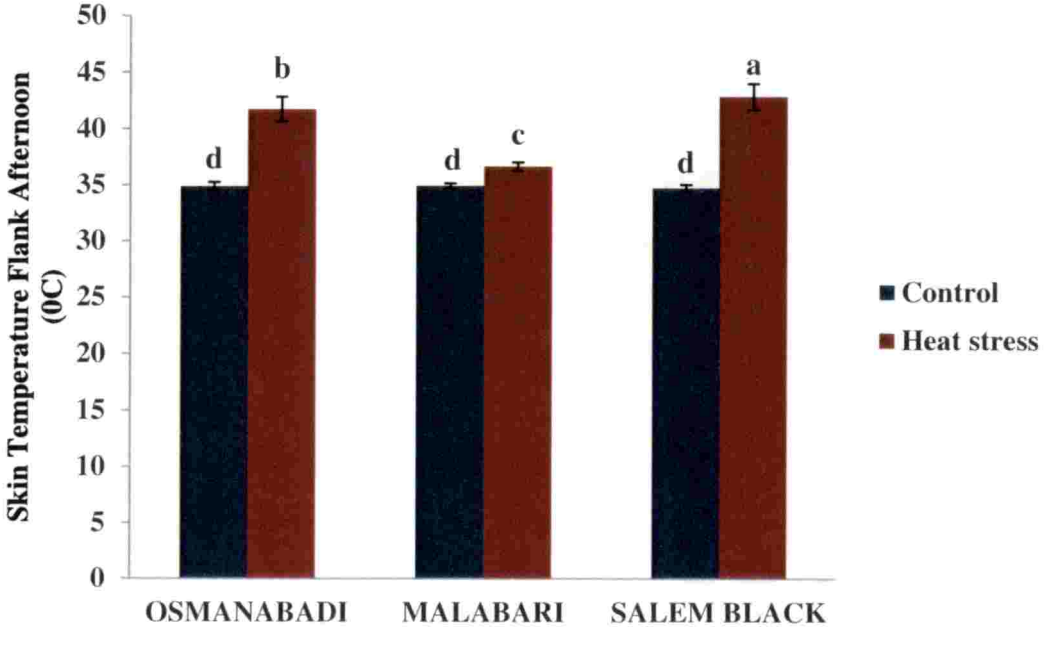


Fig. 4.4(f): Skin temperature flank afternoon

4.5 Blood biochemical responses

The effects of heat stress on blood biochemical responses in Osmanabadi, Malabari and Salem Black with graphical representations are described in table 4.11, 4.12, 4.13 and Fig. 4.5 respectively. Plasma glucose showed significant ($P<0.01$) changes for breed factor. However, plasma glucose did not show significant variation for treatment. Further, experimental days significantly ($P<0.01$) influenced plasma glucose, while the interaction between breed, treatment and experimental days did not influence plasma glucose. Plasma total cholesterol did not show significant variation for breed factor. However, plasma cholesterol showed significant ($P<0.05$) variation for the treatment. Significantly higher cholesterol concentration was found in MHS compared to MCON. In addition, experimental days significantly ($P<0.01$) influenced plasma cholesterol but interaction between breed, treatment and experimental days did not influence plasma total cholesterol significantly. Plasma protein showed significant ($P<0.01$) variation for the breed factor. However, treatment, experimental days and interaction between breed, treatment and experimental days did not influence the plasma protein. Plasma albumin showed a similar trend to that of plasma protein. The breed significantly ($P<0.01$) influenced plasma albumin, while the treatment, experimental days and interaction between breed, treatment and experimental days did not influence the plasma albumin in the study. The globulin showed significant ($P<0.01$) variation for the breed factor. However, the treatment did not influence significantly the plasma globulin. Further, experimental days significantly ($P<0.01$) influenced globulin, while interaction between breed, treatment and experimental days did not influence globulin. A/G ratio did not differ for breed, treatment, and breed, treatment and experimental days interaction, but experimental days significantly ($P<0.01$) influenced A/G ratio in goats. Plasma triglycerides showed significant variation for both the breed ($P<0.01$) and treatment ($P<0.05$) effect. The heat stress treatment significantly influenced plasma triglycerides level in Osmanabadi and Salem Black goats. Significantly lower triglyceride level was found in OHS and SHS compared to OCON and SCON respectively. In addition, both experimental days and interaction between breed, treatment and experimental

days did not significantly influence the plasma triglyceride values in the study. Both plasma urea and PUN did not show significant changes for breed and treatment factor. However, the experimental days significantly ($P<0.01$) influenced both urea and PUN. Further, interaction between breed, treatment and experimental days did not significantly influence the urea and PUN values in goats. The AST showed completely different trend compared to that of the other biochemical parameters, it showed significant variation for breed ($P<0.05$), treatment ($P<0.05$), experimental days ($P<0.01$) and their interaction ($P<0.05$). The ALT differed significantly ($P<0.05$) for breed factor, but the treatment did not significantly influence ALT in the study. In addition, experimental days also significantly ($P<0.05$) influenced ALT in goats. However, these independent factors interaction did not significantly influence the ALT in the study. Further, non-significant correlations were established between THI and all biochemical variables in the study (Table 4.14 and Table 4.15).

Table 4.11: Effect of heat stress on Blood biochemical responses in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
Plasma Glucose (mg/dL)	0	54.95	54.93	52.76	55.41	51.30	51.49	**	NS	**	NS
	15	66.67	69.63	60.11	74.56	68.03	59.09				
	30	52.63	54.46	50.26	51.18	43.83	37.14				
	45	57.17	63.52	45.40	52.94	48.97	44.21				
	Mean	**57.85^{ab}**	**60.64^{a}**	**52.13^{bc}**	**58.52^{ab}**	**53.03^{abc}**	**47.98^{c}**				
	Pooled SE	±2.48	±2.48	±2.48	±2.48	±2.48	±2.48				
Plasma Total Cholesterol (mg/dL)	0	65.06	59.80	58.50	57.05	58.65	58.33	NS	*	**	NS
	15	116.03	86.86	83.97	117.95	109.04	103.21				
	30	76.58	78.97	70.26	83.91	100.71	97.56				
	45	133.65	117.31	76.28	100.96	64.74	54.81				
	Mean	**97.83^{a}**	**85.73^{abc}**	**72.25^{c}**	**89.97^{ab}**	**83.29^{abc}**	**78.48^{bc}**				
	Pooled SE	±4.96	±4.96	±4.96	±4.96	±4.96	±4.96				
Plasma Total Protein (g/dL)	0	5.82	5.95	5.40	5.83	5.91	5.87	**	NS	NS	NS
	15	9.42	8.72	7.50	8.65	8.56	7.93				
	30	6.91	6.95	6.43	7.82	6.78	6.21				
	45	8.31	7.97	7.27	6.06	6.12	4.67				
	Mean	**7.62^{a}**	**7.40^{ab}**	**6.65^{bc}**	**7.09^{ab}**	**6.84^{abc}**	**6.17^{c}**				
	Pooled SE	±0.26	±0.26	±0.26	±0.26	±0.26	±0.26				
Plasma Albumin (g/dL)	0	4.24	4.43	3.06	3.19	4.01	3.92	**	NS	NS	NS
	15	6.67	6.49	3.80	4.83	5.83	5.34				
	30	4.40	4.60	3.54	3.61	4.28	4.13				
	45	5.73	5.72	3.88	3.35	3.39	3.41				
	Mean	**5.26^{a}**	**5.31^{a}**	**3.57^{d}**	**3.74^{cd}**	**4.37^{b}**	**4.20^{bc}**				
	Pooled SE	±0.18	±0.18	±0.18	±0.18	±0.18	±0.18				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS-Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction

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

Table 4.12: Effect of heat stress on Blood biochemical responses in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
Plasma Globulin (g/dL)	0	2.42	2.18	2.86	3.22	2.62	2.74	**	NS	**	NS
	15	2.75	2.23	3.70	3.83	2.73	2.60				
	30	2.50	2.36	2.89	4.20	2.50	2.09				
	45	2.58	2.25	3.39	2.71	1.07	1.26				
	Mean	**2.56^{bc}**	**2.26^{c}**	**3.21^{ab}**	**3.50^{a}**	**2.23^{c}**	**2.17^{c}**				
	Pooled SE	±0.25	±0.25	±0.25	±0.25	±0.25	±0.25				
Albumin/ Globulin Ratio	0	1.82	2.05	1.18	1.02	1.59	1.64	NS	NS	*	NS
	15	2.74	4.44	2.44	1.34	2.28	2.20				
	30	2.00	2.22	1.56	0.94	2.28	2.31				
	45	2.36	2.65	1.25	3.74	4.43	3.25				
	Mean	**2.23^{a}**	**2.84^{a}**	**1.60^{a}**	**1.76^{a}**	**2.65^{a}**	**2.35^{a}**				
	Pooled SE	±0.46	±0.46	±0.46	±0.46	±0.46	±0.46				
Plasma Triglycerides (mg/dL)	0	8.04	8.22	8.57	9.29	8.56	8.41	**	*	NS	NS
	15	9.93	6.72	9.61	10.15	8.55	6.93				
	30	8.71	6.71	8.71	10.61	8.51	6.86				
	45	9.13	6.49	9.51	10.29	8.69	6.39				
	Mean	**8.95^{ab}**	**7.04^{c}**	**9.1^{a}**	**10.09^{ab}**	**8.58^{b}**	**7.15^{c}**				
	Pooled SE	±0.40	±0.40	±0.40	±0.40	±0.40	±0.40				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS-Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction

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

Table 4.13: Effect of heat stress on blood biochemical responses in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
Plasma Urea (mg/dL)	0	42.17	38.88	44.02	43.29	50.08	49.32	NS	NS	**	NS
	45	59.95	63.01	50.28	66.67	47.64	43.42				
	Mean	**51.06^{a}**	**50.94^{a}**	**47.15^{a}**	**54.98^{a}**	**48.86^{a}**	**46.37^{a}**				
	Pooled SE	±3.09	±3.09	±3.09	±3.09	±3.09	±3.09				
PUN (mg/dL)	0	19.69	18.16	20.56	20.22	23.39	23.03	NS	NS	**	NS
	45	28.00	29.43	23.48	31.13	22.25	20.28				
	Mean	**23.85^{a}**	**23.79^{a}**	**22.02^{a}**	**25.68^{a}**	**22.82^{a}**	**21.65^{a}**				
	Pooled SE	±1.44	±1.44	±1.44	±1.44	±1.44	±1.44				
AST (IU/L)	0	65.90	66.33	65.31	68.72	67.85	69.44	*	*	**	*
	45	76.02	93.02	70.30	79.00	72.31	68.14				
	Mean	**70.96^{b}**	**79.67^{a}**	**67.80^{b}**	**73.86^{ab}**	**70.08^{b}**	**68.79^{b}**				
	Pooled SE	±2.17	±2.17	±2.17	±2.17	±2.17	±2.17				
ALT (IU/L)	0	16.69	16.14	17.08	17.14	15.06	15.85	*	NS	*	NS
	45	15.04	14.85	14.66	17.37	14.37	13.08				
	Mean	**15.87^{ab}**	**15.50^{ab}**	**15.87^{ab}**	**17.26^{a}**	**14.71^{b}**	**14.46^{b}**				
	Pooled SE	±0.77	±0.77	±0.77	±0.77	±0.77	±0.77				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; PUN- Plasma Urea Nitrogen; AST- Aspartate aminotransferase; ALT- Alanine aminotransferase

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

Table 4.14: Correlation association between THI and Blood biochemical responses

	THI	Glucose	TP	Albumin	Globulin	A/G ratio	TC	TG
THI	1							
Glucose	0.06	1						
TP	-0.08	0.35**	1					
Albumin	-0.01	0.47**	0.72**	1				
Globulin	-0.03	0.00	0.64**	-0.01	1			
A/G ratio	0.04	0.15	-0.29*	0.14	-0.63**	1		
TC	0.02	0.35**	0.46**	0.43**	0.22*	-0.13	1	
TG	-0.26	0.09	0.09	-0.09	0.21*	-0.03	0.04	1

THI- Temperature humidity index; TP- Total protein; A/G ratio- Albumin/globulin ratio; TC- Total cholesterol; TG- Total triglycerides

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

Table 4.15: Correlation association between THI and Blood biochemical responses

	THI	Urea	PUN	AST	ALT
THI	1				
Urea	0.17	1			
PUN	0.17	1.00**	1		
AST	0.30	0.53**	0.53**	1	
ALT	0.07	0.23	0.23	-0.03	1

THI- temperature humidity index; PUN- plasma urea nitrogen; AST- aspartate aminotransferase; ALT- alanine aminotransferase

**Indicates statistical significance at $P < 0.01$

Figure 4.5: Effect of heat stress on Blood biochemical responses in Osmanabadi, Malabari and Salem Black goat breeds (a) Plasma glucose (b) Plasma total cholesterol (c) Plasma total protein (d) Plasma albumin (e) Plasma globulin (f) A/G ratio (g) Plasma triglycerides (h) Plasma urea (i) Plasma urea nitrogen (j) Aspartate aminotransferase (k) Alanine aminotransferase

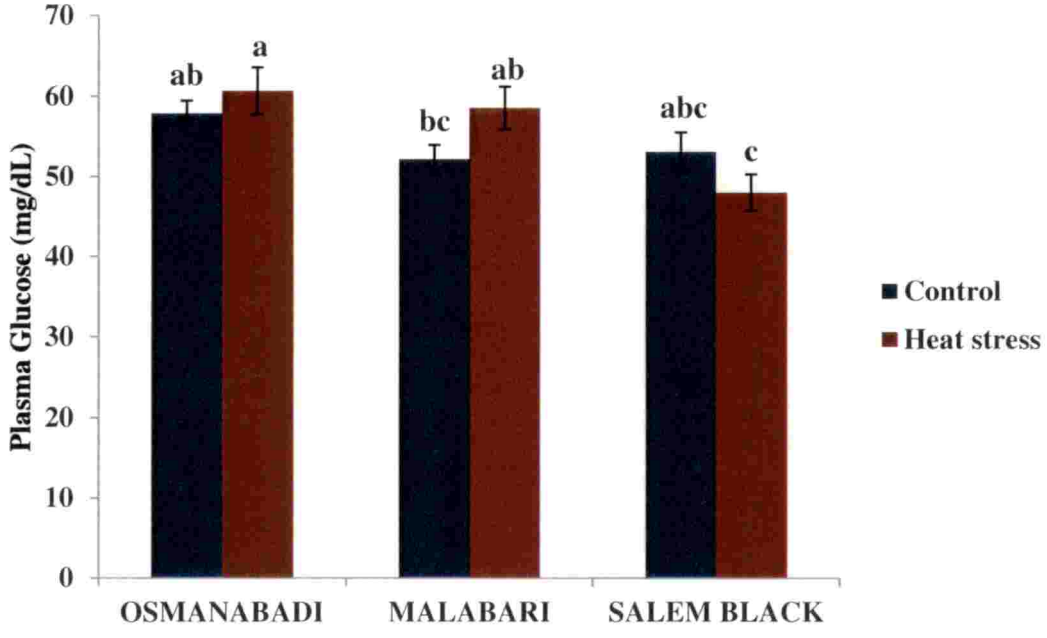


Fig. 4.5(a): Plasma glucose

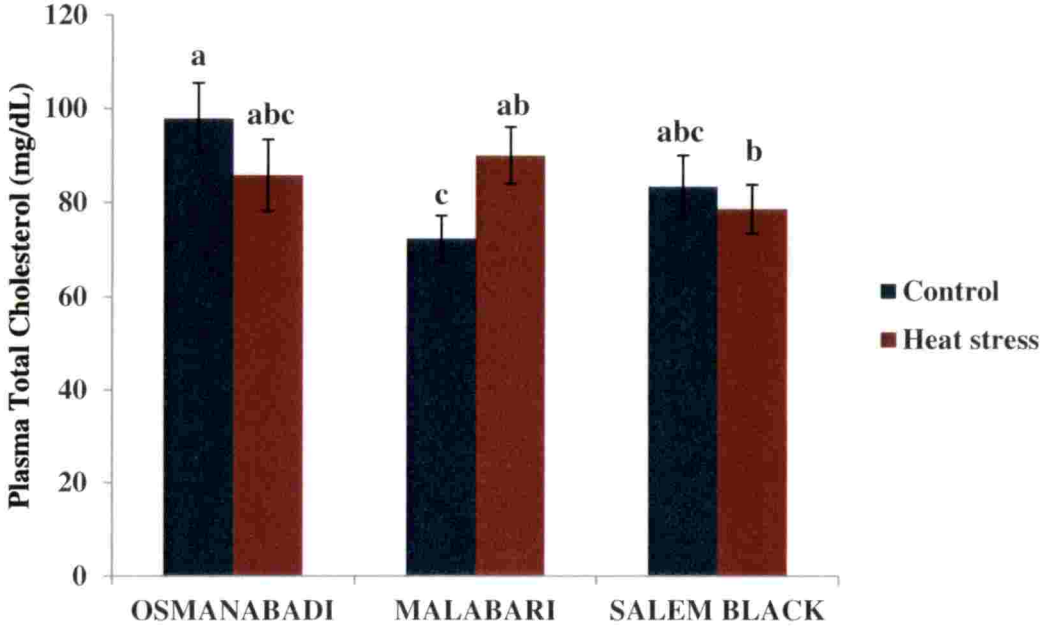


Fig. 4.5(b): Plasma total cholesterol

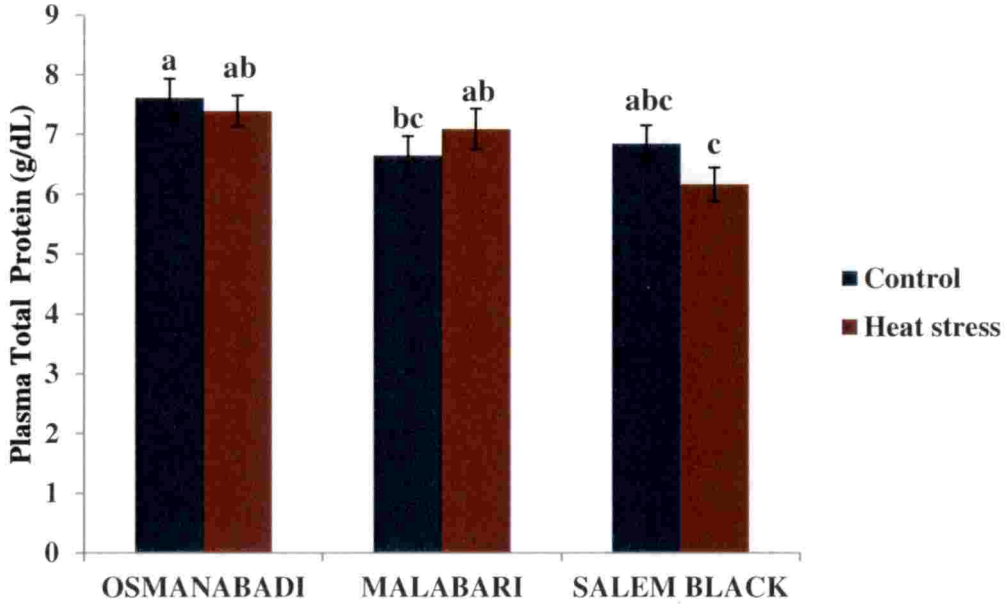


Fig. 4.5(c): Plasma total protein

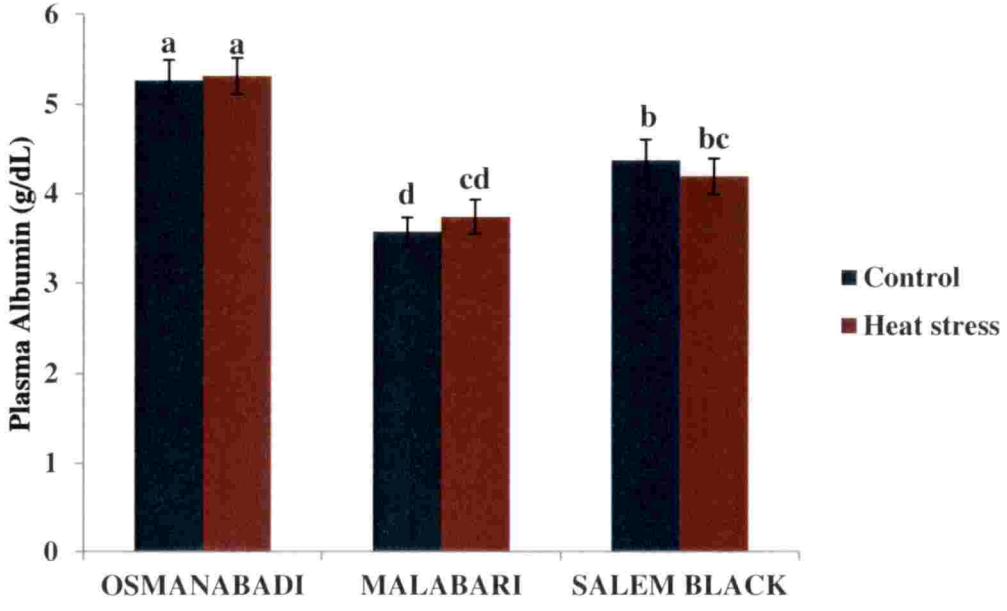


Fig. 4.5(d): Plasma albumin

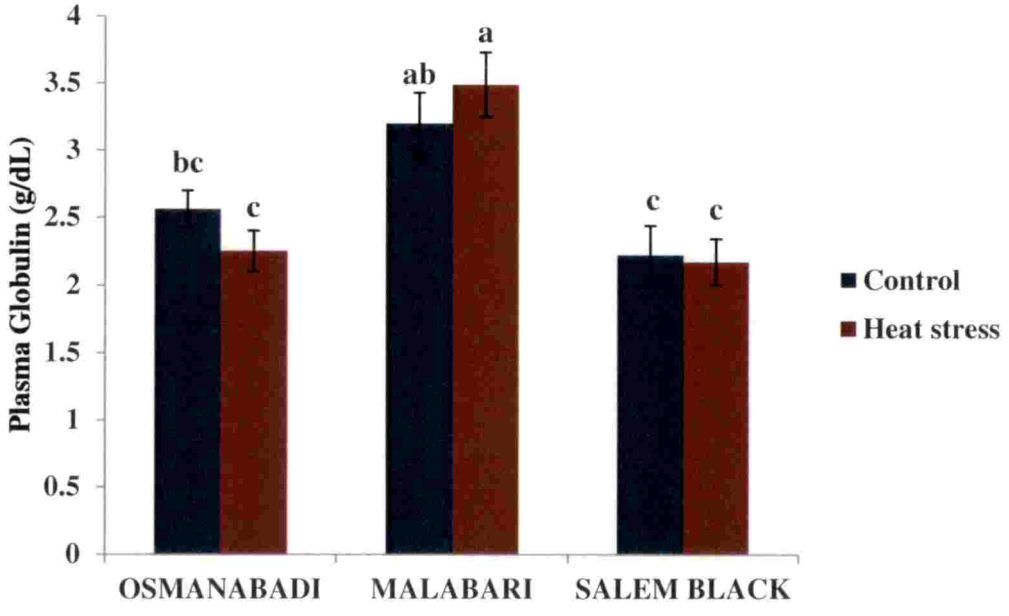


Fig. 4.5(e): Plasma globulin

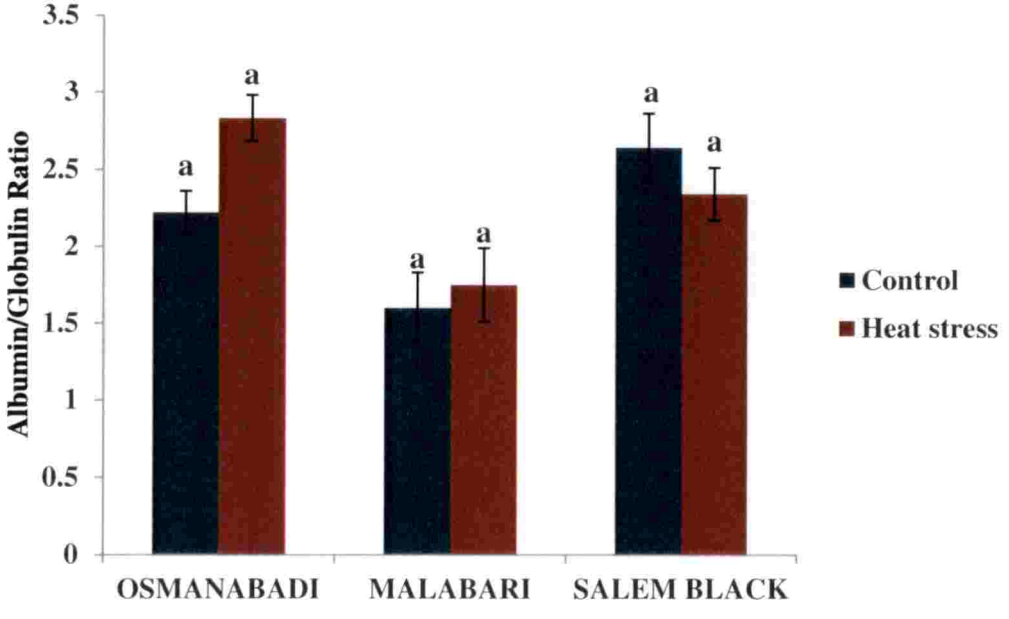


Fig. 4.5(f): A/G ratio

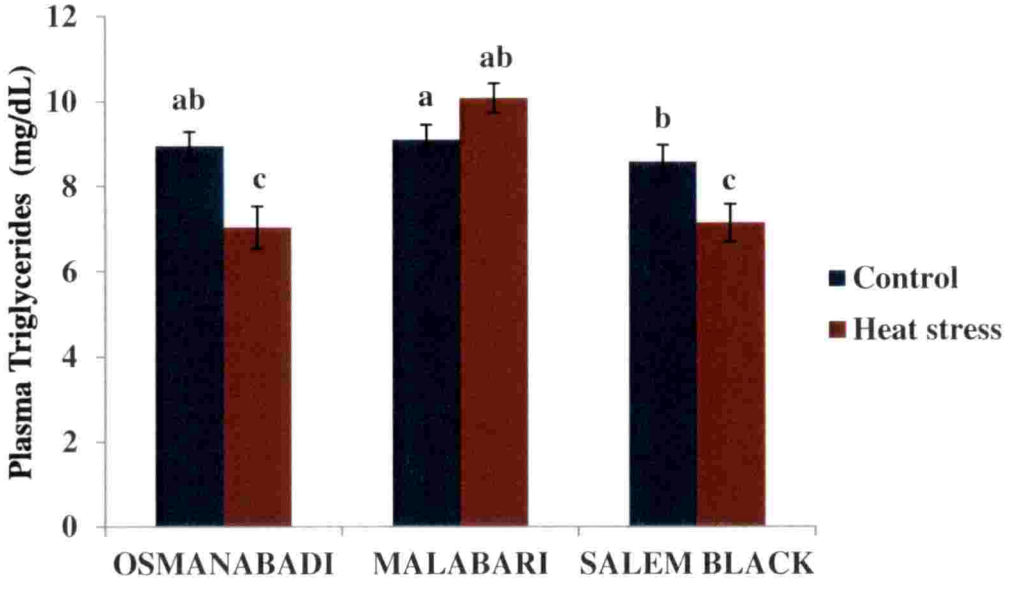


Fig. 4.5(g): Plasma trigycerides

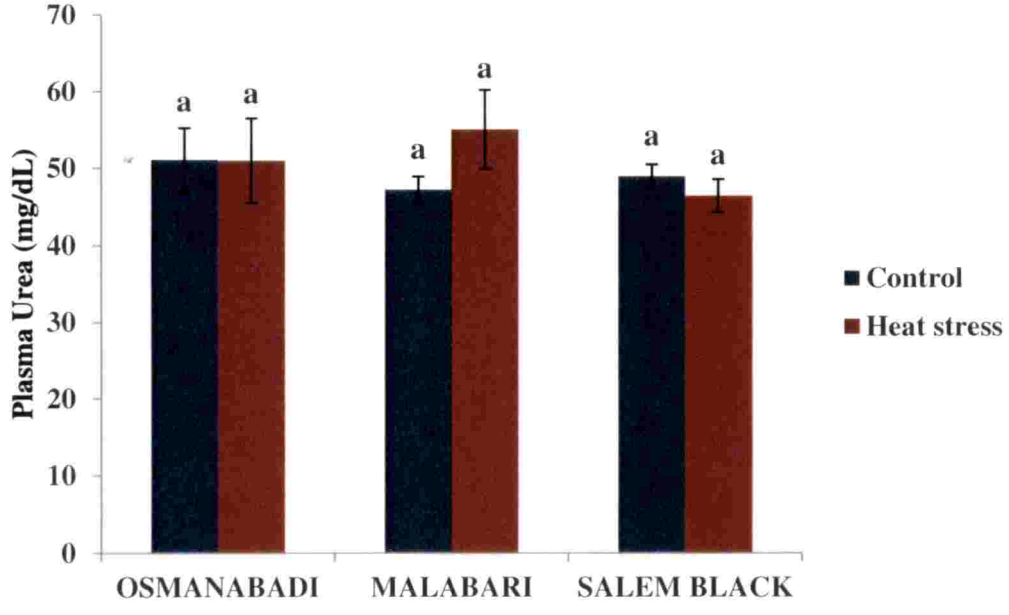


Fig. 4.5(h): Plasma urea

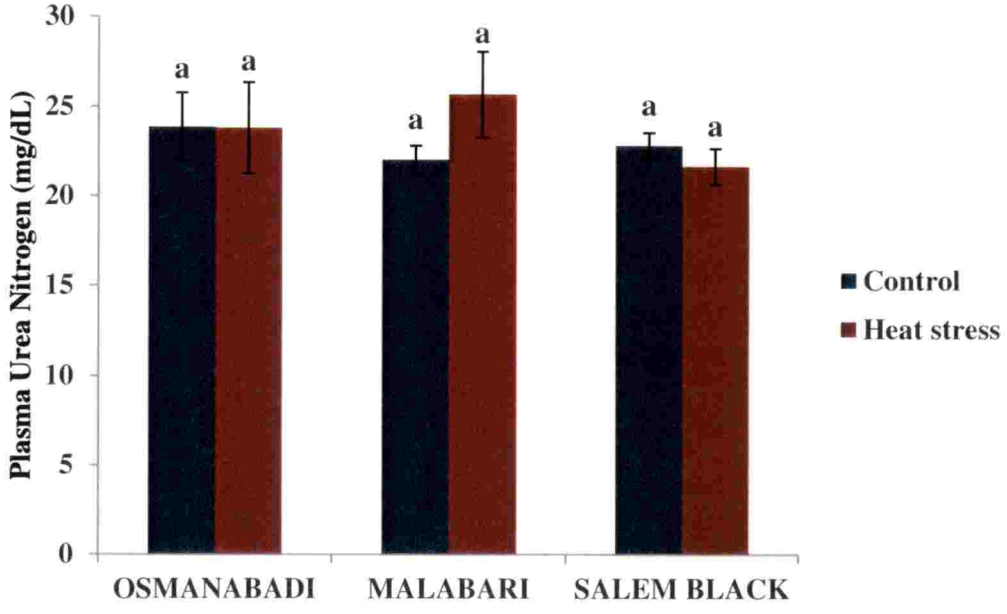


Fig. 4.5(i): Plasma urea nitrogen

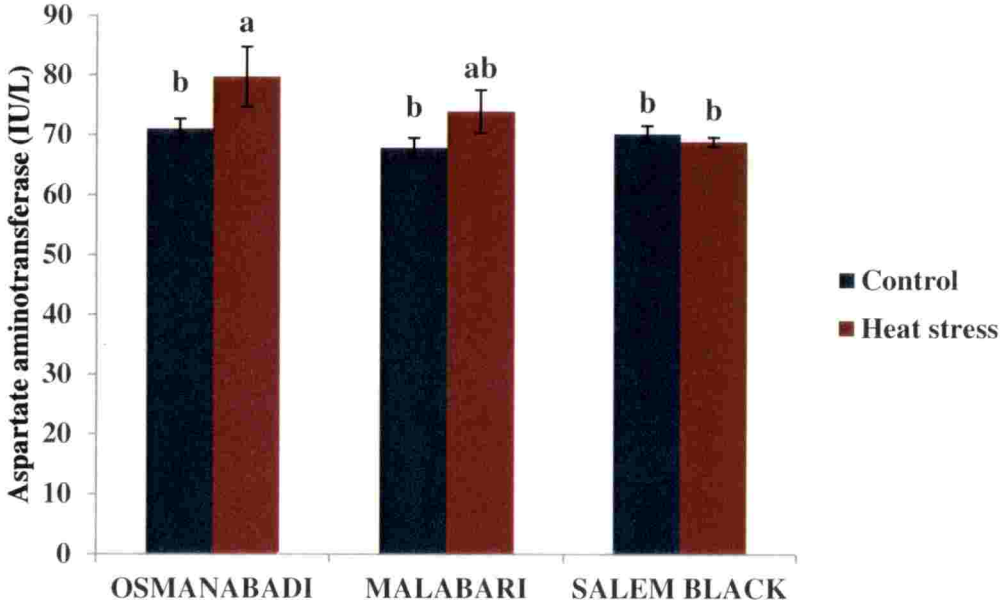


Fig. 4.5(j): Aspartate aminotransferase

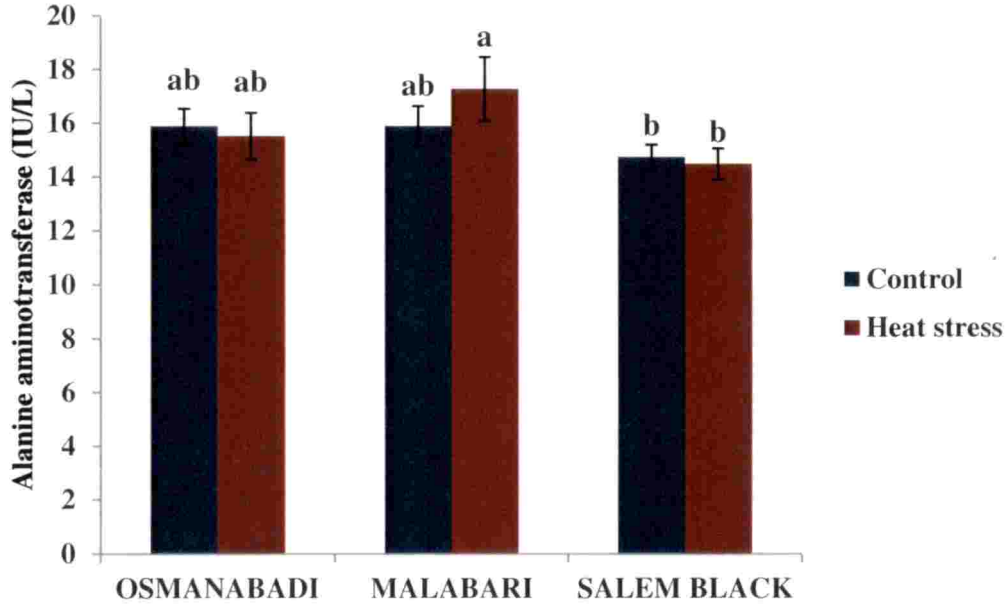


Fig. 4.5(k): Alanine aminotransferase

4.6 Endocrine responses

The effects of heat stress on endocrine responses in Osmanabadi, Malabari and Salem Black goats with graphical representations are presented in table 4.16 and Fig. 4.6 respectively. Plasma cortisol did not show significant variation for breed factor. However, heat stress treatment significantly ($P<0.01$) influenced cortisol level in all three breed goats with higher concentration found in heat stress groups as compared to their respective control groups. Further, both experimental days and interaction between breed, treatment and experimental days did not influence cortisol level in goats. However, plasma aldosterone showed significant variation for both breed ($P<0.01$) as well as heat stress treatment ($P<0.05$). Heat stress significantly ($P<0.05$) increased the plasma aldosterone level in Malabari goats. Further, the experimental days also significantly ($P<0.01$) influenced plasma aldosterone in the study. However, the interaction between breed, treatment and experimental days did not influence plasma aldosterone level. Further, a positive ($P<0.05$) correlation was established between THI and plasma cortisol while a negative ($P<0.01$) correlation was established between THI and plasma aldosterone (Table 4.17).

Table 4.16: Effects of heat stress on Endocrine responses in Osmanabadi, Malabari and Salem Black goat breeds

Attributes	Days	Treatments						Effects			
		OCON	OHS	MCON	MHS	SCON	SHS	Breed	TRT	DAY	Breed* TRT * DAY
Cortisol (µg/dl)	0	16.87	15.96	14.78	16.11	15.30	16.72	NS	**	NS	NS
	15	14.87	18.22	14.44	24.06	18.40	26.11				
	30	17.79	26.03	15.09	31.69	13.53	20.23				
	45	16.60	27.03	16.01	34.19	15.28	15.74				
	Mean	**16.53^{b}**	**21.81^{ab}**	**15.08^{b}**	**26.51^{a}**	**15.63^{b}**	**19.70^{b}**				
	Pooled SE	± 2.21	± 2.21	± 2.21	± 2.21	± 2.21	± 2.21				
Aldosterone (pg/mL)	0	51.08	48.34	109.03	109.10	114.81	110.38	**	*	**	NS
	15	68.04	37.80	113.36	85.57	114.35	87.46				
	30	40.93	33.12	111.78	39.97	89.98	64.19				
	45	53.72	59.72	100.73	65.98	97.60	67.27				
	Mean	**53.44^{cd}**	**44.74^{d}**	**108.72^{a}**	**75.16^{bcd}**	**104.1^{ab}**	**82.32^{abc}**				
	Pooled SE	±10.55	±10.55	±10.55	±10.55	±10.55	±10.55				

OCON- Osmanabadi control; OHS- Osmanabadi heat stress; MCON- Malabari control; MHS- Malabari heat stress; SCON- Salem Black control; SHS- Salem Black heat stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction

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

Table 4.17: Correlation association between THI and Endocrine responses

	THI	Plasma cortisol	Plasma aldosterone
THI	**1**		
Plasma cortisol	**0.35***	**1**	
Plasma aldosterone	**-0.34****	**-0.12**	**1**

THI- Temperature humidity index

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

4.7 PBMC HSP70 gene expression

The effects of heat stress on relative PBMC HSP70 mRNA transcript expression of Osmanabadi, Malabari and Salem Black goat breeds are depicted in Fig 4.7. The results obtained indicated PBMC HSP70 expression in OCON, OHS, MCON, MHS, SCON and SHS to be 1.07, 2.3, 1.04, 2.13, 1.11, 1.13 folds respectively. Comparatively, heat stressed groups of both Osmanabadi and Malabari showed significantly ($P<0.05$) higher expression of PBMC HSP70 mRNA compared to their respective control groups. However, in Salem Black goats, PBMC HSP70 expression did not differ between control and heat stress groups. Further among the stress groups, the level of HSP70 mRNA expression was significantly lower in SHS as compared to OHS and MHS groups.

Figure 4.6: Effect of heat stress on Endocrine responses in Osmanabadi, Malabari and Salem Black goats (a) Plasma cortisol (b) Plasma aldosterone

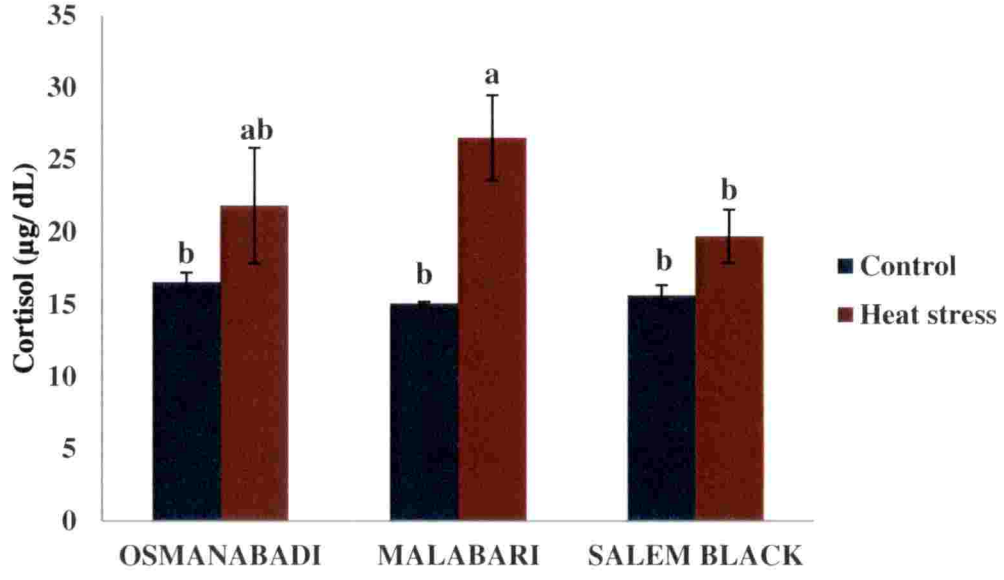


Fig. 4.6(a): Plasma cortisol

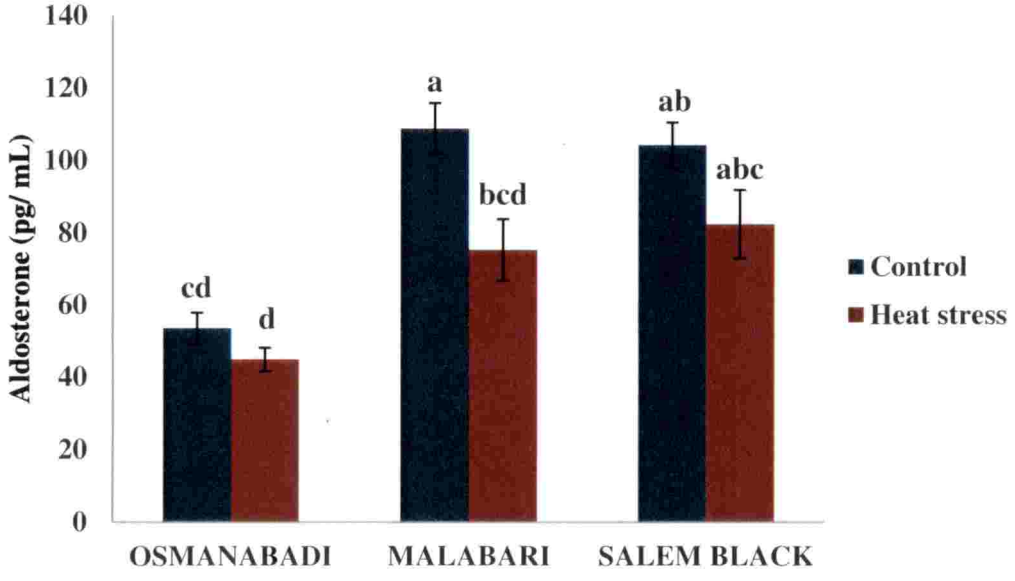


Fig. 4.6(b): Plasma aldosterone

Figure 4.7: Effect of heat stress on relative PBMC HSP70 mRNA expression in Osmanabadi, Malabari and Salem Black goats

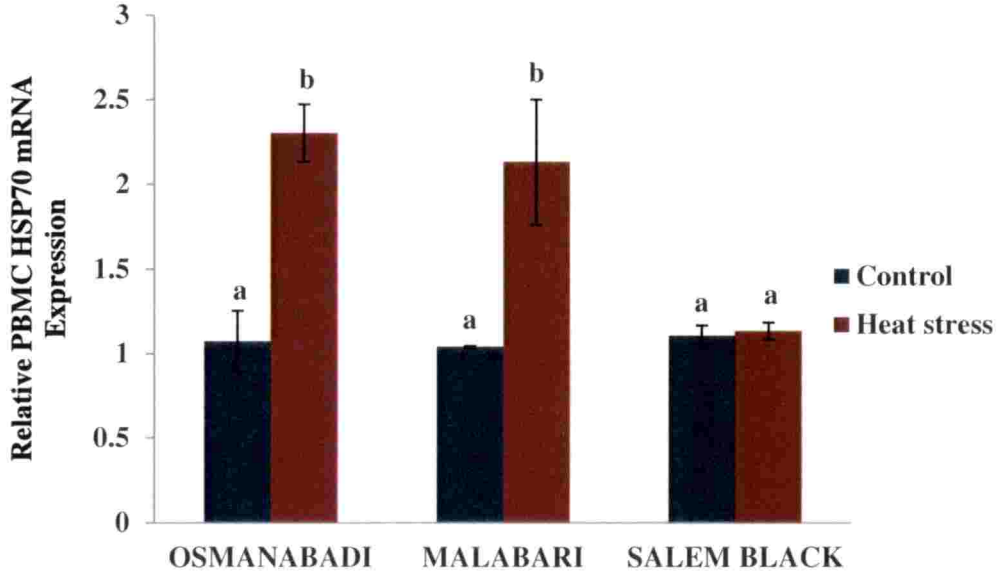


Fig.4.7 PBMC HSP70 mRNA gene expression

DISCUSSION

CHAPTER 5
DISCUSSION

5.1 Behavioural responses

The present study is a novel attempt to compare the adaptive capacity of different indigenous breeds of goat to heat stress. The study targeted assessing the severity of heat stress between the different indigenous goat breeds based on the relative changes in the phenotypic traits. The evaluation of breed differences for ST, LT, DF and DeF did not revealed any differences indicating the indigenous natures of all these three breeds depicting the similarity in their approaches to adapt to harsh climatic condition. Further, the non-significant influence of heat stress on ST, LT, DeF proves the superiority of these breeds to adapt to heat stress challenges although the THI described extreme heat stress exposure for these animals in the study. Similar result of non-significant change in ST and LT was also reported in Osmanabadi bucks exposed to heat stress in the same agro-ecological zone (Shaji *et al.,* 2016). However, generally heat stressed goats tend to be more in standing position to orient themselves in different directions to avoid direct solar radiation as well as the ground radiation (Kamal *et al.*, 2016; Panda *et al.*, 2016). In addition, the standing position also restricts the conductive heat transfer due to the presence of a layer of air adjacent to the skin, thereby facilitating the dissemination of body heat load to air (Darcan *et al.,* 2008). Moreover, small area of contact of animal body with the ground in standing posture allows only minimal conductive heat transfer to the ground (Silanikove, 2000). The reason for the non-significant changes in these cardinal behavioural parameters in the current study on all three breeds could be attributed to their non-reliability on these mechanisms to counter heat stress. However, there are reports indicating significantly increased ST and decreased LT in Osmanabadi (Panda *et al.,* 2016) and Black Bengal goat breeds (Alam *et al.,* 2013) during heat stress condition. This difference in the results with our study could be attributed to the heat stress magnitude in different agro-ecological zone. The recorded weather variables clearly showed higher magnitude of heat stress in the former region (AT- 43.6 ^{0}C, RH- 88%) compared to the current

region (AT- 39.9 ^{0}C, RH- 29.1%) during afternoon hours. This change in behaviour of such indigenous breeds also signifies their wider adaptability to different harsh climatic regions. This shows their inherent ability to cope up to the stressed conditions by executing various adaptation pathways fitting to the existing situation. The DF refers to number of times the animal had access to water resources. The results obtained from the present study exhibited significantly increased DF in heat stressed groups than control groups in all the three breeds. The result was in accordance with the previous experiment conducted in Osmanabadi bucks which also showed significantly increased DF during heat stress condition (Shaji *et al.,* 2016). This result could be due to severe dehydration in goats, caused as a result of enhanced evaporative cooling mechanisms through both respiratory tract as well as skin in heat stressed animals (Panda *et al.,* 2016; Sejian *et al.*, 2014). Consequently, the heat stressed animals tend to show higher DF thereby increased WI to cool their body and also to restore their normal body water level (Garner *et al.,* 2017; Kaliber *et al.,* 2016). Additionally, in comparison to both Osmanabadi and Salem Black breeds Malabari showed less DF and this could be attributed to the less severity experienced by this breed due to their white coat colour. The non-significant difference in DeF was observed in all the three breeds for heat stress treatment. However, in a previous experiment conducted in the same location showed significantly reduced DeF in Osmanabadi breed during heat stress condition (Shaji *et al.,* 2016). The reason for the change in behaviour could be due to the sex variation where the former experiment was in Osmanabadi bucks. Further, Panda *et al.* (2016) reported significantly lower DeF in Osmanabadi goats and this might be due to extreme harsh climatic conditions in the other locality and attributed it to water conservation measures. Similar results of lower DeF were also observed during acute heat stress exposure in Black bengal goats (Alam *et al.,* 2013). There were breed differences for UF with significantly lower UF reported in Salem Black heat stressed goats. The lower UF in Salem Black goats could prove the better adaptive capability of this breed in conserving the body water during heat stress condition. The RuT showed highly significant changes for the breed, heat stress treatment and experimental day influence in the study. The highest value for

RuT was exhibited by Malabari breed compared to both Osmanabadi and Salem Black breeds. The complete absence of RuT in both Osmanabadi and Salem Black breeds might be a metabolic adaptation to reduce internal metabolic heat production (Panda *et al.,* 2016). In contrast the significantly higher RuT in Malabari breed clearly indicates that they were under extreme energy deficiency due to higher energy utilization for enhanced thermoregulatory activities. Further, the non-significant influence of interaction between breed, treatment and experimental days on all the behavioural responses indicates that the indigenous goats showed a constant response for these parameters throughout the experimental period. In addition, a strong positive correlation of both DF and WI with THI indicates the significance of these parameters in assessing the quantum of heat stress in indigenous goat breeds. Furthermore, the negative correlation of DeF with THI signifies the importance of this behavioural variable in conserving the body water when exposed to heat stress condition in indigenous goats. These findings suggest the DF, WI and DeF may be used as indicators to reflect severity of heat stress in goats.

5.2 Physiological responses

Mechanism of physiological adaptation in the livestock is generally indicated by RR, PR and RT (Gupta *et al.,* 2013). Several researches conducted in small ruminants clearly demonstrated the significant influence of heat stress on physiological parameters (Panda *et al.,* 2016; Sejian *et al.*, 2014; Shaji *et al.,* 2016). Further, Banerjee *et al.* (2015) also reported breed differences in Indian goat breeds for physiological variables with RR, PR and RT being lower in breeds adapted to hot climates. Lower respiratory activity in the OHS could be an adaptive mechanism for keeping their physiological activities to a minimum level to cope up to the extreme stressful condition in the afternoon (Marai *et al.,* 2007; Shaji *et al.,* 2016). Further it was also observed from the study that RRA was significantly higher in heat stress groups compared to their respective control groups in all the three breeds. The RRA is the only parameter in the study which was influenced all independent variables (Breed, treatment and experimental days) and their

interaction. This shows the significance of this parameter in determining the adaptive ability of goats irrespective of breeds. The higher respiration rate in heat stressed animals would be directly related to the increased evaporative cooling mechanisms in restoring their thermal balance (Habibu *et al.,* 2016). Similar results of significantly increased RR were also reported in black Bengal, Sokoto and Sahel goats during afternoon (Alam *et al.,* 2013; Habibu *et al.*, 2016). Likewise, in a study, Panda *et al.* (2016) reported higher RR in Osmanabadi goats during afternoon. Moreover, higher RR could also be an adaptive mechanism in heat stressed animals to meet the increased oxygen demand of the vital organs for the adaptation processes (Wojtas *et al.,* 2013). In addition, Shaji *et al.* (2016) and Al-Haidary *et al.* (2012) stated the RR to be a reliable indicator to measure magnitude of heat stress in small ruminants. In comparison to the other two breeds, SHS group showed significantly lower RR which also indicates the superior adaptability of this breed to hot environments and this could be attributed to the much severe tropical climate exposure in their place of origin. This again shows shifting of breeds from much higher harsh climate to comparatively less severe hot environment might prove beneficial in terms of improving their productive potential. Further, RR during both morning as well as afternoon was significantly influenced by interaction between breed treatment and experimental days. This also indicates that the response of the groups varied over time for RR for adapting to the heat stress challenges depicting the higher adaptive capability of these indigenous goat breeds to harsh climatic conditions. Hence the RR can serve as a reliable indicator to evaluate the thermo-tolerant capacity of different indigenous goat breeds. The most noticeable effect of heat stress on heart and blood vessels are evident from increased PR in goats during summer (Gupta *et al.,* 2013). Between the breeds, Malabari breed showed highest PR value during both morning as well as afternoon. Higher PR value in MHS compared to MCON during morning hours clearly proves their higher susceptibility to thermal environments. These animals were unable to restore their proper circulation balance even after the night hours. Further, findings from several researches also proved a direct correlation between heart rate and general metabolic status in livestock (Barkai *et al.,* 2002; Popoola *et al.*, 2014). Hence,

significantly higher PR during morning in MHS could be an adaptive mechanism to ensure constant energy supply for the adaptation process. Further, the non-significant change in PR in heat stressed Osmanabadi and Salem Black compared to their control groups also establishes their higher adaptive capacity to the heat stress challenges. They were able to maintain their metabolic and circulation status even during high stressful conditions. The result from the current study was in agreement with previous studies in small ruminants (Hooda and Upadhyay, 2014; Panda *et al.*, 2016). Increased PR could be directly attributed to redistributed blood flow from core body to the periphery tissues for facilitating heat loss by sensible and insensible means of heat transfer in goats (Okoruwa, 2014; Shaji *et al.,* 2016). The RT is considered as the most common indicator of body temperature in farm animals (Shaji *et al.,* 2016) and also used as a simple and reliable tool for monitoring animal welfare in hot environment (Silanikove, 2000). The RT also showed similar trend to that of PR with significantly higher value in MHS both during morning and afternoon. Significantly higher RT value in Malabari goats indicates their increased vulnerability to the hot environments. Further, the significantly lower RT in SHS compared to SCON shows the superior adaptive capability of Salem Black goats to keep themselves cool in morning hours to cope up to the stressful condition in the afternoon (Shaji *et al.,* 2016). However, heat stress treatment significantly increased the RT in all the three heat stress groups compared to their respective control groups. Similar results were also reported in goats by Habibu *et al.* (2016) and Shaji *et al.* (2016). The increased RT in heat stressed group indicates the inefficient thermoregulatory mechanisms in these goats to maintain thermal equilibrium (Marai *et al.*, 2007). Further, the non-significant influence of interaction between breed, treatment and experimental days on both PR and RT indicates that irrespective of breed, treatment and days the response of these physiological parameters were constant in these indigenous goat breeds throughout the study period. All skin parameters showed significant variation for the treatment. Further, the skin temperatures in different area during morning in heat stress groups was generally significantly lower as compared to the respective control groups while reverse trend was observed during afternoon. These highly

significant changes in skin temperatures between the groups indicate its significance for assessing the adaptive nature of these breeds. The similar results of increased skin temperature due to heat stress were also reported in goats by Hooda and Upadhyay (2014) and Shaji *et al.* (2016). This higher skin temperature could be directly attributed to the vasodilatation of skin capillary bed to enhance the blood flow to the skin periphery for facilitating heat transfer to the surroundings (Shaji *et al.*, 2016). Although Malabari breed showed highly significant changes for most of the physiological indicators, skin temperatures in various regions showed reverse trend in this breed indicating significantly lower skin temperature during heat stress than Salem Black and Osmanabadi goats. This difference could be attributed to the coat color as Malabari breed is pure white while both Salem Black and Osmanabadi goats are pure black in color. Similar results of low temperature in white color goats were also reported by Hagan *et al.* (2012). The goats with light coloured coat have greater advantage over dark coat coloured regarding thermoregulation mechanisms in hot environments as the white colour reflects most of the direct solar radiation falling on them very effectively thereby exhibits better adaptability to hot environments distinguished by intense solar radiation (Hagan *et al.*, 2012). Further, the significant influence of breed treatment and day interaction on both STHA and STFA signify the importance of these parameters on goat adaptation. In addition, a strong positive correlation for all the physiological responses with THI indicates the significance of these variables in assessing the thermo-tolerance capacity of indigenous goats. Furthermore, the negative correlation for respiration rate and rectal temperature in the morning proves that these two variables to be the ideal physiological biomarker for assessing the heat stress in goats.

5.3 Blood biochemical responses

Generally, the blood metabolic profile indicates the health and nutritional status of the livestock (Calamari *et al.*, 2016). Exposure of the animals to heat stress environments significantly alter their general blood biochemical status (Singh *et al.*, 2016; Banerjee *et al.*, 2015). The mean concentration values for the glucose showed breed differences with significantly higher values in Osmanabadi and Malabari goat

breeds compared to the Salem Black breeds. The results obtained in the study were in accordance with previous experiment conducted by Mohammed *et al.* (2016) where they also showed breed variations regarding the glucose level in different goat breeds. The non-significant change in the glucose level in all the three breeds for the heat stress treatment could be attributed to their adaptive potential for these breeds are well known for their survival in the adverse environmental conditions in their native tracks (Banerjee *et al.,* 2015; Shaji *et al.,* 2017). Similar results of the non-significant variations of glucose concentration during heat stress were also reported in heat tolerant indigenous sheep and goats (Singh *et al.,* 2016; Banerjee *et al.,* 2015; Shaji *et al.*, 2017). However, plasma cholesterol showed significant variation among the breeds for heat stress treatment. In addition, the analysis pertaining to the effect of heat stress between the breed indicated that only Malabari breed showed significantly higher cholesterol level as compared to other two breeds. This indicates that on comparative basis Malabari breed was sensitive to the heat stress challenges imposed in the study. The higher cholesterol level in MHS goats could be an effort by this breed to support hepatic gluconeogenesis by mobilizing the fat reserves to maintain the additional glucose supply for the adaptation processes (Sejian *et al.,* 2013a). Further, the increased cholesterol level in MHS goats may also be attributed to the requirement of higher cortisol level to counter the heat stress level given the fact that cholesterol is the precursor material for cortisol synthesis (Ghani *et al.,* 2016). These explanations pertaining to cholesterol level were supported by the findings of Sejian *et al.* (2013a) in heat stressed indigenous Malpura sheep. However, there are also reports indicating decreased cholesterol concentration in ruminants during summer season (Singh *et al.*, 2016; Ocak and Guey, 2010). The highly significant difference in breed factor for total protein, albumin and globulin in the study indicates the different potential of these breeds to use the protein sources efficiently for supporting the adaptive processes to tropical climate. Generally, concentrations of total protein and albumin are used as indices for nutritional status in goats (Attia, 2016). The plasma protein, albumin, globulin and A/G ratio did not show significant variation between groups for the heat stress treatment. However, significant increase of these parameters was

reported in Baladi, Zarabi goats during short term exposure to heat stress (Helal *et al.,* 2010). The non-significant change in the protein level again proves the significant adaptive nature of the indigenous goat breeds to heat stress challenges as they were able to maintain their nutritional status throughout the study. The plasma triglycerides showed significant variation within both Osmanabadi and Salem Black breeds for heat stress treatment with lower values in OHS and SHS compared to their respective control groups. Similar result of significantly reduced triglyceride level was also reported in heat stressed goats, sheep and cattle (Pandey *et al.,* 2012; Singh *et al.,* 2016; Omran *et al.,* 2011). The lower triglyceride level in OHS and SHS could be attributed to the higher hepatic gluconeogenesis for the enhanced energy supply to meet the energy requirements during heat stress condition (e Silva *et al.,* 2016). However, relatively higher triglyceride concentration in the heat stressed Malabari breeds indicates their compromised energy status related higher body triglyceride mobilization as triglyceride act as one of the most important energy sources in ruminants when they are in nutritionally deficient condition (Hashem and El-Zarkouny, 2017). This again shows the vulnerability of the Malabari breeds to hot environments. The non-significant interaction between breed, treatment and experimental days for all the blood biochemical parameters indicates the rigidity of all the three breeds in maintaining the respective effects for heat stress throughout the study period. The non-significant effect of breed and heat stress treatment on both plasma urea and PUN indicates the coping ability of all the three breeds without relying on these parameters to support the adaptation processes. Similar results of non-significant change of urea and PUN during heat stress in male animals of Osmanabadi breed was also reported by Shaji *et al.* (2017). The AST and ALT are generally recognized as indicators for adaptive capability in livestock (Gupta *et al.,* 2013). The AST level was found to be significantly higher in OHS compared to OCON, while within Salem Black and Malabari breeds, non-significant variations were observed. The findings were similar to the observations in Sirohi and Barbari breed where they also did not show significant difference for AST level during summer (Banerjee *et al.,* 2015). However, significantly higher AST concentration was also reported in

ruminants exposed to heat stress condition (Kalmath *et al.,* 2015; Rathwa *et al.,* 2017; Gupta *et al.,* 2013). The significantly higher AST level in OHS could be due to increased hepatic gluconeogenesis or due to hepatic damage cause from deleterious effects of higher heat stress condition (Rathwa *et al.,* 2017). Further, the significantly higher AST only in OHS might be correlated to the requirement for supporting thermo-tolerance as this is the only breed which is native to the locality where the study was conducted. The non-significant influence of heat stress on both MHS and SHS could be attributed to the fact that these breeds are brought from their original native track of much higher magnitude of heat stress to the current locality in the study. Therefore, the AST did not increase in the heat stress groups of these two breeds due to the lower magnitude of heat stress in the current location as compared to their native agro-ecological zone. Additionally, AST is the only biochemical parameter which was significantly influenced by interaction between breed, treatment and experimental days. This clearly indicates the role of this parameter in assessing the adaptive potential in indigenous goat breeds. In addition, the ALT level did not show significant variations in all the three breeds for heat stress treatment. However, higher activity of ALT was reported by Sharma and Kataria (2011), Banerjee *et al.,* (2015) in heat stressed goats. This difference could be attributed to the differences in the magnitude of heat stress between the studies. Further, the non-significant correlation between THI and biochemical variables indicates that the experimental animals did not rely on these parameters to cope to the heat stress condition.

5.4 Endocrine responses

Endocrine modifications are found to be crucial in goats for adapting to the heat stress challenges (Ghassemi Nejad *et al.,* 2017). Among the various endocrine regulators involved in stress response, cortisol is recognized as the primary hormone involved in relieving heat stress in ruminants (Shaji *et al.,* 2017). It is a well established fact in ruminants that during heat stress condition animals tend to synthesis more cortisol as an adaptive response to initiate hepatic gluconeogenesis (Ghassemi Nejad *et al.,* 2017; Shaji *et al.,* 2017). The higher plasma cortisol level

in Malabari heat stress group as compared to its control group could be directly attributed to their sensitivity to heat stress. The higher cortisol level in the heat stressed Malabari goats indicates the higher magnitude of heat stress response in this breed and the higher requirement of cortisol is very essential in these animals to support life sustaining activities through the process of hepatic gluconeogenesis (Baumgard and Rhoads ., 2013). Similar results of heat stress induced higher cortisol concentration were also reported in different breeds of goats (Tajik *et al.,* 2016; Chergui *et al.,* 2017). However, non-significant change in the cortisol concentration in both Osmanabadi and Salem Black goats between their respective control and heat stress groups indicates the superior adaptive capability of these breeds to hot environmental conditions. Although, these goats experienced certain degree of heat stress which was evident from the changes in their behaviour and physiological variables, their higher thermo-tolerant potential was clearly evident from the similar plasma cortisol concentration between their control and heat stressed goats in these two breeds. Blood cortisol level is generally considered an ideal biological marker for quantifying heat stress in ruminant livestock. Therefore, in the current study it can be concluded that cortisol may act as an ideal biomarker for quantifying the heat stress in Malabari breed while in both Osmanabadi and Salem Black breed, cortisol may act as a marker for assessing the thermo-tolerance ability. Further, a strong positive correlation between THI and cortisol level supports this argument.

The results obtained in the current study for plasma aldosterone in heat stressed goats were in contrast to majority of the published reports (Beede and Collier; 1986, Shaji *et al.,* 2017). It is a general observation in many species that when the animals are subjected to heat stress it causes severe dehydration resulting in increased renin-angiotensin-aldosterone response (Olsson and Dahlborn, 1989; Wittenberg *et al.,* 1986; Shaji *et al.,* 2017). However, when the heat stressed animals get access to water sources or in other words if they are rehydrated there are further increase in the renin response which is usually not accompanied by elevated plasma aldosterone concentration (Olsson and Dahlborn, 1989;

Wittenberg *et al.,* 1986). Similarly, in the current study in Malabari breed, the level of plasma aldosterone was significantly lower in heat stressed animals which had *ad libitum* access to water. The non-significant influence of heat stress on plasma aldosterone concentration in both Osmanabadi and Salem Black could be attributed to their extreme adaptive capability in maintaining the water and electrolyte balance. Further, these two breeds are possessing higher thermo-tolerance which was evident from the non-significant changes for plasma cortisol level. Therefore, the non-significant influence of heat stress on both plasma cortisol and aldosterone in Osmanabadi and Salem breeds could prove their superior adaptive capability over Malabari breed goats. However, in another study in Osmanabadi male goats, Shaji *et al.* (2017) reported significantly higher plasma aldosterone concentration in heat stressed group. This difference in aldosterone concentration between the studies could be attributed to the sexual difference. Further, Maltz and Shkolnik (1980) also reported significantly higher plasma aldosterone concentration in heat stressed Bedouin goats which had *ad libitum* water access. These differences in plasma aldosterone concentration across the studies indicate the genetic difference in the ability of the goat breeds to maintain water and electrolyte balance. Further, the primary factors which determine this difference among the breeds for blood aldosterone level could be the magnitude of heat stress the animals are subjected to as well as the accessibility to the water resources. It is a general observation that indigenous breeds rely more on panting mechanism rather than sweating and this imparts an exclusive ability to these animals to maintain water and electrolyte balance. Osmanabadi and Salem Black are also indigenous breeds and well known for their adaptive capability to heat stress and both these breeds relied mostly on the respiratory evaporative cooling mechanism to cope to heat stress. This could be the reason for the non-significant influence of heat stress on the plasma aldosterone concentration in these breeds as they tried to keep intact their electrolyte balance indicating the non-requirement for this hormone. This finding could be supported by the fact that these animals had access to water *ad libitum* and they were provided with balanced ration containing mineral and salt supplementation.

5.5 PBMC HSP70 mRNA gene expression

The HSP70 is recognized as one of the most abundant and important protein in the HSP family playing a very critical role in goats during thermal adaptation (Gupta *et al.,* 2013). Additionally, HSP70 is also identified as a confirmatory cellular marker for heat and humidity stress in ruminants (Shaji *et al.,* 2017; Bharati *et al.,* 2017). Both Osmanabadi and Malabari goats showed a different trend to that of Salem Black goats with significantly higher HSP70 expression in the heat stressed groups compared to their respective control groups. The result obtained was in accordance with other studies which also indicated significantly higher HSP70 expression in heat stressed goats as compared to the control goats (Banerjee *et al,.* 2014; Shaji *et al.,* 2017). Further, the significantly increased HSP70 in the heat stressed Osmanabadi and Malabari goats also indicates the higher magnitude of stress experienced by these two breeds as compared to Salem Black breed. The result also emphasizes the higher HSP70 requirements in these two breeds to counteract the deleterious effects of hyperthermia at the cellular level (Banerjee *et al.,* 2014). The non-significant difference in HSP70 expression between SCON and SHS groups indicated that the stressful condition was not severe enough to create cellular responses in this breed. Further, the level of HSP70 mRNA expression in SBHS group was significantly lower than OHS and MHS groups. These findings clearly indicate the superior adaptive capability of the Salem Black goats to tropical environments. From these results it may be inferred that HSP70 gene can be used effectively for assessing the superior thermo-tolerance ability among the extremely adapted breeds.

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Livestock rearing serve as a major economic activity in the lives and livelihoods of millions of poor and marginal farmers, particularly in the developing countries. Among the various climatic variables, ambient temperature fluctuations are found to be the most intriguing factor affecting livestock production potential. Prolonged exposures of the animals to heat stress condition negatively affects their productive parameters such as growth, milk, meat and reproduction, which in turn results in lower food production and higher economic loss to poor farmers. Considering the importance of selecting highly adapted livestock breeds for the future, comparative studies on different indigenous breeds to assess their potential to survive and produce optimally and this may provide valuable information in identifying superior thermo-tolerant breeds specific to a tropical location. Further, such studies also assist in developing important biological markers which can be utilized in breeding programmes using marker assisted selection (MAS) to sustain livestock production in the hot climatic regions.

Animals having superior productive traits may produce poorly when the production environment is not favourable due to negative interaction between their genetic merit and environmental variables. In most of the cases shifting of exotic breeds from their specific agro-ecological zone failed to become a sustainable strategy mainly due to their compromised production levels in the new region. Hence, the identification of agro-ecological zone specific breeds is considered an important strategy to sustain animal production level optimally in the changing climate scenario. Therefore, the present study was conducted to evaluate the effect of shifting two extremely adapted indigenous breeds in their native tract to another agro-ecological zone and assess their adaptive capabilities in the new locality in comparison to the local breed. For this purpose, Malabari and Salem Black breeds are the two breeds well known for their ability to survive in extremely hot and humid environment were shifted to a new locality where the heat stress was of much

lower magnitude. Their thermo-tolerance ability was compared to the local Osmanabadi breed well known for its survival in the current experimental location. The primary objective of the study was to compare the genetic potential of these breeds to cope to heat stress based on the changes in their behavioral, physiological, biochemical, endocrine responses and peripheral blood mononuclear cell heat shock protein 70 (PBMC HSP70) gene expression patterns.

A study was conducted for a period of 45 days between April-May 2017. Thirty six goats were randomly allocated into six groups of six animals each, OCON (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MCON (n=6; Malabari control), MHS (n=6; Malabari heat stress), SCON (n=6; Salem Black control) and SHS (n=6; Salem Black heat stress). The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40 % concentrate (Maize 36kg, wheat bran 37kg, soybean meal 25kg, mineral mixture 1.5kg, common salt 0.5 kg/ 100kg). The OCON, MCON and SCON animals were maintained in the shed in while OHS, MHS and SHS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h. The OCON, MCON and SCON animals were fed and watered inside the shed while OHS, MHS and SHS animals were fed and watered while they are exposed to summer heat stress in the outside environment.

Both ST and LT did not show any significant variation for both breed and treatment. However, the experimental days significantly influenced both ST ($P<0.01$) and LT ($P<0.05$). The DF also did not show any significant difference for breed variation. However, heat stress groups of all the three breeds (OHS, MHS and SHS) showed significantly ($P<0.01$) higher DF than their control groups (OCON, MCON and SCON). The UF showed significant ($P<0.05$) variation for both the breed and treatment effect. But the UF did not differ between the control groups whereas within heat stress groups, SHS showed significantly lower UF than MHS while OHS did not show any significant variation with other breeds. The RuT showed similar trend to UF. Further, WI showed variation for the treatment with

significantly higher (P<0.01) value in heat stress groups as compared to their respective control groups.

The RRM did not show any significant difference for the breed factor. However, the RRM significantly (P<0.01) reduced in heat stress groups compared to their respective control groups for the treatment. The RRA showed significant (P<0.01) variation between groups for the breed as well as treatment. The SHS group showed significantly (P<0.01) lower RR compared to both OHS and MHS during afternoon. The PRM significantly (P<0.01) differed for the breed factor. However, the PRM did not show any significant variation between the groups for the treatment. The PRA showed significant variations for both the breed and treatment. The RTM also showed similar trend to that of PRA in that the breed (P<0.01), and treatment (P<0.05) significantly influenced the RTM. The RTA showed significant variation for both the breed (P<0.05) and treatment (P<0.01).

The STHM showed significant (P<0.05) variation for the breed as well as treatment. The STHA showed significant (P<0.01) variation for the breed. Additionally, STHA showed significant (P<0.01) variation for the treatment and the effect was significantly higher in the heat stress groups than the control groups. The STSM did not show significant variation for breed factor. However, STSM showed significant (P<0.05) variation for the treatment. The STSA showed significant (P<0.01) variation for both breed and treatment. The STFM showed significant (P<0.01) variation for the breed factor. Further, STFM also showed significant (P<0.01) variation between groups for the treatment. The STFA showed significant (P < 0.01) variation between the breeds. Further, STFA showed significant (P<0.01) variation between groups for the treatment.

Breed factor significantly influenced plasma glucose (P<0.01), total protein (P<0.01), albumin (P<0.01), globulin (P<0.01), triglycerides (P<0.01), AST (P<0.05) and ALT (P<0.05). However, heat stress treatment significantly influenced only plasma cholesterol (P<0.05), triglycerides (P<0.05) and AST (P<0.05). Heat stress significantly increased total plasma cholesterol and AST in

MHS and OHS groups respectively. However, heat stress significantly reduced the plasma triglycerides concentration in both OHS and SHS groups. Further, heat stress treatment significantly ($P<0.01$) influenced plasma cortisol level only in Malabari breed. However, heat stress did not influence plasma cortisol in both Osmanabadi and Salem Black breeds. Further, plasma aldosterone showed significant variation for both breed ($P<0.01$) as well as heat stress treatment ($P<0.05$). Heat stress significantly ($P<0.05$) decreased the plasma aldosterone level only in Malabari goats. In addition, positive correlation ($P<0.05$) was established between THI and plasma cortisol level while strong negative correlation ($P<0.01$) was established between THI and plasma aldosterone concentration.

The results obtained indicated PBMC HSP70 expression in OCON, OHS, MCON, MHS, SCON and SHS to be 1.07, 2.3, 1.04, 2.13, 1.11, 1.13 folds respectively. Comparatively, heat stressed groups of both Osmanabadi and Malabari breeds showed significantly ($P<0.05$) higher expression of PBMC HSP70 mRNA compared to their respective control groups. However, in Salem Black goats, PBMC HSP70 expression did not differ between control and heat stress groups. Further among the stress groups, the level of HSP70 mRNA expression was significantly lower in SHS as compared to OHS and MHS groups.

The results from the study indicated that heat stressed goats were trying to cope to the adverse environmental conditions through alterations in both behavioral and physiological responses. The finding from the study also clearly indicated the non-reliability of all the three breeds on the biochemical response to adapt to the heat stress challenges. Further, the study clearly indicated the differences in the thermo-tolerant ability even among the indigenous breeds. Further, these differences in heat stress response were observed even at cellular level as evident from the differences in HSP70 expression patterns between these breeds. In addition, the study also indicated RR, RT plasma cortisol and PBMC HSP70 to be ideal biological marker for assessing the thermo-tolerance ability of indigenous goats.

The results obtained for various variables clearly indicated that the Malabari breed goats experienced the higher magnitude of heat stress as compared to both Osmanabadi and Salem Black breeds. Therefore, shifting of Malabari breed from extreme hot and humid environment in their native tract to the current experimental location with heat stress of much lower magnitude did not proved advantageous in terms of its adaptive potential. However, significantly lower influence of heat stress on majority of the variables studied in Salem Black breed and the efficiency of this breed in maintaining the adaptive potential more or less similar to Osmanabadi breed clearly indicated the superior ability of this breed to cope to varied tropical environment. Therefore, shifting of Salem Black breed may yield beneficial effects in sustaining their production in different agro-ecological zone. However, future studies are warranted to further test the thermo-tolerant efficiency of this breed in other locations.

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COMPARATIVE ASSESSMENT OF THE ADAPTIVE CAPACITY OF DIFFERENT INDIGENOUS BREED GOATS TO SUMMER HEAT STRESS BASED ON CHANGES IN PHENOTYPIC TRAITS

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to evaluate the differences in the thermo-tolerant abilities of three indigenous goat breeds (Osmanabadi, Malabari and Salem Black) to heat stress challenges. The primary objective of the study was to compare the adaptive ability of two indigenous goat breeds (Malabari and Salem Black) when they were shifted from their native tract to a new agro-ecological zone with the local breed (Osmanabadi) during heat stress exposure. The adaptive capabilities of these breeds were assessed based on the changes in their behavioral, physiological, blood biochemical and endocrine responses and peripheral blood mononuclear cell heat shock protein 70 (PBMC HSP70) gene expression patterns. Thirty six 10 months to one year old female goats were randomly allocated into six groups of six animals each as OCON (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MCON (n=6; Malabari control), MHS (n=6; Malabari heat stress), SCON (n=6; Salem Black control) and SHS (n=6; Salem black heat stress). The OCON, MCON and SCON animals were maintained in the shed while OHS, MHS and SHS animals were exposed to summer heat stress between 10:00 h to 16:00 h. All the animals had access to *ad-libitum* feed and water. The duration of the study was 45 days. Results indicated that among the behavioural variables studied, both drinking frequency (DF) and water intake (WI) were significantly higher ($P<0.01$) in heat stress groups of all the three breeds as compared to their respective control groups. Further, significantly lower ($P<0.05$) urinating frequency (UF) and higher ($P<0.05$) rumination time (RuT) was recorded in MHS as compared to other stress groups. The heat stress treatment significantly ($P<0.05$) lowered the respiration rate (RR) and rectal temperature (RT) in the morning while significantly ($P<0.01$) increased all the physiological variables such as RR, pulse rate (PR) and RT in all the three breeds during afternoon. Further, the results indicated that only breed differences ($P<0.01$) were established for different biochemical variables in the study while the heat stress did not alter these parameters.

However, heat stress significantly (P<0.05) increased the plasma cholesterol in MHS group while significantly lowered (P<0.05) plasma triglyceride in both OHS and SHS groups. Further, aspartate aminotransferase (AST) showed significant variation for both breed (P<0.05) as well as treatment (P<0.05) effect. In addition, heat stress significantly increased (P<0.01) plasma cortisol and significantly decreased (P<0.05) plasma aldosterone in only MHS group. Furthermore, heat stress induced PBMC HSP70 expression was significantly higher (P<0.05) in OHS and MHS as compared to SHS group. Thus, it can be concluded from the results that although Malabari breed which is well known for its survival in harsh environment in its native tract could not adapt well to the new locality with much lower magnitude of heat stress. The less severe influence of heat stress on the various biological functions of Salem Black breed as compared to other two breeds clearly indicated the superior adaptability of this breed to survive in different agro-ecological zones. Further, the study also identified RR, RT, plasma cortisol and PBMC HSP70 to be the reliable biological markers for evaluating the thermo-tolerant capacity of indigenous goat breeds.

Keywords: Adaptation; Climate change; Cortisol; Drinking frequency; Goat; Heat stress; HSP70