COMPARATIVE ASSESSMENT OF CLIMATE RESILIENT CAPACITY OF TWO INDIGENOUS GOAT BREEDS BASED ON CHANGES IN BOTH PHENOTYPIC AND GENOTYPIC TRAITS

By

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(2015-20-008)

THESIS

Submitted in partial fulfillment of the requirements for the degree of

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DECLARATION

I, hereby declare that this thesis entitled **"Comparative Assessment of Climate Resilient Capacity of two Indigenous Goat Breeds based on changes in both Phenotypic and Genotypic 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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CERTIFICATE

I, hereby certify that the thesis entitled "**Comparative Assessment of Climate Resilient Capacity of two Indigenous Goat Breeds based on changes in both Phenotypic and Genotypic Traits**" is a record of research work done independently by Ms. Reshma Nair M R (2015-20-008) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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I do believe in destiny.

Dedicating this thesis to the destiny that made me to meet my Mentor

To my beloved family

&

To all the 24 lives who sacrificed themselves for my study

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Reshma Nair

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SYMBOLS AND ABBREVIATIONS

%- Percentage

µg – Micro gram

4-AAP - 4-Aminoantipyrine

AC- Adaptability coefficient

ACTH- Adrenocorticotrophic hormone

ADG- Average Daily Gain

ADH- antidiuretic hormone

ALT- Alanine tranaminase

ANKLE2- Ankyrin Repeat and LEM Domain Containing 2

ANOVA- Analysis of variance

AR5- Assessment report 5

ASIP- Agouti-signaling protein

AST- Aspartate transaminase

AT-Ambient Temperature

ATP- Adenosine tri phoshphate

B- Blank

BBS7- Bardet-Biedl syndrome 7

BCas3- Breast carcinoma-amplified sequence 3

BCG- Bromocresol Green

bp-Base pair

BxTxD- Breed Treatment Day

CAP- Compensatory accelerated production

CCNA2- Cyclin-A2

CDC25A - Cell division cycle 25 A

cDNA- complementary DNA

CHOD/PAP- Cholesterol oxidase/phenol + Aminophenazone

CHOD-Cholesterol oxidase

cm- Centimeter

CO_2- Carbon dioxide

CRH- Corticotrophin releasing hormone

CSN3- Casein kappa

DBT- Dry bulb temperature

DeF- Defecation frequency

DGCR8- DiGeorge syndrome chromosomal region 8

DGDP- Dairy Goat Development Programme

DGKB- Diacylglycerol kinases

DLC- Differential leukocyte counts

EDTA- Ethylene diamine tetra acetate

ELISA- Enzyme Linked Immuno Sorbent Assay

FAO- Food and Agriculture Organization

FEC- Feacal Egg Count

F- Forward

FGF2- Fibroblast growth Factor 2

FI- Feed intake

fL- Femoliters

F_{ST} - Fixation index

g/dL- Gram per deci litre

g/L- Gram per litre

GAPDH - *G*lyceraldehyde 3-phosphate dehydrogenase

GDP- Gross Domestic Production

GH- Growth hormone

GHG- Greenhouse gas

GIN- Gastrointestinal nematodes

GK- Glycerol Kinase

GNA11- Guanine nucleotide-binding protein subunit alpha-11

GOD-POD- Glucose oxidase Peroxidase

GOLGA3- Golgin A3

GPO- Glycerol phosphate oxidase

GPO-PAP - Glycerol-3-Phosphate Oxidase-Phenol + Aminophenazone

h- Hour

H_2O- Water

H_2O_2- Hydrogen peroxide

Hb- Haemoglobin concentration

HCT- Hematocrit

HGB- Haemoglobin

HLI- Heat load index

HPRT1- Hypoxanthine phosphoribosyl transferase 1

HRP- Hypersensitive reaction and pathogenicity

HSBP1- heat shock transcription factor-binding protein

HSF-1- Heat shock transcription factor 1

HSP- heat shock protein

HSPs- Heat shock proteins

HTC- Heat tolerance coefficient

HTT- Huntingtin

IFCC- International Federation of Clinical Chemistry

IL2- Interleukin 2

IL21- Interleukin 21

IL7- Interleukin 7

IMD- India Meteorological Department

INR- Indian Rupees

IPCC- Inter governmental Panel on Climate Change

IU/L- International units per litre

KAC- Kanni Aadu Control

KAHS- Kanni Aadu Heat Stress

kg- Kilo gram

km/day- Kilo meter per day

KOC- Kodi Aadu Control

KOHS- Kodi Aadu Heat Stress

LT- lying time

LW- Live weight

m- Meter

MAD- Malate Dehydrogenase

MANEA- Mannosidase Endo-Alpha

MAS- Marker Assisted Selection

MaxT- Maximum Temperature

MCH- Mean Corpuscular Haemoglobin

MCHC- Mean Corpuscular Hemoglobin Concentration

MCV- Mean Corpuscular Volume

mg/dL- milli gram per deci litre

mg/dL- milligram per deci litre

MHC- Major histocompatibility complex

MinT- Minimum temperature

ml- milli litre

MT- Mega tonne

n- Number

N/L ratio- Neutrophil to lymphocyte ratio

N_2O- Nitrous Oxide

NAD- Nicotinamide adenine dinucleotide

NCBI- National Center for Biotechnology Information

NFW- Nuclease-free water

nm- Nanometer

NS- Non-significant

^oC- Degree centigrade

OD- Optical Density

OSTM1 - Osteoclastogenesis Associated Transmembrane Protein 1

p mol- Picomole

PBMC- Peripheral blood mononuclear cells

PCDH9 - Protocadherin 9

PCR- Polymerase chain reaction

PCSK5- Proprotein Convertase Subtilisin/Kexin Type 5

PCV- Packed Cell Volume

pg- Picogram

pH- Potential of hydrogen

POD- Peroxidase

PR- Pulse rate

PRA-Pulse rate afternoon

PRE-Pulse rate evening

PRM-Pulse rate morning

PRN-Pulse rate night

PSA- Panting score afternoon

PSD3- Pleckstrin and Sec7 Domain Containing 3

PSMA7- Proteasome 20S Subunit Alpha 7

PS-Panting Score

PST-Pen Surface Temperature

R- Reverse

RBC-Red Blood Cell

RDH16 - Retinol Dehydrogenase 16

RDW- Red Blood Cell Distribution Width

RH- Relative humidity

RNA- Ribonucleic acid

rpm- Revolutions per minute

RR- Respiration rate

RRA-Respiration rate afternoon

RRE-Respiration rate evening

RRM- Respiration rate morning

RRN- Respiration rate night

RT-qPCR - Real-time quantitative polymerase chain reaction

RT- Rectal temperature

RTA- Rectal temperature afternoon

RTE- Rectal temperature evening

RTM- Rectal temperature morning

RTN- Rectal temperature night

RuT- Rumination time

s- Seconds

S- Standard

SA- Serum albumin

SDHA- Succinate dehydrogenase

SkT- Skin temperature

SNF- Solid non-fat

SPP1- Secreted Phosphoprotein 1

SPSS- Statistical Package for the Social Sciences

ST- Standing time

STF- Skin temperature flank

STFA- Skin temperature flank afternoon

STFE- Skin temperature flank evening

STFM- Skin temperature flank morning

STFN- Skin temperature flank night

STH- Skin temperature head

STHA- Skin temperature head afternoon

STHE- Skin temperature head evening

STHM- Skin temperature head morning

STHN- Skin temperature head night

STS- Skin temperature shoulder

STSA- Skin temperature shoulder afternoon

STSE- Skin temperature shoulder evening

STSM- Skin temperature shoulder morning

STSN- Skin temperature shoulder night

T_3- Tri iodothyronine

T_4- Thyroxine

Ta- Annealing temperature

TBX15- T-box transcription factor 15

T_{db}- Dry bulb temperature

TEC- Total erythrocyte count

tF - Desired reporting temperature

THI- Temperature humidity index

TLC- Total leucocyte count

TNZ- Thermo neutral zone

TRT- Treatment

TSP- Total serum protein

T- Test

T_{wb}- Wet bulb temperature

UF- Urinating frequency

UGT8- 2-hydroxyacylsphingosine 1-beta-galactosyltransferase

WAD- West African Dwarf

WBC- White Blood Cell

WBT- Wet bulb temperature

WI- Water intake

α - Alpha

μL- Micro litre

INTRODUCTION

CHAPTER 1

INTRODUCTION

Livestock production is one of the most widely adopted agricultural practices over the entire world. It has emerged as "the engine of agricultural growth" with a contribution of 4.11% to India's total gross domestic production (GDP), which is about 21.58% of the total agricultural GDP of the nation. It has been accounted as the most sensible and powerful tool to achieve food security as the world human population is expected to reach 9.6 billion by 2050 (FAO, 2015). Being an integral part of agriculture, livestock sector plays a significant role in providing livelihood to billions of people throughout the world, especially in developing economies like India (Ahmad and Ma, 2020).

India is one among the emerging nations of world and the major chunk of its economy comes from agriculture and livestock sector. It was reported that the human population in India has grown steadily, reached 1.36 billion and is likely to add 273 million more people by the end of 2050, making the Indian economy more vulnerable to food insecurity. As per the FAO report (2015), most of the developing countries, especially the economies like India have witnessed instability in maintaining food security and eradicating poverty in cause of population explosion. The recent decline in crop productivity associated with the repeated occurrence of extreme events pertaining to changing climate has further posed challenges to the nutritional demand of growing population. Indeed, malnutrition due to inadequate access to staple food results in short term as well as long term impacts on health, productivity and employment potential of

rural economies. However, livestock is established to have the potential to curtail the food insecurity concerned with population explosion. Currently, almost 33 per cent of protein and 16 per cent of energy in human diet is contributed by livestock products (Martin, 2001). As per the World Bank reports (2009), the demand for animal products are likely to increase further by 2050 in developing states.

Apart from bringing nutritional security, livestock contribute immensely to ensure the economic stability of poor and marginal farmers. It has been established that majority of the poor people in India depends on livestock sector for their livelihood. Poor landless farmers find livestock production as a feasible way to ensure their livelihood security. On the other hand, farmers who practice crop cultivation rear livestock as an alternate source of income during scarcity periods. Indeed, livestock acts as a "living bank" for rural communities to minimize the impacts of natural calamities on their livelihood. Additionally, studies reveal that livestock play a significant role in bringing gender equivalence by providing employment opportunities for women.

To a greater extent, livestock sector contributes for eco-intensification and to eliminate the negative impacts of several agricultural practices (Teague *et al.,* 2013). With the increasing global concern about the impacts of urbanization on environment, the livestock sector acts as a solution in reducing the ecological footprint of the whole agriculture sector without compromising the productivity (Vigne *et al.,* 2012). Unlike developed countries, farmers in developing countries mostly rely on small holder farms whose energy demands are satisfied with human as well as animal labour (Tabar *et al.,* 2010). Substituting machineries with human as well as animal labour would decrease the emission of greenhouse gases (GHG), thereby reducing the carbon footprint from the

sector. Through appropriate grazing patterns, livestock production contributes with several ecosystem services, including water infiltration, improving biodiversity, carbon sequestration and ecosystem stability (DeRamus *et al.,* 2003).

Climate change acts as a potential threat to curb the animal agriculture with a direct influence on their growth, milk production, meat production, reproduction and health (Sejian *et al.,* 2013). Apart from the direct effects, climate change has been reported to influence livestock production indirectly by reducing both the pasture and water availability as well as by increasing the frequencies of sudden disease outbreaks (Sejian *et al.,* 2015).

Changing climate impose various kinds of stress on animal agriculture, heat stress being the most alarming one among them (Sejian *et al.,* 2010). Elevated ambient temperature was reported to impair the growth of animals as a result of decrease in anabolic activities with an increase in catabolism due to high catecholamine and glucocorticoid levels. Also, high temperature and humidity were found to decrease the meat quality through increase in meat pH, cooking loss and drip loss (Archana *et al.,* 2017). A further potential effect of heat stress was to alter the reproductive capability of animals. It was well established that heat stress reduced the fertility of animals through the poor expression of estrus thereby reduced their production potential. In addition, changing climate induced various responses which reallocate energy to life sustaining activities, culminates with the reduction in milk production. Models based on different climatic scenarios suggest that milk production will decrease by 1.6 million tonnes by 2020 and by more than 15 million tonnes by 2050 (Ravagnolo *et al.,* 2000).

The unique attribute of animal agriculture is that they are affected by changing climate as well as act as a major contributer to the climate change. It is scientifically evident that the major chunk of human induced radiative forcing is coming from the livestock sector (Chauhan and Ghosh, 2014). Massive share of greenhouse gas emission from the livestock sector is through the enteric methane emission from the ruminants. Other greenhouse gases like N_2O and CO_2 are respectively released from animal manure and the poor management of pasture land (Beauchemin and McGinn, 2005). It was estimated that the annual methane emission from the Indian livestock approaches to be 10.08 MT (Singhal *et al.,* 2008).

The vulnerability of livestock to heat stress varies according to species, genetic potential, life stage and nutritional status. Although animals can adapt to the hot climate, the response mechanisms that ensure their survival have hampered their production potential as metabolic energy gets deviated into life sustaining activities. Heat stress has severe consequences on growth, milk production, meat production as well as reproduction of animal population.

Growth is a complex physiological phenomenon which is mediated by nutritional status of animals, genetic factors and environmental factors. The cellular events like increase in the body mass and cell multiplication may fluctuate with changing environmental factors. Indeed, the economic impacts of heat stress on growth factors of livestock population can be evaluated from their ability to suppress the welfare of the animals. Exposure of animals to elevated ambient temperature may result in reduced voluntary feed intake (FI). The consequences associated with reduced FI are reflected in animal body weight and average daily gain (ADG). Lower body weight may have an

adverse impact on the productivity of the animal. Additionally, thermal stress also affects the immune status of animals thereby making them susceptible to various diseases. Livestock raised under higher ambient temperature may have low body condition score. For instance, the hip width, wither height and trunk length of animals under thermal stress is invariably low when compared with animals reared under comfort zone. The reduction in growth rate due to heat stress also hampers the reproductive ability of livestock. Poor conception rate and lower calving/kidding/lambing percentage have been reported in such animals.

Exposure of animals to higher ambient temperature reduced the concentration of tri iodothyronine (T_3), thyroxine (T_4) and growth hormone (GH). This decrease in the hormone levels has been associated with the adaptive mechanism exhibited by the animal so as to avoid excess generation of heat due to metabolic activities. Milk production and milk quality were identified to get reduced in dairy animals. It has been observed that every one unit rise in temperature humidity index (THI) was found to influence both milk production and its quality. Heat stress was found to reduce both protein and fat content and increases the solid non-fat (SNF) in milk resulting in compromising its quality. Studies found high producing dairy animals to be more sensitive to increased temperature as their metabolic heat production is very high and hence, they find it difficult to cope with heat stress. Thus, the thermo neutrality is found to be skewed to lower temperature in highly prolific dairy cattle.

Heat stress also poses great threat on the overall meat yield as well as on the quality and composition of meat. The exposure of meat animals to increased ambient temperature above the comfort level may cause alteration in physiological variables

resulting in reduced FI which affects the muscle mass development. Moreover, heat stress exacerbates decline in meat quality attributes like muscle pH, water holding capacity and meat color. Thus, heat stress not only affects the meat production but also significantly reduces its quality eventually leading to production losses. In addition, shelf life of meat may reduce in summer months due to bacterial contamination.

Extreme heat stress during the active reproductive phase in animals may act as the major intriguing factor hampering the optimum reproductive efficiency in both sexes. For instance, heat stress impairs the fertility, which ultimately results in reduced reproductive efficiency in farm animals. Heat stress exerts a primary control over the maturation and ovulation of the dominant follicle in goats. The higher ambient temperature and relative humidity can alter the reproductive ability of farm animals by producing poor quality oocytes and embryos in females. Decreased conception rate along with wider days open can influence Furthermore; large numbers of follicles get recruited for development resulting in reduced follicular dominance. Exposure of inseminated cattle to temperature above their comfort zone reduces the chance of pregnancy by 50%. In addition, the consequences of heat stress on reproductive ability of farm animals may result in reduced fetal size at the time of parturition. Studies on the impact of heat stress on reproductive abilities of male animals are also gaining pace for their lesser population when compared to female animals. Heat stress alters the spermatogenesis process causing sperm abnormalities in male. Further, heat stress also reduces the semen quality and concentration. Seasonal influence on semen production and quality also was established in farm animals. For instance, progressive motility of sperms in bull ejaculate is less in summer months when compared to the ejaculate of bulls collected in winter months.

India is recognized as one of the richest countries in terms of their diverse population of goat breeds. As per the available Livestock census data, the goat population in India has touched 140 million. Over time, goat production in India has been considered as a traditional activity and a way of life for rural farmers (Singh *et al.,* 2016). They are reared for a variety of products including milk, meat, skin and fleece. In the context of the anticipated increase in human population, goats can play a major role in catering for the nutritional demands of future generations through the production of milk and meat (Kumar *et al.,* 2010). The demand for goat meat and milk has been rising exponentially and surpassed other livestock species for their purported health benefits and therapeutic values and thus the goat products are becoming popular (Raut and Kurpatwar, 2020). In recent years, goat enterprises have turned out to be of more commercial value as a result of the marketing preference of goat products all over the world (Kumar *et al.,* 2006). Thus, having the potential scope to ensure the nutritional security, goat production serves as an important occupation for the rural poor and marginal farmers in India.

Having the potential for bringing sustenance and food security, livestock sector contributes immensely towards sustainability of livelihood and nutrition of poor and marginal farmers. Climate change consequences on livestock population has been established to be one among the greatest worries of current time for their potential in bringing sustenance and food security in lives of poor and marginal farmers. High ambient temperature along with the effect of humidity impaired availability of forages in terms of their quality and quantity. This is particularly evident in tropical countries where indigenous animals are predominantly distributed. The reasons for the potential depletion of pasture resources are due to the occurrence of extreme events, pandemic disease

outbreaks and elevated CO_2 concentration (IPCC, 2000). This can significantly hamper the nutritional availability of livestock.

Besides being susceptible to the reduced forage availability, animals are exposed to the heavy risk of drought. Livestock which are extensively reared in arid and semi-arid regions are extremely vulnerable to the adverse effects of drought. The indicator for water use, the water footprint, associated with the growth of forages, milking, servicing water and milk production indicates the quantum of water requirement in each component of animal agriculture (Ibidhi and Salem, 2020). Reports suggested that the global livestock water demand is expected to increase 71% by 2025, with a highest consumption in developing countries (Rosegrant *et al.,* 2013; Schmidhuber and Tubiello, 2007). As dry period progress, animals are forced to mobilize their available body fat reserve so as to cope with the nutritional deficiency. Eventually, it results in reduction of livestock production. It is therefore important that the scientific fraternities should put their efforts to find the most climate resilient animal cutting across the livestock species so that it can be disseminated to the poor and marginal farmers to maintain their livelihood in challenging climatic conditions.

In context of climate change in perspectives of water and forage scarcity, farmers cannot afford the maintenance expenses for large ruminants when compared to small ruminants (Sejian *et al.,* 2018). Thus, studies illustrated small ruminants as the go to species in background of changing climate. Among the small ruminants, goats are gaining more primacy for their inherent ability to survive, produce, reproduce and withstand under wide range of environmental conditions. They are considered as the superior animal species to be raised in the changing climatic scenario for their thermo tolerance,

drought tolerance, ability to thrive under low pasture conditions and disease resistance capacity (Pragna *et al.,* 2017).

Goats are opportunistic feeders and thus the depletion of pasture lands may hardly impose an impact on their diet requirement. In addition, selective feeding behavior of goat helps them to consume even the poor quality forages and have the ability to convert them into high quality products (Dossa *et al.,* 2015). Moreover, goats exhibit bipedal stance which helps them to get access to tree leaves and this is considered advantageous as compared to other livestock species. Further, goat has the better feed conversion efficiency than other ruminant species. In addition, goats do not require specialized shelter structures and they could ideally survive in any location with minimum protection from the weather variables. It, therefore is considered to be the most ideal animal to be reared in changing climate scenario.

Goat rearing is an important occupation of the small and marginal farmers in Tamil Nadu. As per the livestock Census data, Tamil Nadu ranks seventh in terms of goat population with a total of 9.27 million goats distributed throughout the state. It accounts for almost 6.6 % of the total goat population of the country. Within a span of 50 years, the goat population in Tamil Nadu has grown positively with an increase of 5.23 million in numbers. Tamil Nadu is endowed with three indigenous goat breeds, namely, Kanni Aadu, Kodi Aadu and Salem Black. These breeds are well adapted to extreme hot and dry weather of Tamil Nadu and perform excellent under various agro-ecological zones of Tamil Nadu. All these breeds are known for their quality meat. Tamil Nadu farmers prefer Kanni Aadu as well as Kodi Aadu over Salem Black for their wider distribution throughout the state.

Literatures project indigenous animals to have greater adaptive capabilities to withstand under challenging environment than the exotic as well as crossbred species. There are diverse reports to verify the superior characteristics of indigenous cattle to withstand heat stress associated challenges namely, thermo-tolerance, drought tolerance, disease resistance and ability to withstand low pasture conditions. On the other hand, goats have always remained as unexplored. There are very few studies on superior characteristics of indigenous goats against environmental fluctuations (Pragna *et al.,* 2017; Archana *et al.,* 2018; Aleena *et al.,* 2018). Even then, these indigenous species fail to adapt to different magnitudes of heat stress when exposed to different agro-ecological zones.

In a series of studies conducted in indigenous Osmanabadi, Malabari and Salem Black goats, attempts were made to test their ability to survive in multiple locations (Pragna *et al.,* 2018b; Afsal *et al.,* 2019; Savitha *et al.,* 2019; Rashamol *et al.,* 2019). Based on several variables such as growth, adaptation, meat production and immune system related variables it was established that Salem Black breed possessed the better ability to survive in multiple locations (Vandana *et al.,* 2019; Pragna *et al.,* 2018a; Archana *et al.,* 2018; Aleena *et al.,* 2018). This breed was able to maintain their growth and meat production apart from adapting to the heat stress challenges. Therefore, it was concluded that among the three breeds, Salem Black breed are more suitable to propagate to different agro-ecological zones in Southern India. Such research efforts pertaining to identifying the best breed among the various indigenous breeds is the need of hour to sustain livestock production in the changing climate scenario. This may pave way for

disseminating most suitable breed which can cope to the climate change oriented environmental extremes and produce optimally in a specific location.

Although we claim indigenous goat as the go to species in perspectives of changing climate, we are yet to explore the genetic merit of this particular species cutting across all the breeds to identify a breed which is superior in adaptation point of view to sustain the demands of growing human population. Considering the importance of identifying a superior thermo-tolerant goat breed, scientific fraternities should focus on comparing the adaptive potential of different goat breeds in terms of their growth, production and immune status when exposed to thermal stress. Further, such studies could yield important biological markers utilized in breeding programs using Marker Assisted Selection (MAS) to evolve thermo-tolerant breed. With these intensions, the present study was conducted to delineate and compare the adaptive capabilities of two indigenous goat breeds viz. Kanni Aadu and Kodi Aadu, well known for their ability to survive under extreme hot climate of Tamil Nadu. With this background, the study was conducted with the following objectives:

1. To observe the impact of heat stress on the behavioral and physiological responses of two indigenous goat breeds
2. To establish the impact of heat stress on the blood biochemical and endocrine responses of two indigenous goat breeds
3. To ascertain the impact of heat stress on the selective thermo-tolerant gene expression patterns in two indigenous goat breeds

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Climate change is a hot topic in the current era with an alarming concern and it is no longer a distant problem. It has been placed as the most challenging issue that human kind had ever faced posing as a threat to both global ecological balance as well as economy (Rashamol *et al.,* 2019). As per the fifth IPCC Assessment report (AR5), global surface temperature is expected to rise by 1-7ºC by the end of 2100 (IPCC, 2013). On the global scale, there are increasing reports on varying temperatures, increase in the melting of glaciers, rising sea level which leads to inundation of the coastal areas, increased risks of droughts and floods, threats to biodiversity, rise in disease outbreaks and potential challenges for public health. Apart from all these, it is the impact of climate change on agriculture and animal husbandry that is of a major concern as it will have a striking effect on the economy and livelihood. However, it is the developing countries that are more vulnerable to the extreme climatic changes as they depend on natural resources like agriculture, animal husbandry and forestry which are climate sensitive (IPCC, 2007; Chauhan and Gosh, 2014). Cline (2007) has predicted agricultural production in developing countries to fall between 10 to 20 per cent and also that global warming would decrease the agricultural capacity of India by 40 per cent if continued unabated.

Nearly 75 per cent of the world's extremely poor (1.2 billion) are estimated to live in rural areas and depend on agriculture and/or agriculture related activities for their daily income (Pica-Ciamarra *et al.,* 2015). The livestock sector contributes 40 per cent of the

world's agriculture GDP and the demand for livestock products is expected to double during the first half of the 21st century (Sejian *et al.,* 2013). During this same period, extreme variations in the climatic conditions are also expected which would adversely affect this sector (Sejian *et al.,* 2013). India, being the largest milk producer in the world is also facing economic losses due to varying climatic conditions. An annual loss of about 1.8 million tonnes of milk (2661.62 crores), which was nearly 2 per cent of the milk production was reported in the country as a consequence of thermal stress on livestock (Chauhan and Ghosh, 2014). Similarly, African countries too have been experiencing severe droughts having a devastating impact on their food security (Ali *et al.,* 2017). Apart from providing essential protein and nutrition to human diet, the livestock sector also plays an important role in utilization of non-edible agricultural by-products. The livestock sector has emerged as a key component of agricultural growth in some of the developing countries especially in India. A compounded growth rate of more than 5 per cent was observed in the Indian livestock production in contrast to the stagnant or marginally increasing growth rate of crop production. Thus, the livestock sector is of economic importance to the developing countries.

The rapidly changing climatic conditions exert various kinds of stress on livestock, heat stress being the prominent one among them. Heat stress affects the performance, productivity and health of livestock thereby leading to severe economic losses (Sejian *et al.,* 2013). The adverse effects on climate change on livestock production includes decreased growth, reduced reproductive efficiency, reduction in milk and meat production and increased susceptibility of animals to diseases (Rashamol *et al.,* 2019). Apart from the direct effects, climate change has been reported to negatively

influence livestock production indirectly by having an impact on soil infertility, reduction in fodder yield and quality, water scarcity and spread of pathogens (Sejian *et al.,* 2015).

Changes in climatic conditions are predicted to increase with time. The rising concern on the impact of climate change on livestock production has led to the involvement various government and non-government agencies to build a plan in order to mitigate the adverse effects of climate change (Dzama, 2016). Among the various ameliorative strategies, selection of climate resilient animal is gaining more focus. Every region has their own indigenous livestock population which have adapted to the local environmental conditions over the years on inhabitation. Hence conserving such breeds and promoting their breeding would be suggestive in the current climatic scenario (Dzama, 2016; Sejian *et al.,* 2018). Though these animals may be less productive when compared to the exotic or crossbred population, their thermo-tolerant and disease tolerant capacity will outshine that of the exotic germplasm (Sejian *et al.,* 2018). Hence the expenditure to maintain the animals during extreme climatic conditions may be reduced thereby saving the poor farmers from further losses.

The rapidly increasing human population adds on the rising concern of climate change. The global human population has already exceeded 7 billion in the year 2011 and is expected to touch between 8.1 billion and 10.6 billion by 2050. Adding on to the increasing population, the rising incomes and increasing food demands is expected to double the global demand for livestock products by 2050 (Godber and Wall, 2014; FAO, 2015; Rashamol *et al.,* 2019). Livestock production inevitably plays a potent role in achieving sustainable food security with ruminant based products increasing the human food supply (Kabubo-Mariara, 2009). Apart from meeting the global food demands the

livestock sector, especially ruminants, plays a significant role in conservation of grasslands, ecosystems, and also improves the fodder and land productivity thereby boosting the agricultural sector (Janzen, 2011; Godber and Wall, 2014). Therefore, ruminants are an integral part in maintaining ecological balance, global economy and sustainable food security and have to be given utmost importance.

Animals follow various strategies to adapt to harsh climatic conditions which includes morphological, physiological, neuro-endocrine, metabolic, molecular and cellular responses (Sejian *et al.,* 2018). These adaptive traits vary between species, breeds and also among individual animals (McManus *et al.,* 2009; Sejian *et al.,* 2018). At the breed level, dairy animals are more sensitive to heat stress when compared to meat breeds and also among the dairy breeds, it is the high producing animals who are more susceptible to thermal stress due to their higher metabolic heat generation (Bernabucci *et al.,* 2010; Kishore *et al.,* 2014). Animals with lighter coat, lose skin, lower hair density, smaller body size and higher sweat gland density survive better in hot and arid regions (Sejian *et al.,* 2018). Buffaloes evidently lack such adaptive traits and thus are more sensitive than other ruminants to heat stress (Singh *et al.,* 2016). Tropical indigenous cattle tend to have a smaller body size when compared to the exotic breeds which help to survive well in such regions (Sejian *et al.,* 2018). However, sheep and goat adapt better to heat stress when compared to all the other livestock. Their compact body shape and size, lighter skin and hair colour, less subcutaneous fat, increased respiration rate (RR), sweating rate and reduced metabolic rate make them more suited for harsh environmental conditions (Berihulay *et al.,* 2019).

Goats, also called as poor man's cow, are preferred by the small scale landless farmers due to their low input and assured higher output system of rearing (Kumar, 2007). The morphological and physiological characteristics of goats give them an advantage over other ruminants to thrive well in extremely harsh environmental conditions (Gaughan *et al.,* 2019). Their small body size, loose skin, floppy ears, browsing behavior, high digestive efficiency, low feed and water requirements and disease resistance help them to survive in harsh climatic conditions when compared to other ruminants (Collier *et al.,* 2008 Dzama, 2016; Gaughan *et al.,* 2019). Goats have the innate ability of browsing unpalatable forages like tree leaves and shrubs which despite being of low quality are utilized by them to with a fairly good feed conversion rate (Assan, 2014). Goats also possess physiological features like large salivary gland, larger absorptive area in ruminal epithelium and capacity to rapidly change the foregut volume in response to environmental changes (Thu, 2018). For having such superior traits goats are considered as a popular replacement for cattle and other livestock especially in the rural livelihood where they are reared mostly by women. In certain arid and semi-arid regions of West Africa, a shift in livestock rearing from cattle to small ruminants have been reported as a consequence of increasing droughts (Dossa *et al.,* 2015; Zougmoré *et al.,* 2015). The farmers preferred rearing small ruminants as they were less costly, hardier, required lower feed and reproduced faster when compared to cattle. Also, the contrasting plasma cortisol levels during multiple stress observed by Sejian *et al.* (2013) and Shilja *et al.* (2017) in Malpura rams and Osmanabadi goats respectively, wherein the former had lower levels, highlights that goats are able to adapt to multiple stress (heat and nutritional stress) more effectively than sheep. All these adaptive traits exhibited by goats

in extremely varying climatic conditions make them the ideal candidate to sustain livestock production in such regions.

2.2 Climate Change and Livestock Production

Climate change and its consequences are baleful to the sustainability of our present and future generations. Reliance of human on rearing livestock for their survival is evident from the history of mankind. Studies have demonstated that livestock sector is worst affected by climate change. According to the IPCC (2013), the anticipated rise in temperature is between 2.6 ^{o}C - 4.8^{o}C with a central estimate of 3.7^{o}C. Rapid increase in temperature along with extreme precipitation events like drought and flood associated with climate change is detrimental to the livestock production (Moss *et al.,* 2000). Consequences of climate change on animal agriculture can be studied according to their direct and indirect influence on animals (Sirohi and Michaelowa, 2007).

Koubkova *et al.* (2002) suggested that, out of all the environmental stresses that affects animals, the one which is being more cautiously studied is heat stress. Livestock maintain more or less constant internal environment which make them homeotherms. When animal is exposed to adverse environment, they may get shifted from their thermoneutral zone which leads to hyperthermia ranging from mild discomfort to death of the animal (Hahn *et al.,* 2002). The THI is a universal formula which can be applied to identify the animal stress due to the sudden upturn in temperature (Upadhyay *et al.,* 2013). With every unit of increase in THI beyond 72 will drop the milk production by 0.2 kg (Ravagnolo *et al.,* 2000). According to Mendelsohn and Seo (2007), small ruminants like goat and sheep gain more demand as temperature increases.

Climate change can have a deleterious impact on animal nutrition by curtailing the pasture availability and reducing the quality of available feed. Thornton *et al.* (2009) in his study estimated that species diversity of grasslands will get altered due to extremes in precipitation and temperature. Subsequently this will influence on their growth, reproduction and nutritional status, thereby making the animal susceptibility to diseases (Rotter and Van de Geijn, 1999).

2.3 Advantages of Goat Rearing

Goat is considered as "the poor man's cow" for the significant role they play in lives of poor and marginal farmers in bringing them sustainable economic return (Kumar *et al.,* 2006). Goat has emerged as a major livestock species and witnessed a population increase by around 50% globally in last twenty years while the total livestock population decreased or maintained their population (FAO, 2015). Owing to the socio-economic significance, the goat population in India also showed a similar tendency as that of the global trend with a steady increase of 1.484 million goats annually (Kumar *et al.,* 2010; Saxena *et al.,* 2019).

Livestock production throughout the world has showed a shift towards the small ruminants and especially favored goats for the management advantages they possess over other ruminants. Records proved that goats can survive under a wide range of environmental conditions, especially in arid and semi-arid regions where crop production is uncertain in context of changing climate pertaining to reduced availability of water and soil fertility (Monau *et al.,* 2020). Additionally, in context of the indirect effects of changing climate related to feed and water availability, rearing goat is considered to be

more economical when compared to large ruminants. Goats are opportunistic feeders and thus the depletion of pasture lands may hardly impose an impact on their diet requirement (Lyu *et al.,* 2020). Goats can survive well with poor quality vegetation where other ruminants find difficult to consume (Dossa *et al.,* 2015). Moreover, goats exhibit bipedal stance which helps them to get access to tree leaves and this is considered advantageous as compared to other livestock species. Further, goat has the better feed conversion efficiency than other ruminant species (Mangwai *et al.,* 2020).

Kumar *et al.* (2010) conducted a survey in semi-arid zones of two goat rearing states of Uttar Pradesh and Rajasthan and identified that most of the rural population involved in goat rearing are either landless farmers or marginal farmers with low economic stability. The study concluded that people adopt goat rearing for the less initial investment and low input requirement of goat production. In addition, goats do not require specialized shelter structures and they could ideally survive in any location with minimum protection from the weather variables. Labor availability is another crucial factor for livestock production, which was not considered to a big constrain in goat production as much of the labor could be met with family members. Indeed, Rokonuzzaman and Islam (2009) in their study revealed that 20 to 48 per cent of women were involved in goat rearing. These species have short generation interval and thus possess the ability to multiply rapidly, which is considered to be profitable for the farmers. In phase of changing climate, goats are raised as a source of additional income and as an insurance against crop failures due to the unexpected occurrence of natural calamities. Additionally, because of their smaller size, goats have been extensively integrated with other components of farming system (Kumar *et al.,* 2010).

Goats are reared for the variety of products they produce, like milk, meat, skins and fleece (Sharma *et al.,* 2018). According to FAO (2015) report, several goat breeds were widely distributed across different agro ecological zones in India. Breeds like Changthangi, Chegu and Gaddi found in Western Himalayas are exclusively grown for the finest fiber they produce. Most of the milch breeds like Jamunapari, Beetal and Jhakhrana were found in North and Northwest India (Kumar *et al.,* 2006). Most of the dual purpose goat breeds like Sirohi, Osmanabadi, Sangamneri, Malabari, Gohilabadi, Kutchi, Mehasana and Zalawadi were grown in Southern and Western part of India. Breeds exclusively grown for meat purpose like Kanni Aadu, Kodi Aadu and Ganjam were found in Eastern coast of India (Kumar *et al.,* 2006).

It was proposed that goat and its products contribute more than 74,777 million INR annually to the Indian economy (FAO stat 2002). Of the total GDP from livestock sector, 7.6 per cent was estimated to be contributed by goats. Of all goat products, meat alone contributes around 50 per cent of total value of GDP, followed by milk, skin and fleece (Morales-Jerrett *et al.,* 2020). When compared to any other meat sources, goat meat has highest price per kilogram of meat for its taste and medicinal value (Tainang *et al.,* 2007). Goat milk is a rich source of Vitamin B1 and is highly nutritious, with high fat and low cholesterol content than cow milk. Thus, goat milk is advisable for people with high blood pressure and diabetics (Kalyankar *et al.,* 2016).

Conclusively, studies highlighted goats as the “living bank” of farmers for the ability they hold in meeting food requirements, economic stability and alternate livelihood during periods of natural calamities.

2.4 Breed Differences for Adaptation in Goats

Livestock adaptation involves morphological, behavioural and genetic capacity of the animal to cope up with the harsh environmental conditions (Sejian *et al.,* 2018). Coat colour, hair density, body conformation, shade seeking nature, digestive and physiological changes, blood biochemistry, neuro endocrine, cellular and molecular mechanisms are the major adaptive strategies exhibited by goats during heat stress (Afsal *et al.,* 2018). These adaptive traits are heritable and thus are under genetic control which is the probable reason for the better adaptation of indigenous breeds than the exotic breeds, to harsh environmental conditions (Niyas *et al.,* 2015).

Boer, Angora, Cashmere, Saanen, Toggenburg, Alpine and Anglo-Nubian are few of the well-known breeds of temperate origin which have been incorporated in crossbreeding programs in most of the developing countries (Capote, 2014). Though these breeds are high producing, they fail to adapt well to the tropical environments due to which they fail to meet up the expected production levels (Capote, 2014). There are fewer reports on studies conducted to compare the productive performances between exotic and indigenous goats. On studying the effect of heat stress on indigenous Balady and exotic Damascus goats in Egypt, Helal *et al.* (2010) reported a significant difference in live body weight changes between the breeds. They observed 2.85 and 3.33 per cent reduction in live body weight of Balady and Damascus goats on exposure to heat stress. Idamokoro *et al.* (2017) compared the milk yield of Nguni (indigenous goat breed), Boer and non-descript goats reared under free ranging system in South Africa. The Nguni goats were observed to have a significantly higher milk yield during all the stages of lactation when compared with the Boer and non-descript goats. The probable reason for

the lower production by the Boer goats could be attributed to the environmental conditions prevailing in the region. Also, among the indigenous goats, variations in adaptability to difference agro-climatic zones have been reported by Pragna *et al.* (2017). They assessed the growth performance of three indigenous goat breeds, Osmanabadi, Malabari and Salem Black, on exposure to heat stress in India. They observed that the Salem Black goats performed much better than the other two breeds of goats.

Breeding programs promote crossbreeding programs to improve the productivity of indigenous and non-descript goats using exotic germplasm. However not always does this yield the expected positive response. Ayalew *et al.* (2003) conducted a study to compare the net benefit of goat farming to small scale farmers in Ethiopia. They studied two flocks, one maintaining indigenous goats (Hararghe Highland and Somali) reared under traditional practices while the other containing mixed flocks of indigenous and Anglo-Nubian×Somali crossbred goats following improved management practices under the Dairy Goat Development Programme (DGDP). Though the mixed flocks had a significantly higher production of unit net benefit than the indigenous flocks, the assumption that this superiority was due to the breed was proven wrong. They reported a positive response from 29 indigenous goat flocks reared under improved management than the traditional system of rearing. The crossbreds alone did not produce higher benefits neither in individual nor mixed flocks. Thus, these results highlight the superior adaptability and productivity of indigenous goats under the local climatic condition.

Morphological traits are of prime importance from adaptation point of view as they directly influence heat exchange mechanism between the animal and surrounding (Sejian *et al.,* 2018). In general, the tropical breeds of goats have a comparatively smaller

body size than the temperate goats (Silanikove, 2000a). Similarly breeds with long and narrow Coat and skin color are also important adaptive traits protecting indigenous goats from solar radiations (Sejian *et al.,* 2018). An example for this is the West African dwarf goats; their small body size, smooth, short and straight hair helps them adapt to hot, humid environments (Daramola and Adeloye, 2009).

Indigenous goats are well renowned for their ability to walk long distances in search of fodder and water while the exotic goats lack this primary behavioral adaptation. It is this trait in indigenous goats that help them to thrive well in hot and arid regions with fodder scarcity compared to the temperate goats which are mostly stall fed or grazed on nearby grasslands (Capote *et al.,* 2004). Differences in physiological responses between indigenous Aradi, exotic Damascus and their crossbreds were studied by Samara *et al.* (2016). On subjecting these goats to short term exposure to heat stress, they observed an alteration in the measured thermophysiological parameters, rectal temperature (RT), RR, heat tolerance coefficient (HTC) and adaptability coefficient (AC), in all the goats, irrespective of their genotype. However, the difference in thermotolerance among the genotypes were highlighted based on the time taken by them to exhibit normal levels of the studied variables. The RT and HTC of purebred Aradi and their crossbreds return to normal levels within a short span of 24 hours while that of Damascus took longer. Also, they observed that the RR and AC of purebred Aradi goats alone had returned to normal when compared with the other genotypes. This highlights the better adaptability of the indigenous goats to the extreme environments than the exotic goats. Syafiqa *et al.* (2018) observed comparatively lower hemoglobulin levels, lymphocyte counts and higher N/L ratio during heat stress in the native Katjang goats than exotic Saanen and Boer breed.

They suggested these changes to be a result of the proactive status of the indigenous goats to heat stress there highlighting their thermotolerance capacity

Over the years, intensive artificial selection practices for economic traits have been followed in temperate breeds. Indigenous goats in the tropics and sub-tropics however were more often ignored and thus underwent natural selection so as to adapt well to diverse environment (Kim *et al.,* 2016). Such differences in intensity and type of selection have resulted in phenotypic and genotypic differences between the temperate and indigenous tropical goats (Hanotte *et al.,* 2010). Kim *et al.* (2016) explored the genomic signatures of natural selection for adaptation to hot arid environment of Egypt in the indigenous Barki goats and compared it with that of five exotic goat breeds, Boer, Myotonic, Spanish, Kiko, and LaMancha, which were reared in temperate regions. Apart from the significant difference in genetic diversity observed between the indigenous and exotic breeds, seven candidate selection sweep regions were observed in Bakri goats using the F_{ST} approach to selection signature. The regions were on chromosomes CHI3, 6, 13, 14, 17 and 25 having 33 genes distributed across them. Similar results were also observed by the iHS approach to selection signature. Though iHS analysis identified selection sweeps in the exotic goats, none of these overlapped with that of Bakri goats, thereby confirming their genomic difference. A number of genes, *UGT8*, *FGF2*, *IL2*, *IL7*, *IL21*, *CSN3*, *PCDH9* and *SPP1*, were identified on the candidate sweep regions. These candidate genes were annotated to influence several traits for survival in hot environments like development and function of nervous system, immune and inflammatory responses and coat color (melanogenesis). These results highlight the putative selection of candidate regions and genes involved in biological and functional

pathways, shaping the genomic architecture of indigenous goats to survive in hot arid environment.

Wang *et al.* (2016) conducted a whole genome study on eight different goat breeds representing different morphological, geographical and production systems, to identify genomic region under selection. Selection signatures were identified on pooling sequences of Taihang Black (black coated breed), Tibetan goat (highland breed), Inner Mongolia Cashmere (cashmere breed), Shaanbei Cashmere (cashmere breed), Angora (mohair breed), Saanen (dairy breed), Boer (meat breed) and Guizhou Small (mini goat breed of small body size). Using the ZH_p and di value approach, they identified 22 genomic regions under selection which would have resulted in the phenotypic differences among these breeds. Genes like *ASIP*, *KITLG*, *HTT*, *GNA11* and *OSTM1* (responsible for coat color); *TBX15*, *DGCR8*, *CDC25A* and *RDH16* (responsible for body size) and few other cashmere and hypoxia adaptation traits were found to be under strong selection.

2.5 Goat as the Ideal Climate Change Animal Model

Among all livestock, goats are gaining more primacy for their inherent ability to survive, produce, reproduce and withstand under wide range of environments. Goats are homeotherms; therefore, possess mechanisms to eliminate excess heat in order to maintain a thermally balanced state. Goats can withstand heat stress, can endure prolonged water deprivation, tolerate drought and have disease resistance capacity.

2.5.1 Superior thermo-tolerant ability over other livestock species

Goats possess certain inherent morphological traits which help them to withstand extreme high temperatures. Their smaller body size and loose skin helps to dissipate more

heat to the external environment. They are well adapted to extreme temperatures with their coats providing an insulation layer to protect them from cold and heat. The rate of radiant absorption as well as reflection is partly determined by the coat characteristics like coat color and coat length and hair follicle density. Coat color has a significant influence on heat tolerance traits. Generally, animals with lighter fur color reflect more radiation which helps them to maintain their body temperature within comfort zone ranges. In contrast, animals with dark coat color are found to have higher RR, pulse rate (PR), RT and heat load index (HLI) values. As an adaptive trait towards seasonal fluctuations in solar radiation, several goat breeds possess multi coat colors so as to maintain their body temperature apparently near comfort zone temperature in extreme climatic conditions.

Coat structures in goats have a notable association with the environment they live which helps them to adapt to different agro-ecological locations. In the semi-arid to humid zones, short coats with coarse fiber support goats to tolerate higher temperature and humidity. Goats inhabiting in the arid zones have long and coarse hairs to protect themselves from warmer day temperatures and colder night temperatures. Furthermore, goats adapted to live in hilly regions of central Asia have a top coat of long coarse fibers and a seasonal undercoat of short, fine fibers to protect against extreme cold. In addition, some goat breeds have enlarged appendages in order to increase the surface area for heat loss.

Goats exhibit certain behavioral responses to cope with adverse environments. The immediate response for higher temperature in goats is reflected in their FI and water intake (WI). In order to reduce the metabolic heat production, goat reduces their FI. In

contrast to decreased FI, goats consume more water to balance the water loss from their body either through respiratory or cutaneous evaporative cooling mechanisms. Further, goats travel shorter distances during grazing and rest more during the hottest and drier hours of the day to cope with extreme temperature stress in arid and semi-arid tropical environments.

Environmental fluctuations results in variations in physiological responses like RR, PR, RT and sweating rate in an effort to maintain normal body temperature within the range of thermo neutral zone temperature. Increased RR is an immediate physiological response of goats to environmental stress. Exposure of goats to higher ambient temperature results in higher sweating rate so as to maintain the body temperature of animal through cutaneous evaporative cooling mechanisms.

2.5.2 Superior drought tolerant ability over other livestock species

The creeping disaster, drought is regarded as one of the greatest limiting factors for animal production pertaining to feed as well as water requirement of animals. In order to maintain the homeostasis of the body fluid, ruminants alter certain behavioral as well as physiological responses like urination, fecal excretion and evaporation which are considered as some of the major source of water loss from the animal body (Kaliber *et al.* 2016). Ruminants, especially goats have evolved various adaptation advantages that help them to escape from the undesirable effects of drought. Since time immemorial, a number of reports have documented the unique capability of goats to survive under water stress.

Goats have developed various behavioral responses that help them to resist the undesirable effects of drought. FI of goat was positively correlated with the availability

and intake of water by the animals (Silanikove, 1985). During the periods of water scarcity, the voluntary intake of dry matter by goat was found to decrease in an effort to maintain an equilibrium with the available body water (Alamer, 2009). Similar result was quoted by Hossaini-Hilali *et al.* (1994) in his study with Moroccan goats. He found that water deprivation resulted in 10% decline in their FI. However, a discrepancy was observed by Maltz and Shkolnik (1984) in their experiment with Black Bedouin goats and found that the FI remained unchanged for 48 hours of dehydration indicating the drought tolerant ability of this goat breed. Moreover, investigation done by Alamer (2009) on Aardi goats reported that the WI to FI had decreased significantly in water deprived goats than their respective control group. Kaliber *et al.* (2016) suggested that the goats reduced their rumination time (RuT), walking duration with an increase in the duration of standing and lying time (LT) in association with water stress. In perspectives of limited water availability, goats have the ability to desiccate their feces and can concentrate the urine thereby reducing water loss through urine. In addition, goats have the ability to reduce evaporative water loss as compared to other ruminants. In association with water deprivation, goats were found to loss their live weight (LW) as a result of the significant loss of body water (Parker *et al.,* 2003). However, the loss in LW got stabilized after an increase during the initial four days, indicating the adaptive capability of goats towards water stress.

Goats were found to launch several physiological responses in an effort to reduce the water stress induced dehydration of body fluids. It was found that the RT had a notable influence on water stress, with an increase of 0.51^{o}C than the control group (Kaliber *et al.,* 2016). Findings from the investigation by El-Nouty *et al.* (1990) on aardi

goats were in agreement with that of Kaliber *et al.* (2016) that the RT was significantly influenced by water deprivation, length of water deprivation and time of deprivation. Additionally, there was significant influence for season on water deprivation. In contrast to the increase in RT, RR had a decreasing trend in water deprived goats. Moreover, the heart rate in water restricted goats were declined so as to reduce their metabolism to conserve water and to compensate for the reduction in FI (Brosh, 2007).

During the periods of water shortage, goats were found to activate various water conservation mechanisms resulted in minimum loss of water from the body, thereby increasing the ability to endure the detrimental effects of water scarcity. One such mechanism was associated with the increase in blood plasma concentrations of glucose, cholesterol, sodium, antidiuretic hormone (ADH) and creatinine. In order to preserve body water, the concentration of ADH and sodium ions were increased in body fluid of goats. Additionally, during the period of unlimited water available, a putative regulatory increase in plasma and body fluid volume were found in goats which helped them escape from dehydration quite effectively (Silanikove, 2000a).

Apart from the behavioral advantages, goats are equipped with several anatomical advantages to cope with water scarcity. The salivary gland, rumen and kidney have significant roles in osmoregulatory mechanisms of goats. The fermentation vat, rumen, acts as a water storage tank during dehydration periods. Maltz and Shkolnik (1984) in his study in black Bedouin goats illustrated the unique ability of goats to endure water scarcity for a maximum of four to five days with the anatomical advantage of rumen. Studies proved that rumen has the capacity to store water for some hours to prevent haemolysis and osmotic shock to tissues. This advantage of rumen helps them to travel

long distance in search of feed even when water availability is apparently nil (Simões and Pires, 2018). Thus, goats exposed to water stress are able to maintain a normal water balance in blood and body tissues to ensure a body water level compatible with life.

Water deprivation resulted in modifying the digestibility of goats. It was suggested that the depression in FI resulted in higher digestibility rate in goats. The reduction in FI was correlated with the decline in salivary flow rate with a parallel increase in the osmolarity. Similar relation between dehydration, FI, rumen outflow and digestibility were identified by Brosh *et al.* (1988) in his study on Bedouin goats.

2.5.3 Ability to survive on low pasture

Among the 14 billion hectors of land available for agriculture, 25% of the land was identified to be utilized for pasture (Tubiello *et al.,* 2007). However, depletion of forage resources in terms of both their quality and quantity is observed as one of the unavoidable negative impacts of climate change on animal agriculture (Rotter and Van de Geijn, 1999). The reasons for the potential depletion of pasture land were mainly associated by means of threshold effects with respect to the occurrence of extreme weather events, disease incidence and elevated CO_2 concentration (IPCC, 2000). Indeed, shrinking pasture lands can significantly hamper the livestock production by reducing their nutritional availability. Several studies have suggested the ability of goats to thrive well on low pasture conditions with their behavioral, anatomical as well as digestive advantages (Aziz, 2010).

Goats possess the ability to survive effectively during the periods of low forage availability though a series of behavioral responses which have evolved in response to

their habitat in arid and semi-arid regions. Unlike any other ruminants, goats exhibit browsing behavior with a wide browsing radius in different agro-ecological zones (Lu, 1988; Papachristou *et al.,* 2005). Browsing behavior of goats along with their potential to travel very long distance in search of forages helps them to ensure feed requirement throughout the year (Lu, 1988). Askins and Turner (1972) in their study on Angona goats identified that they travelled almost 5.5 km/day and 3.5 km/day in winter and summer months respectively. Moreover, it has been reported that goats can consume leaves from the upper vegetation layer by utilizing the unique bipedal stance behavior. Furthermore, goats are known to climb on trees when branch structures permitted.

There is no specific feed requirement for goats, which is beneficial for them to meet their nutritional demand with the available forages (El Aich *et al.,* 2007). In addition, goats can utilize forages which other ruminants reject to consume. There are evidences on the ability of goats to consume thorns and spines which generally were least preferred by other ruminants (Decandia *et al.,* 2008). It was well documented that the goat diet was mainly composed of tannin and lignin rich woody plants than herbaceous ones (El Aich *et al.,* 2007). Investigations on diet preference of goats concluded that they have an affinity towards diverse plants, dry leaves, fruits, flowers and trees while, they have the ability to adapt with the available forage resources (Decandia *et al.,* 2008). However, the affinity of goats towards different forages were evaluated in different ecological zones and found that the diet preference will change according to the seasons (Glasser *et al.,* 2012; Osoro *et al.,* 2013). During dry season, goats prefer to eat woody vegetation than the herbaceous plants for the reason that herbaceous plants contain low amounts of energy and protein when they get dried (Glasser *et al.,* 2012). However,

because of the abundance of herbaceous plants during spring and autumn seasons, goats were reported to change their diet preference to herbaceous species (Osoro *et al.,* 2013).

Moreover, goats have evolved anatomical traits to support their selective feeding behavior. They acquired a unique foraging behavior with their physical body structure. When compared to other ruminants, they are small with low body mass and sharp shaped mouth with mobile upper lips and prehensile tongue (Decandia *et al.,* 2008). They have narrow muzzles which helps them to get access to the forages from the soil level (Aziz, 2010).

Along with behavioral and anatomical advantages, digestive characteristics of goats play a crucial role to make them potent to survive with limited pasture. Digestive system of goat is adapted to utilize even the poor quality forages and can convert in to highly nutritious outputs like milk, meat and secondary products like wool, skin and manure (Kosgey *et al.,* 2008). They have better digestive efficiency than other ruminants for having longer mean retention time of digesta in rumen. Additionally, El-Tarabany *et al.* (2017) reported that they have better feed conversion efficiency than the other ruminants because of the microflora inhabited in rumen. In goats fed with low quality forages, it was found that the lignin undergoes modification, degradation and absorption from gastrointestinal tract. Indeed, this was identified as one of the reasons for the enhanced microbial activity in rumen (Daramola and Adeloye, 2009).

During utmost scarcity of feed resources, they are reported to reduce their metabolic process in order to conserve the energy and there by thrive on minimal FI (Decandia *et al.,* 2008). In agreement with this, Silanikove (2000a) reported that the

energy metabolism of desert goats was lower in comparison to their relatives from non-desert areas.

2.5.4 High disease resistance capacity

Livestock diseases have a huge impact on animal production throughout the world causes high production losses. Apart from this, the transmission of diseases to humans is also a rising concern (FAO, 2007). Among the domestic ruminants, goats are the well known for their disease resistance/tolerance, hardy nature and ability to survive in extreme climatic conditions (Capote, 2014). Fewer studies have been reported comparing the prevalence of diseases between goats and other ruminants. However, there are a few reports indicating the physiological differences and disease tolerance/resistance in goats (Daramola and Adeloye, 2009; Onzima *et al.,* 2017). When compared with other ruminants, goats have more lymphocytes than neutrophils in their circulation thereby suggesting a well-developed immune system in this species (Olusanya *et al.,* 1976; Daramola and Adeloye, 2009). In a review, Daramola and Adeloye (2009), highlighted the physiological mechanisms that enables the West African Dwarf (WAD) goats to be less susceptible and/or resistant to a few diseases. The WAD goats were reported to have a higher Packed Cell Volume (PCV) when compared to the other goat breeds in Nigeria and they also possessed the ability for compensatory accelerated production (CAP) of PCV during infection. This mechanism would allow these animals to return to normal PCV levels following infections thereby enabling better productivity and welfare. Additionally, WAD goats were also reported to have higher white blood cell values when compared to other breeds of ruminants in Nigeria (Tambuwal *et al.,* 2002; Daramola *et*

al., 2005). This also emphasizes on the immune potency of this breed of goat on comparison to other ruminants.

Excessive usage of drugs for treatment of diseases in animals has raised a concern from the public health point of view and hence emphasis has been given to selection animals/breeds possessing disease resistant traits (Chiejina and Behnke, 2011; Imran *et al.,* 2018). Identification of suitable markers are thus necessary to select such animals. McBean *et al.* (2016) suggested Feacal Egg Count (FEC) as the most reliable and useful method to screen goats for resistance against some species of gastrointestinal nematodes (GIN). Haematological and biochemical markers like PCV, haemoglobulin levels, eosinophils level, total serum protein (TSP), serum albumin (SA) and plasma proteins have been reported as markers for resistance against GIN in goats (Imran *et al.,* 2018). Various other members of the major histocompatibility complex (MHC), cytokine family and immunoglobulins have also been reported as important markers for associated with disease resistance in goats (Gopalraj *et al.,* 2014; Mandal *et al.,* 2018).

Fewer researches on vector borne diseases in goats have been reported. Cecchi *et al.* (2017) conducted a study to identify candidate genes for resistance against paratuberculosis in Italian Garfagnina goats. They observed significant SNPs near 13 genes, *DGKB*, *CCNA2*, *PSMA7*, *BCas3*, *GOLGA3*, LOC102187381, *PCSK5*, *BBS7*, *MANEA*, *PSD3*, *ANKLE2*, *TRNAS-AGA* and *TRNAC-ACA*, having a role in disease resistance. The involvement of these genes in the function of Golgi complex and protein kinases (one of the most important enzymes involved to immune response to paratuberculosis) make them the candidate markers for disease resistance in goats.

The WAD goats are endowed with the ability to resist trypanosome and intestinal nematode infections more effectively than any other known breed of goat (Chiejina and Behnke, 2011). Scanty studies have been conducted to identify the mechanism of trypanotolerance in goats when compared to that in cattle. Goats are proposed to be less exposed to tsetse flies, the vector transmitting the protozoal disease, as a result of their grazing pattern and behavioral differences (Geerts *et al.,* 2009). Vale (1977) reported that the feeding success of tsetse flies were higher on cattle (35%) than sheep and goats (1%). They specifically emphasized on the antifeeding behavior exhibited by goats (kicking, stamping, tail and ear flicks and skin rippling) to be the main reason for this. Teshome and Derso (2015) studied the prevalence of major skin diseases in ruminants in Ethopia. The overall prevalence of skin diseases in cattle, sheep and goats observed in their study was 27.68% (142), 42.47% (268) and 38.15% (58) respectively. Among them, the prevalence of ectoparasite infestations was higher in sheep (13.58%) and cattle (7.95%) and lower in goats (2.24%). These results also highlight the lower incidence of diseases in goats.

2.6 Behavioral responses of goats to heat stress

Goats exhibit certain behavioral responses to cope with adverse environments. They show a series of responses on feeding, WI, defecating frequency (DeF) and urinating frequency (UF), LT, standing time (ST) and shade seeking behavior. When goats are exposed to a hot environment, the intake of feed was found to be reduced (Pragna *et al.,* 2018a). Similarly, investigations done on Sardinian and Comisana goats found that their dry matter intake started to decline at temperatures 30-33°C, in temperate climatic conditions with rapid decrease recorded at 35°C (Darcan and

Güney, 2008). Since intake of feed act as a source of metabolic heat production, reduction in FI can help them to decrease the heat production and thereby keeping them under comfort zone.

In contrast to decreased FI, goats consume more water to balance the water loss from their body either through respiratory or cutaneous evaporative cooling mechanisms. Experiment conducted in Osmanabadi goats by Shilja *et al.* (2017) found that the drinking frequency (DF) was higher when exposed to a maximum temperature of 36^{o}C. Similar experiment conducted in three different goat breeds showed that irrespective of breed differences, all three heat stressed groups had higher DF than their respective control groups (Aleena *et al.,* 2018).

The frequency of defecation and urination in goats vary significantly with the environmental factors like temperature and humidity along with the changes in their FI and WI (Alam *et al.,* 2011). Goats exposed to heat stress were found to have high rate of respiratory as well as cutaneous evaporative cooling mechanisms to bring down the body temperature, leading to dehydration which in turn reduce the urination and defecation. Studies conducted in Osmanabadi goats (Shilja *et al.,* 2017; Panda *et al.,* 2016) were in close agreement with the findings of Alam *et al.* (2011).

Heat stress was found to induce changes in standing and lying behavior of goats so as to reduce the additional heat load from the ground as well as to enhance the heat dissipation to the environment (Panda *et al.,* 2016; Alam *et al.,* 2011). Studies on thermal stress induced behavioral changes in goats proved to have an inverse relation between the ST and LT (Shilja *et al.,* 2017; Aleena *et al.,* 2018). The results from the study

conducted by Aleena *et al.* (2018) in three different indigenous goat breeds revealed a discrepancy with the results of Alam *et al.* (2011) showed non significance of heat stress on standing and LT indicated their superiority to withstand heat stress. Further, goats travel shorter distances and seek shade during browsing, rest more during the hottest and drier hours of the day to cope with extreme temperature stress in arid and semi-arid tropical environments

2.7 Physiological responses of goats to heat stress

Environmental fluctuations result in various physiological responses like RR, PR, RT and skin temperature (SkT) in an effort to maintain normal body temperature within the range of thermo neutral zone temperature. There are evidences to prove that the RR in goats increased in exposure to thermal stress (Alam *et al.,* 2011; Panda *et al.,* 2016). The increase in RR was known to be the immediate response by goats to dissipate excess heat load to the surrounding through evaporation (Hamzaoui *et al.,* 2013). Study conducted by Hamzaoui *et al.* (2013) on Murciano-Granadina dairy goats illustrated that the RR was higher in thermal stressed goats during their first week of exposure and later it was found to decrease gradually, indicated the adaptive ability of goats to thermal stress. Similarly, increased RR were also reported in Black Bengal, Sokoto, and Sahel goats during afternoon (Alam *et al.* 2013; Habibu *et al.* 2016). Shilja *et al.* (2016) in their study proved that the RR of Osmanabadi goats under heat stress during the morning and evening hours showed significant difference from that of control animals with a higher value at the afternoon hours as an adaptive mechanism to meet the oxygen demand for the vital adaptation processes. Additionally, with the already existing high temperature, the respiratory frequency was found to have a further increase with the notable elevation in

relative humidity (Alam *et al.,* 2011). Banerjee *et al.* (2015) in their study on four different Indian goat breeds reported that there were breed differences for physiological variables with lower in breeds which are more adapted to heat stress. The results from Shilja *et al.* (2016) were in agreement with the findings of Banerjee *et al.* (2015) that the lower respiratory activity in Osmanabadi goat breed could be regarded as an adaptive efficiency of this breed to cope with extreme stressful conditions. However, study conducted with Osmanabadi, Salem Black and Malabari goat breeds disproved the results of Shilja *et al.* (2016) and showed lower RR to Salem Black heat stress group indicating the superior adaptability of this breed to cope with hot environments (Aleena *et al.,* 2018).

Goats exposed to higher ambient temperature showed higher PR than their respective control group. Gupta *et al.* (2013) reported that goats adopt the mechanism of higher pulsation rate so as to increase the blood flow from the core body to the surface such that more heat is lost by sensible as well as insensible means. Similar conclusion was drawn by Silanikove (2000b) and implicated that the increase in PR and cutaneous blood flow helped goats to reduce the heat load from their body.

In several studies, the RT of goats were found to be elevated when exposed to thermal stress (Banerjee *et al.,* 2015; Panda *et al.,* 2016; Aleena *et al.,* 2018). Similar trend was observed in case of SkT. Irrespective of breed variations, SkT in different regions during morning in heat stressed group was lower than their respective control group while a reverse tendency was observed during afternoon (Aleena *et al.,* 2016). Comparable results on increased SkT due to heat stress in Osmanabadi goats were reported by Shilja *et al.* (2016) and explained as an adaptive mechanism in which skin

capillaries undergo vasodilation to improve the blood flow to the periphery of body for enhancing heat transfer to the environment.

2.8 Blood biochemical responses of goats to heat stress

Goats are observed to have certain blood biochemical changes in response to heat stress. It was found that the haematological parameters like haemoglobin concentration (Hb), total leucocyte count (TLC) and total erythrocyte count (TEC) changed with season. Banerjee *et al.* (2015) claimed that the level of Hb, TLC and TEC in goats decreased during summer season. Gupta *et al.* (2013) suggested that the elevation in ambient temperature would result in greater oxygen consumption as a result of the increased RR. As a result of greater oxygen intake, the concentration of erythrocytes decreased which in turn reduced the concentration of Hb, TLC and TEC in blood.

Hormones like GH, prolactin, glucocorticoids, mineralocorticoids, catecholamines, antidiuretic hormone are involved in bringing either homeorhesis or homeostasis in goats. Sirohi and Barbari goats under heat stress were found to have an elevated cortisol secretion than their respective control groups (Banerjee *et al.,* 2015). This can be effectively correlated with the increase in blood glucose level in heat stressed goats as the level of glucocorticoids shoot up during heat stress. The findings were similar to the observations in Marwari goats (Sejian and Srivastava, 2010a). Similarly, in heat stressed goats, the level of tri iodothyronine (T_3) and thyroxine (T_4) were observed to be declined as an acclimatization process to alleviate heat stress.

Goats exposed to thermal stress were recognized to produce heat shock proteins (HSPs) as a cellular and molecular defense mechanism to cope with the deleterious

effects of heat stress. Extensive studies were conducted on expression pattern of *HSP-70* in heat stressed goat breeds and are identified to play a vital role in bringing homeostasis. Aleena *et al.* (2018) used the expression of *HSP-70* molecular marker in three different indigenous goat breeds, Osmanbadi, Malabari and Salem Black to identify the most climate resilient goat breed and concluded salem Black to be the better adapted breed. Testicular expression of *HSP-70* in Osmanabadi bucks was studied and concluded that the heat stressed group had an up regulated expression than their respective control group. Furthermore, experiment conducted on Malabari goat breed by Afsal *et al.* (2019) proposed a strong correlation between the temperature- humidity index (THI) and *HSP-70* gene expression in liver and uterus while a negative correlation was obtained for *HSP-70* expression between thyroid and lymph node. Collectively, it was concluded that the expression of HSPs varies with breeds and within breeds there were variations at tissue level (Afsal *et al.,* 2019).

2.9 Endocrine responses of goats to heat stress

Upon prolonged exposure to thermal stress, goats employ several life sustaining activities which culminates with the expression of various behavioral, physiological, neuro-endocrine as well as metabolic mechanisms. The immediate behavioral and physiological changes act as an emergency alert in activating the endocrine systems to bring homeostasis. Endocrine is one of the crucial pathways by which the animal, especially goats withstand environmental stressors (Afsal *et al.,* 2018). Indeed, the front line of defense to thermal stress comes from the release of primary endocrine hormones such as glucocorticoids and catecholamine, combines with paracrine secretions to strengthen the endocrine responses. Extensive investigations have been conducted on

ruminants to claim their pineal gland to be highly responsive to temperature variations. Pineal gland mediates the activation of neuro-endocrine responses via its hormones, bringing homeostasis (Kräuchi *et al.,* 2006). For instance, higher ambient temperature stimulates the pineal gland which culminates with the release of hormones resulting in hypothermic and antioxidant effects and thereby ensuring the reduction in deleterious impacts of the heat stress on goats.

Literatures concerning the impact of heat stress on hormonal activities of goats have illustrated a significant depression in thyroid gland activity resulting in a lowering of thyroid hormone serum concentration. Additionally, the activity of adrenal gland gets activated, resulting in an increased level of cortisol blood concentration. Thus, the levels of these hormones are commonly considered as biomarkers to indicate heat stress.

2.11 Biological markers for climate change in goats

Prolonged exposures of goats to higher temperatures induced several life sustaining responses that hamper the health and production potential through both direct as well as indirect means (Marai *et al.,* 2007). A number of studies were conducted on this line to investigate the expression of phenotypic as well as genotypic markers in response to thermal stress. A recent study conducted by Aleena *et al.* (2018) on three indigenous goat breeds of southern India had screened the expression of few phenotypic markers with their exposure to heat stress. Briefly, the study has validated the behavioral indicators exhibited by goats in response with their exposure to heat stress. The ST, LT (LT), DeF, UF and RuT of heat stressed goats were found to have significant difference from their respective control groups.

In addition to the behavioral changes, goats appear to show evident changes in their physiological responses such as RT, RR, PR and SkT. The RT acts as a significant biomarker, indicating the core body temperature of the animal. There are sound evidences available to prove the elevated RR, PR, RT and SkT in the wake of heat stress in goats. In an effort to reduce the heat generation, the metabolic activities along with muscular activities were altered and were reflected in the respiration as well as PR of goats.

Apart from the behavioral and physiological changes, there have been proven records for the activation of certain hormonal mechanisms in goats with an aim to withstand the adversities of heat stress. Expression of hormones like GH, glucocorticoids, mineralocorticoids, antidiuretic hormone, catecholamines and thyroxine were intensively studied to identify their involvement in heat stress adaptation. The changes in the release of such hormones are controlled by hypothalamus-pituitary-adrenal axis as a result of their interaction with environmental variables. The principle stress reliving hormone, cortisol, increases with the exposure of goats to higher ambient temperatures. In contrast to this, the levels of thyroid hormones were found to be decreased during heat stress. Moreover, the release of aldosterone from adrenal medulla falls significantly and was reflected in their electrolyte balancing.

Several investigations give further evidences on significant changes in hematological variables in animals in response to thermal stress. Although there are limited reports available on effect of panting on blood acid-base variables, pH, bicarbonate concentration, hemoglobin concentration and partial pressure of carbon dioxide in goats, studies conducted on other species have proved hematological changes to be a biomarker to confirm heat stress. Sivakumar *et al.* (2010) claimed that increased

rate of respiration resulted in higher partial pressure of oxygen in blood, which in turn lead to reduction in PCV and Hb. Additionally, biochemical changes were found to be taken place in heat stressed goats, which resulted in release of both enzymatic as well as non-enzymatic antioxidants to blood. Release of glutathione decreased the oxygen toxicity in blood so as to maintain the PCV and Hb levels. Studies cited on changes Vitamin C and Vitamin E in heat stressed goats resulting impaired oxidation as well as reduction reactions. Higher ambient temperature influences the blood glucose and cholesterol levels in goats. There are contradicting reports available on influence of heat stress on release of glucose. Ocak and Guey (2010) reported decrease in level of glucose in heat stressed bucks. Additionally, they found significant change in level of cholesterol as a result of reduced availability of body water due to heat stress.

There are profound evidences to prove enzymatic changes as an indicator of heat stress in livestock. There have been several studies conducted to illustrate the changes occurred in metabolic activities of goats to reduce the impacts of thermal stress. In response to heat exposure, goats were found to have lower secretion of thyroid hormones. Studies illustrated aspartate transaminase (AST) and alanine tranaminase (ALT) to be the indicators of welfare of goats in heat stressed conditions.

Heat stress responses in goats can be better interpreted through the changes in genotypic traits of goats. Thermal stress had induced the expression of various heat shock proteins that helps in bringing thermo-tolerance in goats. Although several studies have been conducted on different HSP genes, *HSP-70* was traditionally considered as the best biomarker to track heat stress responses in goats. Apart from *HSP-70*, the expression of

HSP-90, HSP110, *HSF-1* could be attributed to draw a clear outline on adaptation of goats to heat stress.

MATERIALS & METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Location

The experiment was carried out at the experimental livestock farm, National Institute of Animal Nutrition and Physiology, Bengaluru, India, the southern Deccan plateau of the country, located on latitude 77°36'25.3"E, longitude 12°57'04.3"N and at an altitude of 920 m above mean sea level. The mean annual ambient temperature and relative humidity ranges from 15 to 36^{o}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 from May-July. The annual minimum and maximum temperature ranges between 15-22^{o}C and 27-34^{o}C respectively. The annual RH variations during the study period ranged between 28-59 per cent under hot semi-arid environment.

3.2 Animals

Two different indigenous breeds of southern peninsular India, Tamil Nadu (Kanni Aadu and Kodi Aadu) were used in this experiment (Plate 3.1). Both breeds belong to meat type goats and are well known for their adaptive capabilities to different agro-climatic conditions of Tamil Nadu.

Kanni Aadu goats are hardy, tall and are having fast growth with a nature of early maturity. They are generally black in color with typical white or reddish-brown stripes on either side of the faces, underbelly and inner sides of legs. They are distributed in different parts of Tamil Nadu, including Sattur and Sivakasi taluks of Virudhunagar

district, Kovilpatti and Vilattikulam taluks of Thoothukudi and parts of Tirunelveli. The average body weights of adult female and male goats are 28 kg and 35 kg respectively (Acharya, 1995). Kodi Aadu goats are distributed in southern coastal regions of Tamil Nadu and are docile, hardy, meat purpose goats with fast growth rate and early maturity (Mariadas, 1996). They have a predominant white coat color with scattered black and brown patches. The average body weight of Kodi Aadu female ranges between 25-30 kg.

The study was conducted in 24 female goats, 12 each from both Kanni Aadu and Kodi Aadu breeds with average body weights 14.23 kg and 13.98 kg respectively. The animals were kept inside the climate chamber throughout the study period (45 days). Prophylactic measures against goat diseases like enterotoxaemia, endo and ecto parasitic infections were taken time to time as per the consultation of a well-trained medical practitioner to ensure that the health condition of animals throughout the study period.

(a) **(b)**

Plate 3.1: Goat breeds used in the current experiment. (a)Kanni Aadu Goat Breed; (b) Kodi Aadu Goat Breed

3.3 Breed characteristics of Kanni Aadu and Kodi Aadu

Kanni Aadu goats are predominantly black in color with long legs, making them capable to adapt to the hot and dry climatic conditions of Tamil Nadu. They are also known as “Varikan Aadu” for the stripes on their face (Mariadas, 1996; Thiruvenkadan, 1997). Based on coat shade, they are classified as Pal-Kanni and Cheng-Kanni. Pal-Kanni are the typical Kanni goats with two parallel white stripes on either side of their face extending from the base of horn to the corner of muzzle. Apart from this, they have white border lines on the edges of their ears and sides of their neck. Pal-Kanni is having white colored underbelly with sides of their forelegs and thighs having white borders. On the other hand, Cheng-Kanni goats are having reddish brown color of varying intensity instead of white stripes. They have medium to long ears, medium sized tail, slender neck and moderately fleshed shoulder. Both male and female adults are having horns. Males grew beard within 8 to 12 months’ time and females were found to have beard in the later stage of their lifespan. The average body length, height at wither and chest girth of adult Kanni Aadu males and females were found as 71 cm, 84 cm, 77 cm and 67 cm, 76 cm and 70 cm respectively.

The name “Kodi Aadu” was derived from their lean, tall and compact appearance. In general, Kodi goats are predominantly white with scattered black as well as brown patches. They have dark dorsal lines with almost white ventral parts. Based on coat color, they can be of different types namely, Pullai-Porai, Chem-Porai and Karum-Porai. Regardless of coat color differences, all the strains have their extremities including face, ears and legs as dark. They have a medium sized head with medium to long ears. Both male and female adult goats are horned without any typical horn patterns. Their Long

lean legs are meant for their adaptive capacity to travel long distance in search of forages during the periods of low pasture availability. In females, udder is under developed and is attached close to their belly with small teats. The overall body weight of an adult Kodi goat comes around 25-30 kg (Thiruvenkadan *et al.,* 2012). The average body length, height at wither and chest girth of adult Kodi Aadu males and females were found as 74 cm, 82 cm, 77 cm and 75 cm, 78 cm and 73 cm respectively.

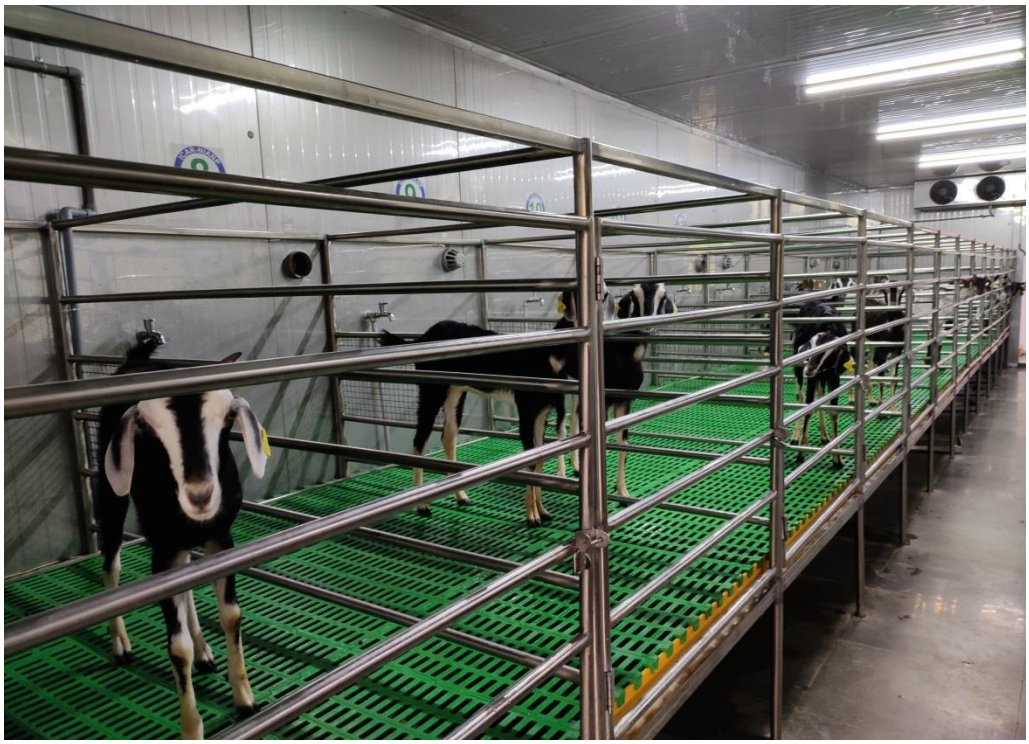
3.4 Technical Design

The study was conducted for a period of 45 days in twenty-four one year old female animals of Kanni and Kodi Aadu goat breeds (12 animals in each breed). The animals were randomly divided into four groups as Kanni Aadu Control (KAC; n=6); Kanni Aadu Heat Stress (KAHS; n=6); Kodi Aadu Control (KOC; n=6); Kodi Aadu Heat Stress (KOHS; n=6). Both KAC and KOC animals were kept in thermo-neutral zone climate chamber exposing them to 24°C while the KAHS and KOHS group animals were kept in heating chamber (Plate 3.2) under simulated heat stress model with a temperature simulated according to the heat stress model (Table 3.1). The control and heat stress group animals were exposed to their respective climatic condition for a period of six hours daily for the 45 days duration of the study. Their behavioural responses were recorded continuously for six hours (10.00 h to 16.00 h) at fortnightly interval. The physiological responses were recorded four times a day (8.00 h; 14.00 h; 20.00 h and 2.00 h) at fortnightly interval. Blood samples were collected at fortnightly interval and separated into three aliquots. The first aliquot was subjected for haematological variable recording while the second aliquot was subjected for plasma separation. The plasma samples were subjected for biochemical and endocrine variables analysis. The third

aliquot of blood samples were subjected for peripheral blood mononuclear cells (PBMC) isolation and subjected total RNA isolation and cDNA conversion for gene expression study. Fig. 3.1 shows the pictorial representation of the technical design.

Plate 3.2: (a) Climate chamber: Thermo-neutral Zone Chamber (left) and Heating/ Cooling Chamber (right) outside view. (b) Kanni Aadu goats inside the chamber (c) Kodi Aadu goats when kept inside the chamber

(a)



(b)

(c)

3.5 Recording of weather variables

All the weather parameters were recorded twice daily (8:00 h and 14:00 h) for the entire period of experiment. The weather parameters like maximum temperature, minimum temperature, dry bulb temperature and wet bulb temperature were recorded manually using maximum thermometer, minimum thermometer, dry bulb and wet bulb thermometers respectively. Both ambient temperature and relative humidity were recorded using thermo-hygrometer. Additionally, Climatic chambers are well equipped with weather sensors (Plate 3.3), which are connected to a server, from which we can collect the data in no time. The following temperature (Table 3.1) model has been used to generate heat stress in heating chamber. The model has been prepared as per the IMD data of Tamil Nadu region.

Plate 3.3: Inside view of Climate chamber; Sensors for recording weather parameters like temperature and humidity.

Table 3.1: The temperature model simulated in the current experiment

Time	10.00 h	11.00 h	12.00 h	13.00 h	14.00 h	15.00 h	16.00 h
Temperature	36^{o}C	37.5^{o}C	38.5^{o}C	38.8^{o}C	40^{o}C	38.3^{o}C	37^{o}C
Relative Humidity	49%	44%	41%	40%	39%	42%	45%

3.6 Calculation of Temperature-Humidity Index (THI)

The THI of the current experiment was calculated with the McDowell (I972) formula:

$$\mathbf{THI = 0.72\ (T_{db} + T_{wb}) + 40.6}$$

T_{db}- Dry bulb temperature

T_{wb}- Wet bulb temperature

3.7 Behavioral responses recording

Climate chambers with which the study was conducted were well equipped with video recording facilities. Four cameras each were installed in the chamber so as to get a close observation on each animal. Using these video recordings, the behavioral responses of animals such as FI (g/w$^{0.75}$/day), WI (L/KgDMI/day), ST (min), LT (min), DeF (no. of times), UF (no. of times) and RuT (min) were calculated for 6 hours (10.00 AM to 4.00 PM). (Plate 3.3)

3.7.1 Feed intake

Individual feeders were provided for all 24 animals. Concentrate as well as roughage was fed to the animal throughout the experimental schedule as per 3.5 per cent

of their body weight (Plate 3.4). FI of a particular day was calculated using the following formula:

Feed intake = Feed offered on previous day - Feed left in the trough

The ingredients and chemical composition of concentrate mixture and hybrid napier hay fed to goats are given in table 3.2.

Plate 3.4: Managemental Practices followed during the study: (a) Weighing Feed (b) Recording Feed Intake (c) Recording Water Intake

(a)

(b)

(c)

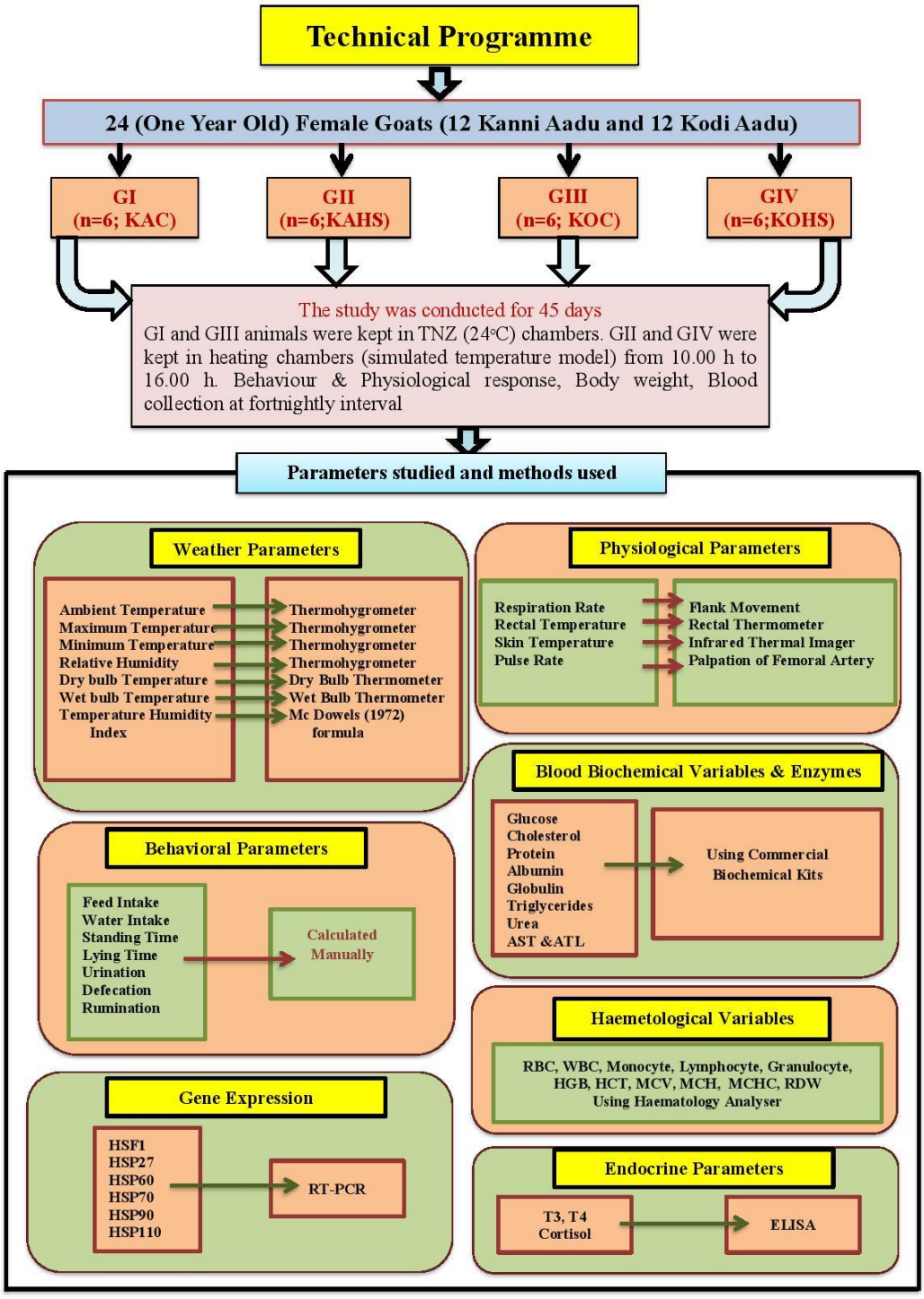


Fig 3.1: Technical design of the current study

Table 3.2: 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	34	-
Wheat bran	38	-
Soybean meal	12	-
Ground nut Cake	14	-
Mineral mixture	1.5	-
Salt	0.5	-
Chemical composition (%)		
Dry matter	95.57	94.41
Organic matter	93.20	89.82
Crude protein	18.55	4.97
Ether extract	1.72	2.86
Crude Fibre	6.72	39.74
Total ash	6.80	10.08
Fibre fractions (%)		
Neutral detergent fibre	30.89	69.80
Acid detergent fibre	8.81	42.37
Nutritive value		
Total digestible nutrients (%)	72	54.22
Digestible energy (kJ/kg)	14.07	9.66
Metabolizable energy (kJ/kg)	11.54	7.95

3.7.2 Water intake (WI)

Individual water troughs were provided for all 24 animals inside their respective chamber compartments. Water was offered to all animals ad libitum (Plate 3.4). WI was calculated with the following formula:

WI= Water offered on previous day- Water remained in the trough next day

3.8 Physiological responses recording

The physiological responses like RR, RT, SkT and PR of both heat stress group and control group kept inside the heat/cooling chamber and thermo-neutral chamber respectively were recorded during the experiment in forthnightly intervals with four collections during 8.00 h, 14.00 h, 20.00 h and 2.00 h. The methodologies for recording each variable are discussed below.

3.8.1 Respiration rate (RR)

RR was recorded by counting the flank movement for one minute using stop watch from a distance of 4-5 m away from the animal. The unit of measurement of RR was in breaths/min. Photograph showing RR recording is shown in Plate 3.5.

3.8.2 Pulse rate (PR)

The PR was recorded by palpating the femoral artery. The animals were restrained for measuring PR. The unit of measurement of PR was in beats/min. Photograph showing PR recording is shown in Plate 3.5.

3.8.3 Rectal temperature (RT)

The RT was recorded using a digital rectal thermometer. It was inserted to the rectum for 6-7 cm, inclined towards the wall of the rectum. The animal has to be gently restrained while recording RT. The unit of measurement of RT was in ^{o}C. Photograph showing RT recording is shown in Plate 3.5.

3.8.4. Skin temperature (SkT)

The SkT at head, shoulder and flank were recorded using an infrared thermometer (B.S.K Technologies, Hyderabad, India). The instrument has to be pointed towards the target site from a distance of 5-15 cm. The unit of measurement of SkT was in ^{o}C. Photograph showing SkT recording is shown in Plate 3.5.

Plate 3.5: Physiological responses recording- (a) Respiration rate, (b) Pulse rate, (c)Rectal temperature, (d) Skin temperature

(a)

(b)

(c)

(d)

3.9 Hematological Variables

Blood samples (8 ml) were collected in fortnight intervals from the jugular vein of each 24 animals. Plastic syringe with 20-gauge sterilized needles and heparin anticoagulant (20 IU per ml of blood) were used for collecting the blood. Collection was conducted at 11.00 h simultaneously from all the four groups. Hematological analyser (Mindray BC 2800) was used to estimate different hematological variables.

4.0 Blood biochemical responses recording

Blood samples (8 ml) were collected in fortnight intervals from the jugular vein of each 24 animals. Plastic syringe with 20-gauge sterilized needles and heparin anticoagulant (20 IU per ml of blood) were used for collecting the blood. Collection was conducted at 11.00 h simultaneously from all the four groups.

Plasma was separated from the blood with the help of a centrifugation technique, at 3500 revolutions per minute (rpm) at room temperature for 20 minutes. The supernatant obtained was straw colored plasma, were separated using a sterile Pasteur pipette and stored in sterilized vials. The plasma was then kept at a temperature of -20 ^{o}C for further analysis.

4.0.1 Glucose

Glucose has been estimated by Glucose oxidase Peroxidase (GOD-POD) method using bio spectrophotometer basis (Eppendorf, Hamburg, Germany). The unit of measurement of glucose was mg/dL. Plasma glucose was estimated using kit method (Autospan, Surat, India). Estimation of glucose using spectrophotometer is shown in Plate 3.6

Assay Principle:

GOD oxidase glucose to gluconic acid and hydrogen peroxide. In the presence of enzyme peroxidases, released hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to form colored quinineimine dye. Absorbance of colored dye is measured at 505 nanometer (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 peroxide respectively.

Procedure:

Blank (B), Standard (S) and Test (T) were prepared and pipetted to the labeled test tubes as shown below.

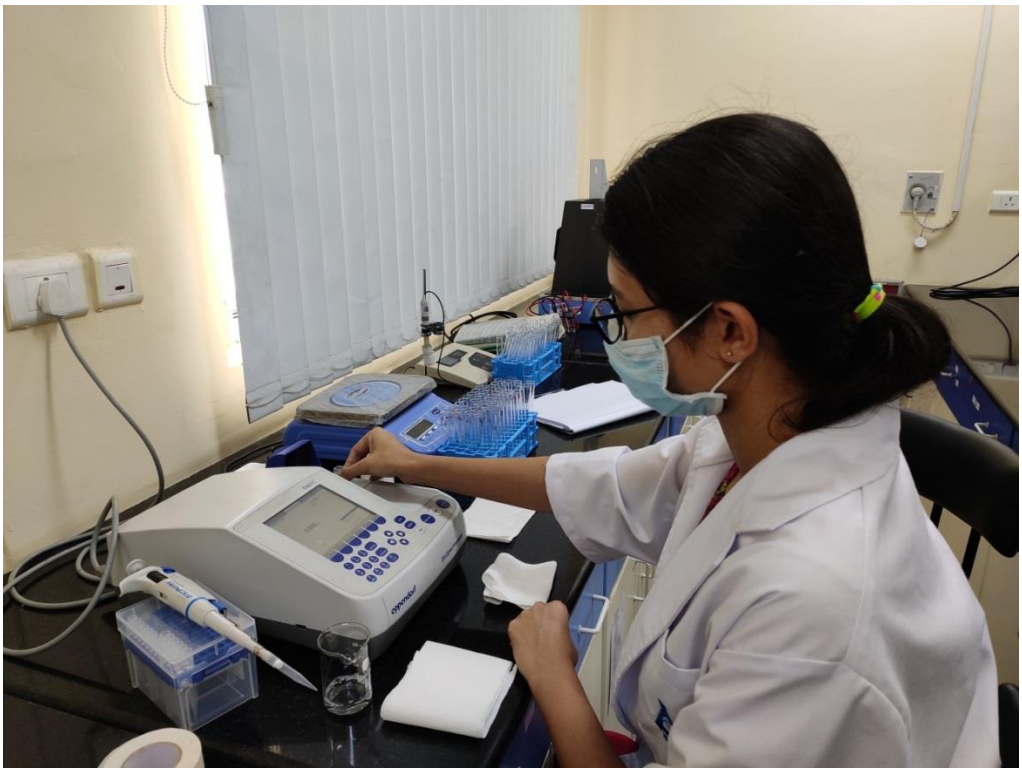
Addition Sequence	Blank	Standard	Test
Serum/ Plasma	-	-	10 µl
Reagent 2	-	10 µl	-
Reagent 1	1000 µl	1000 µl	1000 µl

The solutions were mixed well and incubated at 37°C for 10 minutes.

Calculations:

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

Plate 3.6: Estimation of blood biochemical variables using spectrophotometer



4.0.2 Plasma Cholesterol

Plasma Cholesterol was estimated by cholesterol oxidase/phenol + Aminophenazone (CHOD/PAP) trider's method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The unit of measurement was mg/dL. The total cholesterol was estimated using kit method (Autospan Liquid Gold Cholesterol Kit).

Asssay Principle:

Cholesterol esters are hydrolyzed to cholesterol by cholesterol esterase. The free cholesterol is oxidized 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.

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

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

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

Procedure:

Blank (B), Standard (S) and Test (T) were pipetted out to the labeled 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 505nm

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

4.0.3 Total Plasma Protein

Estimation of total protein was done with VETSCAN@VS2 Chemistry Analyzer (Abaxis Inc., Union City, California, USA). It is a compact and portable veterinary bench-top instrument specific for veterinary applications. The analyzer works on the spectrophotometric principles. The commercially available plastic single-use large animal profile rotors (Abaxis Inc., Union City, California, USA) are used in the analyzer to quantify the total protein. The large animal profile rotor was loaded with 100 µL of whole heparinized blood samples and placed into the analyzer according to the manufacturer's instructions and the analysis was completed within 15 minutes.

4.0.4 Albumin

Bromocresol Green method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany) was adopted to estimate the plasma albumin (g/dL). Kit protocol was followed to estimate total protein (Autospan Liquid Gold Albumin Kit).

Assay Principle:

A green coloured complex is formed in buffered medium when albumin binds with BCG. The absorbance of the complex at 630 nm is directly proportional to the albumin concentration in the sample.

Albumin + Bromocresol Green $\longrightarrow$ Green Albumin BCG Complex

Procedure:

Blank (B), Standard (S) and Test (T) were pipetted out to the labeled 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, the solution has to be incubated for 5 minutes at room temperature. The absorbance of S and T were recorded against B at 630 nm.

Calculation:

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

4.0.5 Globulin

Estimation of Globulin was done with VETSCAN@VS2 Chemistry Analyzer (Abaxis Inc., Union City, California, USA). It is a compact and portable veterinary bench-top instrument specific for veterinary applications. The analyzer works on the spectrophotometric principles. The commercially available plastic single-use large animal profile rotors (Abaxis Inc., Union City, California, USA) are used in the analyzer to quantify the globulin. The large animal profile rotor was loaded with 100 µL of whole heparinized blood samples and placed into the analyzer according to the manufacturer's instructions and the analysis was completed within 15 minutes.

4.0.6 Plasma Triglycerides

Estimation of Plasma Triglycerides was done 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 (Autospan Liquid Gold Triglyceride kit).

Principle:

Serum triglycerides are hydrolyzed to glycerol and free fatty acid by lipoprotein lipase. In the presence of ATP and glycerol Kinase (GK), the glycerol is concerted to glycerol-3-phosphate, which in turn 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 color formed is directly proportional to the tri-glycerides concentration in the sample.

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol+ Free Fatty Acids}$$

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

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

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

Procedure:

Blank (B), Standard (S) and Test (T) were pipetted out to the labeled 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:

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

4.0.7 Urea

Plasma urea was estimated by Urease- Berthlot method using bio-spectrophotometer basic (Eppendorf, Hamburg, Germany). Urea is usually measured in the unit of mg/dL. Urea has been estimated by following the kit protocol (Autospan Urea kit).

Principle:

In presence of urease, urea in plasma is hydrolyzed to ammonia. The ammonia which is formed during this process reacts with hypochlorite phenolic chromogen and result in the formation of a green colored complex. The intensity of the green color formed is directly proportional to the amount of urea present in the sample.

$$\text{Urea} + H_2O \longrightarrow \text{Ammonia} + CO_2$$

4.1 Enzymes

4.1.1 AST

Estimation of AST was done using IFCC method with bio spectrophotometer basic (Eppendorf, Hamburg, Germany). AST is measured in IU/L. Biochemical kit (Autospan Liquid Gold AST Kit) protocol was followed to estimate AST.

Principle:

The function of AST is to catalyze 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.

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

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

Procedure:

The solution and sample were pipetted onto a clean dry test tube labeled as T.

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

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

Calculations:

AST activity in IU/L at 37^{o}C = ΔA/min. x 1746 X tF

Were, tF is the desired reporting temperature.

4.1.2 ALT

The estimation of enzyme ALT was done using mod. IFCC method using bio spectrophotometer basic (Eppendorf, Hamburg, Germany). The measurement of ALT was done in IU/L. Estimation of ALT was done by following the biochemical kit protocol (Autospan Liquid Gold ALT Kit).

Principle:

The function of ALT is to catalyze the transfer of amino group between L-Alanine and α-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 solutions were pipetted into clear dry test tubes labeled as T

Addition Sequence	**Test**
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 using the vortex mixer and the initial absorbance was recorded at 340 nm exactly after 1 minute and the absorbance readings were taken repeatedly after 1, 2 and 3 minutes. The mean absorbance change per minute was calculated (ΔA/min).

Calculations:

$$\text{ALT activity in IU/L at } 37^{o}C = \Delta A/\text{min.} \times 1746$$

5.0 Estimation of Endocrine variables

5.1 Plasma Cortisol

Principle:

The cortisol Quantitative Test is based on widely used immunoassay technique, which utilizes the principle of typical competitive binding. The sample containing unknown amount of cortisol to be assayed (having unlabeled antigen) is added to a standard amount of a cortisol-Horse Radish Peroxidase conjugate (labeled antigen). Both the labeled and unlabeled 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 the bound peroxide can be considered as inversely proportional to the concentration of the cortisol in the given sample. After washing, the substrate solution is added and the enzyme is kept aside for a while for reaction to happen. The enzymatic reaction is then halted by adding the stopping solution. The absorbance is measured on a micro plate reader (Thermo Scientific Multiskan GO, Finland). The intensity of color formed is inversely proportional to the concentration of cortisol in the given sample.

Assay Procedure:

Enzyme Linked Immuno Sorbent Assay (ELISA) method was used to estimate the level of cortisol in the sample (LDN kit, Nordhorn, Germany). All the reagents and samples should be thawed before using them in the reaction. Additionally, all the reagents must be mixed well without foaming. Calibrators, control and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption. The procedure has been done as per the following protocol:

1. Working solution of the cortisol-Horse Radish Peroxidase (HRP) conjugate and wash buffer were prepared.
2. 20 µL of each calibrators, control and specimen samples were pipetted into the corresponding labeled wells in the duplicate.
3. Then 200 µL 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 three times with prepared wash buffer (400 µL/well for each wash) and the plate was struck sharply on the absorbent paper to remove residual droplets (by hand 6 times)
6. 100 µL 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. 100 µL 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 the calibrator was measured
- OD of the unknown sample was read against calibrator curve

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

5.2 Tri iodothyronine (T_3)

Principle:

Goat specific ELISA kits (Puregene Goat Three Original Acid Iodine Thyroid Elisa) were used to estimate the T_3 in plasma samples. Kit contain specialized plate which has been pre-coated with goat T_3 antibody. T_3 present in the sample is added and binds to antibodies coated on the wells. And then, biotinylated goat T_3 antibody is added and binds to T_3 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated T_3 antibody. After incubation, unbound streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of goat T_3. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

Assay Procedure:

- Prepare all the reagents, standard solutions and bring them all to room temperature before starting the estimation.
- Add 50 µl standard to the standard well. After that, add 40 µl sample to the sample wells and then add 10 µl anti-T_3 antibody to sample well along with 50 µl streptavidin-HRP (to all wells). Cover the plate with a seal and incubate it for 60 minutes at 37^{o}C.
- Remove the seal and wash the plates 5 times using wash buffer. Soak the wells with atleast 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blot the plate onto paper towel or other absorbent materials.

- Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
- Add 50 µl stop solution to each well, the blue color will change into yellow immediately.
- Determine the optical density (OD) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculations:

- The mean OD of the calibrator was measured
- OD of the unknown sample was read against calibrator curve
- 4-parameter calibrator curve was drawn using immunoassay software and analyzed for results.

5.3 Thyroxine (T_4)

Principle:

Goat specific ELISA kits (Puregene Goat Thyroxine Elisa) were used to estimate the T_4 in plasma samples. Kit contain specialized plate which has been pre-coated with goat T_4 antibody. T_4 present in the sample is added and binds to antibodies coated on the wells. And then, biotinylated goat T_4 antibody is added and binds to T_4 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated T_4 antibody. After incubation, unbound streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of goat T_4. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

Assay Procedure:

- Prepare all the reagents, standard solutions and bring them all to room temperature before starting the estimation.
- Add 50 µl standard to the standard well. After that, add 40 µl sample to the sample wells and then add 10 µl anti-T_4 antibody to sample well along with 50 µl streptavidin-HRP (to all wells). Cover the plate with a seal and incubate it for 60 minutes at 37^{o}C.
- Remove the seal and wash the plates 5 times using wash buffer. Soak the wells with atleast 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. Blot the plate onto paper towel or other absorbent materials.
- Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37^{o}C in the dark.
- Add 50 µl stop solution to each well, the blue color will change into yellow immediately.
- Determine the optical density (OD) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculations:

- The mean OD of the calibrator was measured
- OD of the unknown sample was read against calibrator curve

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

6.0 Gene expression patterns

6.1 *HSF-1*, *HSP-27*, *HSP-60*, *HSP-70*, *HSP-90* and HSP110

6.1.1 Blood collection and RNA isolation

Blood samples of approximately 5ml were collected in ETDA vials and immediately stored in ice. Immediately the samples were processed after blood collection. To the samples ice-cold 1× RBC lysis buffer was added and incubated at room temperature for 15–20 min. The GeneJET Whole Blood RNA Purification Mini Kit (Thermo Scientific, Lithuania) was used to isolate total RNA from the PMBC pellet and the procedure was done as per manufacturer's protocol with minor modifications as follows: the pellet was resuspended in 600 µL of lysis buffer containing 20 µL of 2-mercaptoethanol, mixed well by vortexing. Further, 600 µL of buffer containing 590 µL of Tris EDTA and 10 µL proteinase K was added which was vortexed and incubated for one hour. Four hundred and fifty microliters of ethanol (96– 100 %) was added and mixed by pipetting. The lysate was added to the purification column and centrifuged at the rate of 12,000 rpm for 2 min, and the flow through was discarded. Then, 700 µL of wash buffer 1 was added to the purification column and centrifuged for 2 min at the rate of 12,000 rpm, and the flow through was discarded. Six hundred microliter of wash buffer 2 was added and centrifuged at the rate of 12,000 rpm for 2 min, and the flow through was discarded. The same wash buffer 2 of 500 µL of was added to the purification column and centrifuged, and the flow through was discarded. The empty purification column was re-spinned at 13,000 rpm for 2 min. The preheated 50-60°C nuclease-free water (NFW) of 20 µL was added at the midpoint of the column and

centrifuged at the rate of 12,000 rpm for 5 min after 5 min of incubation. The RNA eluted was collected in fresh nuclease free microfuge vials and processed immediately (Shilja *et al.,* 2016; Aleena *et al.,* 2018).

6.1.2 Conversion of RNA to cDNA

The total RNA was reverse transcribed into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit for real-time quantitative polymerase chain reaction (RT-qPCR) (Thermo Scientific, Lithuania). This was followed as per the manufacturer's protocol as follows: 4 µL of 5× Reaction Mix, 1 µL RevertAid M-MuLV RT (200 U/µL), 5 µg of template RNA, 15-20 pmol of primer, 1 µL of RiboLock RNase Inhibitor (20 U/µL), 2 µL of 10 mM dNTP Mix and NFW were to make the volume to 20 µL. Then, the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (5 min at 25°C, followed by 42°C for 1h, and the enzymes were inactivated by heating at 70°C for 5 min.

6.1.3 Primer designing and synthesis

Gene-specific new primers were designed using online NCBI primer design software (Primer 3, http://bioinfo.ut.ee/ primer3/). The specificity and efficiency was checked using Primer3 and BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and the primer efficiency was checked using https://imallona.bitbucket.io/efficiency.htmlefficiency.html online and sequences are depicted in table 3.3 (Mallona *et al.,* 2011). The primers were synthesized by Eurofins Genomics (Bangalore, India).

6.1.4 Quantitative real-time polymerase chain reaction (RT-qPCR)

The two housekeeping genes *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) and *HPRT1* (hypoxanthine phosphoribosyl transferase 1) was used as internal control to compare with the relative expression of target genes. The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The cDNA was amplified in triplets each per candidate reference gene and biological sample and on reaction was carried out on the same qPCR plate. The 20-µL reaction was carried out using 50 ng of template and the reaction conditions were enzyme activation at 95 °C for 10 min and amplification cycle (40 cycles; initial denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s). At the end of the reaction the non-specific amplification was ruled out by checking the melt curve analysis. The relative expression patterns of target genes were analyzed as per the formula 2-ΔΔCT (Shilja et at., 2017; Aleena *et al.,* 2018)

7.0 Statistical analysis

The data was analysed using general linear model (GLM) repeated measurement analysis of variance (SPSS 18.0). Effect of fixed factors namely breeds (Kanni Aadu and Kodi Aadu) and treatment (KAC, KAHS, KOC, KOHS) was taken as between subject factor and days (longitudinal time over which the experiment was conducted; day 0, day 15, day 30 and day 45) were taken as within subject factor and also interaction between breed, treatment and experimental days were analysed on the various parameters and studied. The changes in relative expression of PBMC *HSF-1, HSP-27*, *HSP-60*, *HSP-70*, *HSP-90* and *HSP110* mRNA in relation to *GAPDH*, and *HPRT1* as the house keeping

genes were analyzed by one-way analysis of variance (ANOVA) with Tukey's post-hoc analysis to compare the means between the groups by using the average of the three housing keeping genes.

Table. 3.3. Description of primer sequences of target and Housekeeping genes

Gene ID	Primers	Primer sequence (5'–3')	Ta (°C)	Primer length (bp)	Product size (bp)	Accession no.
HSF-1	F	AAAGTGACCAGCGTGTCCA	60	19	115	XM_018058070.1
	R	GTCCATGCTCTCCTGCTTC		20		
HSP-27	F	CCGCCGAAACTGTAACCAAAG	60	21	137	XM_018061298.1
	R	ATCGAAGGATTCATCAGGCCAA		22		
HSP-60	F	AGGTTGGTGGGACAAGTGATG	60	21	139	XM_018061279.1
	R	AAGGCTGGAATGCACCGAAG		20		
HSP-70	F	TGGCTTTCACCGATACCGAG	60	20	167	NM001285703.1
	R	GTCGTTGATCACGCGGAAAG		20		
HSP-90	F	AAGAGCCTGACCAACGACTG	60	20	107	XM_005694067.1
	R	AAAGGAGCTCGTCTTGGGAC		20		
HSP110	F	TACCCACGGCATTTTCACCA	60	20	142	XM_018056621.1
	R	CTCATTAGCCTCGGCATCTGG		21		
HPRTI	F	GCCCCAGCGTGGTGATTAG	60	19	145	XM_018044253.1
	R	ACATCTCGAGCCAGTCGTTC		20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	60	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG		20		

HPRT, SDHA and GAPDH were used as reference gene to normalize the gene expression of target genes. Ta- annealing temperature; bp- base pair, HSP- heat shock protein, F forward, R reverse, *HSF-1*- Heat shock transcription factor 1, HSBP1- heat shock transcription factor-binding protein, HPRT- hypoxanthine phosphoribosyl transferase 1, GAPDH- glyceraldehyde 3-phosphate dehydrogenase

RESULTS

CHAPTER 4

RESULTS

4.1. Weather variables

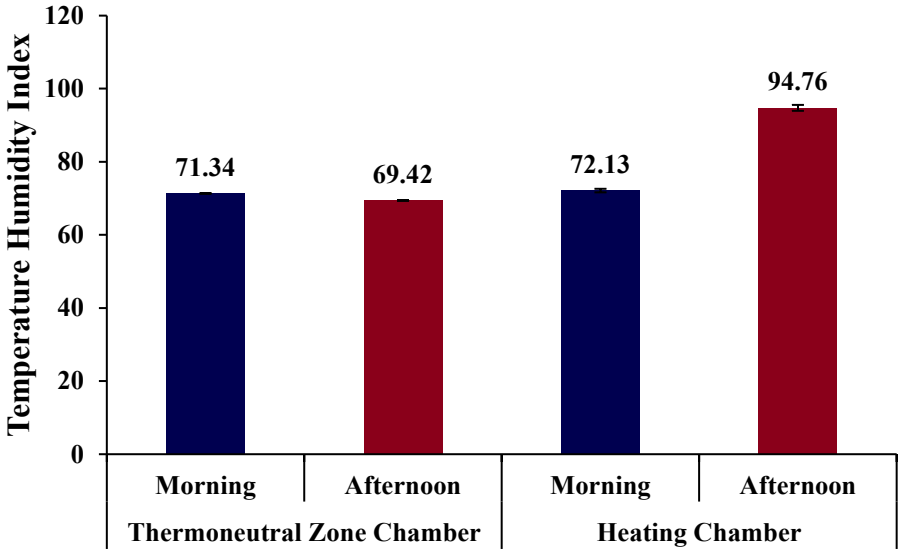
Table 4.1 describes the average weather variables during the study duration of 45 days period. The cardinal weather variables indicated that there were significant differences in the microclimate between the thermo-neutral chamber and heating climate chamber. The ambient temperature, relative humidity, maximum temperature, minimum temperature, dry bulb temperature, and wet bulb temperature were significantly ($P<0.01$) higher in the heating chamber as compared to thermo-neutral chamber. The THI values obtained between thermo-neutral and heating climate chambers both during morning and afternoon hours was described in Fig.4.1. The THI calculated based on temperature and relative humidity also showed similar pattern to other weather variables between the chambers. The THI values obtained both during morning and afternoon in TNZ chamber and heating chambers were 71.34±0.09, 69.42±0.05, 72.13±0.07 and 94.76±0.45, respectively. The THI values were comparable during morning hours in both the climate chambers. However, during afternoon the THI values showed significant ($P<0.01$) variation between the chambers with higher value recorded in the heating chamber.

Table 4.1: Average weather parameters during the entire study

	Time of Recording	DBT (ºC)	WBT (ºC)	MaxT (ºC)	MinT (ºC)	AT (ºC)	RH (%)	PST (ºC)
Thermo-neutral Zone Chamber	Morning (8:00 h)	25.12±0.09	17.57±0.07	27.34±0.09	24.64±0.14	25.81±0.16	66.34±0.58	26.68±0.13
	Afternoon (14:00 h)	23.35±0.05	16.68±0.05	24.19±0.06	23.68±0.07	24.02±0.07	68.88±0.33	22.33±0.39
Heating Chamber	Morning (8:00 h)	25.42±0.09	18.37±0.04	27.74±0.13	24.70±0.06	25.94±0.05	66.64±0.45	26.56±0.31
	Afternoon (14:00 h)	40.54±0.34	34.69±0.31	41.41±0.13	38.64±0.29	41.03±0.32	55.94±0.79	40.13±0.55

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 in Thermo-neutral Chamber and Heating Chamber

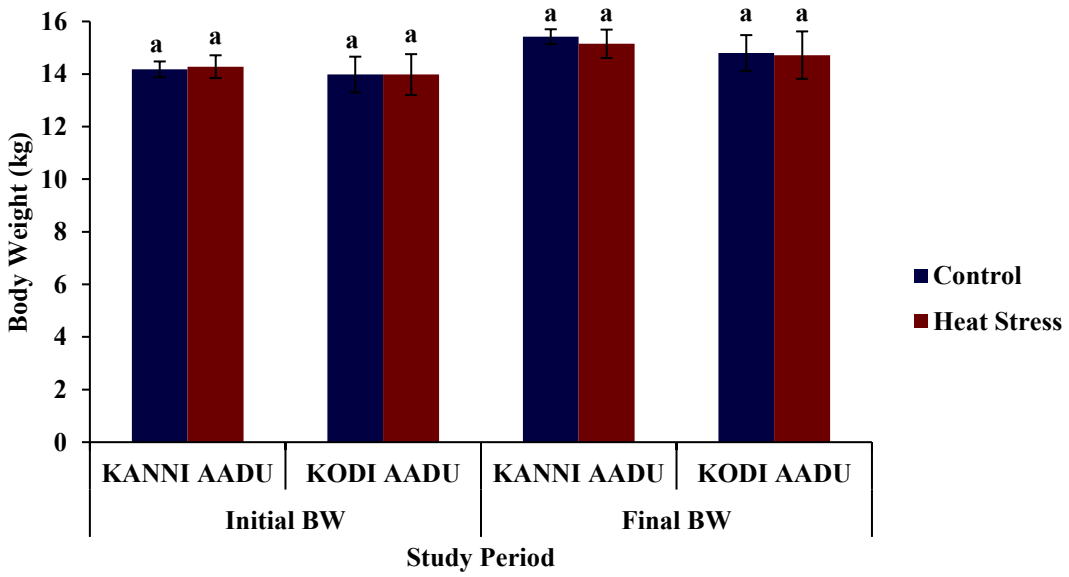


The THI values were calculated as per method described by McDowell (1972). Accordingly, the formula used was THI = 0.72 (T_{db} + T_{wb}) + 40.6 where, T_{db} = Dry bulb temperature in ºC; T_{wb} = Wet bulb temperature in ºC. The THI values 72 and less are considered comfortable; THI values between 75-78 are considered stressful and THI above 78 considered extreme distress. The THI in heating chamber during afternoon is significantly higher than other three values.

4.2. Body Weight Changes

The BW (kg) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.2. The BW in KAC, KAHS, KOC and KOHS groups were 14.80±0.27, 14.72±0.35, 14.39±0.48 and 14.35±0.58, respectively. The breed and treatment factors did not influence the BW during the recording days. Further, the experimental days influenced significantly ($P<0.01$) BW. However, the BxTxD interaction did not influence BW during the study period. The differences in the BW between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig.4.2. The post hoc test did not influence BW across the experimental groups. Further, THI although had a negative correlation with BW still this effect was not statistically significant (Table 4.3)

Fig. 4.2: Effect of heat stress on body weight of both Kanni Aadu and Kodi Aadu goat breeds.



4.3. Behavior Variables

4.3.1 Feed Intake (FI)

The FI ($g/w^{0.75}/day$) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.2. The FI ($g/w^{0.75}/day$) in KAC, KAHS, KOC and KOHS groups were 27.01±1.66, 26.14±1.26, 25.64±1.99 and 25.70±1.78, respectively. The breed and treatment factors did not influence the FI during the recording days. Further, the experimental days influenced significantly ($P<0.01$) FI. However, the BxTxD interaction did not influence FI during the study period. The differences in the FI between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.3. The post hoc test did not influence FI across the experimental groups. Further, THI although had a negative correlation with FI still this effect was not statistically significant (Table 4.3).

4.3.2. Water intake (WI)

The WI (L/KgDMI/day) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.2. The WI (L/KgDMI/day) in KAC, KAHS, KOC and KOHS groups was 5.50±0.44, 6.94±0.58, 7.19±1.02 and 7.46±0.69, respectively. All the factors such breed ($P<0.05$), treatment ($P<0.01$), experimental days ($P<0.01$) and BxTxD interaction ($P<0.01$) influenced significantly WI in this study. The differences in the WI between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig.4.4. The post hoc test did not influence WI across the experimental groups. Further, THI

although had a positive correlation with WI still this effect was not statistically significant (Table 4.3).

Table 4.3: Correlation association between THI, body weight, feed intake and water intake

	THI	Body Weight	Feed Intake	Water Intake
THI	**1**			
Body Weight	**-0.02**	**1**		
Feed Intake	**-0.04**	**0.46****	**1**	
Water Intake	**0.17**	**-0.30***	**-0.65****	**1**

THI- Temperature humidity index; **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$.

Table 4.2: Effect of heat stress on body weight, feed intake and water intake in Kanni Aadu and Kodi Aadu goat breeds

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
BW (kg)	0	14.18	14.28	13.98	13.98	NS	NS	**	NS
	45	15.42	15.15	14.80	14.72				
	Mean	**14.80** [a]	**14.72** [a]	**14.39** [a]	**14.35** [a]				
	Pooled SE	±0.60	±0.60	±0.60	±0.60				
FI ($g/w^{0.75}$/day)	0	25.41	25.27	23.69	24.94	NS	NS	*	NS
	45	28.62	27.00	27.60	26.45				
	Mean	**27.01** [a]	**26.14** [a]	**25.64** [a]	**25.70** [a]				
	Pooled SE	±2.14	±2.14	±2.14	±2.14				
WI (L/KgDMI/day)	0	**6.27**	**5.85**	**9.43**	**6.27**	NS	NS	NS	NS
	45	**4.72**	**8.04**	**4.95**	**8.66**				
	Mean	**5.50** [a]	**6.94** [a]	**7.19** [a]	**7.46** [a]				
	Pooled SE	±0.63	±0.63	±0.63	±0.63				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; BW- Body Weight; FI- Feed Intake; WI- Water Intake.

Pooled SE- Pooled 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.

Fig. 4.3 : Effect of heat stress on Feed Intake of Kanni Aadu and Kodi Aadu goat breeds

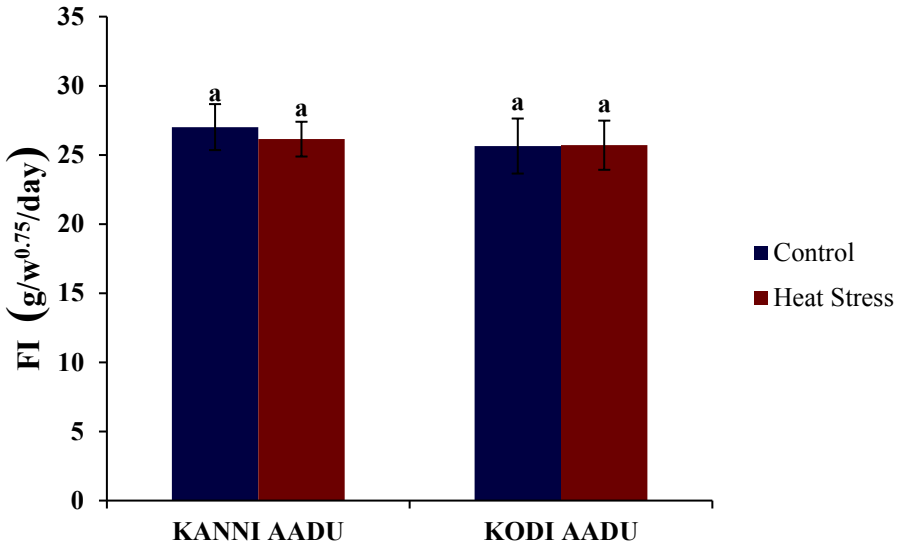


Fig. 4.4 : Effect of heat stress on Water Intake of Kanni Aadu and Kodi Aadu goat breeds

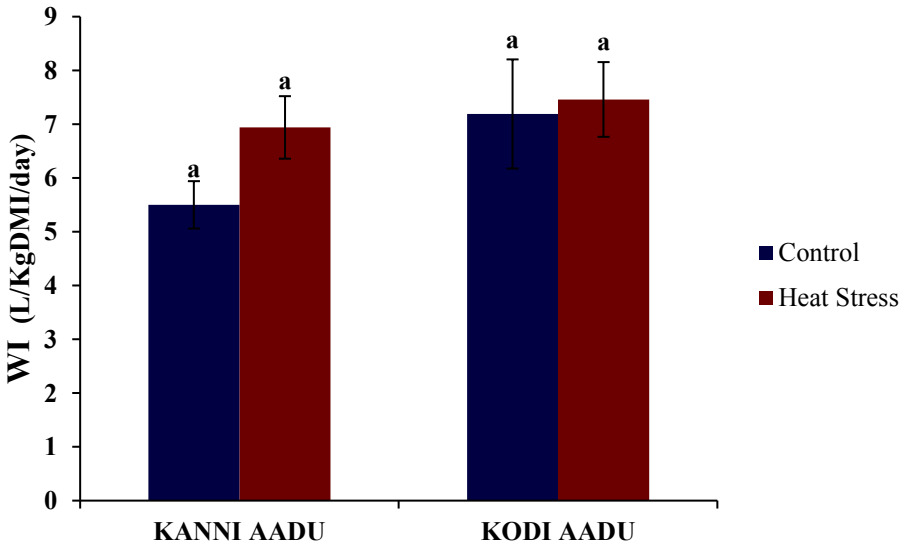


Table 4.4: Effect of heat stress on behavioural responses in Kanni Aadu and Kodi Aadu goat breeds

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
ST (minutes)	0	180.33	184.00	186.50	205.33	NS	**	NS	NS
	15	198.67	123.33	177.83	157.17				
	30	192.33	121.67	186.00	157.33				
	45	230.50	129.00	202.00	146.17				
	Mean	**200.46** [a]	**139.50** [b]	**188.08** [a]	**166.50** [ab]				
	Pooled SE	12.77	12.77	12.77	12.77				
LT (minutes)	0	179.67	167.33	173.50	154.67	NS	**	NS	NS
	15	161.17	234.17	181.83	202.67				
	30	167.67	239.33	174.00	202.67				
	45	129.50	232.50	158.00	213.83				
	Mean	**159.50** [b]	**218.33** [a]	**171.83** [b]	**193.46** [ab]				
	Pooled SE	13.05	13.05	13.05	13.05				
DF (no. of times)	0	0.83	1.33	1.83	1.33	NS	*	**	NS
	15	1.17	1.00	1.17	2.50				
	30	0.67	3.00	2.17	3.33				
	45	1.00	2.00	1.33	3.83				
	Mean	**0.92** [b]	**1.83** [ab]	**1.63** [b]	**2.75** [a]				
	Pooled SE	0.32	0.32	0.32	0.32				
DeF (no. of times)	0	0.50	0.17	0.83	0.67	NS	*	NS	NS
	15	0.00	0.67	0.33	0.00				
	30	0.33	1.50	1.00	0.17				
	45	0.50	0.00	2.33	0.33				
	Mean	**0.33** [b]	**0.58** [ab]	**1.13** [a]	**0.29** [b]				
	Pooled SE	0.19	0.19	0.19	0.19				
UF (no. of times)	0	2.00	2.33	3.00	2.33	NS	NS	NS	NS
	15	2.17	1.83	2.33	2.00				
	30	2.00	2.17	2.67	1.50				
	45	2.50	2.17	3.00	1.50				
	Mean	**2.17** [a]	**2.13** [a]	**2.75** [a]	**1.83** [a]				
	Pooled SE	0.37	0.37	0.37	0.37				
RuT (minutes)	0	63.67	73.67	53.33	43.67	NS	**	NS	NS
	15	73.17	36.17	44.17	13.67				
	30	72.33	36.17	34.67	14.83				
	45	68.00	29.50	81.17	12.83				
	Mean	**69.29** [a]	**43.88** [b]	**53.33** [ab]	**21.25** [c]				
	Pooled SE	6.31	6.31	6.31	6.31				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu 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. Pooled SE- Pooled 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.

4.3.3. Standing Time (ST)

The ST across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4. The ST in KAC, KAHS, KOC and KOHS groups were 200.46±10.33, 139.50±8.85, 188.08±11.02 and 166.50±7.23, respectively. The breed factor did not influence the ST during the recording days. Further, the treatment influenced significantly ($P<0.01$) ST. In addition, both the experimental days BxTxD interaction did not influence ST during the study period. The differences in the ST between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.5 (a). The post hoc test showed significant ($P<0.01$) variation between the groups for the ST. The treatment effect was evident only in Kanni Aadu goat breed as reflected by the significantly lower ST in KAHS group as compared to KAC group. However, the ST was comparable between the KOC and KOHS groups. Further, THI had a strong negative correlation ($P<0.01$) with ST in this study (Table 4.5).

4.3.4. Lying Time (LT)

The LT across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4. The LT in KAC, KAHS, KOC and KOHS groups were 159.50±10.33, 218.33±9.20, 171.83±11.02 and 193.46±7.23, respectively. The breed factor did not influence the LT during the recording days. Further, the treatment influenced significantly ($P<0.01$) LT. In addition, both the experimental days BxTxD interaction did not influence LT during the study period. The differences in the LT between the control and heat stress groups of Kanni

and Kodi Aadu goat breeds were described in Fig. 4.5 (b) The post hoc test showed significant ($P<0.01$) variation between the groups for the LT. The treatment effect was evident only in Kanni Aadu goat breed as reflected by the significantly higher LT in KAHS group as compared to KAC group. However, the LT was comparable between the KOC and KOHS groups. Further, THI had a strong positive correlation ($P<0.01$) with LT in this study (Table. 4.5).

4.3.5. Drinking Frequency (DF)

The DF across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4. The DF in KAC, KAHS, KOC and KOHS groups were 0.92±0.13, 1.83±0.29, 1.63±0.27 and 2.75±0.34, respectively. The breed factor did not influence the DF during the recording days. Further, both the treatment ($P<0.05$) and experimental days ($P<0.01$) influenced significantly DF. However, the BxTxD interaction did not influence DF during the study period. The differences in the DF between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.5 (c) The post hoc test showed significant ($P<0.01$) variation between the groups for the DF. The treatment effect was evident only in Kodi Aadu goat breed as reflected by the significantly increased DF in KOHS group as compared to KOC group. However, the DF was comparable between the KAC and KAHS groups. Further, THI had a strong positive correlation ($P<0.01$) with DF in this study (Table 4.5).

4.3.6. Defecating Frequency (DeF)

The DeF across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4. The DeF in KAC, KAHS, KOC and KOHS groups were 0.33±0.17, 0.58±0.20, 1.13±0.31 and 0.29±0.11, respectively. The breed factor did not influence the DeF. However, the treatment factor influenced significantly ($P<0.05$) the DeF. Further, both the experimental days and the BxTxD interaction also did not influence the DeF level in the study. The differences in the DeF across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.5 (d). The post hoc test showed variation ($P<0.05$) for the DeF across the experimental groups. The treatment effect was evident only in Kodi Aadu goat breed as reflected by the significantly decreased DeF in KOHS group as compared to KOC group. However, the DeF was comparable between the KAC and KAHS groups. Further, THI although had a negative correlation with DeF still this effect was not statistically significant (Table 4.5).

4.3.7. Urinating Frequency (UF)

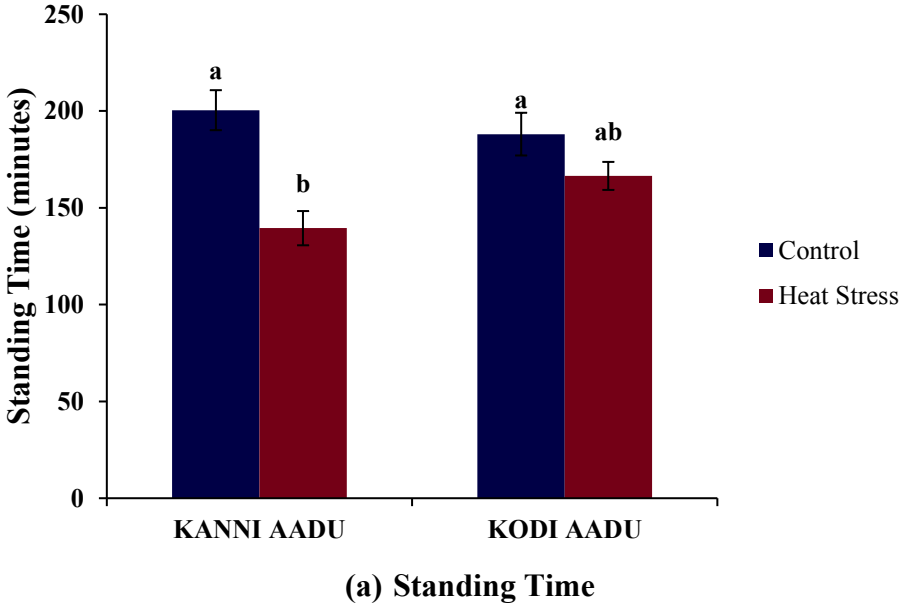
The UF across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4. The UF in KAC, KAHS, KOC and KOHS groups were 2.17±0.21, 2.13±0.29, 2.75±0.25 and 1.83±0.25, respectively. Both the breed and treatment factors did not influence the UF. Further, both the experimental days and the BxTxD interaction also did not influence the UF level in the study. The differences in the UF across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.5 (e). The post hoc test did not show

any variation for the UF across the experimental groups. Further, THI although had a negative correlation with UF still this effect was not statistically significant (Table 4.5).

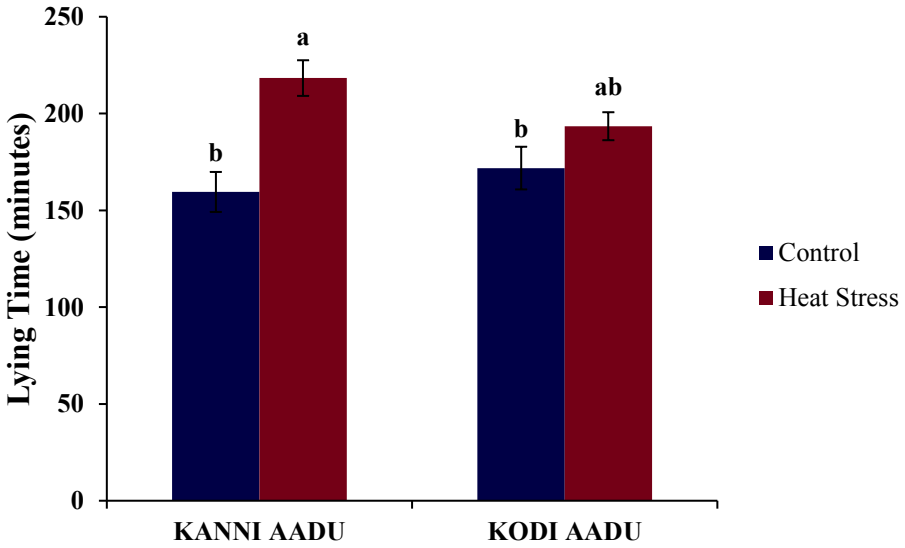
4.3.8. Rumination Time (RuT)

The RuT across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.4**.** The RuT in KAC, KAHS, KOC and KOHS groups were 69.29±5.10, 43.88±5.53, 53.33±7.11 and 21.25±3.61, respectively. The breed factor did not influence the RuT during the recording days. Further, the treatment influenced significantly ($P<0.01$) RuT. In addition, both the experimental days BxTxD interaction did not influence RuT during the study period. The differences in the RuT between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.5 (f). The post hoc test showed significant ($P<0.01$) variation between the groups for the RuT. The treatment effect was significant ($P<0.01$) both in Kanni and Aadu goat breed as reflected by the lower RuT in KAHS and KOHS as compared to KAC and KOC groups, respectively. Further, THI had a strong negative correlation ($P<0.01$) with RuT in this study (Table 4.5).

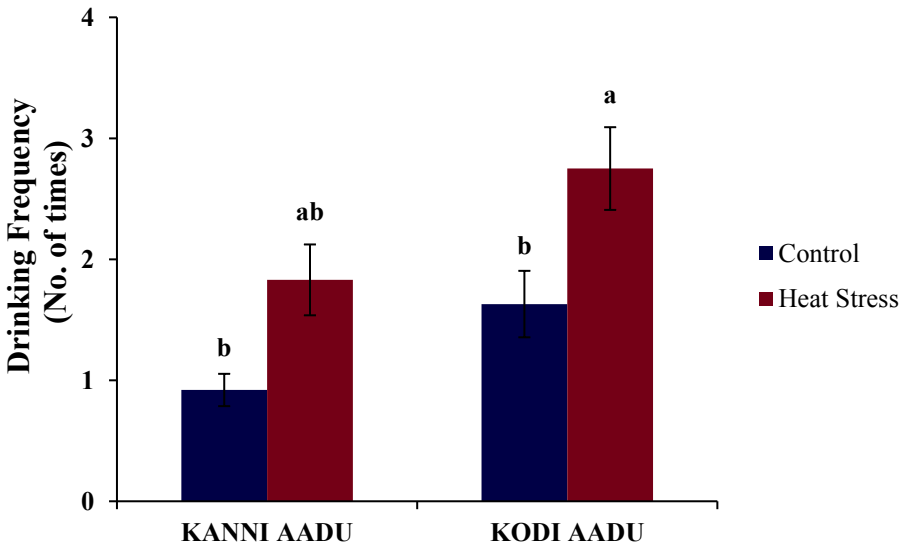
Fig. 4.5: Effect of heat stress on behavioural responses in Kanni Aadu and Kodi Aadu goat breeds (a) Standing Time (b) Lying Time (c) Drinking Frequency (d) Defecating Frequency (e) Urinating Frequency (f) Rumination Time



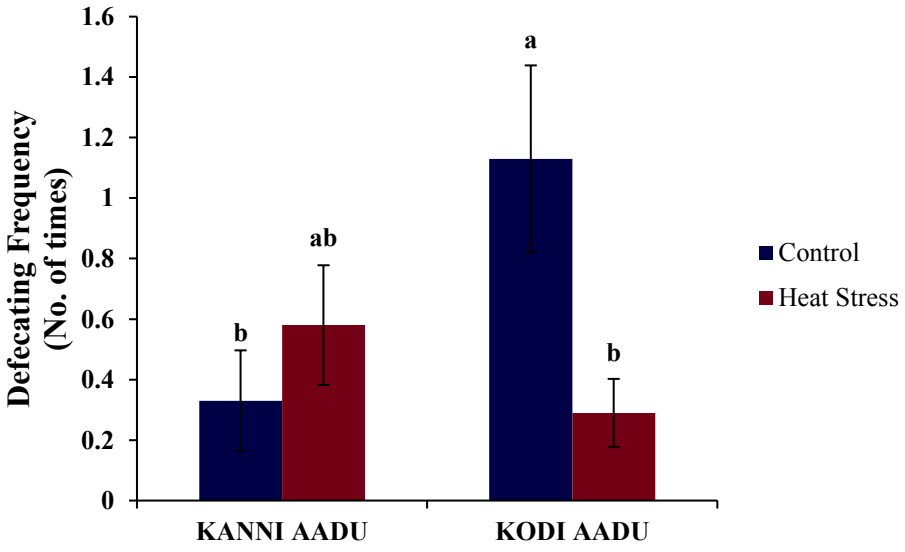
(a) Standing Time



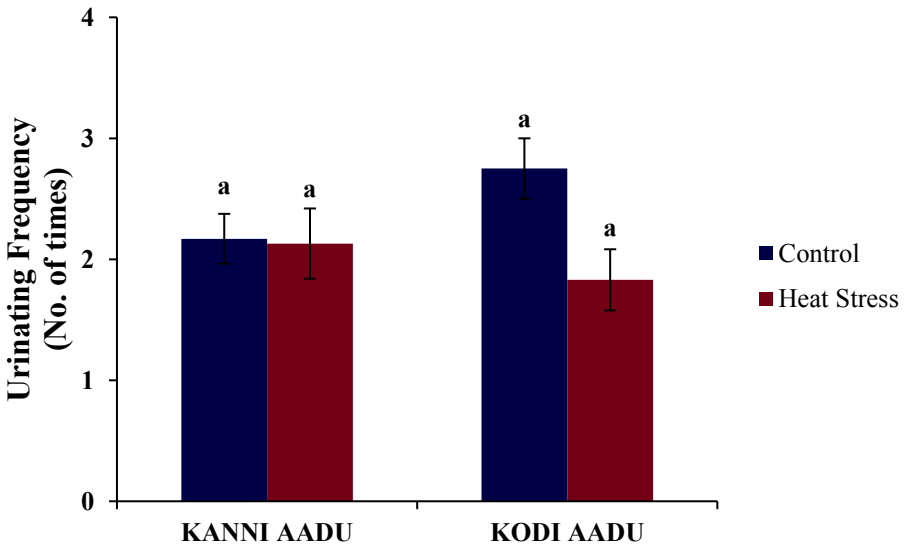
(b) Lying Time



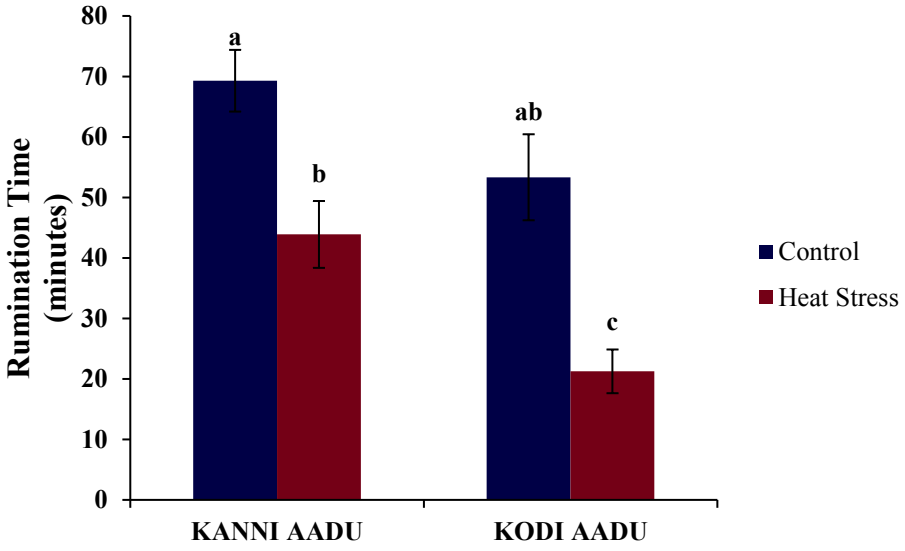
(c) Drinking Frequency



(d) Defecating Frequency



(e) Urinating Frequency



(f) Rumination Time

Table 4.5: Correlation association between THI and behavioural responses

	THI	ST	LT	DF	DeF	UF	RuT
THI	**1**						
ST	**-0.41****	**1**					
LT	**0.39****	**-0.99****	**1**				
DF	**0.35****	**-0.19**	**0.18**	**1**			
DeF	**-0.13**	**0.06**	**-0.06**	**0.12**	**1**		
UF	**-0.17**	**0.16**	**-0.17**	**0.01**	**0.09**	**1**	
RuT	**-0.45****	**0.18**	**-0.18**	**-0.47****	**-0.02**	**0.04**	**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 $P < 0.01$.

4.4. Physiological variables

4.4.1. Respiration Rate (RR)

The RR across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.6. The respiration rate morning (RRM) in KAC, KAHS, KOC and KOHS groups were 21.83±0.64, 20.00±0.76, 20.83±0.72 and 19.17±0.83, respectively. Similarly, the respiration rate afternoon (RRA) in KAC, KAHS, KOC and KOHS groups were 24.83±0.80, 90.42±8.61, 23.83±0.92 and 90.67±8.24, respectively. Further, the respiration rate evening (RRE) in KAC, KAHS, KOC and KOHS groups were 22.00±0.43, 21.83±0.64, 21.75±0.68 and 24.00±0.64, respectively. Likewise, the respiration rate night (RRN) in KAC, KAHS, KOC and KOHS groups were 18.75±0.45, 20.67±0.67, 18.83±0.56 and 18.17±0.93, respectively. The breed factor did not influence the RR at any time point during the recording days. Further, the treatment influenced significantly RRA ($P<0.01$), RRE ($P<0.05$) and RRN ($P<0.05$) but did not influence RRM. In addition, the experimental days significantly ($P<0.01$) influenced RR at all time point except during morning hours. Moreover, the breed x treatment x day (BxTxD) interaction significantly ($P<0.01$) influenced RR only during evening and night hours. However, both during morning and afternoon hours, interaction did not influence the RR. The differences in the RR across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.6 (a-d). The post hoc test showed significant ($P<0.01$ variation among the groups for the RR at all time points except morning hours. Further, the THI had a

negative correlation ($P<0.05$) with RRM, strong positive ($P<0.01$) correlation for RRA while non significant correlation with RRE and RRN in this study (Table 4.8).

Table 4.6: Effect of heat stress on respiration rate in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
RRM (breaths/minute)	0	21.33	22.00	21.33	24.67	NS	NS	NS	NS
	15	20.67	18.00	18.67	18.00				
	30	22.67	22.67	22.67	16.00				
	45	22.67	17.33	20.67	18.00				
	Mean	**21.83** ^{a}	**20.00** ^{ab}	**20.83** ^{ab}	**19.17** ^{b}				
	Pooled SE	±0.72	±0.72	±0.72	±0.72				
RRA (breaths/minute)	0	29.33	30.00	30.00	34.00	NS	**	**	NS
	15	21.33	138.00	22.67	140.67				
	30	26.67	103.67	22.00	95.33				
	45	22.00	90.00	22.67	92.67				
	Mean	**24.83** ^{b}	**90.42** ^{a}	**23.83** ^{b}	**90.67** ^{a}				
	Pooled SE	±2.42	±2.42	±2.42	±2.42				
RRE (breaths/minute)	0	22.00	24.67	24.67	26.67	NS	*	**	**
	15	20.00	22.67	21.00	22.67				
	30	23.33	20.67	22.00	23.33				
	45	22.67	19.33	19.33	23.33				
	Mean	**22.00** ^{b}	**21.83** ^{b}	**21.75** ^{b}	**24.00** ^{a}				
	Pooled SE	±0.51	±0.51	±0.51	±0.51				
RRN (breaths/minute)	0	20.67	22.00	18.00	24.67	NS	*	**	**
	15	18.33	19.33	18.00	16.00				
	30	18.67	22.67	20.67	16.00				
	45	17.33	18.67	18.67	16.00				
	Mean	**18.75** ^{b}	**20.67** ^{a}	**18.83** ^{b}	**18.17** ^{b}				
	Pooled SE	±0.54	±0.54	±0.54	±0.54				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; RRM- Respiration rate morning; RRA-Respiration rate afternoon; RRE-Respiration rate evening; RRN- Respiration rate night; Pooled SE- Pooled 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

Fig. 4.6: Effect of heat stress on respiration rate in Kanni Aadu and Kodi Aadu goat breeds (a) Respiration rate morning (b) Respiration rate afternoon (c) Respiration rate evening (d) Respiration rate night

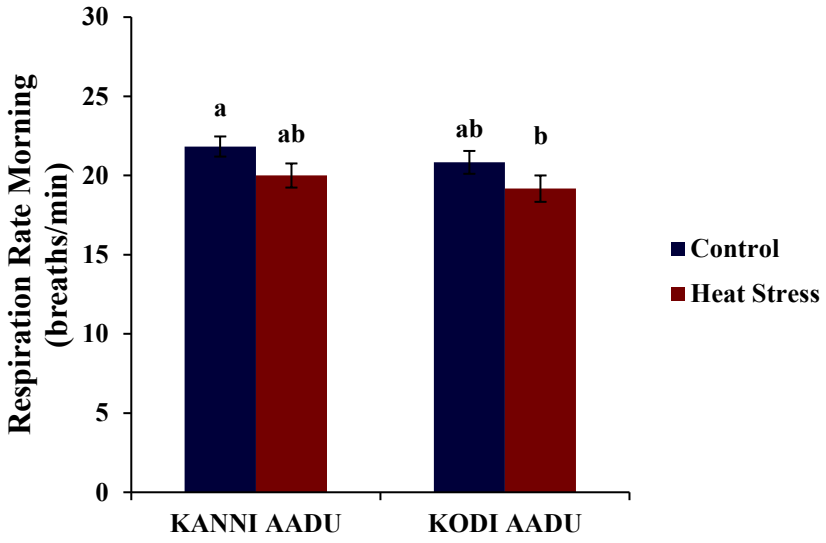


Fig (a): Respiration rate morning

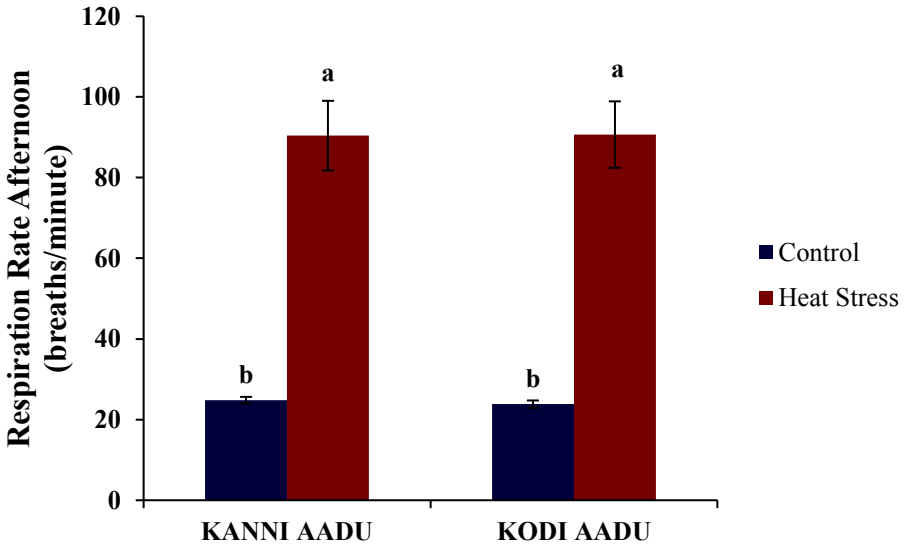


Fig (b): Respiration rate afternoon

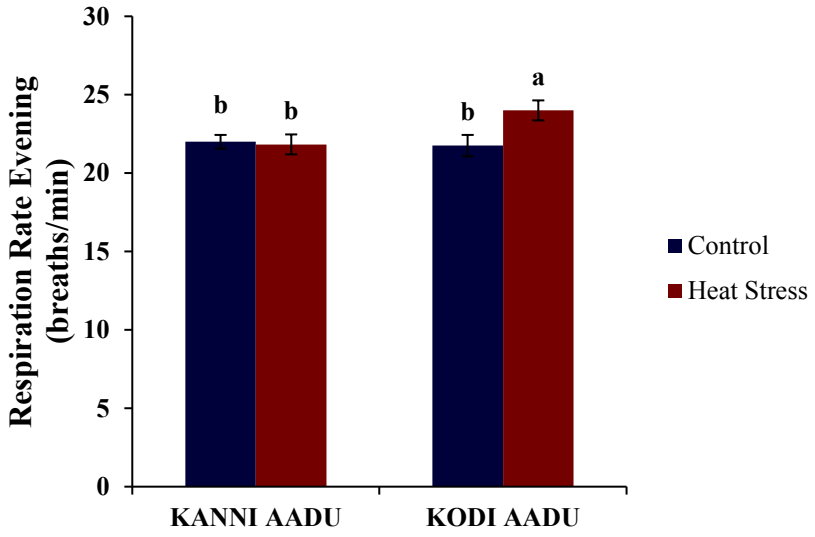


Fig (c): Respiration rate evening

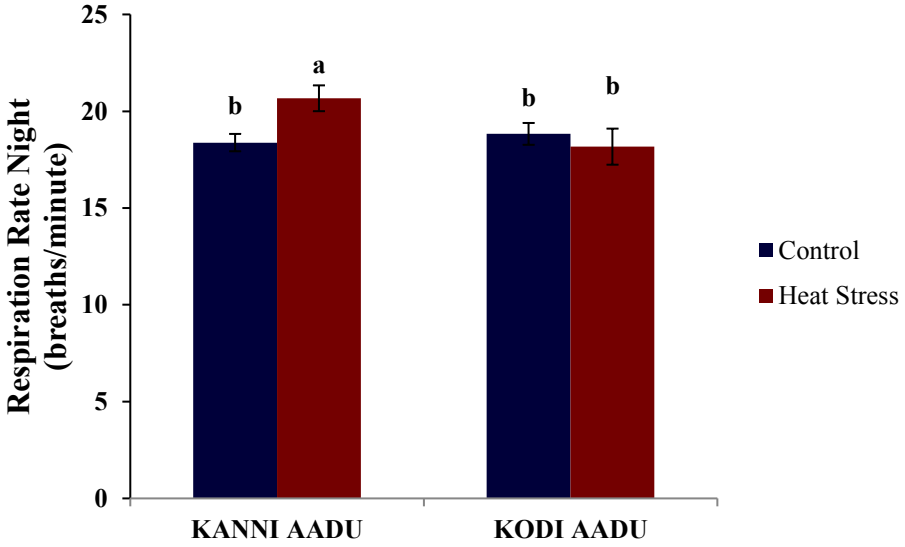


Fig (d) Respiration rate night

4.4.2. Panting Score (PS)

The PS across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.7. The pulse rate afternoon (PSA) in KAC, KAHS, KOC and KOHS groups were 0.00±0.00, 1.67±0.66, 0.00±0.00 and 1.63±0.65, respectively. The breed factor did not influence the PSA during the recording days. Further, the treatment effect influenced significantly (P<0.01) PSA. In addition, the experimental days significantly (P<0.01) influenced PSA during the recording days. However, the BxTxD interaction did not influence PSA. The differences in the PSA between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig.4.7. The post hoc test showed significant P<0.01) variation among the groups for the PSA. Further, the THI had a strong positive (P<0.01) correlation with PSA in this study (Table 4.8).

Table 4.7: Effect of heat stress on Panting Score in Kanni Aadu and Kodi Aadu goat breeds during afternoon.

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
Panting Score	0	0.00	0.00	0.00	0.00	NS	**	**	NS
	15	0.00	3.17	0.00	3.17				
	30	0.00	2.00	0.00	1.50				
	45	0.00	1.50	0.00	1.83				
	Mean	**0.00** ^{b}	**1.67** ^{a}	**0.00** ^{b}	**1.63**^{a}				
	Pooled SE	±0.08	±0.08	±0.08	±0.08				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; Pooled SE- Pooled standard error. **Indicates statistical significance at $P < 0.01$; NS- Indicates non-significant; Values bearing different superscripts within a row differ significantly with each other

Fig. 4.7: Effect of heat stress on Panting Score in Kanni Aadu and Kodi Aadu goat breeds during afternoon.

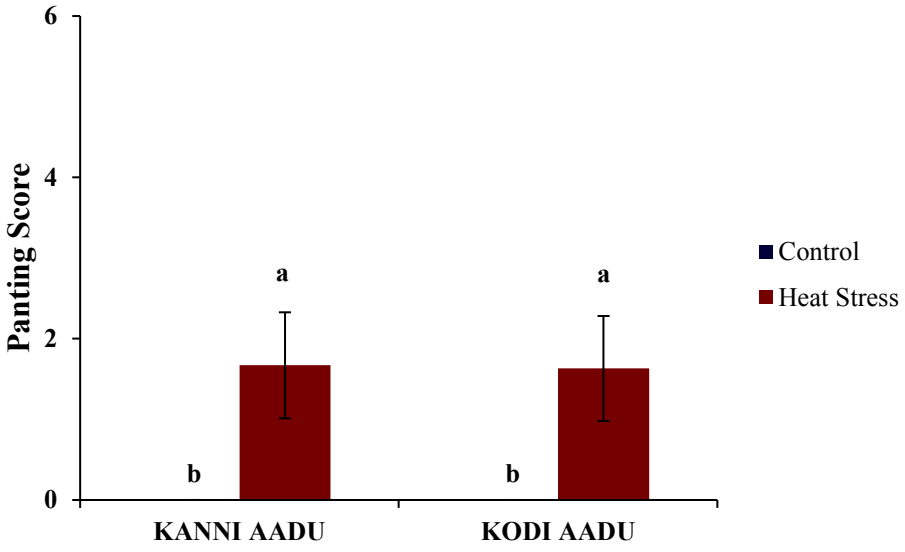


Table 4.8: Correlation association between THI and RR with panting score during morning, afternoon, evening and night.

	THI	RRM	RRA	RRE	RRN	PS
THI	**1**					
RRM	**-0.24***	**1**				
RRA	**0.72****	**-0.45****	**1**			
RRE	**0.17**	**0.10**	**0.00**	**1**		
RRN	**0.09**	**0.28****	**-0.23***	**0.05**	**1**	
PS	**0.69****	**-0.39****	**0.97**	**-0.04**	**-0.19**	**1**

THI-Temperature Humidity Index; RR- respiration rate; RRM- respiration rate morning; RRA- respiration rate afternoon; RRE- respiration rate evening; RRN- respiration rate night; PS- Panting Score **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

4.4.3. Pulse Rate

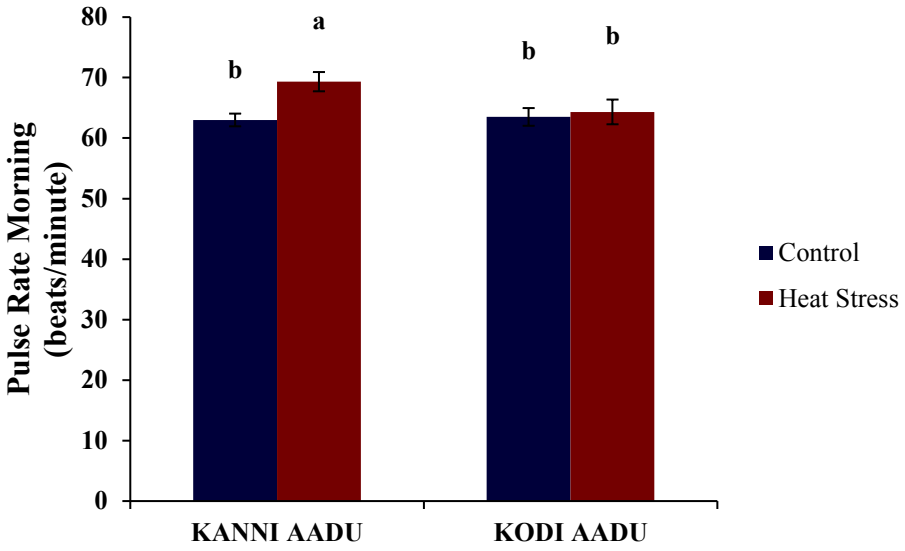
The PR across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.9. The pulse rate morning (PRM) in KAC, KAHS, KOC and KOHS groups were 63.00±1.05, 69.33±1.59, 63.50±1.47 and 64.33±2.04, respectively. Similarly, the pulse rate afternoon (PRA) in KAC, KAHS, KOC and KOHS groups were 67.21±1.59, 80.33±3.36, 71.08±1.71 and 85.67±2.68, respectively. Further, the pulse rate evening (PRE) in KAC, KAHS, KOC and KOHS groups were 65.00±1.63, 75.00±3.36, 66.67±1.88 and 72.67±2.07, respectively. Likewise, the pulse rate night (PRN) in KAC, KAHS, KOC and KOHS groups were 60.75±1.17, 66.50±1.58, 68.00±1.59 and 69.17±2.12, respectively. The breed factor did not influence the PR at any time point during the recording days. Further, the treatment influenced significantly PRM ($P<0.05$), PRA ($P<0.01$), PRE ($P<0.01$) and PRN ($P<0.01$). In addition, the experimental days significantly ($P<0.01$) influenced PR at all time points on the recording days. However, the BxTxD interaction did not influence PR at any time point during the recording days. The differences in the PR across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.8. The post hoc test showed significant ($P<0.01$) variation among the groups for the PR at all time points during the recording days. Further, the THI had a positive correlation with PRA ($P<0.01$) and PRN ($P<0.05$), while non-significant correlation with PRM and PRE in this study (Table 4.10).

Table 4.9: Effect of heat stress on Pulse Rate in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

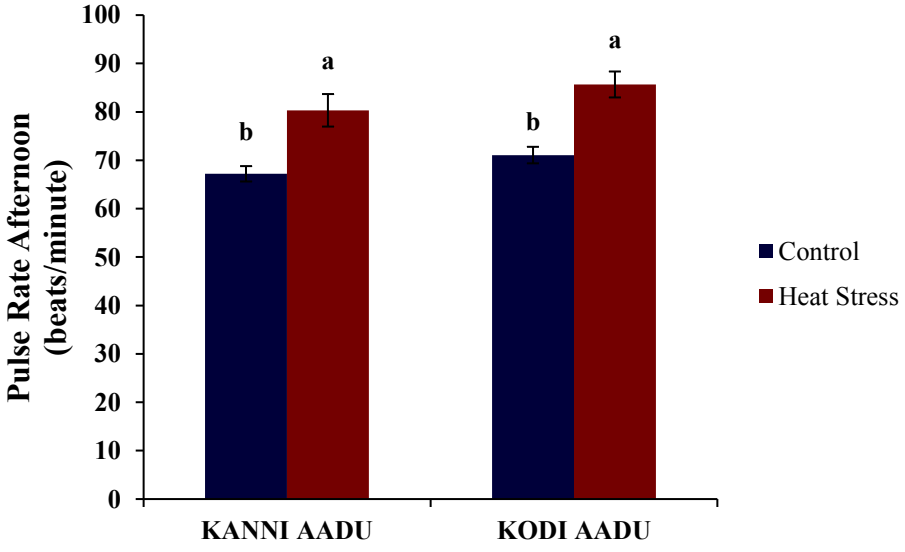
Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
PRM	0	61.67	63.33	60.67	56.00	NS	*	**	NS
(beats/minute)	15	59.67	72.67	58.67	68.67				
	30	60.00	64.67	62.67	60.00				
	45	70.67	76.67	72.00	72.67				
	Mean	**63.00** [b]	**69.33** [a]	**63.50** [b]	**64.33** [b]				
	Pooled SE	±1.40	±1.40	±1.40	±1.40				
PRA	0	75.33	71.33	76.33	67.33	NS	**	**	NS
(beats/minute)	15	58.33	74.00	63.67	89.33				
	30	64.67	82.00	67.33	90.67				
	45	70.50	94.00	77.00	95.33				
	Mean	**67.21** [b]	**80.33** [a]	**71.08** [b]	**85.67** [a]				
	Pooled SE	±2.22	±2.22	±2.22	±2.22				
PRE	0	61.00	61.33	59.67	58.67	NS	**	**	NS
(beats/minute)	15	60.33	70.67	63.00	72.67				
	30	62.67	96.67	63.33	80.67				
	45	76.00	71.33	80.67	78.67				
	Mean	**65.00** [b]	**75.00** [a]	**66.67** [b]	**72.67** [a]				
	Pooled SE	±1.2	±1.2	±1.2	±1.2				
PRN	0	57.33	57.33	65.67	61.33	NS	**	**	NS
(beats/minute)	15	59.00	66.00	60.33	72.67				
	30	57.33	74.67	76.67	76.00				
	45	69.33	68.00	69.33	66.67				
	Mean	**60.75** [b]	**66.50** [a]	**68.00** [a]	**69.17** [a]				
	Pooled SE	±1.15	±1.15	±1.15	±1.15				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; PRM-Pulse rate morning; PRA-Pulse rate afternoon; PRE-Pulse rate evening; PRN-Pulse rate night; Pooled SE- Pooled 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

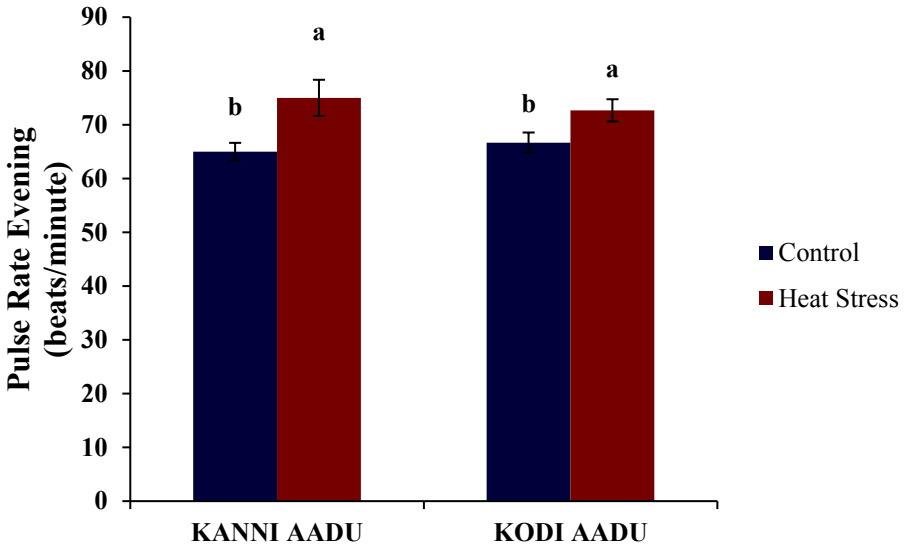
Fig. 4.8: Effect of heat stress on pulse rate in Kanni Aadu and Kodi Aadu goat breeds (a) Pulse rate morning (b) Pulse rate afternoon (c) Pulse rate evening (d) Pulse rate night



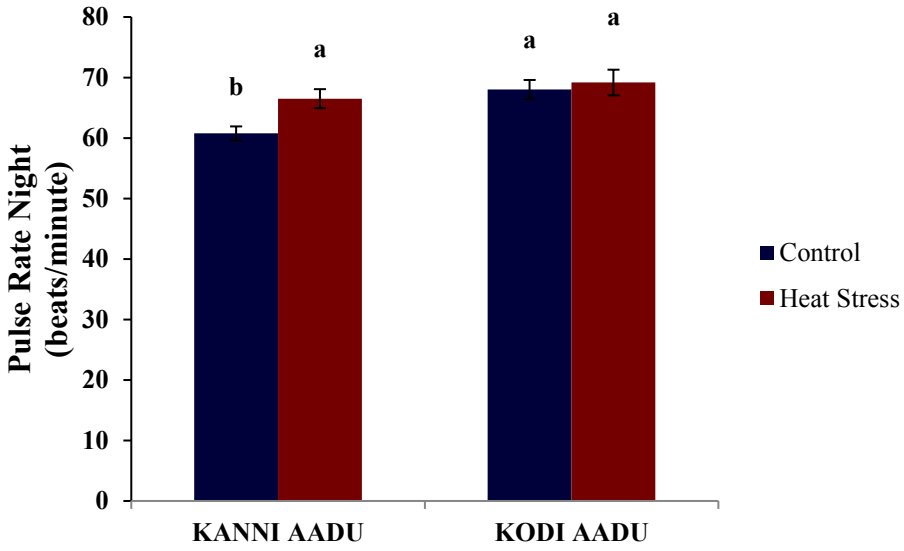
(a) Pulse rate morning



(b) Pulse rate afternoon



(c) Pulse rate evening



(d) Pulse rate night

Table 4.10: Correlation association between THI and Pulse Rate during morning, afternoon, evening and night.

	THI	PRM	PRA	PRE	PRN
THI	**1**				
PRM	**0.23***	**1**			
PRA	**0.50****	**0.42****	**1**		
PRE	**0.34****	**0.35****	**0.35****	**1**	
PRN	**0.20***	**0.28****	**0.33****	**0.50****	**1**

THI-Temperature Humidity Index; PRM- Pulse rate morning; PRA- Pulse rate afternoon; PRE- Pulse rate evening; PRN- Pulse rate night **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

4.4.4. Rectal Temperature

The RT across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.11. The rectal temperature morning (RTM) in KAC, KAHS, KOC and KOHS groups were 38.40±0.06, 38.48±0.10, 38.55±0.05 and 38.35±0.09, respectively. Similarly, the rectal temperature afternoon (RTA) in KAC, KAHS, KOC and KOHS groups were 38.46±0.07, 39.47±0.09, 38.62±0.09 and 39.44±0.09, respectively. Further, the rectal temperature evening (RTE) in KAC, KAHS, KOC and KOHS groups were 38.66±0.06, 38.75±0.08, 38.63±0.07 and 38.40±0.07, respectively. Likewise, the rectal temperature night (RTN) in KAC, KAHS, KOC and KOHS groups were 38.45±0.05, 38.48±0.07, 38.52±0.05 and 38.37±0.06, respectively. The breed factor did not influence the RT at any time point during the recording days. Further, the treatment influenced significantly ($P<0.01$) RT only at 14.00 h. In addition, the experimental days significantly ($P<0.01$) influenced RT at all time points except at 14.00 h on the recording days. However, the BxTxD interaction did not influence RT at

any time point during the recording days. The differences in the RT across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.9. The post hoc test showed significant (P<0.01) variation among the groups for the RT both at 14.00 h and 20.00 h on the recording days. Further, the THI had a strong positive correlation (P<0.01) with RTA while non-significant correlation with RTM, RTE and RTN in this study (Table 4.12).

Table 4.12: Correlation association between THI and rectal temperature during morning, afternoon, evening and night.

	THI	RTM	RTA	RTE	RTN
THI	**1**				
RTM	**-0.00**	**1**			
RTA	**0.74****	**0.08**	**1**		
RTE	**-0.03**	**0.47****	**0.19**	**1**	
RTN	**-0.07**	**0.40****	**0.16**	**0.50****	**1**

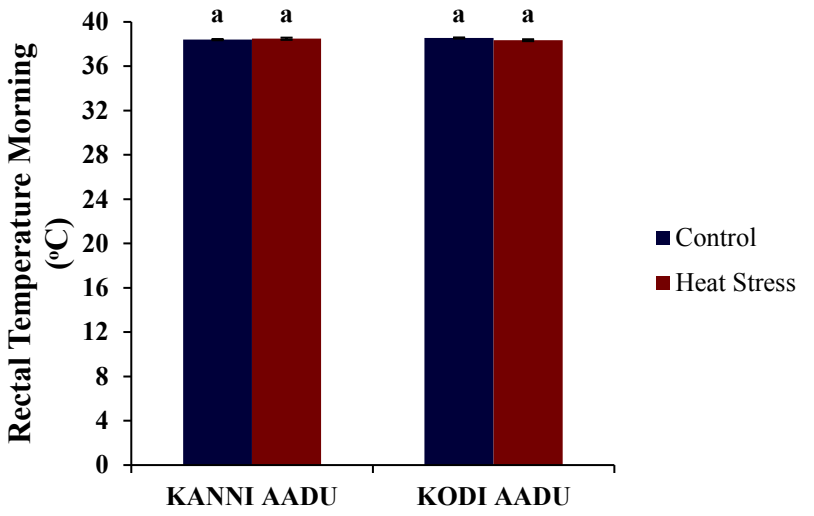
THI-Temperature Humidity Index; RTM- Rectal temperature morning; RTA- Rectal temperature afternoon; RTE- Rectal temperature evening; RTN- Rectal temperature night
**Indicates statistical significance at $P < 0.01$.

Table 4.11: Effect of heat stress on rectal temperature in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

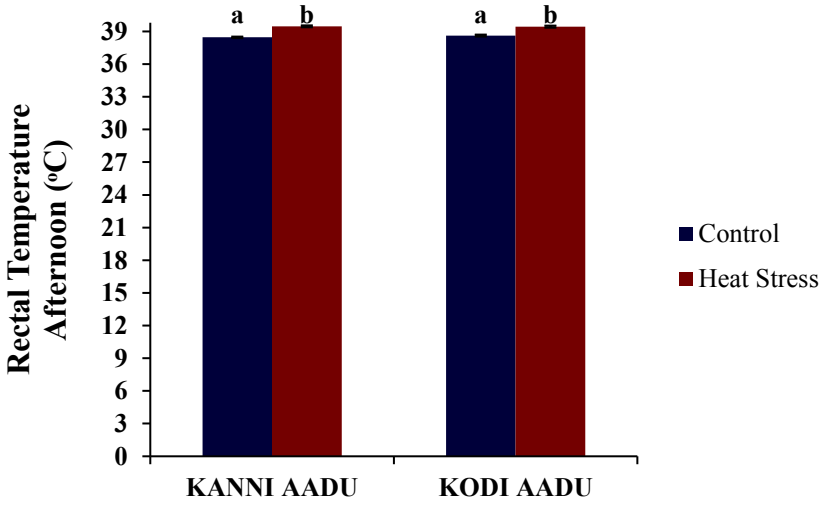
Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
RTM	0	38.43	38.88	38.75	38.80	NS	NS	**	NS
(°C)	15	38.37	38.45	38.60	38.20				
	30	38.45	38.15	38.48	38.23				
	45	38.33	38.42	38.38	38.18				
	Mean	**38.40** [a]	**38.48** [a]	**38.55** [a]	**38.35** [a]				
	Pooled SE	±0.08	±0.08	±0.08	±0.08				
RTA	0	38.88	39.03	39.08	39.00	NS	**	NS	NS
(°C)	15	38.35	39.72	38.82	39.87				
	30	38.28	39.43	38.37	39.42				
	45	38.33	39.68	38.20	39.47				
	Mean	**38.46** [b]	**39.47** [a]	**38.62** [b]	**39.44** [a]				
	Pooled SE	±0.09	±0.09	±0.09	±0.09				
RTE	0	38.80	38.83	38.65	38.82	NS	NS	**	NS
(°C)	15	38.60	38.65	38.65	38.32				
	30	38.77	38.80	38.83	38.27				
	45	38.47	38.73	38.37	38.20				
	Mean	**38.66** [a]	**38.75** [a]	**38.63** [ab]	**38.40** [b]				
	Pooled SE	±0.08	±0.08	±0.08	±0.08				
RTN	0	38.60	38.57	38.62	38.60	NS	NS	**	NS
(°C)	15	38.28	38.45	38.53	38.32				
	30	38.58	38.55	38.65	38.48				
	45	38.33	38.33	38.27	38.08				
	Mean	**38.45** [a]	**38.48** [a]	**38.52** [a]	**38.37** [a]				
	Pooled SE	±0.07	±0.07	±0.07	±0.07				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; RTM-Rectal temperature morning; RTA-Rectal temperature afternoon; RTE-Rectal temperature evening; RTN-Rectal temperature night; Pooled SE- Pooled standard error. **Indicates statistical significance at $P < 0.01$; NS- Indicates non-significant; Values bearing different superscripts within a row differ significantly with each other

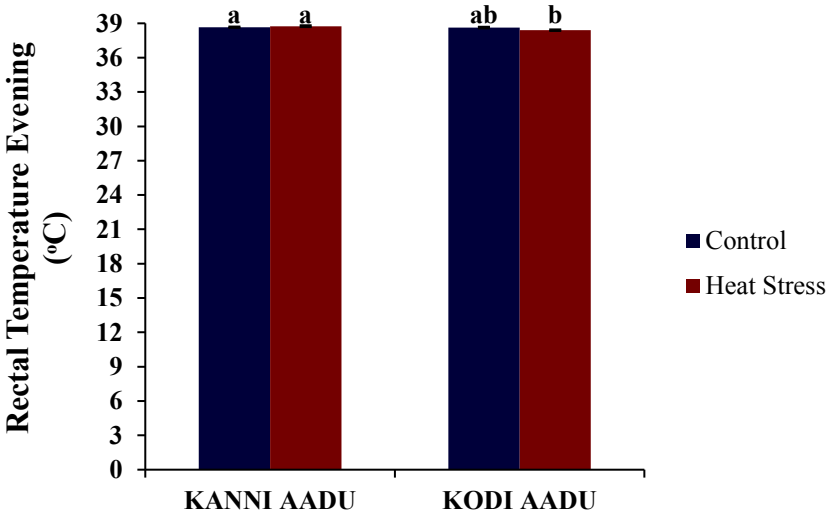
Fig. 4.9: Effect of heat stress on rectal temperature in Kanni Aadu and Kodi Aadu goat breeds (a) Rectal temperature morning (b) Rectal temperature afternoon (c) Rectal temperature evening (d) Rectal temperature night



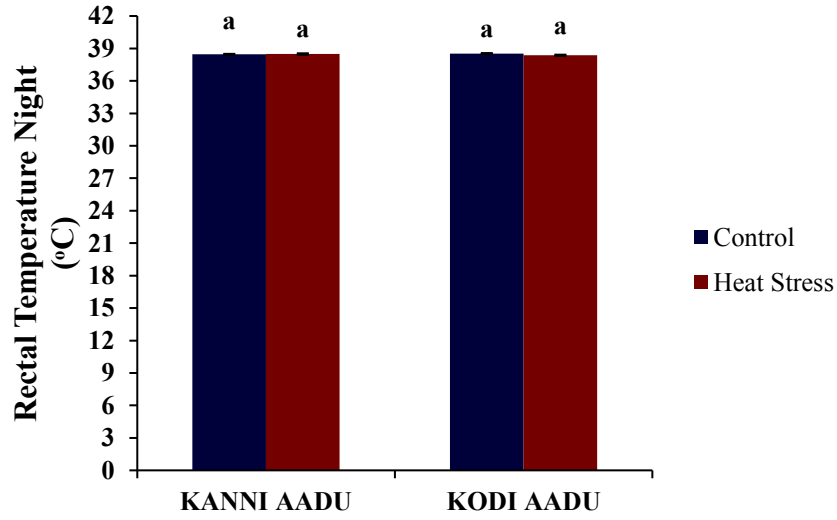
(a) Rectal temperature morning



(b) Rectal temperature afternoon



(c) Rectal temperature evening



(d) Rectal temperature night

4.4.5. Skin Temperatures

4.4.5.1. Skin Temperature Head (STH)

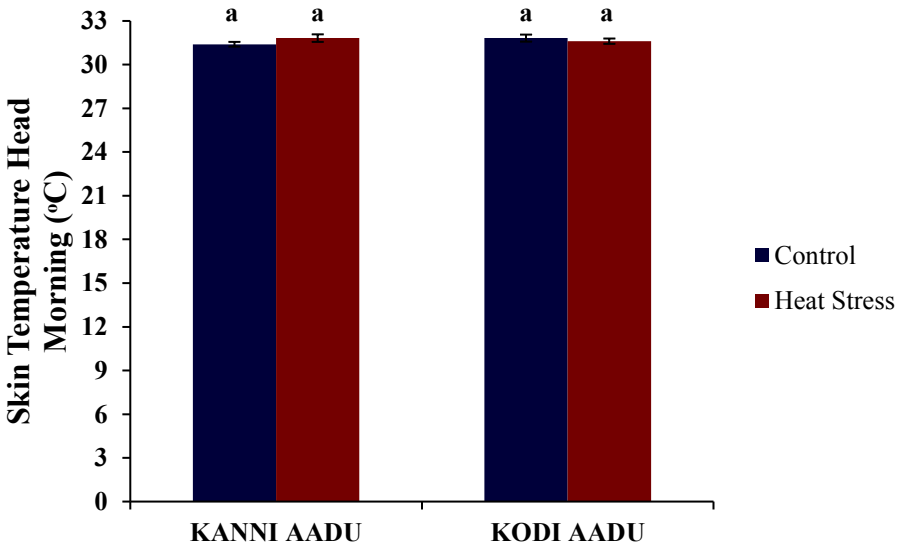
The STH across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.13. The skin temperature head morning (STHM) in KAC, KAHS, KOC and KOHS groups were 31.40±0.16, 31.82±0.26, 31.82±0.24 and 31.61±0.18, respectively. Similarly, the skin temperature head afternoon (STHA) in KAC, KAHS, KOC and KOHS groups were 29.01±0.61, 38.25±0.59, 29.88±0.56 and 38.29±0.67, respectively. Further, the skin temperature head evening (STHE) in KAC, KAHS, KOC and KOHS groups were 32.00±0.39, 32.85±0.19, 32.30±0.21 and 32.45±0.33, respectively. Likewise, the skin temperature head night (STHN) in KAC, KAHS, KOC and KOHS groups were 31.79±0.27, 31.91±0.36, 31.30±0.40 and 31.80±0.31, respectively. The breed factor did not influence the STH at any time point during the recording days. Further, the treatment influenced significantly ($P<0.01$) STH only at 14.00 h. In addition, the experimental days as well as the BxTxD interaction did not influence STH at any time point during the recording days. The differences in the STH across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.10. The post hoc test showed significant ($P<0.01$) variation among the groups for the STH only at 14.00 h on the recording days. Further, the THI had a strong positive correlation ($P<0.01$) with STHA while non-significant correlation with STHM, STHE and STHN in this study (Table 4.14).

Table 4.13: Effect of heat stress on Skin temperature (head) in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

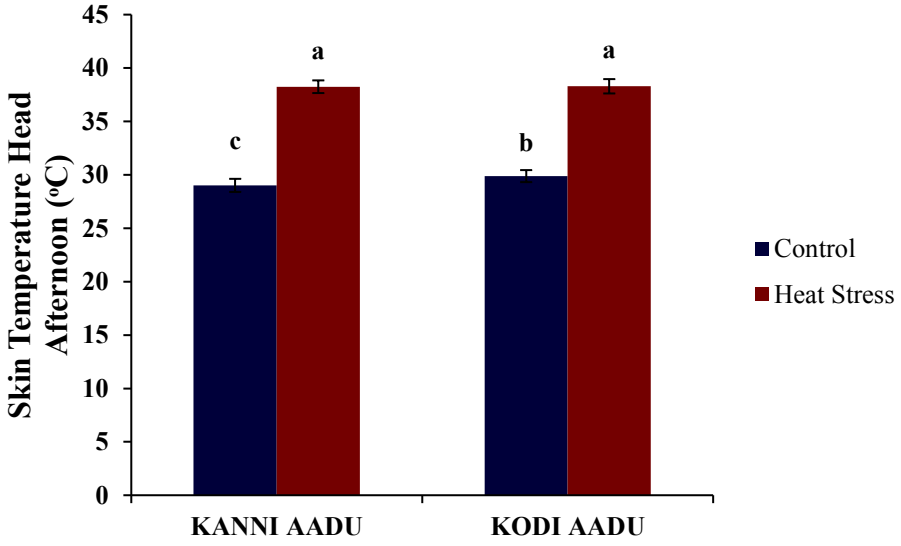
Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
STHM	0	31.90	33.08	32.33	32.70	NS	NS	NS	NS
(°C)	15	30.60	31.28	31.55	30.77				
	30	31.57	30.82	31.18	31.27				
	45	31.52	32.08	32.20	31.72				
	Mean	**31.40** [a]	**31.82** [a]	**31.82** [a]	**31.61** [a]				
	Pooled SE	±0.15	±0.15	±0.15	±0.15				
STHA	0	33.42	33.40	33.58	32.88	NS	**	NS	NS
(°C)	15	27.87	39.80	29.88	40.27				
	30	27.53	40.10	28.65	40.02				
	45	27.23	39.70	27.38	40.00				
	Mean	**29.01** [c]	**38.25** [a]	**29.88** [b]	**38.29** [a]				
	Pooled SE	±0.24	±0.24	±0.24	±0.24				
STHE	0	31.10	32.88	32.17	32.55	NS	NS	NS	NS
(°C)	15	31.63	32.33	32.30	31.90				
	30	32.60	33.13	32.63	32.45				
	45	32.67	33.05	32.10	32.88				
	Mean	**32.00** [a]	**32.85** [a]	**32.30** [a]	**32.45** [a]				
	Pooled SE	±0.31	±0.31	±0.31	±0.31				
STHN	0	31.72	32.52	31.57	30.68	NS	NS	NS	NS
(°C)	15	30.92	31.42	31.23	31.9				
	30	32.60	31.95	31.65	32.73				
	45	31.93	31.75	30.77	31.87				
	Mean	**31.79** [a]	**31.91** [a]	**31.30** [a]	**31.80** [a]				
	Pooled SE	±0.35	±0.35	±0.35	±0.35				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; STHM-Skin temperature head morning; STHA-Skin temperature head afternoon; STHE-Skin temperature head evening; STHN-Skin temperature head night; Pooled SE- Pooled standard error. **Indicates statistical significance at $P < 0.01$; NS- Indicates non-significant; Values bearing different superscripts within a row differ significantly with each other

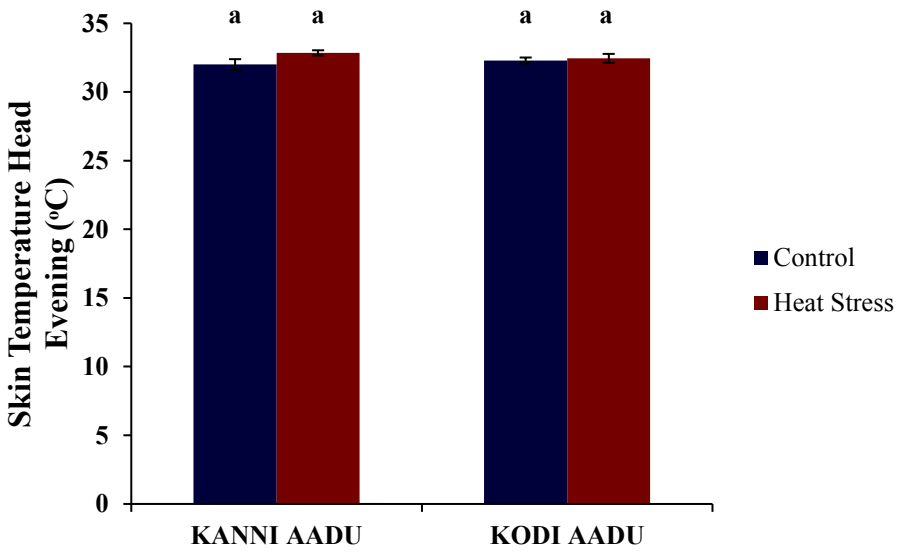
Fig. 4.10: Effect of heat stress on skin temperature (head) in Kanni Aadu and Kodi Aadu goat breeds (a) Skin temperature (head) morning (b) Skin temperature (head) afternoon (c) Skin temperature (head) evening (d) Skin temperature (head) night



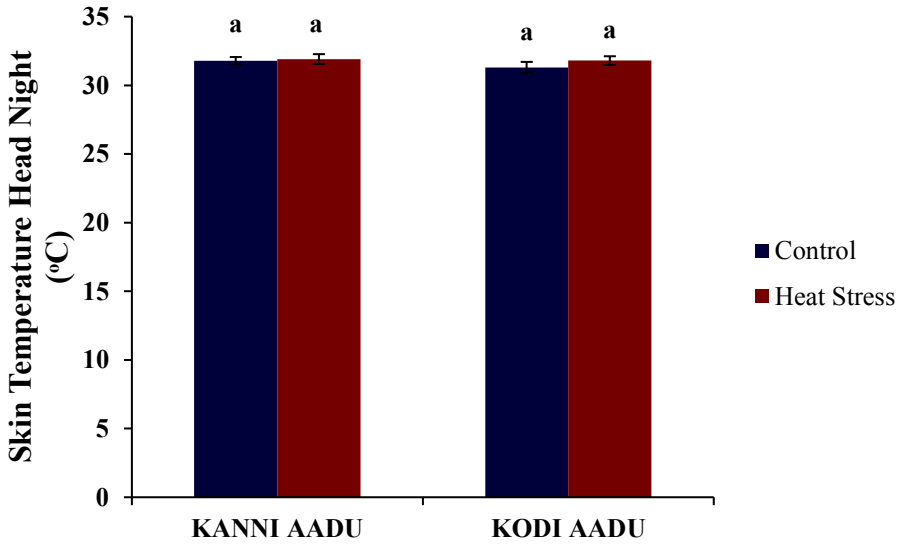
(a) Skin temperature (head) morning



(b) Skin temperature (head) afternoon



(c) Skin temperature (head) evening



(d) Skin temperature (head) night

Table 4.14: Correlation association between THI and Skin temperature (head) during morning, afternoon, evening and night.

	THI	STHM	STHA	STHE	STHN
THI	**1**				
STHM	**0.04**	**1**			
STHA	**0.83****	**-0.08**	**1**		
STHE	**0.17**	**0.10**	**0.11**	**1**	
STHN	**0.09**	**-0.01**	**0.15**	**-0.12**	**1**

THI-Temperature Humidity Index; STHM- Skin temperature head morning; STHA- Skin temperature head afternoon; STHE- Skin temperature head evening; STHN- Skin temperature head night **Indicates statistical significance at $P < 0.01$.

4.4.5.2. Skin Temperature Shoulder (STS)

The STS across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.15. The skin temperature shoulder morning (STSM) in KAC, KAHS, KOC and KOHS groups were 32.79±0.20, 33.55±0.19, 32.88±0.20 and 33.70±0.20, respectively. Similarly, the skin temperature shoulder afternoon (STSA) in KAC, KAHS, KOC and KOHS groups were 31.27±0.46, 38.02±0.47, 31.55±0.40 and 38.14±0.43, respectively. Further, the skin temperature shoulder evening (STSE) in KAC, KAHS, KOC and KOHS groups were 33.38±0.19, 34.46±0.10, 33.32±0.18 and 34.29±0.14, respectively. Likewise, the skin temperature shoulder night (STSN) in KAC, KAHS, KOC and KOHS groups were 33.25±0.22, 33.78±0.15, 33.25±0.14 and 33.78±0.12, respectively. The breed factor did not influence the STS at any time point during the recording days. Further, the treatment influenced significantly STSM (P<0.01), STSA (P<0.01), STSE (P<0.01) and STSN (P<0.05).

However, during 8.00 h and 20.00 h the treatment factor did not influence STS. In contrast, the experimental days significantly influenced STS only during 8.00 h ($P<0.05$) and 20.00 h ($P<0.01$). In addition, the BxTxD interaction significantly influenced STS only at 8.00 h during the recording days. The differences in the STS across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.11. The post hoc test showed significant ($P<0.01$) variation among the groups for the STS at all time points except during 2.00 h on the recording days. Further, the THI had a strong positive correlation ($P<0.01$) with STSM, STSA, STSE and STSN in this study (Table 4.16).

Table 4.15: Correlation association between THI and Skin temperature (shoulder) during morning, afternoon, evening and night.

	THI	STSM	STSA	STSE	STSN
THI	**1**				
STSM	**0.39****	**1**			
STSA	**0.85****	**0.29****	**1**		
STSE	**0.52****	**0.50****	**0.53****	**1**	
STSN	**0.28****	**0.54****	**0.36****	**0.41****	**1**

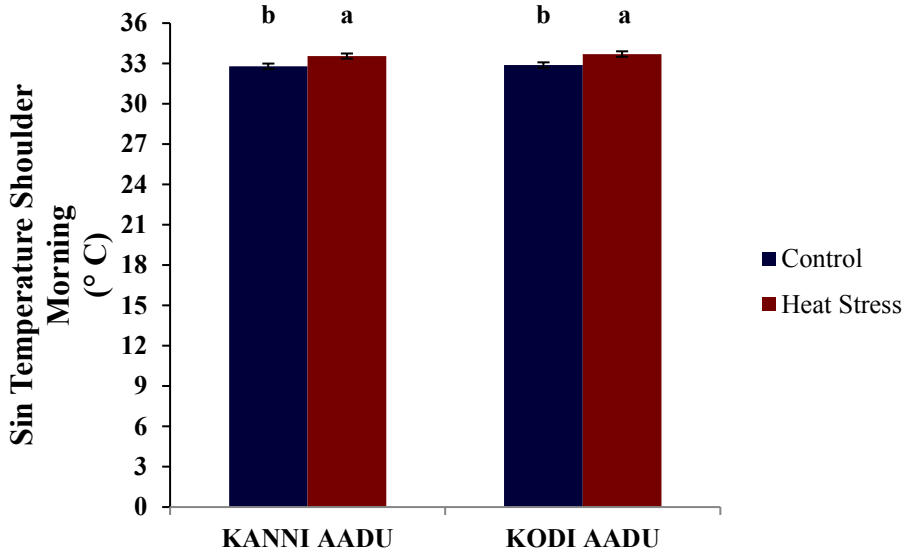
THI-Temperature Humidity Index; STSM- Skin temperature shoulder morning; STSA- Skin temperature shoulder afternoon; STSE- Skin temperature shoulder evening; STSN- Skin temperature shoulder night **Indicates statistical significance at $P < 0.01$.

Table 4.16: Effect of heat stress on Skin temperature (shoulder) in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

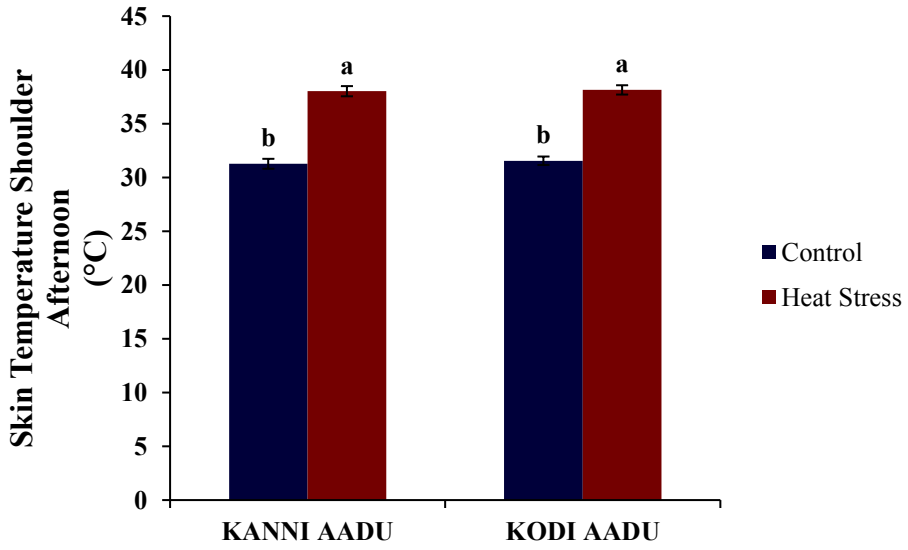
Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
STSM	0	33.60	34.47	34.03	34.85	NS	**	*	NS
(°C)	15	31.57	32.45	31.70	33.08				
	30	32.92	33.28	32.80	33.10				
	45	33.08	33.98	33.00	33.77				
	Mean	**32.79** [b]	**33.55** [a]	**32.88** [b]	**33.70** [a]				
	Pooled SE	±0.14	±0.14	±0.14	±0.14				
STSA	0	34.55	34.42	34.60	34.80	NS	**	NS	NS
(°C)	15	29.40	39.22	31.27	40.08				
	30	31.28	40.17	30.27	38.83				
	45	29.63	38.28	30.07	38.83				
	Mean	**31.27** [b]	**38.02** [a]	**31.55** [b]	**38.14** [a]				
	Pooled SE	±0.14	±0.14	±0.14	±0.14				
STSE	0	33.42	34.33	34.18	34.13	NS	**	**	**
(°C)	15	32.22	34.58	32.22	33.80				
	30	33.68	34.57	33.28	34.50				
	45	34.18	34.35	33.58	34.72				
	Mean	**33.38** [b]	**34.46** [a]	**33.32** [b]	**34.29** [a]				
	Pooled SE	±0.12	±0.12	±0.12	±0.12				
STSN	0	34.15	33.80	33.80	34.03	NS	NS	NS	NS
(°C)	15	32.30	33.20	32.68	33.17				
	30	33.57	34.35	33.68	33.98				
	45	32.97	33.78	32.82	33.93				
	Mean	**33.25** [a]	**33.78** [a]	**33.25** [a]	**33.78** [a]				
	Pooled SE	±0.18	±0.18	±0.18	±0.18				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day-breed treatment and day interaction; STSM-Skin temperature shoulder morning; STSA-Skin temperature shoulder afternoon; STSE-Skin temperature shoulder evening; STSN-Skin temperature shoulder night; Pooled SE- Pooled 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

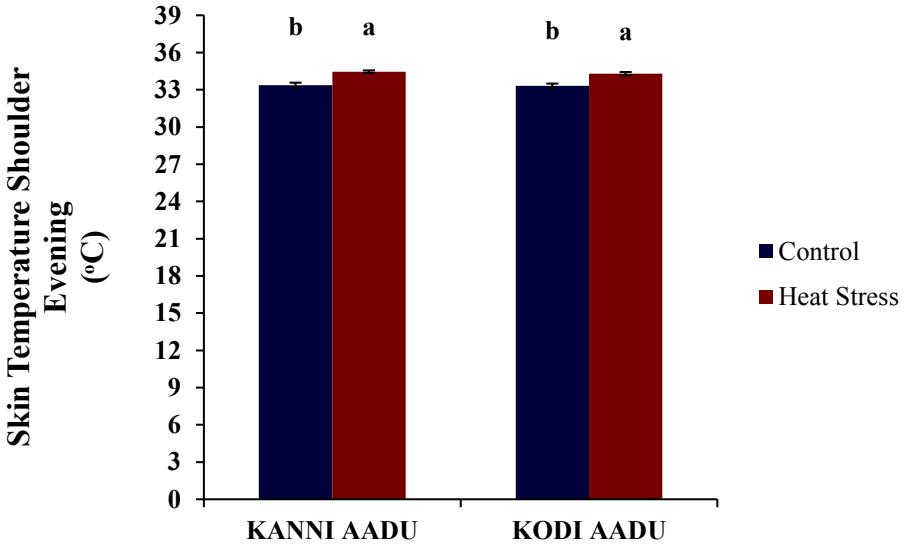
Fig. 4.11: Effect of heat stress on skin temperature (shoulder) in Kanni Aadu and Kodi Aadu goat breeds (a) Skin temperature (shoulder) morning (b) Skin temperature (shoulder) afternoon (c) Skin temperature (shoulder) evening (d) Skin temperature (shoulder) night



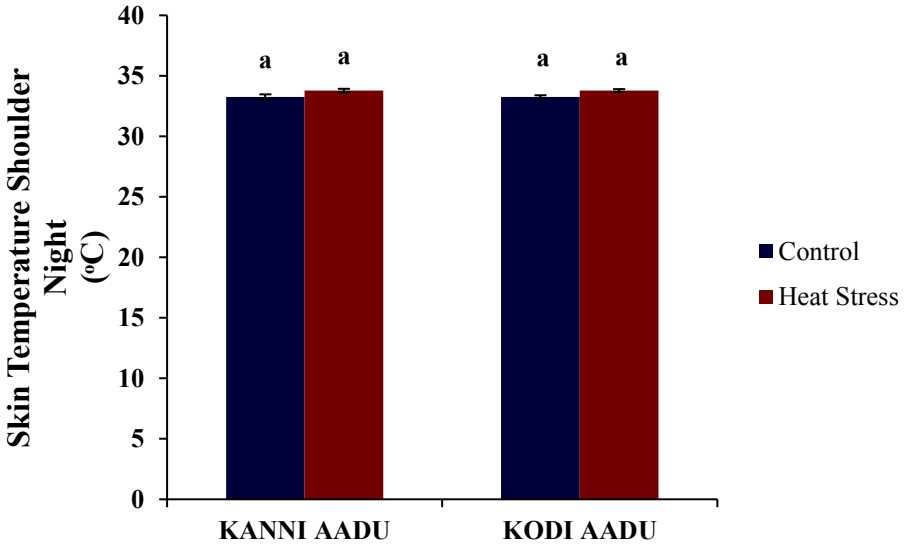
(a) Skin temperature (shoulder) morning



(b) Skin temperature (shoulder) afternoon



(c) Skin temperature (shoulder) evening



(d) Skin temperature (shoulder) night

4.4.5.3. Skin Temperature Flank (STF)

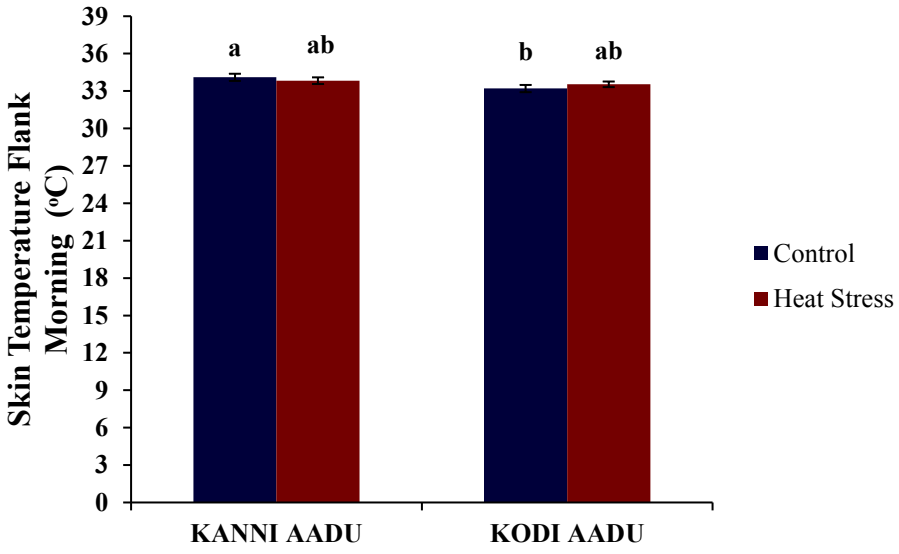
The STF across the experimental groups during morning, afternoon, evening and night hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.17. The skin temperature flank morning (STFM) in KAC, KAHS, KOC and KOHS groups were 34.10±0.29, 33.83±0.27, 33.20±0.29 and 33.54±0.22, respectively. Similarly, the skin temperature flank afternoon (STFA) in KAC, KAHS, KOC and KOHS groups were 32.10±0.57, 38.30±0.49, 32.27±0.42 and 38.18±0.49, respectively. Further, the skin temperature flank evening (STFE) in KAC, KAHS, KOC and KOHS groups were 34.05±0.22, 34.33±0.18, 33.52±0.23 and 34.28±0.20, respectively. Likewise, the skin temperature flank night (STFN) in KAC, KAHS, KOC and KOHS groups were 34.32±0.19, 34.22±0.25, 33.64±0.24 and 33.67±0.23, respectively. The breed factor did not influence the STF at any time point during the recording days. Further, the treatment influenced significantly ($P<0.01$) STF only at 14.00 h. In addition, the experimental days significantly influenced the STF at all time points during the recording days at 1.0 % level at 14.00 h, 20.00 h and 2.00 h while it influenced STF at 8.00 h at 5.0 % level. However, the BxTxD interaction did not influence STF at any time point during the recording days. The differences in the STF across all the time points between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.12 The post hoc test showed significant ($P<0.01$) variation among the groups for the STF at all time points except at 20.00 h on the recording days. Further, the THI had a positive correlation with STFA ($P<0.01$) and STFE ($P<0.05$), while non-significant correlation with STFM and STFN in this study (Table 4.18).

Table 4.17: Effect of heat stress on skin temperature (flank) in Kanni Aadu and Kodi Aadu goat breeds during morning, afternoon, evening and night.

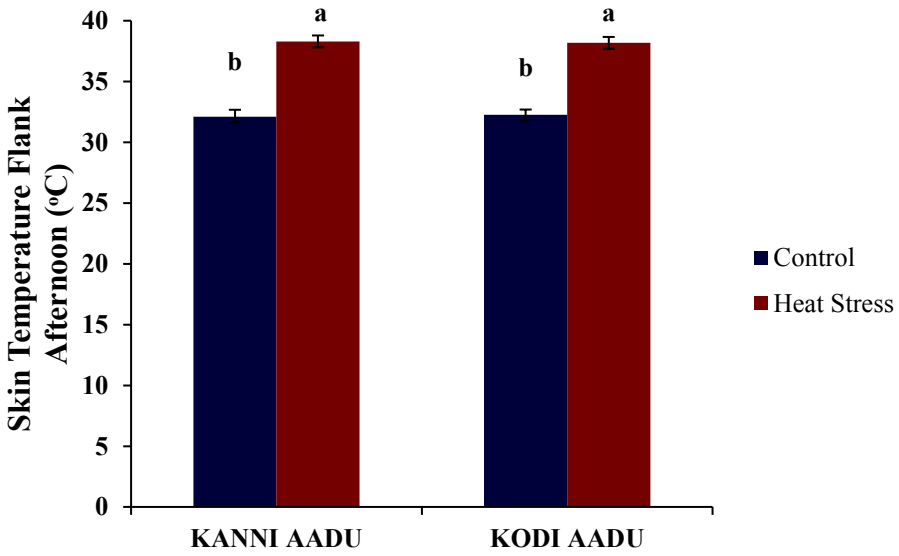
Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
STFM	0	34.63	34.25	33.72	34.08	NS	NS	*	NS
(°C)	15	32.87	32.30	32.37	32.97				
	30	33.10	33.67	32.52	32.90				
	45	35.78	35.08	34.22	34.22				
	Mean	**34.10** [a]	**33.83** [ab]	**33.20** [b]	**33.54** [ab]				
	Pooled SE	±0.25	±0.25	±0.25	±0.25				
STFA	0	34.70	34.57	34.72	34.38	NS	**	**	NS
(°C)	15	29.87	39.63	31.65	40.27				
	30	31.47	40.33	30.07	38.83				
	45	32.38	38.65	32.63	39.22				
	Mean	**32.10** [b]	**38.30** [a]	**32.27** [b]	**38.18** [a]				
	Pooled SE	±0.33	±0.33	±0.33	±0.33				
STFE	0	34.62	34.13	33.33	33.78	NS	NS	**	NS
(°C)	15	33.03	33.57	33.40	33.90				
	30	34.52	34.67	33.07	34.45				
	45	34.02	34.93	34.27	34.98				
	Mean	**34.05** [a]	**34.33** [a]	**33.52** [a]	**34.28** [a]				
	Pooled SE	±0.28	±0.28	±0.28	±0.28				
STFN	0	34.82	32.78	33.48	32.88	NS	NS	**	NS
(°C)	15	33.22	34.15	32.97	33.63				
	30	34.55	34.18	33.57	33.9				
	45	34.70	35.77	34.53	34.25				
	Mean	**34.32** [a]	**34.22** [a]	**33.64** [b]	**33.67** [b]				
	Pooled SE	±0.19	±0.19	±0.19	±0.19				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; STFM-Skin temperature flank morning; STFA-Skin temperature flank afternoon; STFE-Skin temperature flank evening; STFN-Skin temperature flank night; Pooled SE- Pooled 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

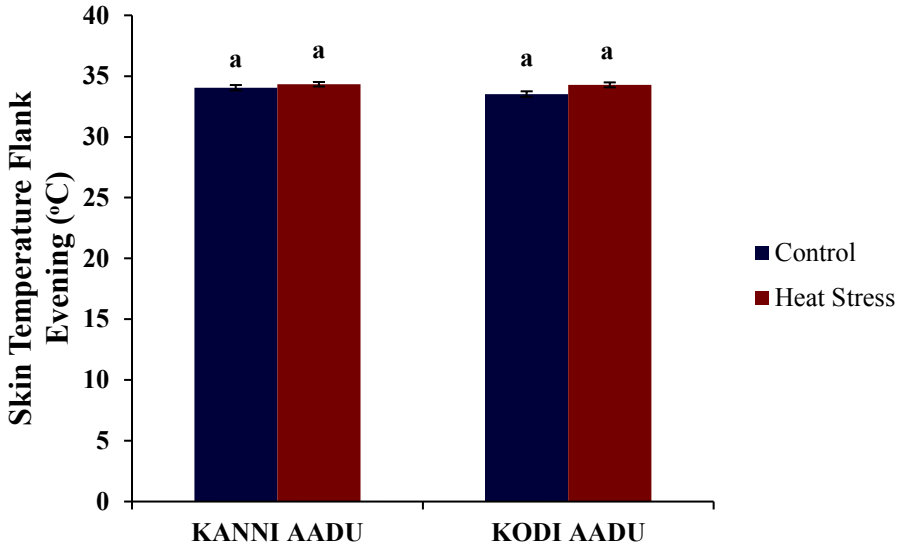
Fig. 4.12: Effect of heat stress on skin temperature (Flank) in Kanni Aadu and Kodi Aadu goat breeds (a) Skin temperature (Flank) morning (b) Skin temperature (Flank) afternoon (c) Skin temperature (Flank) evening (d) Skin temperature (Flank) night.



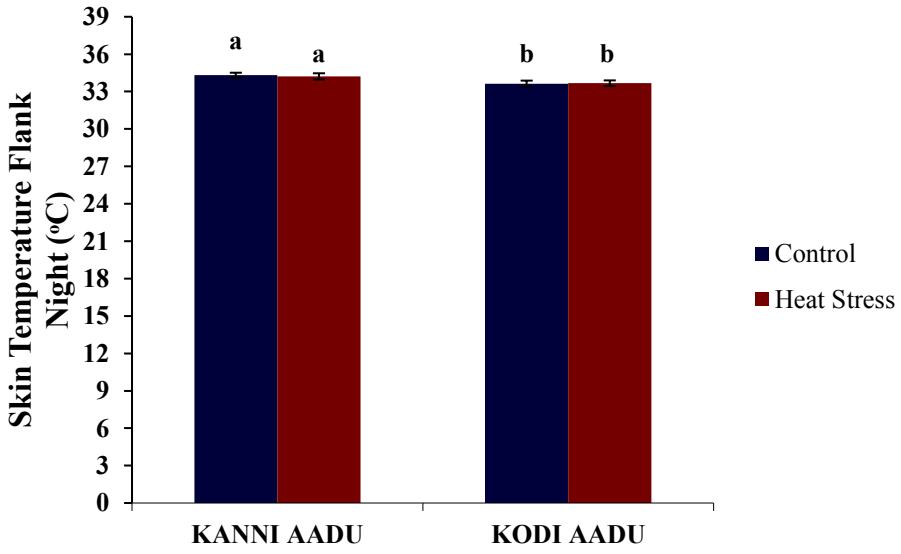
(a) Skin temperature (Flank) morning



(b) Skin temperature (Flank) afternoon



(c) Skin temperature (Flank) evening



(d) Skin temperature (Flank) night.

Table 4.18: Correlation association between THI and skin temperature (flank) during morning, afternoon, evening and night.

	THI	STFM	STFA	STFE	STFN
THI	**1**				
STFM	**-0.10**	**1**			
STFA	**0.78****	**0.04**	**1**		
STFE	**0.24***	**0.34****	**0.30****	**1**	
STFN	**-0.03**	**0.40****	**0.16**	**0.47****	**1**

THI-Temperature Humidity Index; STFM- Skin temperature flank morning; STFA- Skin temperature flank afternoon; STFE- Skin temperature flank evening; STFN- Skin temperature flank night **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

4.5. Haematological Variables

4.5.1. Red Blood Cells (RBC)

The RBCs across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The RBCs in KAC, KAHS, KOC and KOHS groups were 17.46±0.31, 16.39±0.34, 17.94±0.37 and 16.33±0.34, respectively. The breed factor did not influence the RBCs at any time point during the recording days. However, the treatment influenced significantly ($P<0.05$) the RBCs. In addition, the experimental days also significantly ($P<0.01$) influenced RBCs. However, the BxTxD interaction did not influence the RBCs. The differences in the RBCs across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (a). The post hoc test did not show any variation

among the groups for the RBCs. Further, the THI had a strong negative correlation ($P<0.01$) with RBC (Table 4.20).

4.5.2. White Blood Cells (WBC)

The WBCs ($10^{3/}\mu L$) across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The WBCs ($10^{3/}\mu L$) in KAC, KAHS, KOC and KOHS groups were 18.88±0.66, 17.89±0.96, 18.06±0.68 and 15.78±0.65, respectively. Among the various factors, only breed influenced significantly ($P<0.01$) the WBCs. However, the BxTxD interaction did not influence the WBCs. The differences in the WBCs across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (b). The post hoc test did not show any variation among the groups for the WBCs. Further, the THI had a negative correlation ($P<0.05$) with WBC (Table 4.20).

Table 4.19: Effect of heat stress on haematological variables in Kanni Aadu and Kodi Aadu goat breeds

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
RBC (10^6/µL)	0	15.82	15.82	15.92	15.84	NS	NS	**	NS
	15	18.20	16.31	18.39	16.72				
	30	17.98	16.41	18.71	16.28				
	45	17.84	17.01	18.73	16.49				
	Mean	**17.46** [a]	**16.39** [a]	**17.94** [a]	**16.33** [a]				
	Pooled SE	±0.54	±0.54	±0.54	±0.54				
WBC (10^3/µL)	0	20.30	19.10	18.68	13.98	**	NS	NS	NS
	15	17.70	19.43	15.42	16.68				
	30	18.73	16.10	17.45	15.35				
	45	18.77	16.93	20.70	16.28				
	Mean	**18.88** [a]	**17.89** [a]	**18.06** [a]	**15.78** [a]				
	Pooled SE	±1.04	±1.04	±1.04	±1.04				
Lymphocyte (10^3/µL)	0	7.38	8.40	7.52	5.68	**	NS	*	NS
	15	5.42	7.93	5.63	5.90				
	30	7.28	6.00	7.97	6.15				
	45	7.72	6.85	10.27	8.15				
	Mean	**6.95** [a]	**7.30** [a]	**7.85** [a]	**6.47** [a]				
	Pooled SE	±0.90	±0.90	±0.90	±0.90				
Monocyte (10^3/µL)	0	2.65	2.18	2.37	1.92	NS	NS	*	NS
	15	2.38	2.23	2.10	2.12				
	30	2.23	2.07	2.15	2.15				
	45	1.97	2.07	1.88	1.75				
	Mean	**2.31** [a]	**2.14** [a]	**2.13** [a]	**1.98** [a]				
	Pooled SE	±0.12	±0.12	±0.12	±0.12				
Granulocyte (10^3/µL)	0	9.78	7.73	8.22	6.57	NS	NS	NS	NS
	15	9.63	8.20	7.83	8.02				
	30	8.07	8.15	7.10	5.55				
	45	8.37	7.45	7.58	5.72				
	Mean	**8.96** [a]	**7.88** [ab]	**7.68** [ab]	**6.46** [b]				
	Pooled SE	±0.59	±0.59	±0.59	±0.59				
Lymphocyte %	0	35.88	45.73	43.02	42.38	NS	NS	*	NS
	15	29.80	40.27	35.42	35.67				
	30	40.95	37.42	46.72	41.45				
	45	43.72	41.67	51.95	52.03				
	Mean	**37.59** [a]	**41.10** [a]	**44.28** [a]	**42.88** [a]				
	Pooled SE	±3.78	±3.78	±3.78	±3.78				

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
Monocyte %	0	13.82	12.75	12.82	14.08	NS	NS	**	NS
	15	13.77	12.43	13.60	13.48				
	30	13.07	12.55	12.42	16.35				
	45	10.87	12.75	9.73	11.37				
	Mean	**12.88** [a]	**12.62** [a]	**12.14** [a]	**13.82** [a]				
	Pooled SE	±0.56	±0.56	±0.56	±0.56				
Granulocyte %	0	50.30	42.22	44.17	43.60	NS	NS	NS	NS
	15	56.43	47.30	50.88	50.85				
	30	45.98	50.03	40.87	39.97				
	45	45.42	45.58	38.32	36.60				
	Mean	**49.53** [a]	**46.28** [a]	**43.56** [a]	**42.75** [a]				
	Pooled SE	±3.56	±3.56	±3.56	±3.56				
HGB (g/dL)	0	8.65	8.53	8.48	8.40	NS	NS	**	NS
	15	9.95	8.83	10.05	9.00				
	30	10.02	8.85	10.12	8.70				
	45	9.70	9.18	10.12	8.88				
	Mean	**9.58** [a]	**8.85** [a]	**9.69** [a]	**8.75** [a]				
	Pooled SE	±0.36	±0.36	±0.36	±0.36				
HCT (%)	0	23.25	23.30	23.50	22.93	NS	NS	**	NS
	15	27.72	25.08	27.60	25.07				
	30	27.27	24.58	27.93	24.48				
	45	26.27	25.05	27.42	24.08				
	Mean	**26.13** [a]	**24.50** [a]	**26.61** [a]	**24.14** [a]				
	Pooled SE	±1.07	±1.07	±1.07	±1.07				
MCV (fL)	0	14.72	14.82	14.82	14.48	NS	NS	NS	NS
	15	15.25	15.44	15.05	14.95				
	30	15.18	15.05	14.98	14.83				
	45	14.73	14.82	14.70	14.60				
	Mean	**14.97** [a]	**15.03** [a]	**14.89** [a]	**14.72** [a]				
	Pooled SE	±0.41	±0.41	±0.41	±0.41				
MCH (pg)	0	5.42	5.35	5.27	5.25	NS	NS	NS	NS
	15	5.42	5.37	5.42	5.32				
	30	5.52	5.33	5.37	5.27				
	45	5.37	5.38	5.35	5.33				
	Mean	**5.43** [a]	**5.36** [a]	**5.35** [a]	**5.29** [a]				
	Pooled SE	±0.10	±0.10	±0.10	±0.10				

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
MCHC (g/dL)	0	37.27	36.53	36.10	36.55	NS	NS	NS	NS
	15	35.92	35.22	36.33	35.90				
	30	36.75	35.95	36.17	35.88				
	45	37.00	36.60	36.88	36.88				
	Mean	**36.73** [a]	**36.08** [a]	**36.37** [a]	**36.30** [a]				
	Pooled SE	±0.45	±0.45	±0.45	±0.45				
RDW (%)	0	22.32	22.48	22.05	21.55	NS	NS	NS	*
	15	22.00	20.47	21.50	21.23				
	30	21.83	20.65	20.75	21.40				
	45	22.40	21.52	21.20	21.55				
	Mean	**22.14** [a]	**21.28** [a]	**21.38** [a]	**21.43** [a]				
	Pooled SE	±0.33	±0.33	±0.33	±0.33				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day-breed treatment and day interaction; WBC-White Blood Cell; RBC- Red Blood Cell; HGB- Haemoglobin; HCT- Hematocrit; MCV- Mean Corpuscular Volume; MCH- Mean Corpuscular Haemoglobin; MCHC- Mean Corpuscular Hemoglobin Concentration; RDW- Red Blood Cell Distribution Width.
Pooled SE- Pooled 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.

4.5.3. Lymphocytes

The lymphocytes ($10^{3/}$µL) across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The lymphocytes (103/µL) in KAC, KAHS, KOC and KOHS groups were 6.95±0.64, 7.30±0.64, 7.85±0.54 and 6.47±0.56, respectively. Among the various factors, breed ($P<0.01$) and experimental days ($P<0.05$) influenced significantly the lymphocytes. However, the BxTxD interaction did not influence the lymphocytes. The differences in the lymphocytes across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (c). The post hoc test did not show any variation among the groups for the lymphocytes. Further, THI although had a negative correlation with lymphocyte still this effect was not statistically significant (Table 4.20).

4.5.4. Monocytes

The monocytes ($10^{3/}$µL) across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19**.** The monocytes (10^3/µL) in KAC, KAHS, KOC and KOHS groups were 2.31±0.08, 2.14±0.12, 2.13±0.11 and 1.98±0.08, respectively. Among the various factors, only experimental days ($P<0.05$) influenced significantly the monocytes. However, the breed, treatment and BxTxD interaction did not influence the monocytes. The differences in the monocytes across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (d). The post hoc test did not show any

variation among the groups for the monocytes. Further, THI although had a negative correlation with monocyte still this effect was not statistically significant (Table 4.20).

4.5.5. Granulocytes

The granulocytes ($10^{3/}\mu L$) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The granulocytes ($10^{3/}\mu L$) in KAC, KAHS, KOC and KOHS groups were 8.96±0.54, 7.88±0.47, 7.68±0.50 and 6.46±0.47, respectively. Both the breed and treatment factors did not influence the granulocytes. Further, both the experimental days and the BxTxD interaction also did not influence the granulocytes level in the study. The differences in the granulocytes across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (e). The post hoc test did not show any variation for the granulocytes across the experimental groups. However, the granulocyte concentration differed significantly between KAC and KOHS groups. Further, THI had a negative correlation $P<0.05$) with granulocyte (Table 4.20).

4.5.6. Lymphocytes (%)

The lymphocytes (%) across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The lymphocytes (%) in KAC, KAHS, KOC and KOHS groups were 37.59±2.95, 41.10±2.94, 44.28±2.67 and 42.88±2.80, respectively. Among the various factors, only experimental days ($P<0.05$) influenced significantly the lymphocytes. However, the breed, treatment and BxTxD interaction did not influence the lymphocytes. The differences in the lymphocytes across the experimental groups of

Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (f). The post hoc test did not show any variation among the groups for the lymphocytes. Further, THI although had a positive correlation with lymphocyte (%) still this effect was not statistically significant (Table 4.20).

4.5.7. Monocytes (%)

The monocytes (%) across the experimental groups during afternoon hours and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The monocytes (%) in KAC, KAHS, KOC and KOHS groups were 12.88±0.44, 12.62±0.43, 12.14±0.45 and 13.82±0.63, respectively. Among the various factors, only experimental days ($P<0.01$) influenced significantly the monocytes. However, the breed, treatment and BxTxD interaction did not influence the monocytes. The differences in the monocytes across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (g). The post hoc test did not show any variation among the groups for the monocytes. Further, THI although had a positive correlation with monocyte (%) still this effect was not statistically significant (Table 4.20).

4.5.8. Granulocytes (%)

The granulocytes (%) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The granulocytes (%) in KAC, KAHS, KOC and KOHS groups were 49.53±2.80, 46.28±2.81, 43.56±2.41 and 42.75±2.56, respectively. Both the breed and treatment factors did not influence the granulocytes. Further, both the experimental days and the

BxTxD interaction also did not influence the granulocytes level in the study. The differences in the granulocytes across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (h). The post hoc test did not show any variation for the granulocytes across the experimental groups. Further, THI although had a negative correlation with granulocyte (%) still this effect was not statistically significant (Table 4.20).

4.5.9 Haemoglobin (HGB)

The HGB (g/dL) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The HGB (g/dL) in KAC, KAHS, KOC and KOHS groups were 9.58±0.21, 8.85±0.18, 9.69±0.22 and 8.75±0.24, respectively. Both breed and treatment factors did not influence the HGB during the recording days. Further, experimental days ($P<0.01$) influenced significantly HGB. However, the BxTxD interaction did not influence HGB during the study period. The differences in the HGB between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (i). The post hoc test did show significant variation across the groups for the HGB concentration. Further, the THI had a strong negative correlation ($P<0.01$) with HGB (Table 4.20).

4.5.10. Haematocrit (HCT)

The HCT (%) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The HCT (%) in KAC, KAHS, KOC and KOHS groups were 26.13±0.67, 24.50±0.47, 26.61±0.57 and 24.14±0.70, respectively. Both breed and treatment factors did not

influence the HCT during the recording days. Further, experimental days (P<0.01) influenced significantly HCT. However, the BxTxD interaction did not influence HCT during the study period. The differences in the HCT between the control and heat stress groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (j). The post hoc test did not show any significant variation across the groups for the HCT. Further, the THI had a strong negative correlation (P<0.01) with HCT (Table 4.20).

4.5.11. Mean Corpuscular Volume (MCV)

The MCV (fL) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The MCV (fL) in KAC, KAHS, KOC and KOHS groups were 14.97±0.21, 15.03±0.22, 14.89±0.19 and 14.72±0.22, respectively. Both the breed and treatment factors did not influence the MCV. Further, both the experimental days and the BxTxD interaction also did not influence the MCV level in the study. The differences in the MCV across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (k). The post hoc test did not show any variation for the MCV across the experimental groups. Further, THI although had a negative correlation with MVC still this effect was not statistically significant (Table 4.20).

4.5.12. Mean Corpuscular Haemoglobin (MCH)

The MCH (pg) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The MCH (pg) in KAC, KAHS, KOC and KOHS groups were 5.43±0.05, 5.36±0.05, 5.35±0.04 and 5.29±0.06, respectively. Both the breed and treatment factors did not

influence the MCH. Further, both the experimental days and the BxTxD interaction also did not influence the MCH level in the study. The differences in the MCH across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (l) The post hoc test did not show any variation for the MCH across the experimental groups. Further, THI although had a negative correlation with MCH still this effect was not statistically significant (Table 4.20).

4.5.13. Mean Corpuscular Haemoglobin Concentration (MCHC)

The MCHC (g/dL) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The MCHC (g/dL) in KAC, KAHS, KOC and KOHS groups were 36.73±0.33, 36.08±0.29, 36.37±0.25 and 36.30±0.20, respectively. Both the breed and treatment factors did not influence the MCHC. Further, both the experimental days and the BxTxD interaction also did not influence the MCHC level in the study. The differences in the MCHC across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (m) The post hoc test did not show any variation for the MCHC across the experimental groups. Further, THI although had a negative correlation with MCHC still this effect was not statistically significant (Table 4.20).

4.5.14. Red Blood Cell Distribution Width (RDW)

The RDW (%) across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.19. The RDW (%) in KAC, KAHS, KOC and KOHS groups were 22.14±0.21, 21.28±0.26, 21.38±0.27 and 21.43±0.22, respectively. The breed, treatment and experimental day

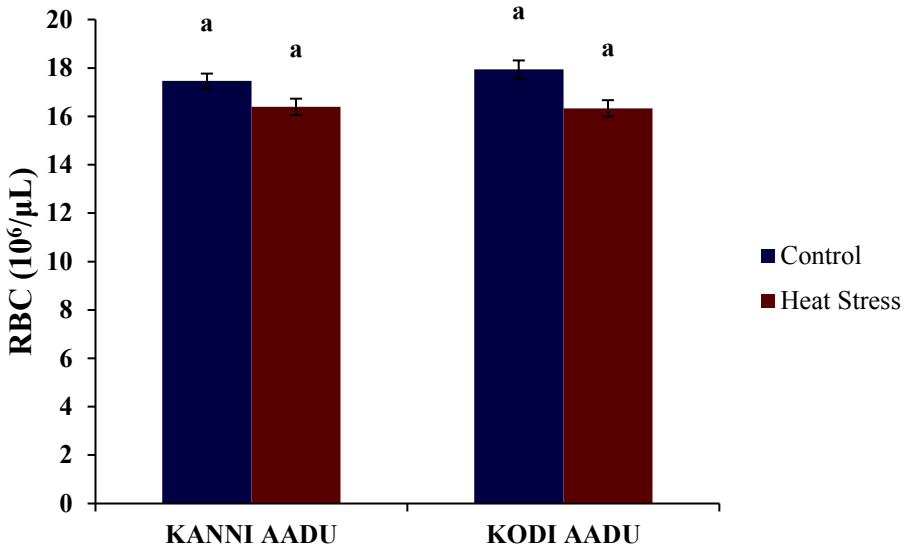
factors did not influence the RDW. However, the BxTxD interaction was the only factor which significantly ($P<0.05$) influenced the RDW level in the study. The differences in the RDW across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.13 (n) The post hoc test did not show any variation for the RDW across the experimental groups. Further, THI although had a negative correlation with RDW still this effect was not statistically significant (Table 4.20).

Table 4.20: Correlation association between THI and haematological variables

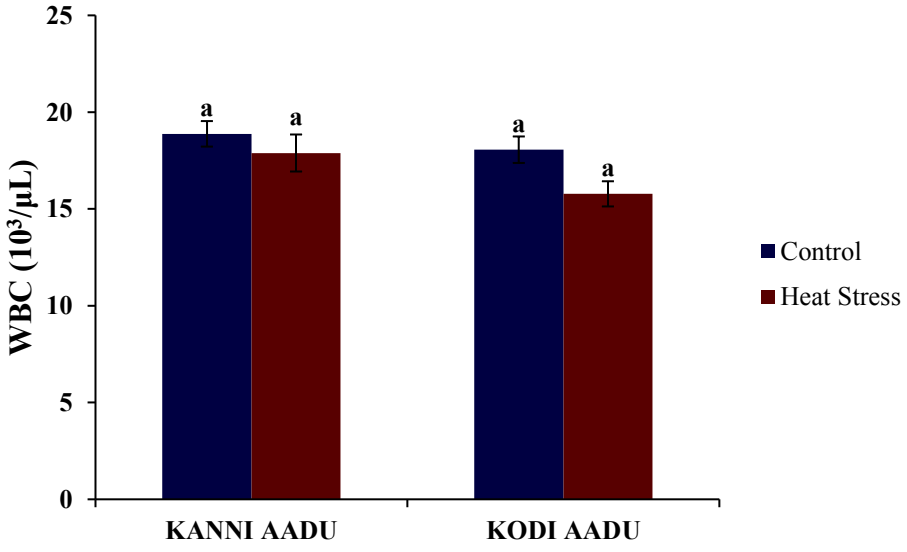
	THI	RBC	WBC	Lym	Mon	Grn	Lym%	Mon%	Grn%	HGB	HCT	MCV	MCH	MCHC	RDW
THI	1														
RBC	-0.38**	1													
WBC	-0.23*	0.10	1												
Lym	-0.09	0.12	0.58**	1											
Mon	-0.16	0.04	0.46**	0.09	1										
Grn	-0.23*	-0.00	0.33**	-0.39**	0.52**	1									
Lym%	0.04	0.06	0.24*	0.88**	-0.23*	-0.72**	1								
Mon%	0.15	-0.10	-0.33**	-0.51**	0.48**	0.06	-0.47**	1							
Grn%	-0.08	-0.03	-0.19	-0.84**	0.15	0.77**	-0.98**	0.30**	1						
HGB	-0.38**	0.93**	0.09	0.08	0.10	0.02	-0.01	-0.00	0.02	1					
HCT	-0.33**	0.84**	0.00	0.01	0.08	-0.03	-0.05	0.09	0.05	0.96**	1				
MCV	-0.03	-0.01	-0.14	-0.16	0.09	-0.12	-0.18	0.28	0.15	0.32	0.54	1			
MCH	-0.13	0.15	0.02	-0.07	0.21*	0.09	-0.16	0.22*	0.14	0.50**	0.60**	0.87**	1		
MCHC	-0.14	0.22*	0.29**	0.18	0.06	0.17	0.11	-0.26**	-0.06	0.06	-0.23*	-0.77**	-0.36**	1	
RDW	-0.17	0.02	0.17	0.23*	-0.03	-0.03	0.21*	-0.20*	-0.19	-0.15	-0.27**	-0.50**	-0.43**	0.40**	1

THI- Temperature humidity index; WBC-White Blood Cell; RBC- Red Blood Cell; HGB- Haemoglobin; HCT- Hematocrit; MCV- Mean Corpuscular Volume; MCH- Mean Corpuscular Haemoglobin; MCHC- Mean Corpuscular Hemoglobin Concentration; RDW- Red Blood Cell Distribution Width.
**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$.

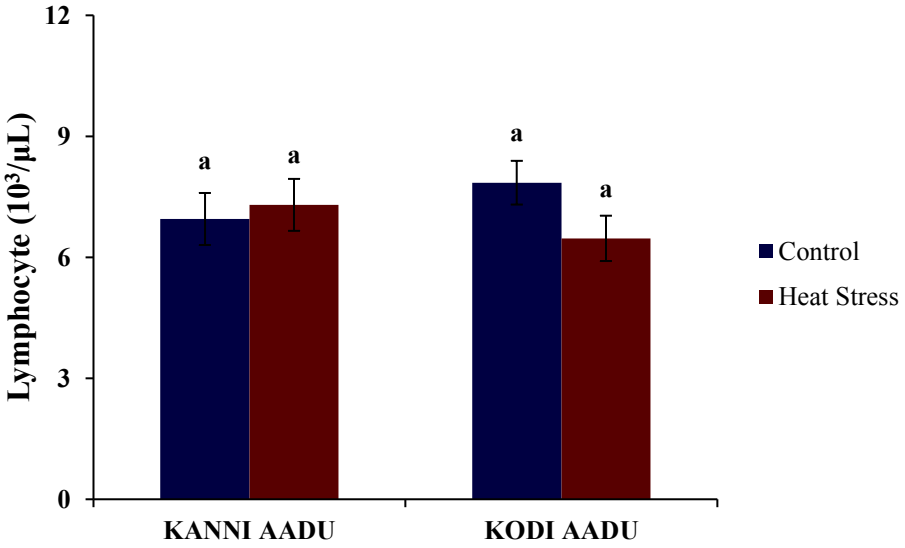
Fig. 4.13: Effect of heat stress on haematological variables in Kanni Aadu and Kodi Aadu goat breeds. (a) Red Blood Cells (b) White Blood Cells (c) Lymphocytes (d) Monocytes (e) Granulocytes (f) Lymphocytes % (g) Monocytes % (h) Granulocytes % (i) Haemoglobin (j) Haematocrit (k) Mean Corpuscular Volume (l) Mean Corpuscular Haemoglobin (m) Mean Corpuscular Haemoglobin Concentration (n) Red Blood Cell Distribution Width (%).



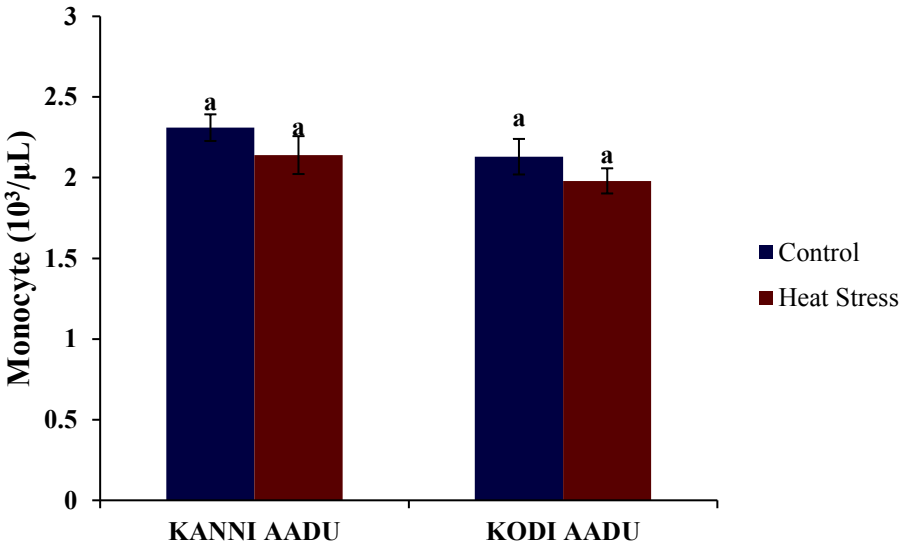
(a) Red Blood Cells



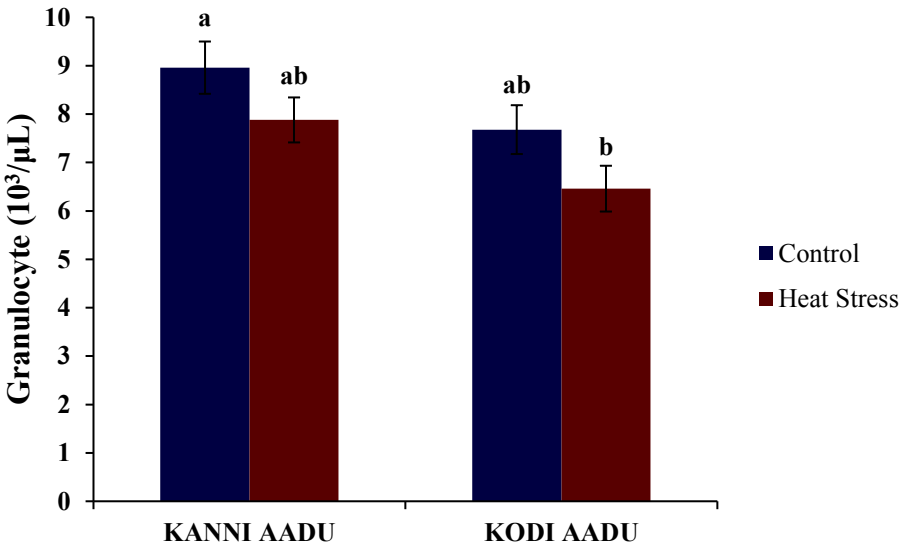
(b) White Blood Cells



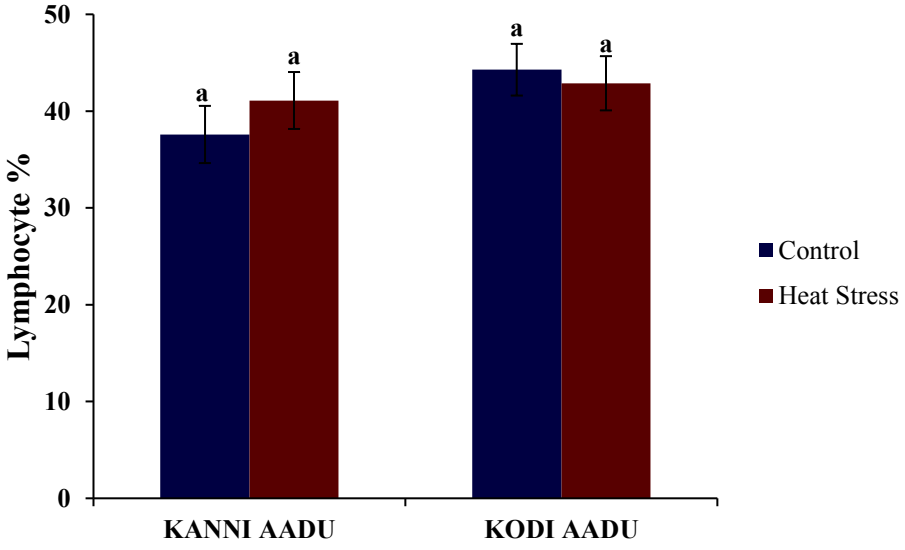
(c) Lymphocytes



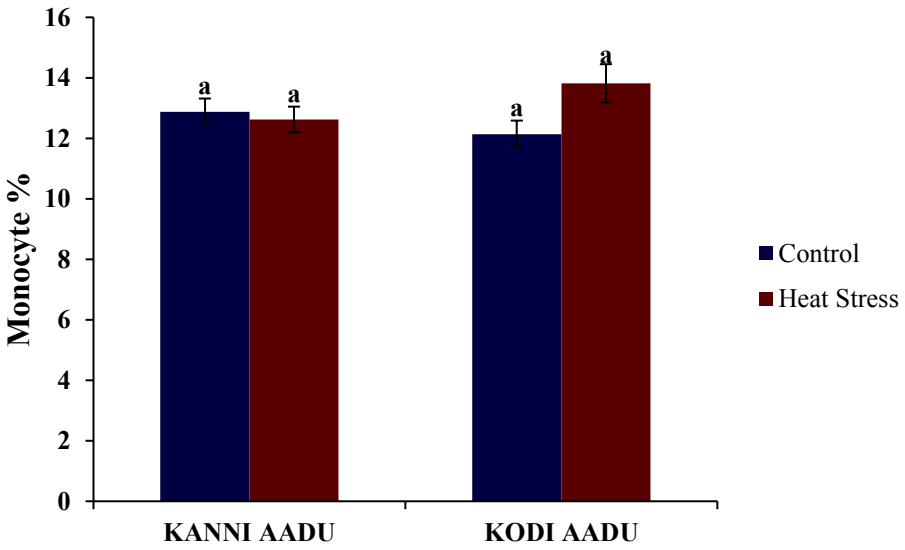
(d) Monocytes



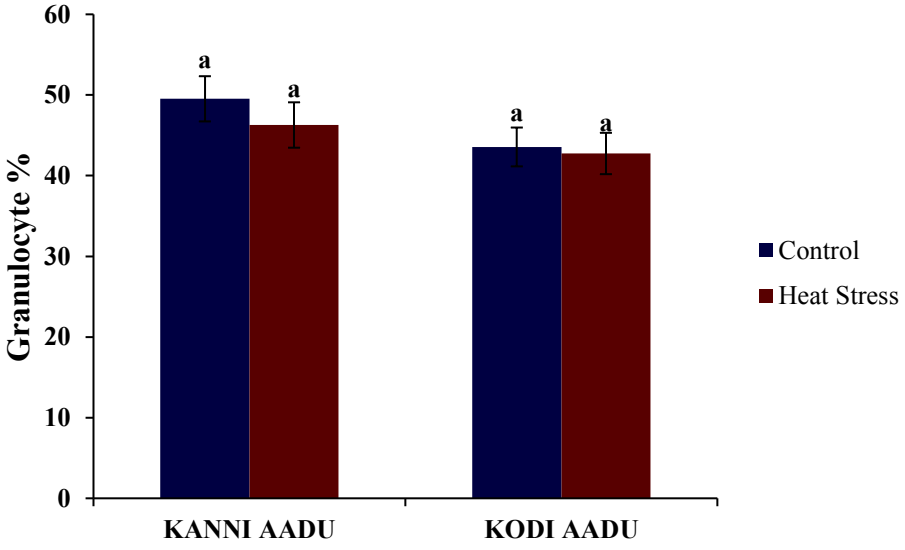
(e) Granulocytes



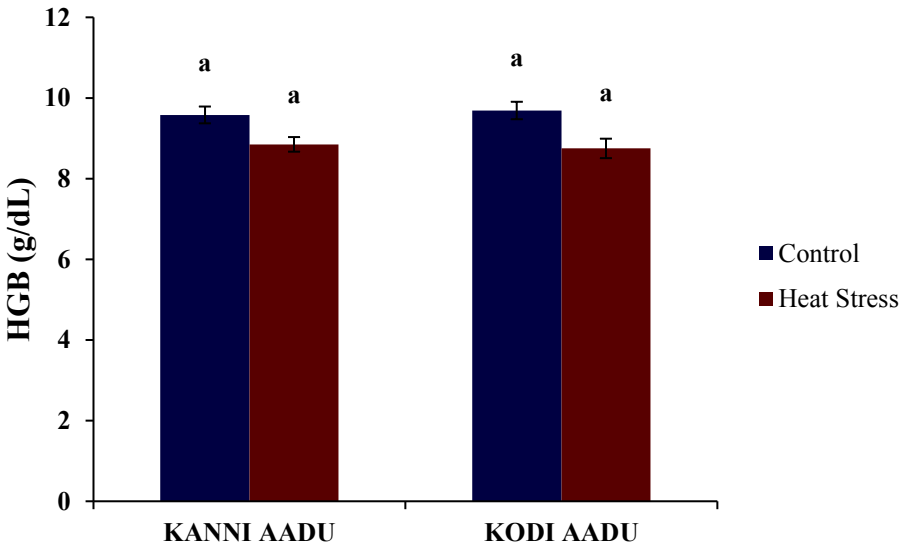
(f) Lymphocytes %



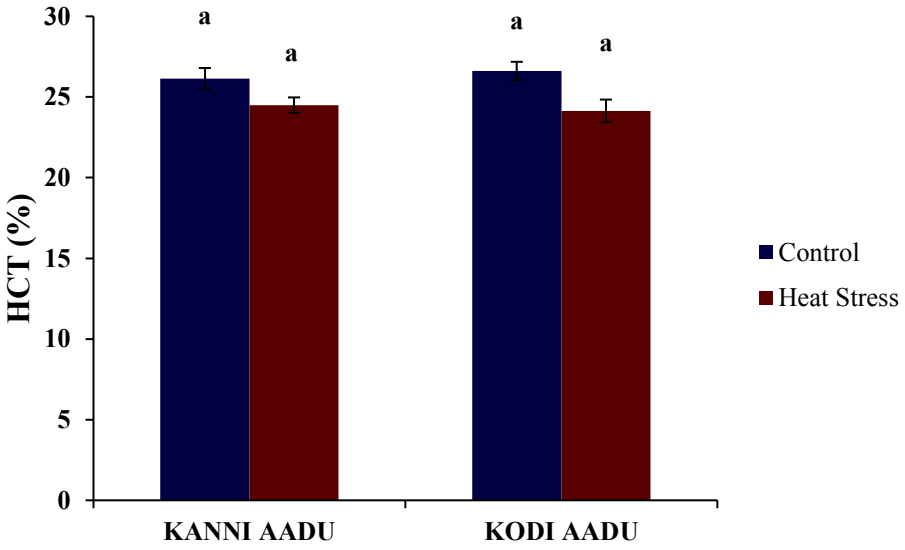
(g) Monocytes %



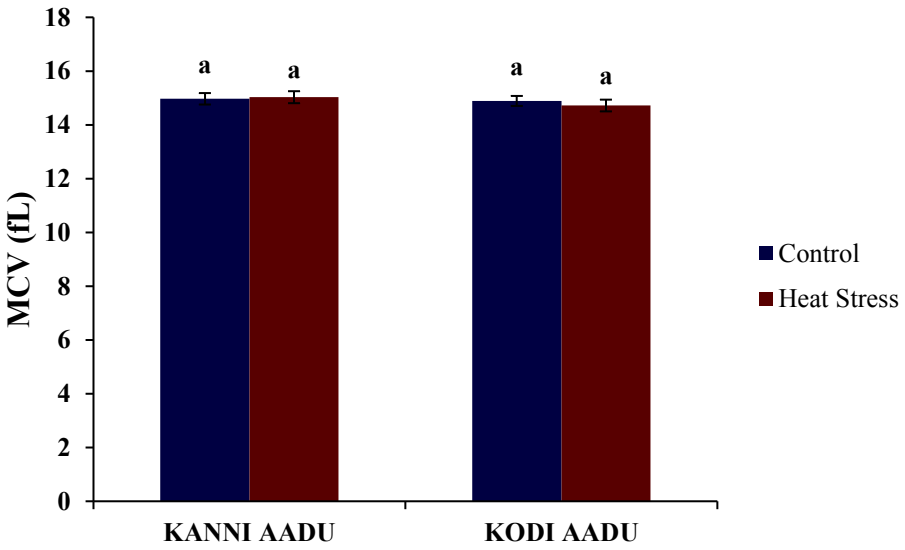
(h) Granulocytes %



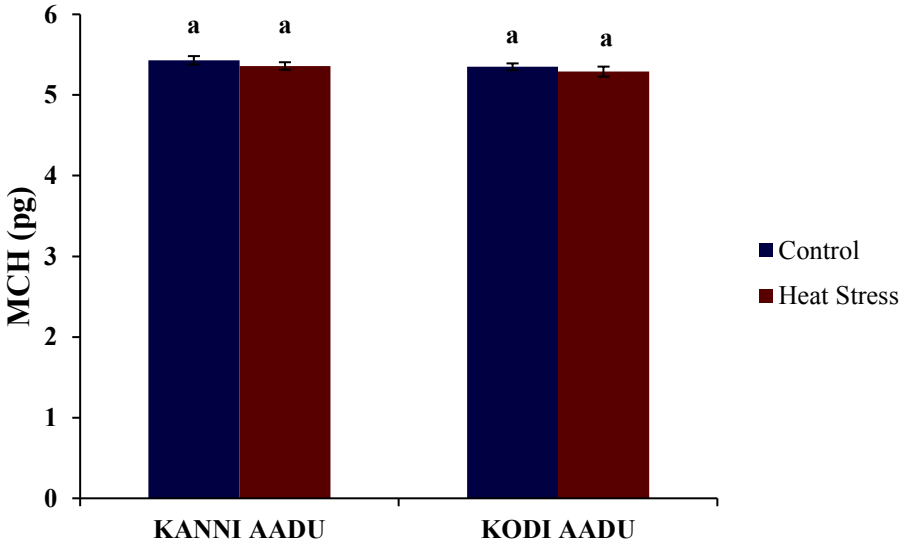
(i) Haemoglobin



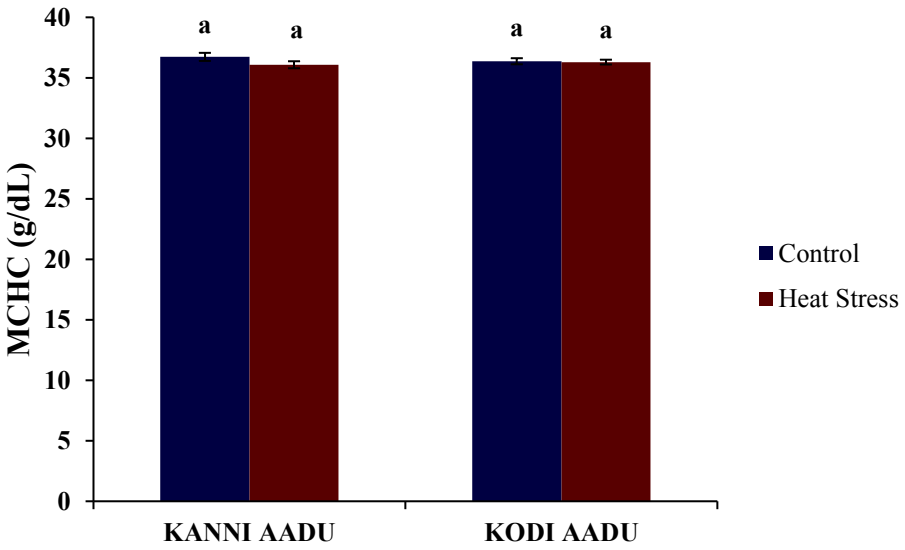
(j) Haematocrit



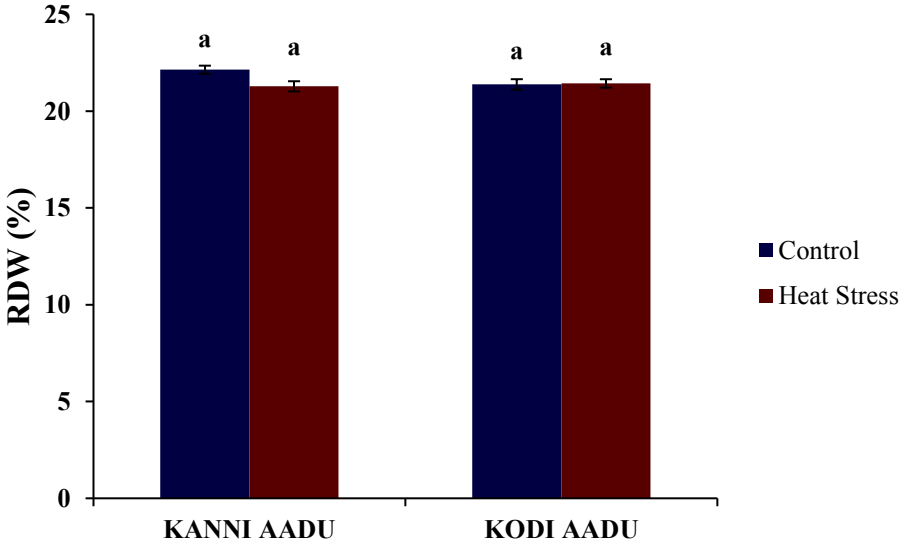
(k) Mean Corpuscular Volume



(l) Mean Corpuscular Haemoglobin



(m) Mean Corpuscular Haemoglobin Concentration



(n) Red Blood Cell Distribution Width (%)

4.6. Blood Biochemical Variables

4.6.1. Plasma Glucose

The plasma glucose across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma glucose in KAC, KAHS, KOC and KOHS groups were 57.01±1.87, 56.66±1.54, 55.89±1.68 and 52.52±1.63, respectively. Both the breed and treatment factors did not influence the plasma glucose concentration. However, the experimental days significantly ($P<0.01$) influenced the plasma glucose level. But, the BxTxD interaction also did not influence the plasma glucose level in the study. The differences in the plasma glucose concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (a) The post hoc test did not show any variation for the plasma glucose concentration across the experimental groups. Further, THI although had a negative correlation with plasma glucose still this effect was not statistically significant (Table 4.22).

4.6.2. Plasma Total Cholesterol

The plasma total cholesterol across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma total cholesterol in KAC, KAHS, KOC and KOHS groups were 76.20±5.01, 58.16±3.03, 51.66±3.53 and 50.46±3.53, respectively. The breed factor significantly ($P<0.05$) influenced the total cholesterol concentration. However, the treatment factor did not influence the total cholesterol concentration. But, the experimental days significantly ($P<0.01$) influenced the plasma total cholesterol

concentration. Likewise, the BxTxD interaction also influenced the plasma total cholesterol level significantly (P<0.05) in the study. The differences in the plasma total cholesterol concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (b). The post hoc test showed significant (P<0.05) variation in the plasma total cholesterol concentration across the experimental groups. Further, the THI had a negative correlation (P<0.05) with plasma total cholesterol (Table 4.22.).

4.6.3. Plasma total Protein

The plasma total protein across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21**.** The plasma total protein in KAC, KAHS, KOC and KOHS groups were 6.97±0.10, 6.56±0.11, 6.79±0.08 and 6.68±0.09, respectively. Both the breed and treatment factors did not influence the plasma total protein concentration. Further, both the experimental days and the BxTxD interaction also did not influence the plasma total protein level in the study. The differences in the plasma total protein concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (c) The post hoc test did not show any variation for the plasma total protein concentration across the experimental groups. Further, the THI had a negative correlation (P<0.05) with plasma total protein (Table 4.22).

4.6.4. Plasma Albumin

The plasma albumin across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21.

The plasma albumin in KAC, KAHS, KOC and KOHS groups were 4.22±0.11, 3.98±0.10, 4.06±0.11 and 4.08±0.09, respectively. Both the breed and treatment factors did not influence the plasma albumin concentration. However, the experimental days significantly ($P<0.05$) influenced the plasma albumin concentration. But the BxTxD interaction also did not influence the plasma albumin level in the study. The differences in the plasma albumin concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (d). The post hoc test did not show any variation for the plasma albumin concentration across the experimental groups. Further, THI although had a negative correlation with plasma albumin still this effect was not statistically significant (Table 4.22).

4.6.5. Plasma Globulin

The plasma globulin across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma globulin in KAC, KAHS, KOC and KOHS groups were 2.68±0.12, 2.71±0.10, 2.53±0.08 and 2.39±0.07, respectively. Both the breed and treatment factors did not influence the plasma globulin concentration. Further, both the experimental days and the BxTxD interaction also did not influence the plasma globulin level in the study. The differences in the plasma globulin concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (e) The post hoc test did not show any variation for the plasma globulin concentration across the experimental groups. Further, THI although had a negative correlation with plasma globulin still this effect was not statistically significant (Table 4.22).

4.6.6. Plasma Triglycerides

The plasma triglycerides across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma triglycerides in KAC, KAHS, KOC and KOHS groups were 26.70±2.25, 23.62±2.66, 25.71±4.25 and 26.31±3.57, respectively. The breed factor significantly ($P<0.01$) influenced the plasma triglycerides concentration. However, the treatment factor did not influence the plasma triglycerides concentration. But, the experimental days significantly ($P<0.01$) influenced the plasma triglycerides concentration. Likewise, the BxTxD interaction also influenced the plasma triglycerides level significantly ($P<0.01$) in the study. The differences in the plasma triglycerides concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (f). The post hoc test showed significant ($P<0.01$) variation in the plasma triglycerides concentration across the experimental groups. Further, THI although had a negative correlation with plasma triglycerides still this effect was not statistically significant (Table 4.22).

4.6.7. Plasma Urea

The plasma urea across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21**.** The plasma urea in KAC, KAHS, KOC and KOHS groups were 67.05±2.41, 58.85±1.53, 64.70±2.08 and 60.98±1.85, respectively. Both the breed and treatment factors did not influence the plasma urea concentration. Further, both the experimental days and the BxTxD interaction also did not influence the plasma urea level in the study. The

differences in the plasma urea concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (g). The post hoc test did not show any variation for the plasma urea concentration across the experimental groups. Further, the THI had a strong negative correlation ($P<0.01$) with plasma total protein (Table 4.22).

4.6.8. Plasma Aspartate Amino Transferase

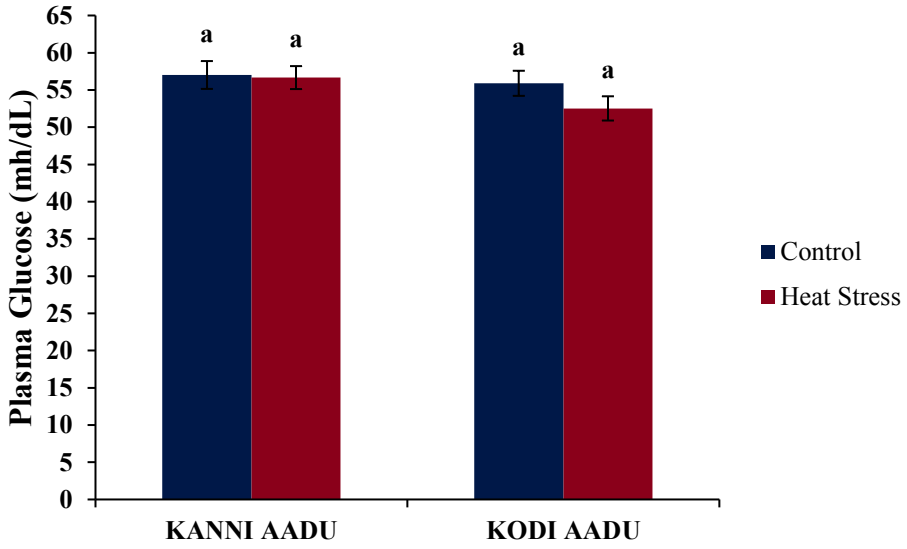
The plasma AST across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma AST in KAC, KAHS, KOC and KOHS groups were 51.86±2.45, 46.78±3.52, 63.50±6.87 and 51.27±4.14, respectively. Both the breed and treatment factors did not influence the plasma AST concentration. However, the experimental days significantly ($P<0.01$) influenced the plasma AST level. But, the BxTxD interaction also did not influence the plasma AST level in the study. The differences in the plasma AST concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (h). The post hoc test did not show any variation for the plasma AST concentration across the experimental groups. Further, THI although had a negative correlation with plasma AST still this effect was not statistically significant (Table 4.22).

4.6.9. Plasma Alanine Amino Transferase

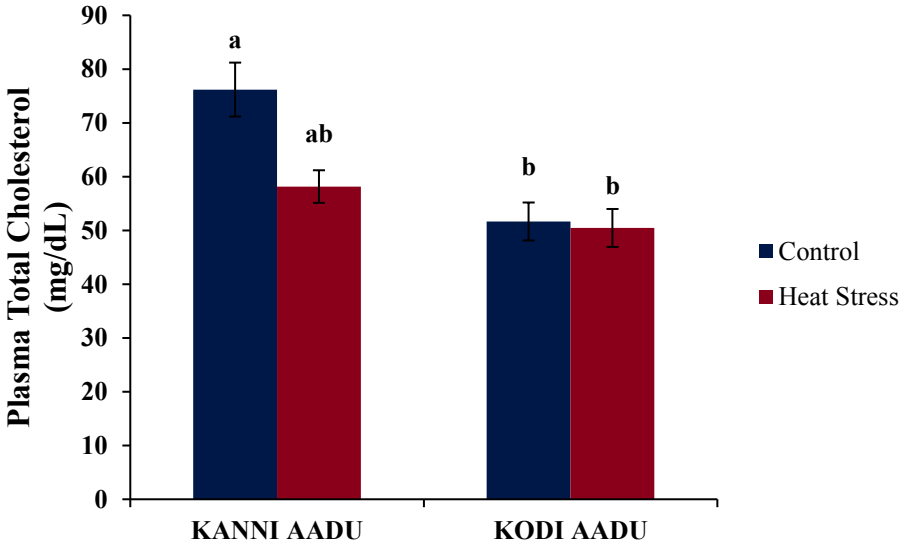
The plasma ALT across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.21. The plasma ALT in KAC, KAHS, KOC and KOHS groups were 74.33±2.09, 71.33±4.04, 81.44±11.35 and 66.50±2.06, respectively. Both the breed and treatment factors did not influence the plasma ALT concentration. Further, both the experimental days and the

BxTxD interaction also did not influence the plasma ALT level in the study. The differences in the plasma ALT concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.14 (i) The post hoc test did not show any variation for the plasma ALT concentration across the experimental groups. Further, THI although had a negative correlation with plasma ALT still this effect was not statistically significant (Table 4.22).

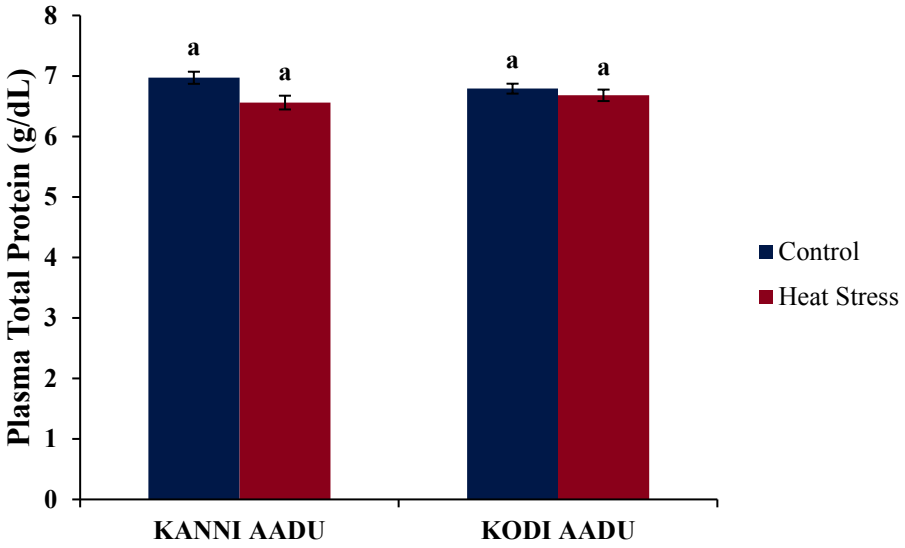
Fig. 4.14: Effect of heat stress on blood biochemical responses in Kanni Aadu and Kodi Aadu goat breeds (a) Plasma glucose (b) Plasma total cholesterol (c) Plasma total protein (d) Plasma albumin (e) Plasma globulin (f) Plasma triglycerides (g) Plasma urea (h) Aspartate aminotransferase (i) Alanine aminotransferase.



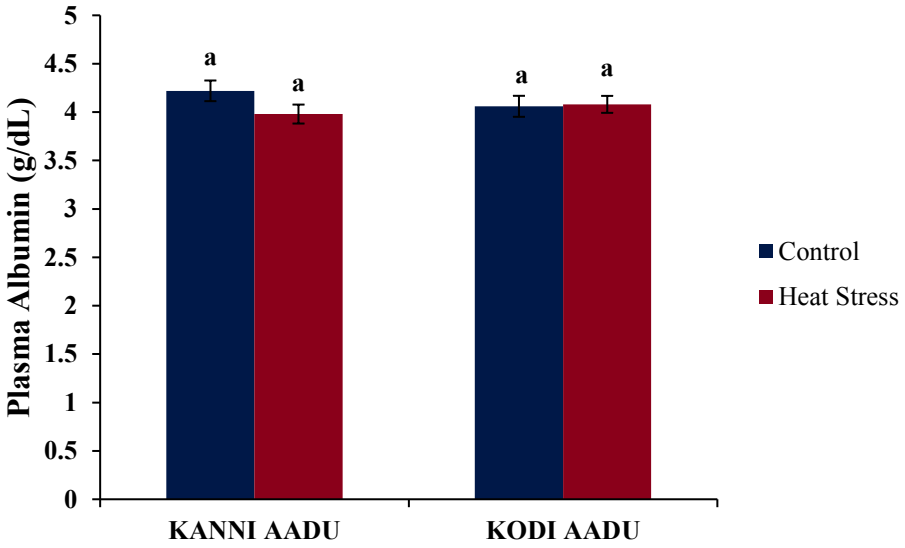
(a) Plasma glucose



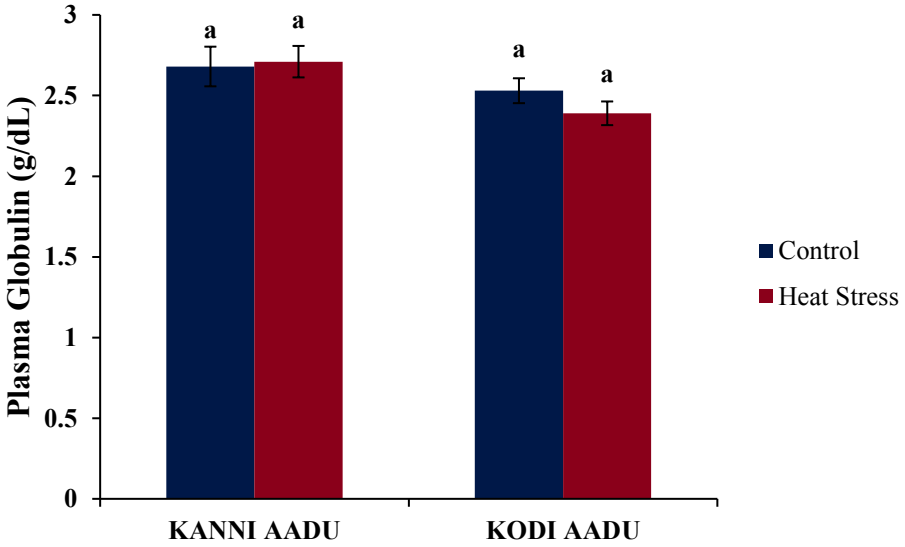
(b) Plasma total cholesterol



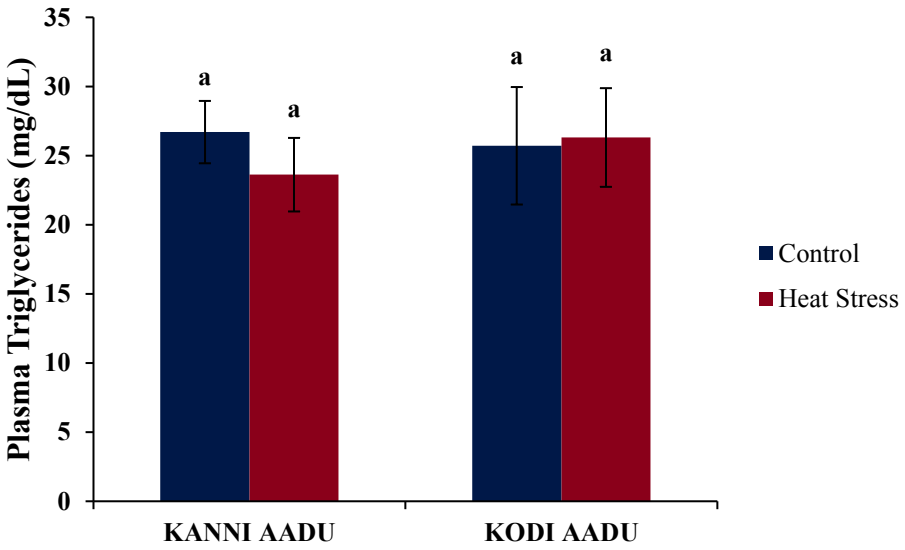
(a) Plasma total protein



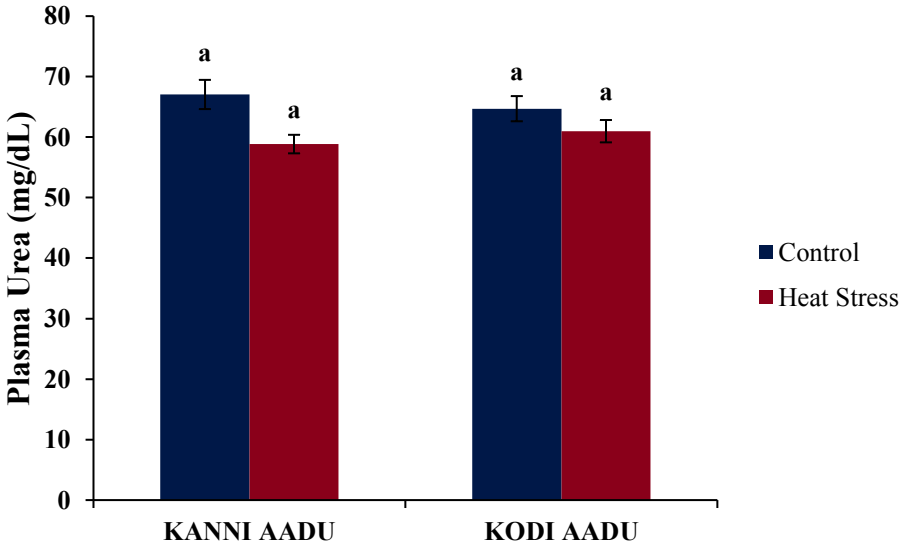
(b) Plasma albumin



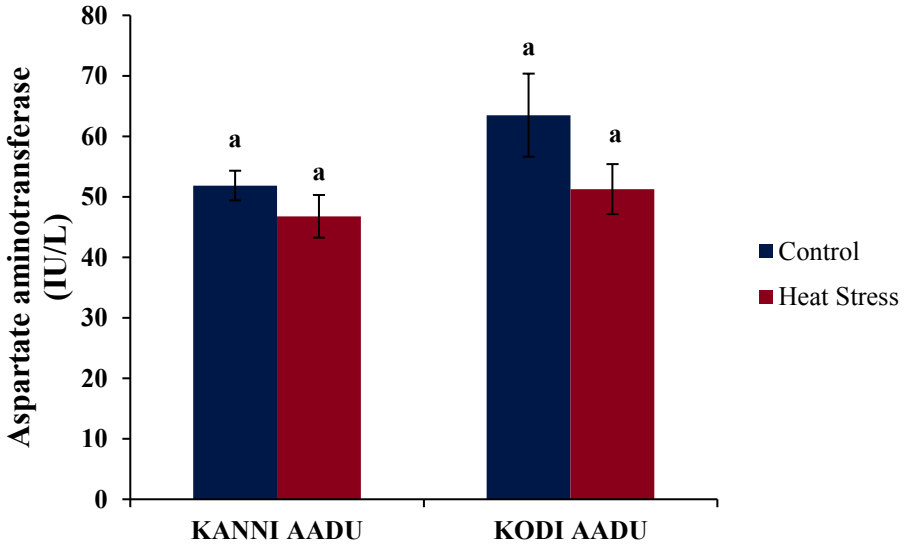
(c) Plasma globulin



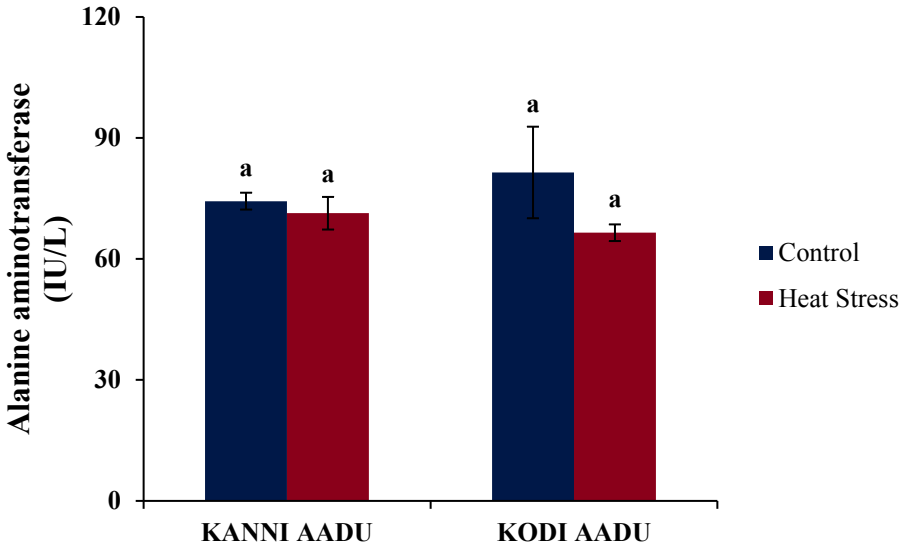
(d) Plasma triglycerides



(e) Plasma urea



(h) Plasma Aspartate aminotransferase



(i) Plasma Alanine aminotransferase

Table 4.21: Effect of heat stress on Blood biochemical responses in Kanni Aadu and Kodi Aadu goat breeds.

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
Plasma Glucose (mg/dL)	0	60.86	60.10	54.60	57.01	NS	NS	**	NS
	15	52.16	56.33	56.81	51.68				
	30	63.74	62.48	61.30	56.87				
	45	51.26	47.72	50.86	44.50				
	Mean	**57.01** [a]	**56.66** [a]	**55.89** [a]	**52.52** [a]				
	Pooled SE	±1.64	±1.64	±1.64	±1.64				
Plasma Total Cholesterol (mg/dL)	0	82.47	53.31	39.65	46.28	*	NS	**	*
	15	75.43	59.84	46.48	48.22				
	30	63.71	54.33	59.02	51.48				
	45	83.18	65.14	61.47	55.86				
	Mean	**76.20** [a]	**58.16** [ab]	**51.66** [b]	**50.46** [b]				
	Pooled SE	±6.15	±6.15	±6.15	±6.15				
Plasma Total Protein (g/dL)	15	6.92	6.60	6.75	6.73	NS	NS	NS	NS
	30	6.97	6.55	6.90	6.68				
	45	7.03	6.52	6.72	6.63				
	Mean	**6.97** [a]	**6.56** [a]	**6.79** [a]	**6.68** [a]				
	Pooled SE	±1.66	±1.66	±1.66	±1.66				
Plasma Albumin (g/dL)	0	4.04	4.13	3.67	4.11	NS	NS	*	NS
	15	4.29	3.76	4.12	3.80				
	30	4.42	3.96	3.93	4.31				
	45	4.15	4.08	4.50	4.12				
	Mean	**4.22** [a]	**3.98** [a]	**4.06** [a]	**4.08** [a]				
	Pooled SE	±0.15	±0.15	±0.15	±0.15				
Plasma Globulin (g/dL)	15	2.68	2.80	2.52	2.47	NS	NS	NS	NS
	30	2.68	2.75	2.63	2.38				
	45	2.68	2.57	2.45	2.33				
	Mean	**2.68** [a]	**2.71** [a]	**2.53** [a]	**2.39** [a]				
	Pooled SE	±1.58	±1.58	±1.58	±1.58				

Plasma Triglycerides (mg/dL)	0	28.18	21.82	15.15	28.64	**	NS	**	**
	15	34.39	20.91	16.97	22.27				
	30	20.44	20.73	17.97	15.22				
	45	23.77	31.01	52.75	39.13				
	Mean	**26.70** [a]	**23.62** [a]	**25.71** [a]	**26.31** [a]				
	Pooled SE	±3.35	±3.35	±3.35	±3.35				
Plasma Urea (mg/dL)	0	62.28	56.14	61.04	62.78	NS	NS	NS	NS
	15	76.46	60.59	68.75	63.40				
	30	62.28	60.76	64.81	58.45				
	45	67.17	57.94	64.19	59.29				
	Mean	**67.05** [a]	**58.85** [a]	**64.70** [a]	**60.98** [a]				
	Pooled SE	±3.03	±3.03	±3.03	±3.03				
Plasma AST (IU/L)	0	40.37	40.66	52.45	45.08	NS	NS	**	NS
	15	54.81	43.02	51.27	40.96				
	30	54.22	54.22	62.76	69.25				
	45	58.05	49.21	87.52	49.80				
	Mean	**51.86** [a]	**46.78** [a]	**63.50** [a]	**51.27** [a]				
	Pooled SE	±5.32	±5.32	±5.32	±5.32				
Plasma ALT (IU/L)	15	72.83	69.33	68.83	66.33	NS	NS	NS	NS
	30	78.50	76.83	74.33	67.67				
	45	71.67	67.83	101.17	65.50				
	Mean	**74.33** [a]	**71.33** [a]	**81.44** [a]	**66.50** [a]				
	Pooled SE	±7.29	±7.29	±7.29	±7.29				

KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; AST-Aspartate aminotransferase; ALT- Alanine aminotransferase.

Pooled SE- Pooled 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 4.22: Correlation association between THI and blood biochemical responses

	THI	Glucose	Cholesterol	Total Protein	Albumin	Globulin	Triglyceride	Urea	AST	ALT
THI	**1**									
Glucose	**-0.11**	**1**								
Cholesterol	**-0.23***	**0.07**	**1**							
Total Protein	**-0.28****	**0.08**	**0.35****	**1**						
Albumin	**-0.11**	**0.03**	**0.08**	**0.35****	**1**					
Globulin	**-0.04**	**0.03**	**0.32****	**0.48****	**-0.22***	**1**				
Triglyceride	**-0.04**	**-0.33****	**0.19**	**-0.01**	**0.05**	**0.10**	**1**			
Urea	**-0.30****	**-0.08**	**-0.06**	**0.22***	**0.02**	**0.18**	**0.19**	**1**		
AST	**-0.19**	**-0.06**	**-0.02**	**0.22***	**0.33****	**-0.04**	**-0.06**	**0.01**	**1**	
ALT	**-0.16**	**-0.02**	**0.28****	**0.44****	**0.11**	**0.21***	**-0.04**	**0.03**	**0.12**	**1**

THI- Temperature humidity index; AST- aspartate aminotransferase; ALT- alanine aminotransferase. **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

4.7. Endocrine Variables

4.7.1. Plasma Cortisol

The plasma cortisol across the experimental groups and the influence of factors breed, treatmen, day and their interactions during the study period were described in table 4.23. The plasma cortisol in KAC, KAHS, KOC and KOHS groups were 7.24±0.34, 8.15±0.77, 6.58±0.59 and 7.04±0.53, respectively. Both the breed and treatment factors did not influence the plasma cortisol concentration. Further, both the experimental days and the BxTxD interaction also did not influence the plasma cortisol level in the study. The differences in the plasma cortisol concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.15 (a). The post hoc test did not show any variation for the plasma cortisol concentration across the experimental groups. Further, THI although had a positive correlation with plasma cortisol still this effect was not statistically significant (Table 4.24).

4.7.2. Plasma Tri-iodo-thyronine (T_3)

The plasma T_3 across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.23. The plasma T_3 in KAC, KAHS, KOC and KOHS groups were 8.40±1.66, 9.05±1.25, 11.08±1.84 and 6.70±0.62, respectively. Both the breed and treatment factors did not influence the plasma T_3 concentration. Further, both the experimental days and the BxTxD interaction also did not influence the plasma T_3 level in the study. The differences in the plasma T_3 concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.15 (b) The post hoc test did not show any variation

for the plasma T_3 concentration across the experimental groups. Further, THI although had a negative correlation with plasma T_3 still this effect was not statistically significant (Table 4.24).

4.7.3. Plasma Thyroxin (T_4)

The plasma thyroxin across the experimental groups and the influence of factors breed, treatment, day and their interactions during the study period were described in table 4.23. The plasma T_4 in KAC, KAHS, KOC and KOHS groups were 98.75±9.27, 109.46±12.74, 142.77±28.81 and 86.03±10.06, respectively. Both the breed and treatment factors did not influence the plasma thyroxin concentration. However, the experimental days significantly ($P<0.01$) influenced the plasma thyroxin level. But, the BxTxD interaction also did not influence the plasma thyroxin level in the study. The differences in the plasma thyroxin concentration across the experimental groups of Kanni and Kodi Aadu goat breeds were described in Fig. 4.15 (c) The post hoc test did not show any variation for the plasma thyroxin concentration across the experimental groups. Further, THI although had a negative correlation with plasma T_4 still this effect was not statistically significant (Table 4.24).

Table 4.24: Correlation association between THI and endocrine responses

	THI	**Cortisol**	**T_3**	**T_4**
THI	**1**			
Cortisol	**0.10**	**1**		
T_3	**-0.16**	**0.49****	**1**	
T_4	**-0.16**	**0.43****	**0.76****	**1**

THI- Temperature humidity index; T_3- Tri iodothyronine; T_4- Thyroxine
**Indicates statistical significance at $P < 0.01$.

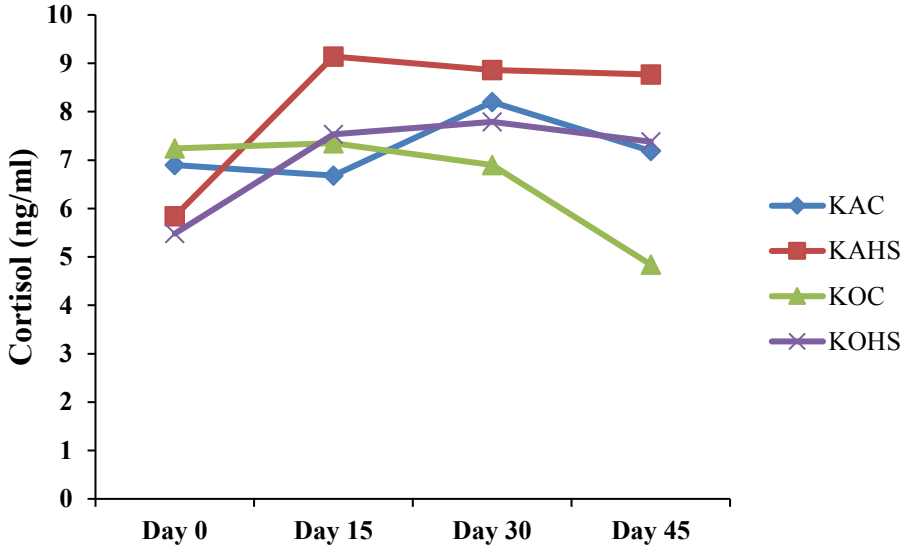
Table 4.23: Effects of heat stress on Endocrine responses in Kanni Aadu and Kodi Aadu goat breeds

Attributes	Days	Treatments				Effects			
		KAC	KAHS	KOC	KOHS	Breed	TRT	DAY	Breed* TRT * DAY
Cortisol (ng/ml)	0	6.90	5.85	7.25	5.48	NS	NS	NS	NS
	15	6.68	9.14	7.35	7.53				
	30	8.20	8.85	6.90	7.79				
	45	7.19	8.77	4.84	7.38				
	Mean	**7.24** [a]	**8.15** [a]	**6.58** [a]	**7.04** [a]				
	Pooled SE	±1.14	±1.14	±1.14	±1.14				
T_3 (ng/ml)	0	3.67	6.06	7.05	5.07	NS	NS	NS	NS
	15	10.14	11.53	12.74	6.54				
	30	11.12	10.63	15.35	8.00				
	45	8.67	7.99	9.18	7.21				
	Mean	**8.40** [a]	**9.05** [a]	**11.08** [a]	**6.70** [a]				
	Pooled SE	±1.74	±1.74	±1.74	±1.74				
T_4 (nmol/L)	0	71.74	72.95	60.79	56.04	NS	NS	**	NS
	15	110.68	128.80	144.67	97.12				
	30	111.12	124.88	178.93	92.52				
	45	101.47	111.20	186.67	98.43				
	Mean	**98.75** [a]	**109.46** [a]	**142.77** [a]	**86.03** [a]				
	Pooled SE	±23.48	±23.48	±23.48	±23.48				

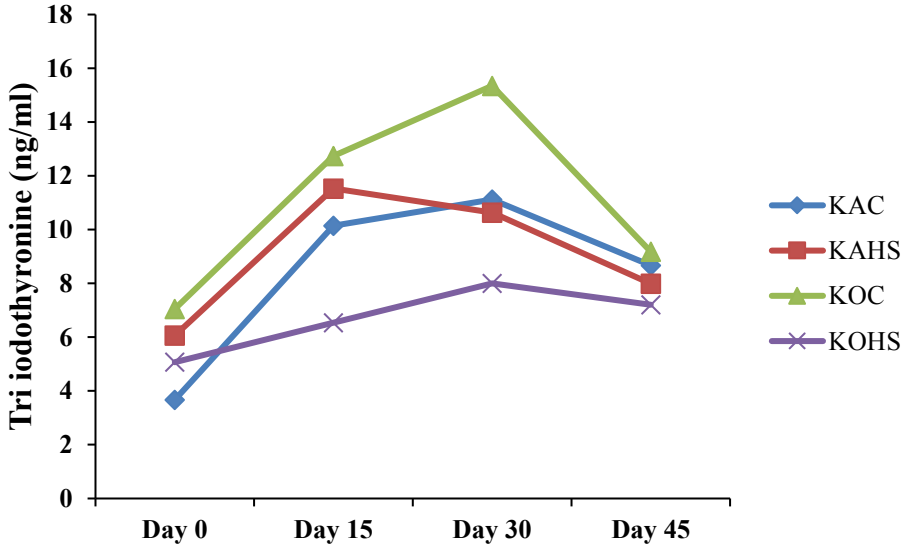
KAC- Kanni Aadu Control; KAHS- Kanni Aadu Heat Stress; KOC- Kodi Aadu Control; KOHS- Kodi Aadu Heat Stress; TRT- treatment; Breed*TRT* Day- breed treatment and day interaction; T_3- Tri iodothyronine; T_4- Thyroxine; Pooled SE- Pooled standard error.

**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

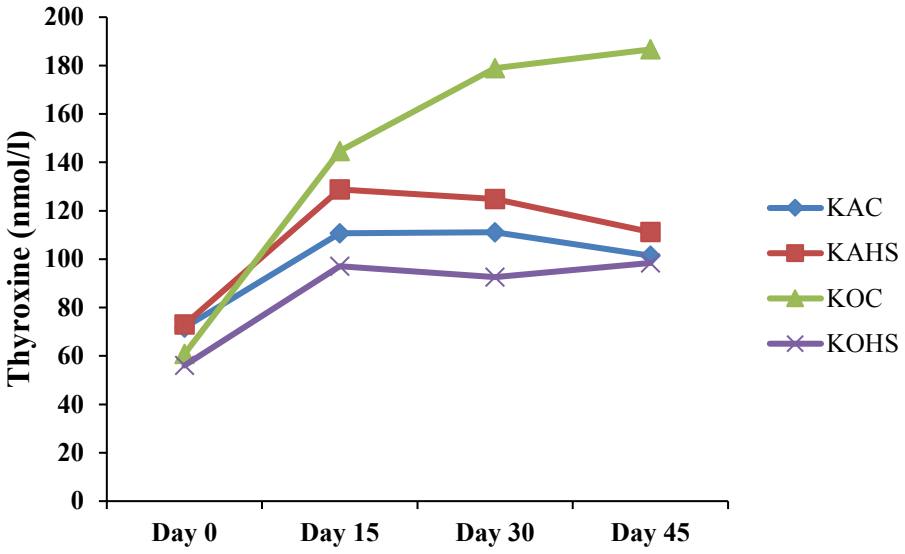
Fig. 4.15: Effect of heat stress on Endocrine responses in Kanni Aadu and Kodi Aadu goat breeds (a) Plasma cortisol (b) T_3 (c) T_4



a) Plasma cortisol



(b) T_3



(c) T_4

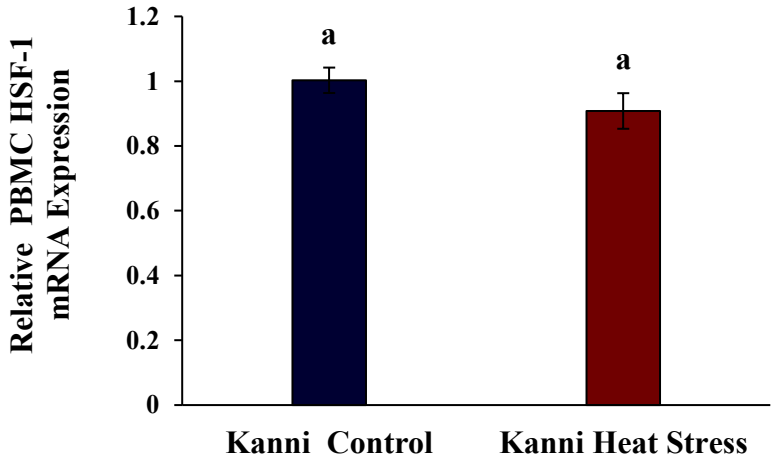
4.8. Gene Expression Variables

4.8.1. Heat Shock Factor 1

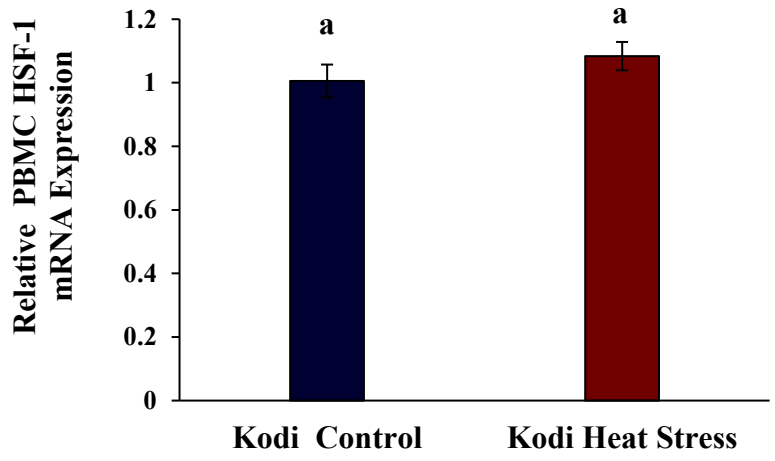
The differences in the *HSF-1* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.16 (a) and Fig. 4.16 (b). The *HSF-1* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.00±0.03, 0.91±0.03, 1.01±0.03, and 1.08±0.15, respectively. The *HSF-1* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. Similarly, the expression pattern of *HSF-1* gene was also comparable between the breeds. Likewise, the heat stress induced *HSF-1* mRNA expression pattern was also similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Although non-significant, the *HSF-1* gene expression showed increasing trend in KOHS

group as compared to KAHS group. Further, THI although had a negative correlation with *HSF-1* still this effect was not statistically significant (Table 4.25).

Fig. 4.16: Effect of heat stress on *HSF-1* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSF-1* mRNA expression in Kanni Aadu goat breed.

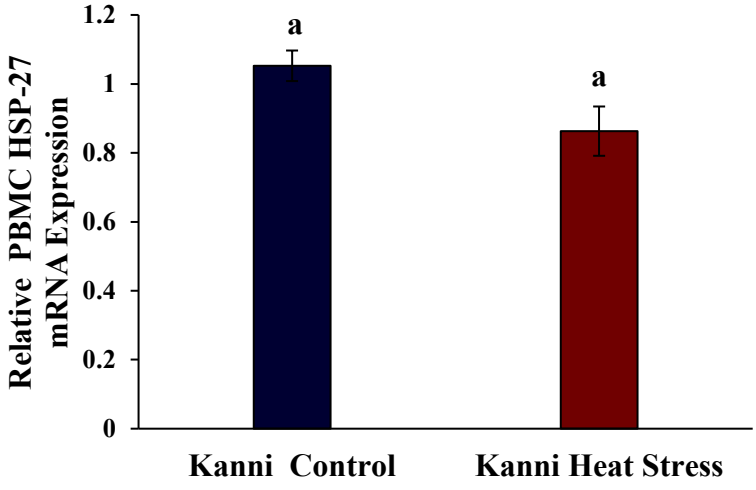


(b) Relative PBMC *HSF-1* mRNA expression in Kodi Aadu goat breed

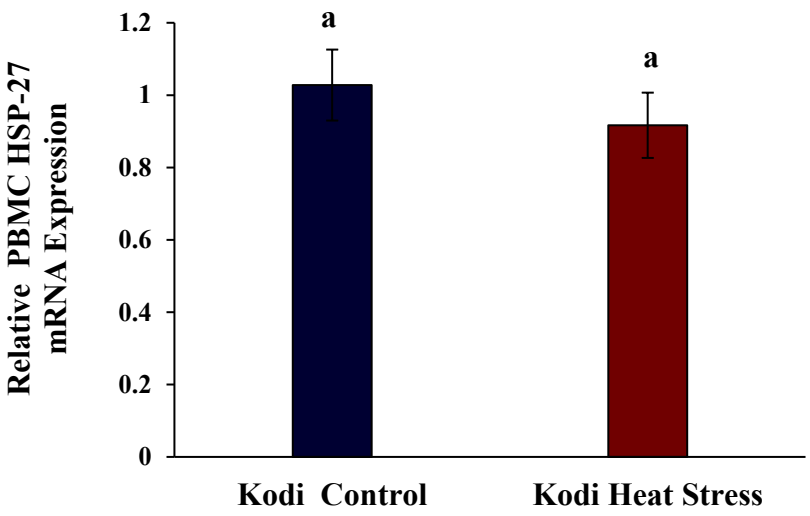
4.8.2. Heat Shock Protein 27

The differences in the *HSP-27* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.17 (a) and Fig. 4.17 (b). The *HSP-27* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.05±0.22, 0.86±0.18, 1.03±0.03, and 0.92±0.15, respectively. The *HSP-27* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. Similarly, the expression pattern of *HSP-27* gene was also comparable between the breeds. Likewise, the heat stress induced *HSP-27* mRNA expression pattern was also similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Although non-significant, the *HSP-27* gene expression showed decreasing trend in both KAHS and KOHS groups. Further, THI although had a negative correlation with *HSP-27* still this effect was not statistically significant (Table 4.25).

Fig. 4.17: Effect of heat stress on *HSP-27* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSP-27* mRNA expression in Kanni Aadu goat breed.



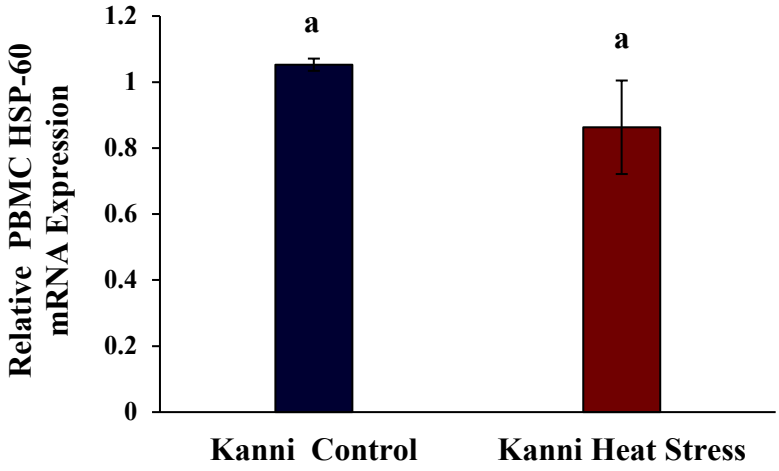
(b) Relative PBMC *HSP-27* mRNA expression in Kodi Aadu goat breed

4.8.3. Heat Shock Protein 60

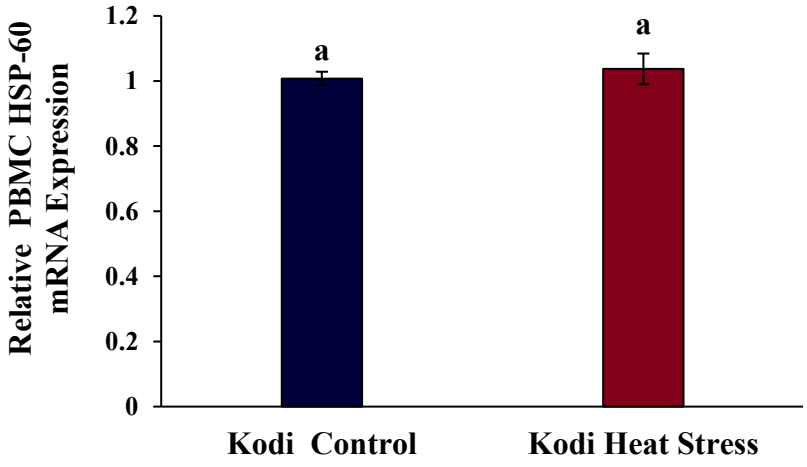
The differences in the *HSP-60* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.18 (a) and Fig. 4.18 (b). The *HSP-60* gene expression pattern showed similar pattern like that of *HSF-1* gene expression in the study. The *HSP-60* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.05±0.15, 0.86±0.10, 1.01±0.06, and 1.04±0.20, respectively. The *HSP-60* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. Similarly, the expression pattern of *HSP-60* gene was also comparable between the breeds. Likewise, the heat stress induced *HSP-60* mRNA expression pattern was also similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Although non-significant, the *HSP-60* gene expression showed increasing trend in KOHS

group as compared to KAHS group. Further, THI although had a negative correlation with *HSP-60* still this effect was not statistically significant (Table 4.25).

Fig. 4.18: Effect of heat stress on *HSP-60* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSP-60* mRNA expression in Kanni Aadu goat breed.

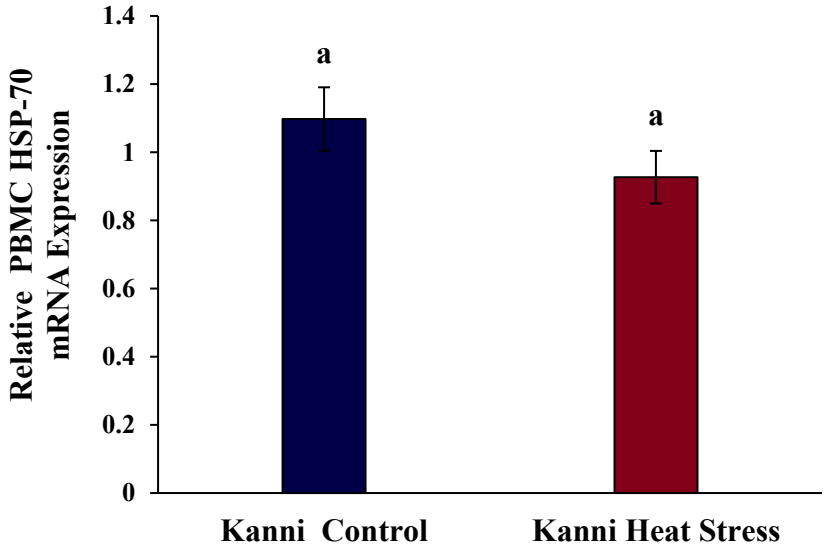


(b) Relative PBMC *HSP-60* mRNA expression in Kodi Aadu goat breed

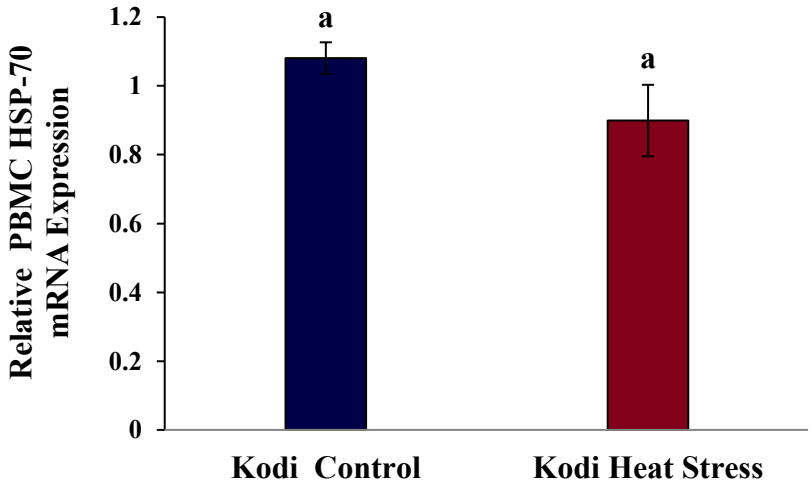
4.8.4. Heat Shock Protein 70

The differences in the *HSP-70* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.19 (a) and Fig. 4.19 (b). The *HSP-70* gene expression pattern showed similar pattern like that of *HSP-27* gene expression in the study. The *HSP-70* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.11±0.24, 0.93±0.07, 1.08±0.15, and 0.91±0.12, respectively. The *HSP-70* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. Similarly, the expression pattern of *HSP-70* gene was also comparable between the breeds. Likewise, the heat stress induced *HSP-70* mRNA expression pattern was also similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Although non-significant, the *HSP-70* gene expression showed decreasing trend in both KAHS and KOHS groups. Further, THI although had a negative correlation with *HSP-70* still this effect was not statistically significant (Table 4.25).

Fig. 4.19: Effect of heat stress on *HSP-70* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSP-70* mRNA expression in Kanni Aadu goat breed.

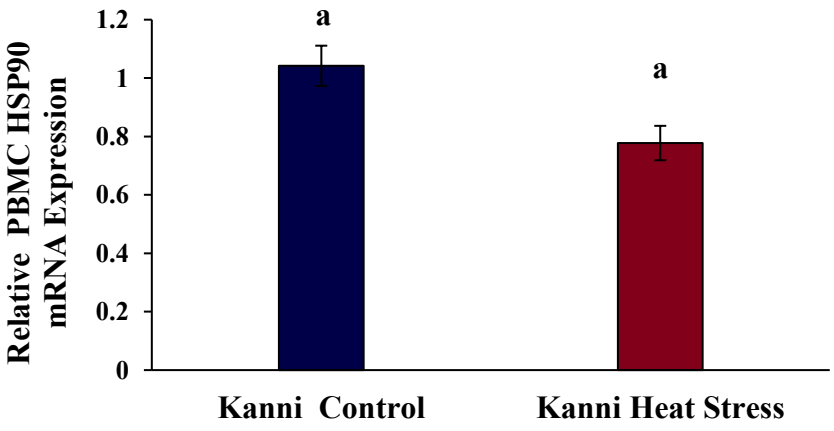


(b) Relative PBMC *HSP-70* mRNA expression in Kodi Aadu goat breed

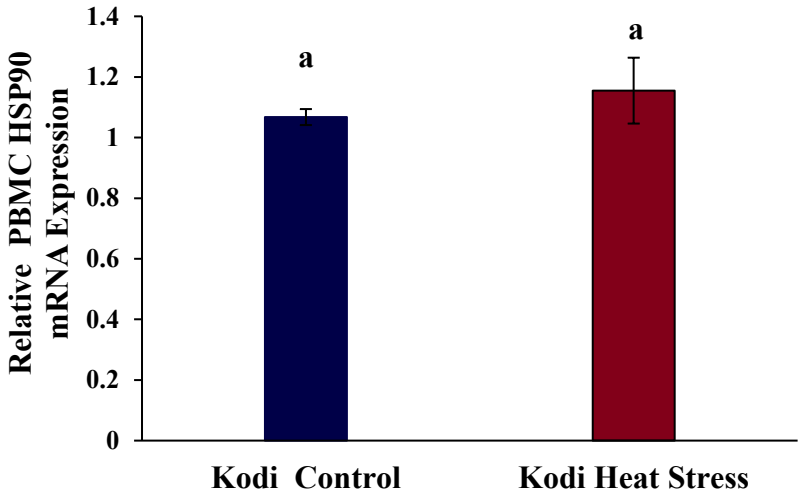
4.8.5. Heat Shock Protein 90

The differences in the *HSP-90* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.20 (a) and Fig. 4.20 (b). The *HSP-90* gene expression pattern showed similar pattern like that of *HSF-1* and *HSP-60* gene expression in the study. The *HSP-90* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.04±0.14, 0.78±0.03, 1.07±0.25, and 1.16±0.06, respectively. The *HSP-90* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. Similarly, the expression pattern of *HSP-90* gene was also comparable between the breeds. Likewise, the heat stress induced *HSP-90* mRNA expression pattern was also similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Although non-significant, the *HSP-90* gene expression showed increasing trend in KOHS group as compared to KAHS group. Further, THI although had a negative correlation with *HSP-90* still this effect was not statistically significant (Table 4.25).

Fig. 4.20: Effect of heat stress on *HSP-90* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSP-90* mRNA expression in Kanni Aadu goat breed.

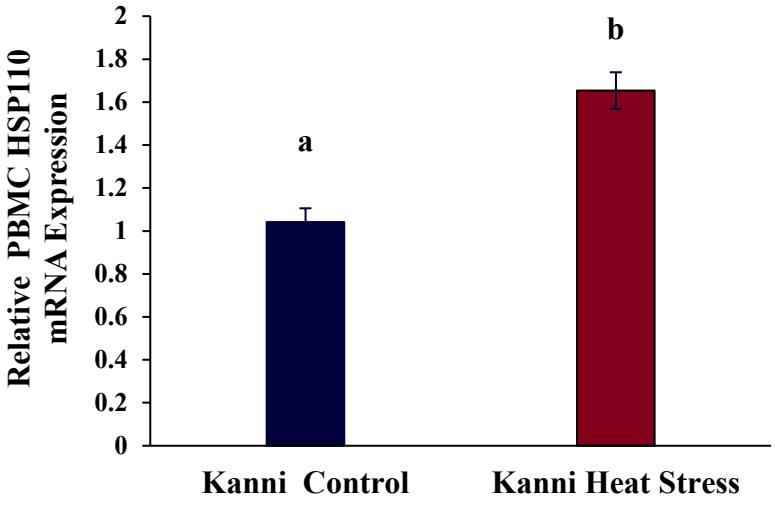


(b) Relative PBMC *HSP-90* mRNA expression in Kodi Aadu goat breed

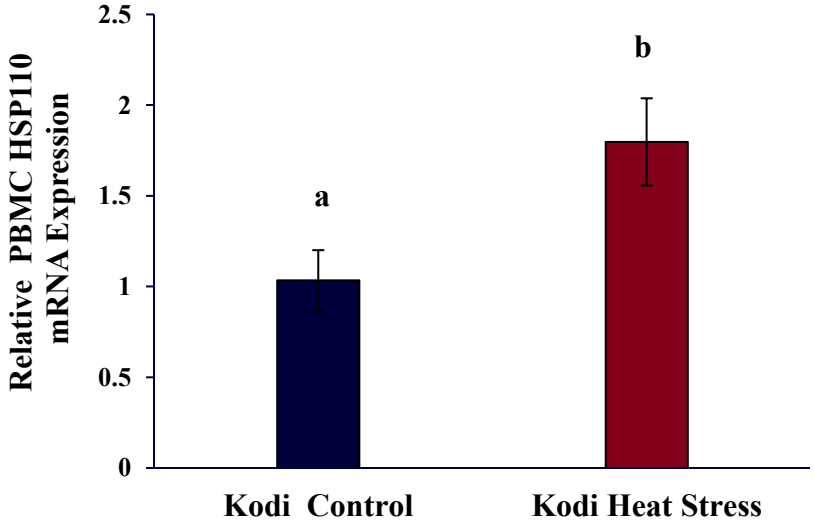
4.8.6. Heat Shock Protein 110

The differences in the *HSP-110* mRNA expression pattern between the control and heat stress groups of Kanni and Kodi Aadu goat breeds are described in Fig. 4.21 (a) and Fig. 4.21 (b). The *HSP-110* gene expression pattern showed unique trend as compared to other targeted gene expression in the study. The *HSP-110* mRNA expression in KAC, KAHS, KOC and KOHS groups were 1.04±0.81, 1.65±0.32, 1.03±0.38, and 1.81±0.47, respectively. The *HSP-110* mRNA expression pattern was similar between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds. The heat stress significantly ($P<0.05$) increased the expression pattern of *HSP-110* gene in both the breeds. However, the heat stress induced *HSP-110* mRNA expression pattern was similar between the breeds as evident from the non-significant difference in the expression pattern between the KAHS and KOHS groups. Further, the THI had a positive correlation ($P<0.05$) with *HSP-110* (Table 4.25).

Fig. 4.21: Effect of heat stress on *HSP-110* mRNA expression pattern in Kanni Aadu and Kodi Aadu goat breeds.



(a) Relative PBMC *HSP-110* mRNA expression in Kanni Aadu goat breed.



(b) Relative PBMC *HSP-110* mRNA expression in Kodi Aadu goat breed

Table 4.25: Correlation association between THI and gene expression

	THI	*HSF-1*	*HSP-27*	*HSP-60*	*HSP-70*	*HSP-90*	*HSP-110*
THI	**1**						
HSF-1	**-0.03**	**1**					
HSP-27	**-0.11**	**0.55**	**1**				
HSP-60	**-0.13**	**0.56**	**-0.08**	**1**			
HSP-70	**-0.37**	**0.34**	**-0.07**	**0.44**	**1**		
HSP-90	**-0.18**	**0.36**	**-0.01**	**0.45**	**0.60***	**1**	
HSP-110	**0.64***	**0.46**	**0.11**	**0.55**	**-0.10**	**0.07**	**1**

DISCUSSION

CHAPTER 5

DISCUSSION

Climate change threatens the world food security and particularly the developing parts of the world are expected to be highly vulnerable to this effect. Therefore, the world is looking for solution to tackle the issue of feeding the growing human population which is expected to be 9.6 billion by 2050 (FAO, 2015). Climate change associated heat and nutritional stresses poses serious threat to sustain large ruminant production. Among the various agriculture sectors, livestock are established to be highly climate resilient and are expected to play a significant role in ensuring food security by 2050 (Gaughan *et al.,* 2019). However, the projected climate change associated feed and fodder resources reduction poses serious threat to large ruminant livestock production. Hence, small ruminants and particularly goats are being projected as ideal climate animal model to cope with climate change associated adversities due to their higher thermo-tolerance capacity (Berihulay *et al.,* 2019). However, such information on the indigenous breeds is very less. Given the significance of goat to food security in future it becomes essential to generate more baseline information governing thermo-tolerance in unexplored native track goat breeds (Aleena *et al.,* 2018). Such efforts could help the researchers to identify agro-ecological zone-specific goat breeds which could play a significant role to ensure livelihood securities of the poor and marginal farmers (Silanikove and Koluman, 2015). Thus, the current study is one such attempt in establishing the thermo-tolerance in two indigenous goat breeds Kanni Aadu and Kodi Aadu. The results from the study present

some unique findings in establishing the superior thermo-tolerance of both the targeted breeds in this study.

Induced microclimate inside the climate chambers

The microclimate condition prevailed in both the climatic chambers induced thermo-neutral condition and heat stress condition for KAC and KOC groups and KAHS and KOHS groups, respectively throughout the study period. This was evident from the significantly higher values for all meteorological variables recorded during the exposure duration of six hours in the heating chamber as compared to TNZ chamber. Further, the temperature and relative humidity derived THI (McDowell, 1972) established this difference in microclimate across the chambers. The comparable THI values during morning between the chambers and significantly higher THI value of 94.76 as compared to 70.57 in the TNZ chamber during afternoon clearly demonstrates the differences in the microclimate across the chambers. As per McDowell (1972) formula the THI values above 78 are considered extreme distress while any value below 72 and less are considered comfortable condition. This shows that the desired microclimate was induced in either of the chambers to prove the hypothesis of the study. Similar results were established in hot semi-arid condition using the same formula in the external and shed environment (Shilja *et al.,* 2016; Aleena *et al.,* 2018). Further, the simulated heat stress model followed in the study could able to mimic the natural environmental stress condition inside the chamber. This was in agreement with the previous report of Indu *et al.* (2014) who followed similar simulated heat stress model in the chambers to induce natural heat stress environment inside the climatic chamber for sheep. Therefore, following simulated heat stress model in the climate chambers could be of practical

relevance to mimic the natural environmental stress condition. Further, such simulated model also would be preferred from ethical angle as constant heat stress model in the climate chamber could be more severe and may not be favoured from animal welfare point of view. This shows that the established weather variables inside the climate chamber induced extremely severe heat stress to the animals in this study.

Body Weight Changes

The non-significant influence of heat stress on body weight in the current study clearly establishes the superior adaptability of both Kanni and Kodi Aadu goat breeds to heat stress challenges. However, there are several reports which established significant influence of heat stress on body weight in exotic (Helal *et al.,* 2010) as well as indigenous goat breeds (Niyas *et al.,* 2017; Pragna *et al.,* 2018b). Hamzaoui *et al.* (2013) observed that the reduction in body weight in heat stress animals could be attributed to the deviation of energy from the productive pathway towards adaptation pathway to help in their survival. Therefore, the non-significant influence of heat stress on body weight clearly demonstrates the excellent climate resilience capacity of these indigenous goat breeds targeted in this particular study. Further, there were no significant correlation established between THI and BW in this study again indicates better adaptive ability of these breeds to tropical environment.

Behaviour Response

Goats exhibit certain behavioural responses to cope with adverse environments. They show a series of responses on feeding, WI, defecating and UF, LT, ST and shade seeking behaviour (Shilja *et al.,* 2016). The most immediate response by

which the animals tries to cope with heat stress is by reducing their FI. This strategy was adopted by the animals in an effort to keep their metabolic heat production under check to cope with external heat stress challenges. Therefore, reduction in FI was considered the most consistent behavioural adaptive mechanisms exhibited by the heat stressed farm animals (Darcan and Güney, 2008; Pragna *et al.,* 2017). However, in the current study, heat stress did not induce decrease in FI in either of the breeds. This shows the extreme adaptive nature of both Kanni and Kodi Aadu goat breeds to cope with heat stress exposure. Similarly, both the breeds did not show any significant difference in WI and this was evident from the non-significant difference in WI between KAC and KAHS groups as well as between KOC and KOHS groups. However, there are reports in goats which established heat stress induced WI in goats (Darcan and Guney, 2008; Shilja *et al.,* 2016). Similarly, Aleena *et al.* (2018) established significantly increased WI in heat stressed Osmanabadi, Malabari and Salem Black goat breeds. Thus, it may be inferred that there could be breed variation for WI when goats are subjected to heat stress. The result obtained in the current study thus could point towards the drought tolerant capacity of both Kanni and Kodi Aadu breeds.

Heat stress was found to induce changes in standing and lying behavior of goats so as to reduce the additional heat load from the ground as well as to enhance the heat dissipation to the environment (Panda *et al.,* 2016; Alam *et al.,* 2011). Both ST and LT showed reverse trends during heat stress exposure in Kanni and Kodi Aadu breeds. Studies on heat stress induced behavioral changes in goats proved to have an inverse relation between the ST and LT (Shilja *et al.,* 2016; Aleena *et al.,* 2018). The ST was

significantly lower while LT was significantly higher in Kanni Aadu breed. However, both ST and LT did not differ significantly during heat stress exposure in Kodi Aadu breed. This shows that Kodi Aadu breed was adapting slightly better than Kanni Aadu breed based on changes in both ST and LT. But the DF showed reverse trend in this study with significantly increased DF in Kodi Aadu breed while in Kanni Aadu breed there was no significant difference established between KAC and KAHS groups.

Experiment conducted in Osmanabadi goats by Shilja *et al.* (2016) found that the DF was higher when exposed to heat stress. Similar experiment conducted in three different goat breeds Osmanabadi, Malabari and Salem Black showed that irrespective of breed, heat stress increased the DF (Aleena *et al.,* 2018). However, in Kanni Aadu breed in this study the DF did not differ between the groups while Kodi Aadu breed the DF was higher in heat stress group pointing towards better adaptability of Kanni Aadu than Kodi Aadu breed. However, this difference in DF did not reflect in the total WI between breeds on subjecting them to heat stress. Like DF, DeF also showed the same trend with significantly lower DeF recorded in Kodi Aadu breed while it was non-significant in Kanni Aadu breed. However, the UF did not differ between the groups in both the breeds indicating heat stress did not induce changes in these breeds for UF. Generally, the heat stressed animals reduce both the DeF and UF in an effort to conserve body water as in these animals the loss of water through both respiratory and evaporative cooling mechanisms would be active and therefore to compensate these animals reduce their DeF and UF (Alam *et al.,* 2011; Panda *et al.,* 2016). The non-significant difference in DeF in Kanni Aadu breed shows the better adaptability of this breed to heat stress challenges.

However, RuT differed significantly in both the breeds with significantly lower value recorded in the heat stress groups of both the breeds. But the magnitude of this difference was higher in Kodi Aadu breed. However, the no difference in FI in this study and significantly lower RuT established that both the breeds exhibited the behaviour of postponing their rumination of consumed feed probably in an effort to prevent the coincidal occurrence of peak metabolic heat production and severe heat stress exposure in the climate chamber. In a similar study, Aleena *et al.* (2018) also established significantly lower RuT in Osmanabadi and Salem Black breeds while did not observe any changes in Malabari breed. Thus, these results point towards the fact that every indigenous breed has their own way of behaviourally adapting to the heat stress exposure. This was evident in the current study also as both Kanni and Kodi Aadu goat breeds showed different level of changes in ST, LT, DF, DeF and RuT indicating their own unique way to behaviourally counter heat stress challenges. However, ultimately the most crucial behaviour response of FI and WI did not differ between their respective control and heat stress groups reflecting the different approaches exhibited by these breeds to reach the common target of maintaining both FI and WI which was considered the ultimate variables for maintaining the thermal balance in these breeds. Further, a strong correlation between THI and ST, LT, DF and RuT clearly indicate the significance of behavioural response in both these breeds to adapt to adverse environmental condition.

Physiological variables reflecting climate resilience in both breeds

Goats rely predominantly on respiratory evaporative cooling mechanisms to dissipate body heat during heat stress exposure (Silanikove and Koluman, 2015). RR have been widely established to be the reliable biological marker for quantifying heat

stress response in goats (Hamzaoui *et al.,* 2013; Panda *et al.,* 2016). Heat stress induced in the study was able to elicit heat stress response in both the breeds and this was evident from the significantly higher RRA in both KAHS and KOHS groups from the second week onwards until end of the study duration. This increase in RRA gradually reduced until end of the experiment in both the breeds. This shows both the breeds performed in similar fashion to counter heat stress with initial higher level of RR which reduced gradually as the animals adapted to the situation. This was evident from the significantly lower RRE in KAHS group as compared to KOHS group. Similar observation was made by Banerjee *et al.* (2015) in their study on four different Indian goat breeds which reported breed differences for RR with lower values in breeds which are more adapted to heat stress. Similarly, the study conducted in three indigenous Osmanabadi, Salem Black and Malabari goat breeds established lower RR in Salem Black breed during heat stress exposure reflecting the superior adaptability of this breed to cope with hot environment (Aleena *et al.,* 2018). In addition, the significantly lower RRN in KOHS group as compared to KAHS group establishes the better recovery efficiency of Kodi Aadu breed as usually the breed with higher recovery efficiency keep their RR at a lower level to cope with high heat exposure during day time. However, the comparable RRM across the heat stress groups in both the breeds shows the similar levels of thermo-tolerance capacity of these breeds. This was supported by the finding that breed factor did not influence RR in this study. However, both experimental days as well as BxTxD interaction influenced RRE and RRN reflecting the extreme climate resilience capacity and recovery efficiency of both the breeds. Thus, the study points towards the fact that RR could be a reliable biomarker for heat stress in goats.

The PS during afternoon also significantly increased in both KAHS and KOHS groups. However, no panting was observed in both breed control groups. Goats predominantly rely on respiratory evaporative cooling mechanisms and therefore PS could be a reliable indicator for heat stress in goats (Lebacq *et al.,* 2013). Therefore, the magnitude of heat stress on goats can be very well predicted and determined using PS. Literatures have clearly stated that alterations in the respiration dynamics can act as important input in developing PS index that gives a clear remark on severity of heat stress impacts on goats (Brown-Brandl *et al.,* 2006; Darcan *et al.,* 2007). The findings from the study also coincided with above findings wherein the heat stress significantly increased the PS in both the breeds. Similar findings were also reported by Darcan *et al.* (2007) to ascertain the heat load with PS in goats. Similar to other physiological variables, breed and BxTxD interaction did not influence PSA reflecting the same level of panting behaviour being exhibited by both the breeds. However, the significant influence of experimental days on PSA clearly indicated that the magnitude of PS response got altered as the experiment progressed. Like behavioural response, the THI showed very strong positive correlation all the physiological variables during afternoon showed that both the breeds relied heavily on their physiological adaptive mechanisms to cope with heat stress challenges.

The higher PRA established in both KAHS and KOHS groups as compared to their respective control groups exhibited the better heat dissipation mechanisms in both these breeds. Similar observation of higher pulsation rate during heat stress exposure was also established in goats by Gupta *et al.* (2013) and these authors attributed this to the increased blood flow from the core body to the surface so that more heat is lost both by

sensible as well as insensible means. Likewise, Silanikove (2000c) also implicated that the increase in PR increased the cutaneous blood flow which helped goats to reduce the heat load from their body. Again, the higher PRM, PRE and PRN in KAHS indicate the better adaptive efficiency of Kanni Aadu breed. The significant influence of experimental days on PR at all time point indicates that both the goat breeds were trying to cope with heat stress challenges. The non-significant influence of BxTxD interaction suggests that the recovery efficiency persisted in both these breeds.

The RT is another important indicator for heat stress in farm animals (Silanikove, 2000a; Marai *et al.,* 2007). In several studies, the RT of goats were found to be elevated when exposed to thermal stress (Banerjee *et al.,* 2015; Panda *et al.,* 2016; Aleena *et al.,* 2018). The significantly higher RTA in both KAHS and KOHS groups indicates that RT could be the ideal biomarker for measuring heat stress in these breeds. Similar observations were made in other goat breeds reflecting the reliability of this variable in assessing the heat stress magnitude cutting across breeds in goat (Habibu *et al.,* 2016; Shilja *et al.,* 2016). The comparable RTA in the heat stress groups in both Kanni as well as in Kodi Aadu breeds indicated that both the breeds countered heat stress in similar way. This was further supported by the fact that both RTN and RTM showed non-significant difference across the experimental groups. Further, this argument was supported by the fact that both breed effect and BxTxD interaction did not influence RT in the current study indicating the similar climate resilience capacity in both the breeds. However, the lower RTE in KOHS group as compared to KAHS group reflects the better potential of Kodi Aadu breed in maintaining the core body temperature.

Shilja *et al.* (2016) established SkT to be a reliable variable to reflect heat stress effects and they attributed this to adaptive mechanism in which skin capillaries undergo vasodilation to improve the blood flow to the periphery of body for enhancing heat transfer to the environment. Recording SkT in head, shoulder and flank was established to be a reliable method to establish thermo-tolerance in goats (Aleena *et al.,* 2018). The SkT recorded at head, shoulder and flank are significantly different among the groups during afternoon. This again reflects the superior thermo-tolerance of both these breeds. The higher SkT in KAHS and KOHS groups over the other groups reflects the magnitude of heat stress experienced by both these breeds. Further, similar level of increase in SkT at head, shoulder and flank region reflects the extreme climate resilience of both Kanni and Kodi Aadu breeds. Generally, the light coat color breeds have less SkT than black coat breeds (Hagan *et al.,* 2012; Aleena *et al.,* 2018). This could be due to higher reflective capacity of solar radiation in light coat coloured breeds. Although Kanni Aadu is black coat coloured while Kodi Aadu is light coat coloured breeds still the SkT on all locations were similar during heat stress exposure. This difference with other studies could be due to absence of solar radiation in climate chambers as compared external heat stress studies in outside environment. Further, the non-significant change in SkT at head and flank region during morning, evening and night hours in contrast to the significant increase in SkT observed at shoulder region during these periods reflects the active involvement of shoulders in both these breeds for adapting to heat stress. In addition, the breed and BxTxD interaction also did not influence the SkTs at various locations reflecting the similar behaviour exhibited by both breeds for adapting to heat stress

environment. However, the experimental days influenced the SkT indicating that as the study progressed both the breeds were adapting to the situation.

Haematological Variables

Haematological indices are considered as reliable indicators of the health status of animals and aid in assessing stress levels due to various stressors (Onasanya *et al.,* 2015). There are numerous factors such as breed, sex, age, nutrition, diseases, physiological stage and environmental conditions may influence the haematological pattern of goats (Al-Eissa *et al.,* 2012; Bhat *et al.,* 2011). Both RBC and WBC concentration did not get altered due to heat stress in the study. Both these variables are very crucial for maintaining both the adaptation as well as the health status (Ribeiro *et al.,* 2016). The non-significant influence of heat stress on both these crucial haematological variables could reflect their better adaptation as RBCs are involved in carrying oxygen molecules for inducing effective RR well as their ability to keep intact their health as WBCs are involved in this process. Similar to RBC, the HGB, HCT, MVC, MCH, MCHC also did not differ across the experimental groups. This could be attributed to no difference in RBC across the groups as most of these variables are linked with this blood variable. However, there are reports which suggest heat stress affecting the quantitative and morphological features of blood cells such as the alterations in HCT values, RBC, HGB, MCV and MCHC (Ribeiro *et al.,* 2016, Habibu *et al.,* 2018). Similarly, Alam *et al.* (2011) reported higher number of RBC, HCT, HGB, and WBC in heat stressed goats. An elevated value of HGB was also established in heat stressed southern Nigeria dwarf goats which could be attributed to higher HGB requirement in the animal to meet the increased oxygen circulation during panting (Okoruwa, 2014; Sejian *et al.,* 2018). The no

difference in any of these variables in the current study suggests the immense potential of both Kanni and Kodi Aadu goat breeds in maintaining the blood cells which could aid in respiratory evaporative cooling mechanisms. Both HGB and HCT are considered important indicators of heat stress in animals and both these variables are expected to increase due to decrease in plasma volume (Ribeiro *et al.,* 2018). However, in this study no influence of heat stress was noticed in either of these variables suggesting the better thermo-tolerance of both these breeds.

Both the WBCs as well as differential leukocyte counts (DLC) did not differ across the experimental groups in this study. Heat stress activates the both the hypothalamo pituitary adrenal axis as well as sympatho adreno medullary axes to increase the neutrophils and decrease lymphocytes. Generally, the ratio of neutrophils to lymphocytes acts as a biomarker for heat stress in farm goats (Davis *et al.,* 2008; Habibu *et al.,* 2018). The non-significant difference in this ratio in the current study reflects the coping ability of both Kanni and Kodi aadu breeds to heat stress. There are conflicting reports on heat stress influence on both WBCs as well as DLCs (Alam *et al.,* 2011; Okoruwa, 2014). Alam *et al.* (2011) reported increased DLC in heat stressed goats with elevated levels of neutrophil, esonophil, lymphocyte and monocyte %. Further, the lower level of neutrophils in the circulation during heat stress indicates the compromised immune functions in heat stressed animals (Gupta and Mondal, 2019). Further, reduced DLCs during heat stress could be attributed to the elevated cortisol concentration in heat stressed animals (Caroprese *et al.,* 2012; El-Tarabany *et al.,* 2017) thus reflecting the compromised immune system of heat stressed animals. Such intact WBCs as well as DLCs in the current study suggests the supreme potential of these two breeds to keep

intact their health status during heat stress exposure. Further, the non-significant influence of heat stress on the plasma cortisol concentration in this study could be the reasons for the intact WBCs as well as DLCs in this study. Thus, the result on the haematological variables in the study establishes both the supreme adaptive potential of both Kanni and Kodi Aadu goat breeds by strongly supporting the respiratory evaporative cooling mechanisms as well as their ability to keep their immune system functions intact. Further, the non-significant correlation of THI with majority of haematological variables reflected the supremacy of both the breeds of targets to cope with heat stress. However, the significant negative correlation established for THI with RBC, WBC and granulocytes, HGB and HCT pointing towards the fact that these variables could be potential indicators for heat stress in these breeds. But the non-significant influence of heat stress on these variables clearly describes climate resilient capacity of both these breeds.

Blood biochemical variables

Alterations in the blood biochemical variables are expected as the animals tries to adapt to harsh climatic conditions thus reflecting the both the nutritional as well as health status of the animals (Singh *et al.,* 2016; Banerjee *et al.,* 2015; Aleena *et al.,* 2018). Plasma glucose did not differ between any of the groups indicating the effective adaptive efficiency of both the breeds in maintaining blood glucose level to support life sustaining activities. Adaptive mechanisms are biologically very costly process and demands continuous glucose supply to support both respiratory and cutaneous evaporative mechanisms. Thus, there are several reports on the impacts of heat stress on the plasma glucose level in farm animals (More and Sahni 1980; Sejian and Srivastava, 2010b; Shilja

et al., 2016). Further, breed factor also did not influence plasma glucose level indicating no difference in the adaptive potential of both the breeds. However, contrasting reports of significant breed factor influence on plasma glucose during heat stress exposure was reported in several studies (Mohammed *et al.,* 2016; Shilja *et al.,* 2016; Aleena *et al.,* 2018). Further both treatment and BxTxD interaction also did not influence plasma glucose concentration indicating that the blood glucose level remained constant without much alteration throughout the study period. Further, the non-significant influence in plasma cortisol concentration whose result will be discussed later in this report also supports the finding of non-influence of heat stress on blood glucose concentration. All these results point towards better climate resilient capacity of both Kanni and Kodi Aadu goat breeds.

Plasma total cholesterol was the only biochemical variable wherein all factors breed, treatment, experimental days and BxTxD interaction influenced its level in both the breeds. This shows that breed differences were established for the total cholesterol concentration. Therefore, the differences in plasma total cholesterol in the current study could be attributed primarily to the breed variation during heat stress exposure. This could be attributed to the fact that plasma cortisol did not show any variation for heat stress in the study and cholesterol is the precursor materials for cortisol biosynthesis. However, heat stress could not alter total cholesterol concentration between the stress groups KAHS and KOHS. However, contrasting findings of significant influence of heat stress was reported on the blood cortisol concentration in goats (Pandey *et al.,* 2012). In addition, heat stress induced similar effects for total cholesterol concentration in either breeds. This was evident from the no difference in cholesterol concentration between the

KAC and KAHS groups as well as between KOC and KOHS groups. This was in contrast to the previous findings in our laboratory wherein heat stress significantly increased cholesterol concentration in Malabari breed indicating the sensitivity of this breed to heat stress challenges (Aleena *et al.,* 2018).

Plasma total protein, albumin and globulin concentration did not differ between the groups. Further breed, treatment and BxTxD interaction also did not influence any of these variables indicating that the non-significant influence of heat stress on these variables persisted throughout the study duration. Similar observation was made by Aleena *et al.* (2018) in Osmanabadi, Malabari and Salem Black goat breeds and they attributed this to the extreme adaptive nature of these breeds to heat stress challenges. These results point towards the higher adaptive potential of both Kanni and Kodi Aadu breeds to heat stress. However, contrasting reports of significant influence of heat stress on total protein, albumin and globulin level was reported in Baladi, Zarabi goats (Helal *et al.,* 2010).

Like total cholesterol, the plasma triglycerides also showed similar findings and except treatment rest all factors including the interaction effect influenced its level in the study. This shows that heat stress did not influence plasma triglycerides in the study and the differences were primarily due to breed effect. However, heat stress induced decrease in triglycerides level was reported in small ruminants (Nazifi *et al.,* 2003; Pandey *et al.,* 2012). Similar observation of reduced triglycerides level was established by Aleena *et al.* (2018) in a recent study in heat stressed Osmanabadi and Salem Black goat breeds. The contrasting findings of non-significant influence of heat stress on triglyceride concentration in the current study reflects the extreme adaptive potential of both Kanni

and Kodi Aadu goat breeds compared to other breeds in above mentioned studies. In contrast to triglyceride variable, none of the factors influenced plasma urea concentration in the study. Similar non-significant influence of heat stress on plasma urea concentration was reported in other indigenous goat breeds (Shilja *et al.,* 2016; Aleena *et al.,* 2018).

Both blood AST and ALT are found to be associated with the thermo-tolerance in goats (Banerjee *et al.,* 2015). Heat stress did not influence either of these variables in the current study. This was in agreement with the findings of Aleena *et al.* (2018) in who reported no influence of heat stress on both AST and ALT in Malabari and Salem Black goat breeds. These authors attributed this to the low magnitude of heat stress experienced by these breeds being indigenous nature. However, in the present study although the breeds were exposed to very high magnitude (THI 94.76) of heat stress still this could not induce changes in both AST and ALT. Since these enzymes are strongly correlated with thermo-tolerance it could be inferred that both Kanni and Kodi Aadu goat breeds to be extremely adapted breeds with superior thermo-tolerance capacity. Likewise, Banerjee *et al.* (2015) also reported similar findings of non-significant influence of heat stress on AST in Sirohi and Barbari goats. However, these same authors reported significantly increased concentration of ALT in these breeds attributing this difference to breed variation.

These findings again indicate that heat stress was not able to induce much changes in the blood biochemical variables in the current study in both Kanni and Kodi Aadu goat breeds reflecting the intact nutritional status of these breeds even when exposed to extremely severe heat stress condition. This was in contrast to many findings on other goat breeds as discussed above which showed atleast certain degrees of changes

in the blood biochemical variables on subjecting them to heat stress. The significant observation here is that in comparison to other studies the magnitude of heat stress in the current study was extremely severe. But even this very high magnitude of heat stress could not induce any changes in their biochemical responses reflecting the potential of both Kanni and Kodi Aadu goat breeds to cope with heat stress challenges. Further, the non-significant influence of breed, treatment and day interaction on most of the biochemical variables indicated that this extreme adaptive nature in terms of maintaining constant levels of biochemical variables reflects the sound nutritional as well as health status of these breeds again reflecting the extreme climate resilience capacity of both Kanni and Kodi Aadu goat breeds. Further, the non-significant correlation of THI with majority of biochemical variables reflected the supremacy of both the breeds of targets to cope with heat stress. However, the significant negative correlation established for THI with plasma total cholesterol, total protein and urea pointing towards the fact that the heat stress was influencing these variables but not to the extent to induce significant changes in their concentration.

Endocrine Variables

The endocrine responses are considered crucial adaptive responses in farm animals (Sejian *et al.,* 2010; Shilja *et al.,* 2017; Sejian *et al.,* 2018). This adaptive pathway usually provides concrete indicators for heat stress in farm animals. Blood cortisol is considered the principal indicator for heat stress in goats (Ali and Hayder, 2008). Cortisol are secreted from adrenal cortex in response to heat stress under the stimulation of corticotrophin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) from hypothalamus and anterior pituitary, respectively (Ghassemi Nejad *et al.,* 2017;

Sejian *et al.,* 2018). The heat stress did not influence the plasma cortisol production in the current study. Irrespective of breed, the level of plasma cortisol was comparable between the experimental groups. Further, the breed, treatment, experimental day factors and their interactions also did not influence the plasma cortisol concentration. This indicates that the trends obtained in the plasma cortisol concentration remained the same throughout the study. Thus, it was evident from the study that the heat stress of very high magnitude also could not induce any changes in the plasma cortisol concentration. This shows that even a THI difference of over 25.34 between the control (69.42) and heat stress (94.76) groups in either breed did not influence the cortisol production. This reflects the extreme adaptive nature of both these breeds and they were able to cope with heat stress quite efficiently. Similarly, such results of heat stress induced higher cortisol concentration were also reported in other goat breeds (Tajik *et al.,* 2016; Chergui *et al.,* 2017). In a recent study on three different indigenous goat breeds, Aleena *et al.* (2018) established breed differences for cortisol concentration in response to heat stress. Heat stress significantly increased the plasma cortisol concentration only in Malabari breed as compared to Osmanabadi and Salem Black breed. These authors attributed this to the extreme adaptive nature of Salem Black and Osmanabadi goat breed. However, in the current study although the heat stress induced to both Kanni and Kodi Aadu goat breeds was of very high magnitude as compared to Aleena *et al.* (2018) study, still this could not bring in any changes in plasma cortisol level across the experimental groups. From this result it could be inferred that both Kanni and Kodi Aadu goat breeds could be better climate resilient breeds even than Osmanabadi and Salem Black breed.

Thyroid hormones are metabolic activity controlling hormones and their concentration in the blood could serve as indicators of heat stress (Pragna *et al.,* 2017). Further, the levels of both T_3 and T_4 could also serve as indicators for the nutritional status of the animals (Sejian *et al.,* 2014). Similar to plasma cortisol concentration, heat stress did not influence the levels of both plasma T_3 and T_4. Further, the breed, treatment and their interaction also did not influence thyroid hormone concentrations indicating that the non-significant effect persisted throughout the study period. The results on the thyroid hormone concentration in the study clearly demonstrates that both Kanni and Kodi Aadu goat breeds did not rely on metabolic adaptation. This is evident from the comparable levels of both T_3 and T_4 levels across the experimental groups. In a recent study conducted in three indigenous goat breeds it was established that heat stress significantly reduced T_3 but did not altered T_4 (Pragna *et al.,* 2017). These authors established that T_3 could act as ideal indicator to reflect heat stress response in indigenous Osmanabadi, Malabari and Salem Black goat breeds. These results show that there are breed variations in utilizing the metabolic response to cope with heat stress. The most important observation here is that inspite of very high magnitude of heat stress in the current study as compared to the stress level in Pragna *et al.* (2017) study, the non-significant effect of heat stress on both T_3 and T_4 concentration in this study it could be inferred that both Kanni and Kodi Aadu may be considered better thermo-tolerant breeds than Osmanabadi, Malabari and Salem Black breeds. Further, the non-significant correlation of THI with any of the endocrine variables in the study clearly demonstrates that both Kanni and Kodi Aadu goat breeds are extremely climate resilient.

Molecular mechanisms

The *HSP* genes are highly conserved and temperature sensitive (Yadav *et al.,* 2016). Their expression was breed specific, species specific and tissue specific (Afsal *et al.,* 2019). The HSPs are essential for processing of stress- denatured proteins (Rout *et al.,* 2016). To establish the resilience capacity of an indigenous animals, research efforts are needed based on quantification of these genotypic traits as these could serve as ideal biological makers for reflecting their heat tolerant capacity. In this line, the study provided some valuable inputs for higher resilience capacity of both Kanni and Kodi Aadu goat breed based on changes in the genotypic traits associated with heat shock response. The *HSF-1* is linked to intrinsic properties of the molecular conformation and is regulated by several factors and heat stress was found to play a crucial role in this regulation (Madhusoodan *et al.,* 2020). The results showed non-significant changes for the expression pattern of HSF-1 even at 94.76 THI value reflecting that the HSF-1 machinery was not activated in both Kanni and Kodi Aadu goat breeds. Similar results but with less heat stress magnitude of THI value of 86.5 was established in Salem Black goat breeds (Madhusoodan *et al.,* 2020). The *HSF-1* primarily triggers the heat shock response in goat by stimulating the production of *HSPs* and this acts as the stimulus for regulating the cellular stress response in heat stressed animals (Madhusoodan *et al.,* 2020). Further, the *HSF-1* was found to be associated with heat tolerance in farm animals (Das *et al.,* 2016; Li *et al.,* 2011). The comparable levels of *HSF-1* expression pattern between the control and heat stress groups in both Kanni and Kodi Aadu goat breeds proves that the *HSF-1* machinery was not activated in both these breeds.

Similarly, the expression pattern of *HSP-27* mRNA expression also did not differ between the control and heat stress groups of both Kanni and Kodi Aadu goat breeds again reflecting the extreme adaptive potential of both these breeds to heat stress challenges. Zhang *et al.* (2010) reported role for HSP-27 in reducing reactive oxygen species through increased production of glutathione to prevent oxidative stress. Further, Parida *et al.* (2020) established *HSP-27* to be a marker to protect myocardial cells during heat stress exposure in goats. In addition, Archana *et al.* (2017) reviewed and identified that *HSP-27* is one of the important biomarkers for heat stress in farm animals and they observed that this gene has a cellular protective role during heat stress. However, in our study heat stress did not influence *HSP-27* expression pattern in both the goat breeds. This shows that there are breed variation in the expression pattern of *HSP-27* gene during heat stress exposure. The non-significant influence of heat stress on *HSP-27* gene expression in both Kanni and Kodi Aadu goat breeds even after exposing them to heat stress of very high magnitude clearly establishes their climate resilience capacity. Further, the study establishes that the heat stress induced in the study did not induce cellular changes in both the breeds.

Like *HSP-27*, there was no difference in the expression pattern of *HSP-60* mRNA expression between the control and heat stress groups of both the targeted breeds in this study. The *HSP-60* was established to be the most important mitochondrial chaperones which participate in environmental stress responses (Shi *et al.,* 2016). Further, *HSP-60* was observed to be activated to prevent protein denaturation under heat stress (Martin *et al.,* 1992). In addition, Sahu *et al.* (2019) has observed increased mRNA expression pattern of *HSP-60* in heat stressed goat myocardial cells. These findings point towards

the active involvement of *HSP-60* in heat stress response and also suggest that there could be breed variation for its involvement during heat stress. Considered a very important chaperone which takes part in heat stress response and whose level was not altered in this study in either of the breeds suggests the supreme thermo-tolerance of Kanni and Kodi Aadu goat breeds.

Similarly, there were no significant differences among the experimental groups for *HSP-70* expression pattern in the current study. The level of *HSP-70* expression was comparable in both Kanni and Kodi Aadu goat breeds both under thermo-neutral as well as heat stress condition. The *HSP-70* gene was considered ideal biological marker for quantifying heat stress response in domestic livestock and infact this particular molecular chaperone was considered the cellular thermometer in heat stressed animals (Gaughan *et al.,* 2013; Manjari *et al.,* 2015; Niyas *et al.,* 2017). In a recent study in heat stressed Salem Black goat breed Madhusoodan *et al.* (2020) demonstrated significantly lower expression pattern of hepatic *HSP-70* gene. However, majority of heat stress studies in goat have observed significant upregulation of *HSP-70* gene expression irrespective of its target tissue for its expression (Rout *et al.,* 2016; Niyas *et al.,* 2017; Aleena *et al.,* 2018). However, in the current study heat stress of very high magnitude also could not induce changes in the *HSP-70* gene expression pattern between the control and heat stress groups across both the breeds. These findings prove that there could be strong breed variation in *HSP-70* expression pattern. The result shows the extreme adaptive capacity of both Kanni and Kodi Aadu goat breeds to heat stress challenges.

The *HSP-90* mRNA expression pattern also showed similar pattern like that of other HSPs in this study. There were no any variations observed on the expression pattern of

HSP-90 gene between the control and heat stress groups in both the targeted breeds in this study. The HSP-90 gene was closely associated with HSP-70 gene to determine thermo-tolerance in most of the farm animals (Yu *et al.,* 2008; Belhadj Slimen *et al.,* 2016; Shilja *et al.,* 2016). Further, there are reports in goats which established increased expression pattern for HSP-90 gene after heat stress exposure (Gupta *et al.,* 2013; Sharma *et al.,* 2013). In addition, *HSP-90* gene was also considered an important molecular marker to reflect heat stress impact in farm animals *(*Belhadj Slimen *et al.,* 2016; Archana *et al.,* 2017). However, in our study even very severe heat stress also could not induce changes in the expression pattern of *HSP-90* gene. This was in contrast to the previous findings discussed above on the expression pattern of *HSP-90* gene expression. From these findings we can ascertain that both Kanni and Kodi Aadu goat breeds possess extreme climate resilience capacity which may provide them the ability to survive in tropical environment.

In contrast to all other *HSP* gene expression patterns in this study, HSP-110 showed significant variation in its expression pattern. In both the breeds, heat stress significantly increased the expression pattern of HSP-110 gene. This was evident from the significant upregulation of HSP-110 gene expression in the KAHS and KOHS groups as compared to KAC and KOC groups respectively. Not much research reports are available relating heat stress with HSP-110 in farm animals. However, in laboratory animal models HSP-110 was found to be associated in protecting heat-denatured proteins and it thus confers cellular thermo resistance during heat stress condition (Oh *et al.,* 1997). Further, there are few reports which established increased expression pattern of HSP-110 after heat stress exposure in goats (Gupta *et al.,* 2013; Sharma *et al.,* 2013). This was similar to the

findings reported in this study. This shows that *HSP-110* gene could play a significant role during heat stress in goats. This was a very unique finding in this study and therefore it could be inferred that *HSP-110* may play a significant role in determining the thermo-tolerance in both Kanni and Kodi Aadu goat breeds. Thus, *HSP-110* gene could be considered ideal molecular marker for quantifying heat stress response in both indigenous Kanni and Kodi Aadu goat breeds. Further, the non-significant correlation of THI with most of the HSP genes in the study clearly demonstrates that both Kanni and Kodi Aadu goat breeds are extremely climate resilient. The HSP-110 is the only HSP gene which showed positive correlation with THI could indicate that this gene could be the ideal molecular marker for quantifying heat stress response in both Kanni and Kodi Aadu.

Closing remarks

The study is first of its kind to prove the hypothesis that goats are the ideal climate animal model. The results from the study point towards the better climate resilience capacity of both the breeds targeted in this study. Both the breeds performed mostly similarly for some of the vital adaptive variables which imparts them the potential to survive in hot tropical environment. The stress model induced in the study could able to successfully subject the animals to extremely severe heat stress which was evident from the 94.76 THI in the heating climatic chamber. However, still this extremely severe heat stress could not elicit completely the stress response in both the breeds. Both the breeds exhibited excellent climate resilience by coping with the extremely severe heat stress. Both the breeds achieved this target principally through behavioural and physiological mechanisms. Within the set of these behavioural and physiological variables also both the breeds adopted certain unique way to reach to the ultimate common pathway of

maintaining the thermal balance. However, comparatively both the breeds showed a lot of differences with other breeds of goat with the study conducted elsewhere in different tropical climate. Further, to our knowledge the heat stress induced in this study was of very high magnitude as compared to other studies. This establishes a lots of breed variation within goat to cope with heat stress challenges. Since even the very high magnitude of heat stress could not induce heat stress response at blood biochemical, endocrine and molecular mechanisms and both the breeds were able to maintain thermo-tolerance based on only behavioural and physiological responses. This shows their extreme climate resilience capacity. The study also established that RR, RT and *HSP-110* could be the most reliable biological markers for quantifying heat stress response in both Kanni and Kodi Aadu goat breeds. Further, the physiological responses clearly indicated the better recovery efficiency from heat stress in Kodi Aadu goat breed as compared to Kanni Aadu breed. On comparative analysis of the current study results with other studies in other indigenous goat breeds in southern India clearly establishes the supremacy of both these breeds to adapt to the extreme climate condition. Thus, it could be inferred that both Kanni and Kodi Aadu goat breeds could be ideal breeds to survive in Southern India. However, more detailed study involving Omics technologies could prove this hypothesis in a concrete way. On comparing the results with other studies, lots of breed variations were established even with indigenous goat breeds. This necessitates the need to study the climate resilience in all indigenous goat breeds as this approach could help to identify agro-ecological zone-specific goat breeds which could help farming community to get rich dividends for their input costs by sustaining the productivity of these specific

adapted breeds in a particular locality. Therefore, promoting such breeds for breeding as well as disseminating to the local farmers may ensure their livelihood security.

SUMMARY & CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Among the various agriculture sectors, livestock are established to be highly climate resilient and are expected to play a significant role in ensuring food security by 2050. However, the projected climate change associated feed and fodder resources reduction poses serious threat to large ruminant livestock production. Hence, small ruminants and particularly goats are being projected as ideal climate animal model to cope with climate change associated adversities due to their higher thermo-tolerance capacity. However, such information on the indigenous breeds is very less. Given the significance of goat to food security in future it becomes essential to generate more baseline information governing thermo-tolerance in unexplored native track goat breeds. Such efforts could help the researchers to identify agro-ecological zone-specific goat breeds which could play a significant role to ensure livelihood securities of the poor and marginal farmers. Thus, the current study is one such attempt in establishing the thermo-tolerance in two indigenous goat breeds Kanni Aadu and Kodi Aadu.

To prove this hypothesis a study was conducted for a period of 45 days in twenty four one year old female animals of Kanni and Kodi Aadu goat breeds (12 animals in each breed). The animals were randomly divided into four groups as Kanni Aadu Control (KAC; n=6); Kanni Aadu Heat Stress (KAHS; n=6); Kodi Aadu Control (KOC; n=6); Kodi Aadu Heat Stress (KOHS; n=6). Both KAC and KOC animals were kept in thermo-neutral zone climate chamber exposing them to 24°C while the KAHS and KOHS group

animals were kept in heating climate chamber under simulated heat stress model with a temperature range of 36°C to 40°C and relative humidity range of 35-65% to simulate the natural heat stress condition of their origin. The control and heat stress group animals were exposed to their respective climatic condition for a period of six hours daily for the 45 days duration of the study. Both the group animals were fed and watered individually in their respective chambers. The animals were offered with the diet composition of 60% roughage and 40% concentrate mixture. Their behavioural responses were recorded continuously for six hours (10.00 h to 16.00 h) at fortnightly interval. The physiological responses were recorded four times a day (8.00 h; 14.00 h; 20.00 h and 2.00 h) at fortnightly interval. Blood samples were collected at fortnightly interval and separated into three aliquots. The first aliquot was subjected for haematological variable recording while the second aliquot was subjected for plasma separation. The plasma samples were subjected for biochemical and endocrine variables analysis. The third aliquot of blood samples were subjected for peripheral blood mononuclear cells (PBMC) isolation and subjected total RNA isolation and cDNA conversion for gene expression study.

The microclimate condition prevailed in both the climatic chambers induced thermo-neutral condition and heat stress condition for KAC and KOC groups and KAHS and KOHS groups, respectively throughout the study period. This was evident from the significantly higher values for all meteorological variables recorded during the exposure duration of six hours in the heating chamber as compared to TNZ chamber. Further, the temperature and relative humidity derived THI established this difference in microclimate across the chambers. The comparable THI values during morning between the chambers and significantly higher THI value of 94.76 as compared to 70.57 in the TNZ chamber

during afternoon clearly demonstrates the differences in the microclimate across the chambers.

The non-significant influence of heat stress on BW in the current study clearly demonstrates that the animals were not getting affected by the extreme THI they were exposed. This was evident from the fact that the animals did not deviated the energy from the production to adaptive pathway as usually was the case with heat stress affected animals compromising their BW they maintain heat dissipation mechanisms. This reflects the better climate resilient capacity of both Kanni and Kodi Aadu goat breeds.

Goats exhibit certain behavioural responses to cope with adverse environments. The results from the current study point towards the fact that every indigenous breed has their own way of behaviourally adapting to the heat stress exposure. This was evident in the current study also as both Kanni and Kodi Aadu goat breeds showed different level of changes in ST, LT, DF, DeF and RuT indicating their own unique way to behaviourally counter heat stress challenges. However, ultimately the most crucial behaviour response of FI and WI did not differ between their respective control and heat stress groups reflecting the different approaches exhibited by these breeds to reach the common target of maintaining both feed and WI which was considered the ultimate variables for maintaining the thermal balance in these breeds.

Heat stress induced in the study was able to elicit heat stress response in both the breeds and this was evident from the significantly higher RRA in both KAHS and KOHS groups from the second week onwards until end of the study duration. The higher PRA established in both KAHS and KOHS groups as compared to their respective control

groups exhibited the better heat dissipation mechanisms in both these breeds. Likewise, the significantly higher RTA in both KAHS and KOHS groups indicates that RT could be the ideal biomarker for measuring heat stress in these breeds. The SkT recorded at head, shoulder and flank are significantly different among the groups only during afternoon but non-significant during morning, evening and night. Heat stress significantly increased both SR and PS in the afternoon in both the breeds as compared to their respective control groups. This increase in SR and PS was again comparable between KAHS and KOHS groups indicating that there is no difference between the breeds for both SR and PS on exposure to heat stress.

Both RBC and WBC concentration did not get altered due to heat stress in the study. Similar to RBC, the HGB, HCT, MVC, MCH, MCHC also did not differ across the experimental groups. This could be attributed to no difference in RBC across the groups as most of these variables are linked with this blood variable. Both the WBCs as well differential leukocyte counts (DLC) did not differ across the experimental groups in this study. Such intact WBCs as well as DLCs in the current study suggests the supreme potential of these two breeds to keep intact their health status during heat stress exposure. Thus the result on the haematological variables in the study establishes the supreme adaptive potential of both Kanni and Kodi Aadu goat breeds by strongly supporting the respiratory evaporative cooling mechanisms as well as their ability to keep their immune system functions intact.

Plasma total cholesterol was the only biochemical variable wherein all factors breed, treatment, experimental days and their interaction influenced its level. However, plasma glucose, total protein, albumin, globulin, triglycerides, urea concentration did not

differ between the groups reflecting the extreme adaptive potential of both Kanni and Kodi Aadu goat breeds. Similarly, heat stress did not influence both plasma AST and ALT in the current study. Since these enzymes are strongly correlated with thermo-tolerance and no influence of heat stress on these variables could reflect the superior thermo-tolerance capacity in both Kanni and Kodi Aadu goat breeds.

The heat stress did not influence the plasma cortisol production in the current study. Irrespective of breed, the level of plasma cortisol was comparable between the experimental groups. This reflects the extreme adaptive nature of both these breeds and they were able to cope with heat stress quite efficiently. Similar to plasma cortisol concentration, heat stress did not influence the levels of both plasma T_3 and T_4. The results on the thyroid hormone concentration in the study clearly demonstrates that both Kanni and Kodi Aadu goat breeds did not relied on metabolic adaptation. This is evident from the comparable levels of both T_3 and T_4 levels across the experimental groups.

The results showed non-significant changes for the expression pattern of *HSF-1, HSP-27, HSP-60, HSP-70* and *HSP-90* genes even at 94.76 THI value reflecting that the heat shock machinery was not activated in both Kanni and Kodi Aadu goat breeds. The non-significant influence of heat stress on majority of heat shock protein genes expression in both Kanni and Kodi Aadu goat breeds even after exposing them to heat stress of very high magnitude clearly establishes their climate resilience capacity. In contrast to all other *HSP* gene expression patterns in this study, *HSP-110* showed significant variation in its expression pattern. In both the breeds, heat stress significantly increased the expression pattern of *HSP-110* gene. This was evident from the significant upregulation of HSP-110 gene expression in the KAHS and KOHS groups as compared

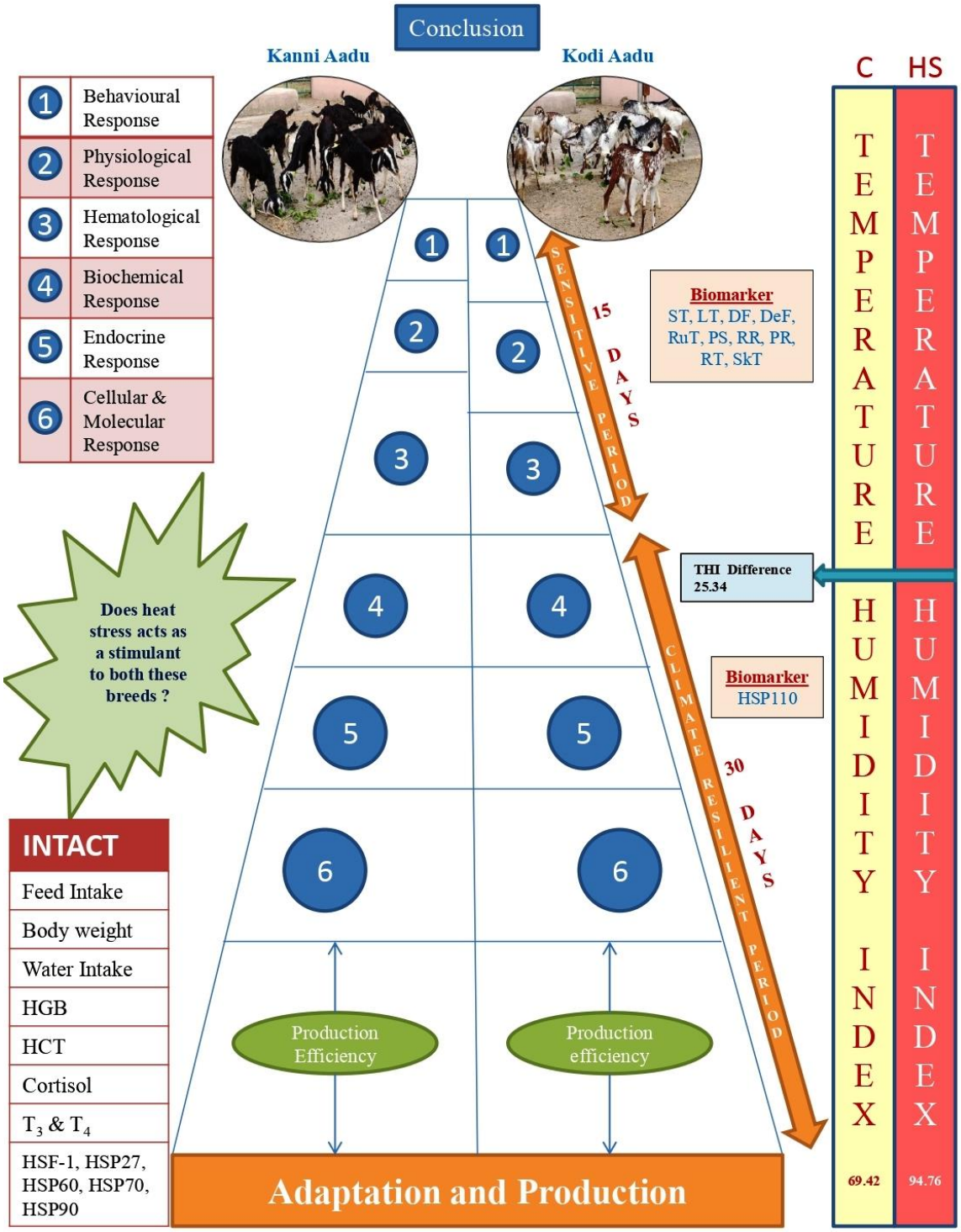
to KAC and KOC groups respectively. This was a very unique finding in this study and therefore it could be inferred that *HSP-110* may play a significant role in determining the thermo-tolerance in both Kanni and Kodi Aadu goat breeds. Thus, *HSP-110* gene could be considered ideal molecular marker for quantifying heat stress response in both indigenous Kanni and Kodi Aadu goat breeds.

The finding from this study provides crucial scientific information with respect to promoting goat as ideal climate animal model. The study provides strong evidence for extreme adaptive nature of indigenous goat breeds to heat stress challenges. The study is first of its kind in establishing crucial basic information on various adaptive mechanisms of indigenous Kanni and Kodi Aadu goat breeds which imparts them the potential to survive in harsh tropical environment. Both the breeds performed mostly similarly for some of the vital adaptive variables which imparts them the potential to survive in hot tropical environment. The stress model induced in the study could able to successfully subject the animals to extremely severe heat stress which was evident from the 94.76 THI in the heating climatic chamber. However, still this extremely severe heat stress could not elicit completely the stress response in both the breeds. Both the breeds exhibited excellent climate resilience by coping with the extremely severe heat stress. Both the breeds achieved this target principally through behavioural and physiological mechanisms. Within the set of these behavioural and physiological variables also both the breeds adopted certain unique way to reach to the ultimate common pathway of maintaining the thermal balance. However, comparatively both the breeds showed a lot of differences with other breeds of goat with the study conducted elsewhere in different tropical climate. Further, to our knowledge the heat stress induced in this study was of

very high magnitude as compared to other studies. This establishes a lots of breed variation within goat to cope with heat stress challenges. Even the very high magnitude of heat stress could not induce heat stress response at blood biochemical, endocrine and molecular mechanisms levels and both the breeds were able to maintain thermo-tolerance based on only behavioural and physiological responses. This shows their extreme climate resilience capacity.

The study also established that RR, RT and *HSP-110* could be the most reliable biological markers for quantifying heat stress response in both Kanni and Kodi Aadu goat breeds. Further, the physiological responses clearly indicated the better recovery efficiency from heat stress in Kodi Aadu goat breed as compared to Kanni Aadu breed. On comparative analysis of the current study results with other studies in other indigenous goat breeds in southern India clearly establishes the supremacy of both these breeds to adapt to the extreme climate condition. Thus, it could be inferred that both Kanni and Kodi Aadu goat breeds could be ideal breeds to survive in Southern India. However, more detailed study involving Omics technologies could prove this hypothesis in a concrete way. On comparing the results with other studies, lots of breed variations were established even with indigenous goat breeds. This necessitates the need to study the climate resilience in all indigenous goat breeds as this approach could help to identify agro-ecological zone-specific goat breeds which could help farming community to get rich dividends for their input costs by sustaining the productivity of these specific adapted breeds in a particular locality. Therefore, promoting such breeds for breeding as well as disseminating to the local farmers may ensure their livelihood security.

This strong baseline information generated in this study would be very valuable in promoting both these breeds as ideal breeds to survive in hot semi-arid environment. It remains to be seen though how these animals performed for the other cellular and molecular changes associated with heat stress through advanced genomics and proteomics approach. With the projected adverse impacts of climate change on livestock production and goat has been projected as ideal climate animal model, the finding from this study has greater significance in terms of providing conclusive scientific evidence to support this argument. This warrants more such research efforts to generate such valuable information on other indigenous goat breeds. Such efforts could be the way forward in establishing agro-ecological zone-specific goat breeds which could be propagated among the local farmers to ensure their livelihood security in the changing climate scenario. It is also very essential to establish similar simulated heat stress effect on the productive variables such as growth, meat production, reproduction as well as maintaining the immune status as this could provide evidence for whether these breeds adapt by compromising their production. If their production potential remains intact then these breeds could be the ideal ones for breeding to evolve animals with supreme adaptive potential. This warrants more research efforts in trying to establish the impact of simulated hot semi-arid environment on the productive potential of both Kanni and Kodi Aadu breeds. Such information would be very valuable from a climate change perspectives to propagate these breeds among the poor and marginal farmers for providing sustained income to these economically weaker population. The conclusion from the current study is depicted in Fig. 4.22.



ST-Standing time; LT-Lying time; DF-Drinking Frequency; DeF-Defecating Frequency; RuT- Rumination time; PS- Pulse Rate; RT-Rectal temperature; RR-Respirtion rate; SkT-Skin temperature; THI- Temperature humidity index; HSP-Heat Shock Protein; C-Control; HS-Heat stress; HGB-Hemoglobin; HCT-Hematocrit.

Fig. 4.22: Conclusion of the current study

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COMPARATIVE ASSESSMENT OF CLIMATE RESILIENT CAPACITY OF TWO INDIGENOUS GOAT BREEDS BASED ON CHANGES IN BOTH PHENOTYPIC AND GENOTYPIC TRAITS

By

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Abstract of Thesis

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ABSTRACT

Given the significance of goat to food security in future it becomes essential to generate more baseline information governing thermo-tolerance in unexplored native track goat breeds. Therefore, this study is one such attempt in establishing the thermo-tolerance in two indigenous goat breeds Kanni Aadu and Kodi Aadu. The study was conducted for a period of 45 days in twenty four one year old female animals. The animals were randomly divided into four groups as KAC (Kanni Aadu Control; n=6); KAHS (Kanni Aadu Heat Stress; n=6); KOC (Kodi Aadu Control; n=6); KOHS (Kodi Aadu Heat Stress; n=6). Both KAC and KOC animals were kept in thermo-neutral zone climate chamber while the KAHS and KOHS group animals were kept in heating climate chamber. Both the group animals were fed and watered individually in their respective chambers. The animals were offered with the diet composition of 60% roughage and 40% concentrate mixture. The weather variables and the derived THI values clearly demonstrated extreme heat stress condition being imposed on the heat stress groups of the respective breeds. The non-significant influence of heat stress on BW in the current study clearly demonstrates that the animals were not getting affected by the extreme THI they were exposed. Further, significantly ($P<0.01$) increased standing time (ST), lying time (LT), drinking frequency (DF), defecating frequency (DeF), rumination time (RuT), respiration rate (RR) and rectal temperature (RT) in the heat stress groups of both the breeds clearly indicated the stressful condition experienced by these animals. However, comparatively both the breeds performed almost similar with respect to adapting to the simulated hot semi-arid condition. The study is first of its kind to prove the hypothesis

that goats are the ideal climate animal model which was evident from the fact that even a very high THI did not influence most of the adaptive variables. Almost negligible differences were obtained for the levels of most of the indicators of heat stress such as RR, RT, panting score (PS) and skin temperature (SkT) between the KAHS and KOHS groups. Further, most of the blood biochemical and endocrine variables did not differ across the groups. In addition, except *HSP110* gene, most of the heat tolerant gene expression patterns were comparable in the heat stress group animals of both these breeds. Thus, the results from the study point towards the better climate resilience capacity of both Kanni and Kodi Aadu goat breeds which imparts them the ability to survive in hot and humid tropical environment.

Keywords: Adaptation; Climate; Drought-tolerance; Goat; HSP110; Thermo-tolerance