

**ASSESSING THE DIFFERENCES IN BODY WEIGHT CHANGES,
RUMEN FERMENTATION PROFILE AND METABOLIC ACTIVITY
BETWEEN DIFFERENT INDIGENOUS BREEDS OF GOATS
SUBJECTED TO SUMMER HEAT STRESS**

by

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(2012-20-119)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

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ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

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DECLARATION

I, hereby declare that this thesis entitled “**ASSESSING THE DIFFERENCES IN BODY WEIGHT CHANGES, RUMEN FERMENTATION PROFILE AND METABOLIC ACTIVITY BETWEEN DIFFERENT INDIGENOUS BREEDS OF GOATS SUBJECTED TO SUMMER HEAT STRESS**” 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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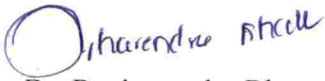
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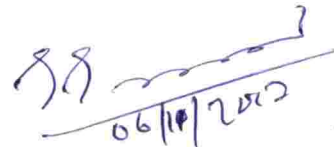
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*Dedicated to
my mentor
and
my family*

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SYMBOLS AND ABBREVIATIONS

(NH₄)₂SO₄- Ammonium Sulfate

ACP- Acid Phosphatase

ADG- Average Daily Gain

ALP- Alkaline Phosphatase

ANOVA- Analysis of variance

AT- Ambient Temperature

BCS- Body Condition Score

BL- Body Length

BMI- Body Mass Index

bp- base pair

BW- Body Weight

CD- Chest Depth

cDNA- complementary DNA

CH₄- Methane

CO₂- Carbon dioxide

DMI- Dry Matter Intake

EDTA- Ethylenediaminetetraacetic Acid

FAO- Food and Agriculture Organization

g- gram

GAPDH- Glyceraldehyde 3-phosphate dehydrogenase

GC- Gas Chromatography

GDP- Gross Domestic Product

GH- Growth Hormone

GHR- Growth Hormone Receptor

H₂- Hydrogen

H₂O- Water

HG- Heart Girth

HgCl₂- Saturated Mercuric Chloride

hGH- Human Growth Hormone

HPA- Hypothalamic-Pituitary-Adrenal Axis

HRP- Horse Radish Peroxidase

HW- Hip Width

ICAR- - Indian Council of Agricultural Research

IGF-1- Insulin like Growth Factor

IPCC- Intergovernmental Panel on Climate Change

IU- International Unit

IU/L- International Units Per Litre

Kg- kilogram

KR- Kleiber Ratio

LCD- Liquid Crystal Display

LH- Luteinizing Hormone

m- Metre

MaxT-Maximum Temperature

MC-Malabari Control

mg- Milligram

MHS- Malabari Control

MinT-Minimum Temperature

mm- Millimetre

mmol/L- Millimoles per litre

mRNA- messenger RNA

NaOH- Sodium Hydroxide

ng- Nanogram

ng/ml- Nanogram/milliliter

NH₃-N- Ammoniacal Nitrogen

NRC- National Research Council

°C- Degree Celsius

OC- Osmanabadi Control

OD- Absorbance

OHS- Osmanabadi Heat Stress

PBMC- Peripheral Blood Mononuclear Cell

PCR- Polymerase Chain Reaction

PNPP-DEA- P-Nitrophenyl Phosphate- Diethanolamine

PST-Pen Surface Temperature

R²- Coefficient of Determination
RGR- Relative Growth Rate
RH- Relative Humidity
RNAase- Ribonuclease
rpm- Revolutions per minute
RTqPCR- real-time quantitative polymerase chain reaction
RuH- Rump Height
SBC- Salem Black Control
SBHS- Salem Black Control
SE- Standard Error
SRH- Somatotropin Releasing Hormone
SRL- Strained Rumen Liquor
STH- Somatostatin
T₃- Triiodothyronine
T₄- Thyroxine
Tdb- Dry bulb temperature
THI- Temperature Humidity Index
TMB- 3,3',5,5'-Tetramethylbenzidine
TRH- Thyrotropin-Releasing Hormone
TRT- Treatment
TSH- Thyroid-Stimulating Hormone
Twb- Wet bulb temperature

USA- United States of America

UV- Ultra Violet

VFA- Rumen volatile Fatty Acid

TVFA- Total Rumen volatile Fatty Acid

WH- Wither Height

μg - Microgram

μL - Microlitre

μM - Micrometre

INTRODUCTION

CHAPTER 1

INTRODUCTION

Livestock sector plays an important role in global economy. It accounts 40% of the world's agricultural Gross Domestic Product (GDP). Livestock is a main source of income for poor people around the globe and it provides employment for more than 1.3 billion people (FAO, 2009). Livestock sector contributes milk, meat, wool, hides, egg, manure etc. However, currently production from livestock is decreasing due to the increased frequency of extreme weather events. Climate change affects livestock sector through many ways by altering feed-grain production- price and availability, quality of pastures and forage crop production, water availability and quality, pest and disease outbreak, and by affecting directly the animal production, reproduction and health (IPCC, 2007).

Indigenous breeds are well adapted to the agro-climatic conditions where they have evolved and are hardy enough to thrive well on poor forage and stressful conditions where high yielding exotic and crossbred animals succumb (Akinyi, 2008). Further, the indigenous breeds play a vital role in the economy and food security of poor and marginal farmers around the globe. In addition, the smaller size of indigenous goat breeds as compared to exotic and crossbred goats is a genetic adaptive strategy synchronized with the available nutrient resources (Nyamushamba *et al.*, 2017). Although the indigenous breeds are expected to adapt better due to their inherent genetic potential, their superior adaptive ability has not been validated in several indigenous breeds (Sansthan and Köhler-Rollefson, 2005). Even though the production potential of indigenous breeds are less compared to exotic and crossbred goats they possess a stable production during testing conditions where high producing animals fail to maintain their productive performance.

Growth of an animal is influenced by environmental and genetic factors. Importance of growth performance in livestock has gained wider attention from the

researchers around the world since last two to three decades (Bourdon, 2000). Growth, particularly the pre-weaning growth is a major selection criterion for the meat breed animals and is considered a crucial factor which determines the meat production. Reduction in growth performance due to the variation in the environmental factors is an important limiting factor that threatens meat industry in the tropical region (Mpofu *et al.*, 2017).

Among the various environmental stressors, heat is a major stressor that affects the production performance of livestock species, particularly in the tropical region. Elevated ambient temperature combined with high relative humidity (RH) during the summer season is a major threat that challenges the livestock production in the tropical regimes of the world (Naqvi and Sejian, 2011). Generally, animals reduce the growth and other productive and reproductive functions during the summer season in order to adapt to the hot environment during the summer (Darcan and Silanikove, 2017). Summer season is also associated with reduced feed intake and altered endocrine responses in the animal. Hyperthermia during the summer season is a major threat in the livestock economy (Alam *et al.*, 2013).

Reduced growth performance associated with summer heat stress is a common phenomenon in the tropical and sub-tropical regions of the globe. Decreased anabolic activity due to the reduced feed intake and enhanced tissue catabolism are the major reasons for the growth retardation in livestock. Several researches have reported alteration in the growth parameters during thermal exposure (Popoola *et al.*, 2014; Habibu *et al.*, 2016; Niyas *et al.*, 2015). Among the several growth variables, body weight (BW) is the first and foremost factor which gets affected due to the heat stress in livestock. In a study conducted in West African dwarf goats exposed to summer heat stress, average daily gain (ADG) was reported to be reduced which could be attributed to the decrease in feed intake in these animal (Popoola *et al.*, 2014). Further, Habibu *et al.* (2016) reported a significant reduction in body mass index (BMI) in Sahel and Red

Sokoto kids after thermal exposure. Furthermore, Niyas (2015) reported a significant reduction in body condition score (BCS) in heat stressed Osmanabadi goats.

Indigenous breeds are more adapted to sudden environmental fluctuations and disease outbreaks than exotic and crossbred goats (Alamer, 2003). There are several unique adaptive mechanisms in these indigenous breeds that help them to survive in a stressful environment. Altering the metabolic activities in an effort to minimize the internal heat load to cope to the external hot environment is a common phenomenon in the indigenous breeds (Wheelock *et al.*, 2010). There are also reports suggesting the seasonal rhythmicity in thyroid gland function in altering the metabolic activity in domestic ruminants (Piccione *et al.*, 2012; Dardente *et al.*, 2014). Regardless of species and breed all animals' exhibit seasonality in their metabolic functions that reflects the ability of animal's endogenous adaptive mechanisms to react with the regular environmental changes associated with seasons (Duarte *et al.*, 2010).

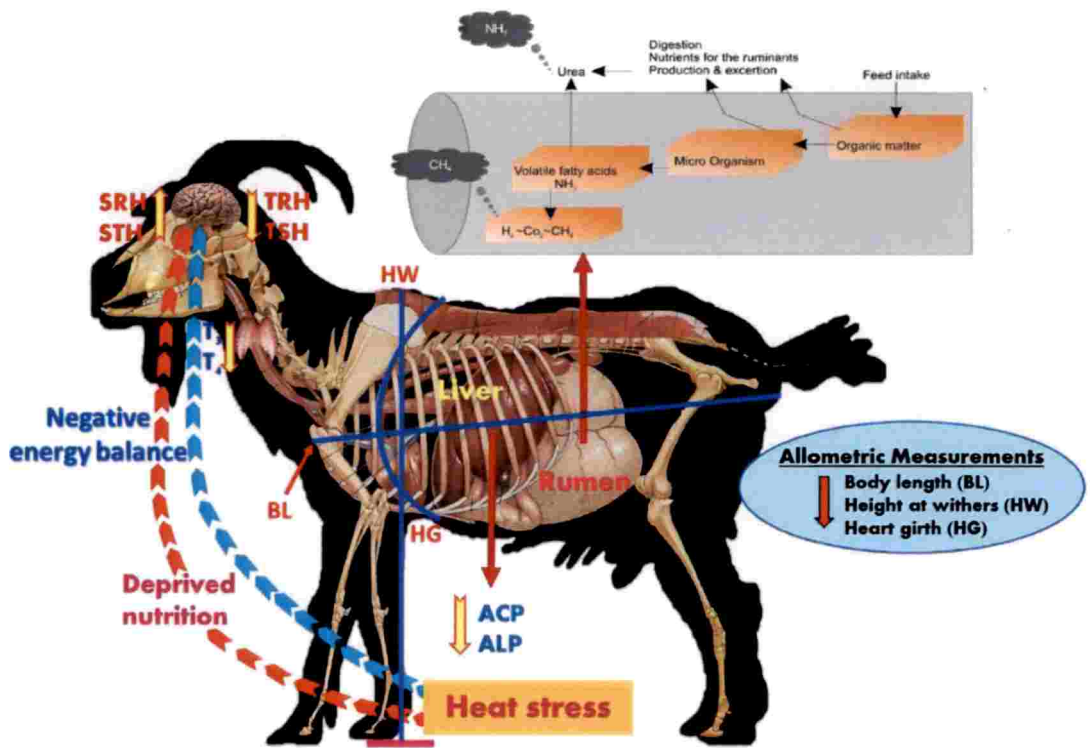
Among the domesticated ruminant species goat has been projected as the ideal animal that can withstand the detrimental effects of climate change. Exposure of goats to high ambient temperature augments the thermoregulation by reducing the production of metabolic enzymes and hormones. Generally the heat stressed animals reduces their feed intake and slows down their basal metabolism causing hypo-function of thyroid gland in order to prevent the additional metabolic heat production (McManus *et al.*, 2009). Further, the animals reduce the production of triiodothyronine (T₃) and thyroxine (T₄) during both short and long term exposure to high ambient temperatures (Rhoads *et al.*, 2009).

Despite the general awareness that indigenous breeds are more adaptable to testing environmental conditions, not much attention was focused on identifying the hidden intricacies of different adaptive mechanisms of the indigenous breeds to heat stress conditions. Further, very limited reports are available establishing the seasonal rhythmicity in metabolic adaptive mechanisms in livestock and goats in particular. It

has been widely established that different indigenous breeds exhibit superior adaptive capabilities in their respective agro-ecological zones and among these, only few breeds possess the ability to thrive well in different agro-ecological zones due to their higher genetic merit (Helal *et al.*, 2010).

Despite the general awareness that indigenous breeds are more adaptable to testing environmental conditions, not much attention has been focused on how different indigenous breeds respond to heat stress of different magnitude while exposing them to different agro-ecological zones. This approach is very crucial as the scientific community seeks solution to sustain livestock production in the changing climate scenario. Such efforts can help the researchers to identify the appropriate breed which can survive in multiple environmental conditions. 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. Therefore, the present study was conducted to evaluate the effect of shifting two extremely adapted indigenous breeds from their native tract to another agro-ecological zone and assess their growth performance in the new locality in comparison to the local breed. For this purpose, Malabari and Salem Black breeds, the two breeds well known for their ability to survive in extremely hot and humid environment, were shifted to a new locality where the heat stress was of much lower magnitude. Their growth performance was compared to the local Osmanabadi breed well known for its survival in the current experimental location. With this background, the study was conducted with the following objectives:

1. To evaluate the differences in body weight changes between different indigenous breed goats subjected to heat stress.
2. To compare the rumen fermentation profile and metabolic activity between different indigenous breed goats exposed to heat stress.
3. To determine the differences in growth and metabolic activity related endocrine profile between different indigenous breed goats exposed to heat stress.



ACP- Acid Phosphatase, ALP- Alkaline Phosphatase, TRH- Thyrotropin Releasing Hormone, TSH- Thyroid Stimulating Hormone, SRH- Somatotropin Releasing Hormone, STH- Somatotropin, T₃- Tri-Iodothyronine, T₄- Thyroxine, NH₃- Ammonia, CH₄- Methane

Fig 1: Concept figure of the present study.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Small ruminants, in particular goats and sheep are considered as an important source of income and nutrition for poor and marginal farmers around the world. In the tropical regions, this sector accounts 15% of the family's income (Chokerah, 2012). Low initial investment and high turnover rate for small ruminant production are the primary reasons behind the promotion of small ruminant production (Pollott and Wilson, 2009). Another reason for their primary importance is their small body size, so that they can be easily integrated into different types of farming systems (Amankwah *et al.*, 2012). Their reduced body size favours the farmers as it needs only less investment and reduced risk of economic loss and their high reproductive efficiency over large ruminants (Omoike, 2006; Aphunu, 2011). Small ruminants are often referred to as village banks in some rural areas without banks where the villagers invest their money on purchasing small ruminants and consider it as an adequate way to save money for the future (McDermott *et al.*, 2010; Oluwatayo and Oluwatayo, 2012). Among all the livestock species, sheep and goat hold the second and third position, 1078.2 and 861.9 million heads respectively (FAO, 2015; Aziz, 2010). Recent trends showed an increased demand for dairy products from small ruminants particularly in the developing countries where they act as a substitute for dairy products from large ruminants for human dietary needs (Lerias *et al.*, 2014). Goat and sheep are the most significant components of several agro-pastoral systems because of their constant source of protein during and immediately following the drought period (Kumar *et al.*, 2011).

Small ruminants are the morphologically versatile species that adapt themselves to the changing climate trend more readily than the other ruminant species. Besides, they are well known for their suitability to small farming systems (Feleke *et al.*, 2016). Much of the small ruminant population is concentrated in the arid and semi-

arid agro-ecological zones that have a high tendency for droughts and famines (Kumar and Roy, 2013). Still, these species are reported to be less affected by the harsh climate and continues to reproduce comparatively fairer than the large ruminants that are highly sensitive to subtle changes in the surrounding environmental fluctuations. Due to the detrimental effects of climate change and extreme climate events, African farmers shifted their preference from beef cattle, dairy cattle and chickens towards rearing of sheep and goat (Seo and Mendelsohn, 2008). Hence, small ruminant rearing is a major means of nutrition and also the means of economic stability for many small and marginal farmers since they provide meat, wool and manure which are the three major sources of income (Kumar and Roy, 2013).

The goat and sheep husbandry is the most demanding enterprise of tropical and subtropical region, but their contribution to world GDP is very less compared to that of large ruminants (Vieira *et al.*, 2009). Milk, meat, manure, wool and skin are the important sources of income from the small ruminant sector (Pollott and Wilson, 2009). Among the goat products meat and fibre are are considered to be of high market value (Yangilar, 2013). Products from the small ruminants are relatively easy to market because of their high demand and less availability (Mogashoa, 2015). In Ethiopia about 1/4th of the domestic meat consumption and half of the wool requirements are met from the small ruminant sector (Mengesha and Tsega, 2012). In Nigeria and Africa, more than 50% of the milk used for human consumption is from the small ruminants (Adedeji, 2012). Similarly, Oluwatayo and Oluwatayo (2012) also reported that 35% of total meat consumed in Nigeria are from the small ruminant sector. Likewise in Ethiopia, an increase in the per capita meat consumption and annual growth rate of goat and sheep meat was 0.5 as compared to the total countries production 1.3 (FAO, 2009; Mengesha and Tsega, 2012). In Asian continent the growth rate of small ruminant population (5.3%) is very high as compared to that of large ruminants (1.8; Chowdhury *et al.*, 2002).

Goat and sheep possess the ability to thrive in the of harsh environments semi-arid and arid regions. They can effectively survive the periods of less water availability, prevailing in these environments (Erasmus, 2000; Silanikove, 2000a). Though in the arid and semi-arid regions there are constraints for the fodder resources, because of their browsing habit, an anatomical advantage of the upper lips, the small ruminants thrive well with limited feedstuffs (Yami and Merkel, 2008). In addition, they also have a physiological advantage where they efficiently utilize the poor quality feedstuff and produce appreciably good output in terms of milk, meat, wool, and also manure (Kosgey *et al.*, 2008). During utmost scarcity of feed resources, they are reported to reduce their metabolic process in order to conserve the energy resources and thereby thriving on minimal feed intake (Yadav *et al.*, 2013). In areas of uncultivable land adjacent to cultivable land, the integration system of perennial tree crops can be adopted to sustain the animal farming. Small ruminants being the best suited species for such integrated farming systems, stand out as the major source of survival for small farmers (Sánchez, 1995; Stringer *et al.*, 2009). Integrated system of farming is projected as the most suitable method of farming in the climate change scenario.

2.1. Climate change impact on small ruminant production

Climate change has emerged as the major threat to the ecosystems. The Intergovernmental panel on climate change (IPCC) has projected a global surface temperature increase to the tune of 1.4°C to 5.8°C by the end of 2100 (Cubasch, 2013). Climate change can have both direct and indirect impacts on livestock production (Rojas-Downing *et al.*, 2017). Indirect impacts include quantity and quality deterioration of pasture resources (Chapman *et al.*, 2012; Polley *et al.*, 2013), reduction in the quantity and quality of water resources (Rosegrant *et al.*, 2002) and outbreak of pest and diseases (Patz *et al.*, 2000; Perry and Sones 2009; Chauhan and Ghosh, 2014). Direct impacts include the variations in the length of photoperiod (Baumgard *et al.*, 2012), escalated drought periods (Thornton *et al.*, 2014), and increased frequency of extreme weather events such as heat waves, cold waves and heavy downpours

(Thornton *et al.*, 2014; Bett *et al.*, 2017). Among all these stressors, heat seems to be the major stressor which affects the production potential of livestock species (Rivington *et al.*, 2009). It can create significant financial burden to the farmers by reducing quality and quantity of the animal products such as meat, milk and fiber (Al-Dawood, 2017). In addition to the production parameters heat stress also affects the growth and the reproductive potential of the animals. Even though heat stress is a global phenomenon, its effects are more prominent in tropical and subtropical regions.

Elevated ambient temperature is the foremost limiting factor in dairy production, particularly in the hot and humid regions. Decreased feed intake during the stress period is responsible for half of the reduction in the milk production (Rhoads *et al.*, 2009; NRC, 2007). In addition to the milk production, milk composition was also found to be reduced in lactating small ruminants. Lactating animals respond to heat stress mainly through two mechanisms (Hamzaoui *et al.*, 2013). One is the local mechanism (Silanikove, 2000b) which associates the plasmin-plasminogen system to the autocrine inhibition of lactation, whereas the other is the systemic mechanism wherein the role of hypothalamic-pituitary-adrenal (HPA) axis in governing the rate of milk secretion is involved (Matteri *et al.*, 2000). During stress condition, HPA axis is activated in the animals, which will be followed by up shooting of cortisol level in the blood (Silanikove, 2000b). This activates the release of plasmin activator from mammary epithelial cells into the mammary cistern. This in turn would activate the plasmin system comprising of β -casein which would produce 1–28 β -casein as the residue. This is otherwise termed as proteoso-peptone channel blocking. Ion channels in the apical membranes of the mammary epithelia would be blocked during this event and hence acts as an inhibitory factor for lactose and monovalent ion secretion, which ultimately result in reduction of milk volume (Sevi *et al.*, 2001; Dwyer and Bornett, 2004). During stress condition, prolactin synthesis is also hampered due to dopamine release. There will be surplus metabolic energy only for a short period due to reduction in energy output by the milk and increase in mobilization of stored energy. This results

from the increased glucocorticoid secretion and increased insulin uptake of adipose tissue (Kandemir *et al.*, 2013).

Goat and sheep in early lactating stage are more affected by heat stress. Williams (2012) reported a decline in milk production from normal 1.68kg to 1.53kg during heat stress conditions; he also reported a decrease in the milk fat and protein content in heat stressed goats. Similarly Sano *et al.* (1985) also observed a milk production reduction in Saanen goats exposed to moderate or severe heat stress (THI-81 or 89) for 4 days, 3 or 13%, respectively. Further in another study, the authors recorded 9% and 3% decline in milk yield in early and late lactating dairy goats exposed to high ambient temperature (Hamzaoui *et al.*, 2013; Das *et al.*, 2016). Likewise, Brasil *et al.* (2000) also observed 5.4% reduction in milk production in heat stressed Alpine goats. Further, Sevi *et al.* (2001) also reported significant changes in the fat, casein, and clot firmness content of heat stressed Comisana ewes.

Heat stress jeopardizes the meat industry by affecting the quantity and quality of meat from small ruminants. There are several reports stating the impact of heat stress on the quantitative traits of goat and sheep meat (Kadim *et al.*, 2006; Kadim *et al.*, 2008; Rana *et al.*, 2014a). Kadim *et al.* (2006) reported a reduction in live weight, carcass weight and marbling or intramuscular fat content in Omani goats transported during high ambient temperatures (37°C), probably due to the moisture loss and tissue shrinkage. However, non-significant influence of heat stress in pre-slaughter, slaughter and carcass weight have also been reported in indigenous Bangladesh sheep (Rana *et al.*, 2014a). Heat stress has also been reported to have profound impact on the meat quality by altering its physiochemical and sensory attributes (Kadim *et al.*, 2008; Hashem *et al.*, 2013; Chulayo and Muchenje, 2013). Rana *et al.* (2014a) reported darker meat and high meat pH in heat exposed sheep as result of increased vasodilatation and muscle glycogenolysis due to elevated adrenaline activity. Similarly, Liu *et al.* (2012) also reported an increase in meat pH and darkness of meat in Ujumqin wool sheep exposed to heat stress. Increase in the darkness of meat has

also been reported in dehydrated and heat stressed animals through myofibrillar shrinkage due to dryness. Further, Casey and Webb (2010) studied significant reduction in the juiciness of meat in goats transported during peak summer hours. Apart from the quality and quantity deterioration, meat safety is also getting hampered due to the increase in enteric pathogens in their body and gut surface during protracted heat exposure (Rana *et al.*, 2014a; Gregory, 2010).

Reproduction, considered a major measure of animal production contributes immensely to the economy of the livestock sector. Elevated ambient temperature is the major factor which affects the reproductive potential of the male and female animals (Silanikove, 2000b). Heat stress alters the estrous cycle and fertility in female animals (Hansen, 2009). Further, thermal stress affects the development of follicles and oocyte by altering the neuroendocrine balance of the female animals (Ozawa *et al.*, 2005). Regarding the endocrine imbalance, Ozawa *et al.* (2005) had reported a significant reduction in follicular plasma and estradiol concentration and reduced luteinizing hormone (LH) receptor level. Likewise, Siddiqui *et al.* (2010) reported delayed ovulation mainly due to the reduced pre-ovulatory Luteinizing hormone surge during heat exposure. Poor estrus expression, fertility and abnormal ovum are the main detrimental effects of heat stress (Sejian *et al.*, 2011). Heat stress can also affect endometrial functions and its secretory activities, which will further lead to the termination of the pregnancy (Wolfenson *et al.*, 2000). In addition to female fertility, heat stress also has profound effects on the male fertility. Hansen (2009) reported a decrease in fertility and sexual desire in heat stressed animals and this could be mainly due to the reduction in testosterone secretion. Further, the same report also revealed a significant decrease in semen production and sperm motility and an increased number of abnormalities in spermatozoa in animals exposed to heat stress. In rams and bucks Gimenez and Rodning (2007) had reported the negative influence of heat stress on the semen attributes such as ejaculate volume, pH, spermatic concentration, sperm abnormalities, and sperm motility.

2.2. Impact of heat stress on the growth performance of small ruminants

Growth, the irreversible positive change in the measured dimensions of the body is controlled by genetic and environmental factors (Sejian *et al.*, 2012). Among the environmental factors, heat stress is the major factor that hampers the growth performance of the small ruminants. Particularly in the tropical regions, animals which are raised in an extensive system of rearing are more prone to heat related hazards. Studies conducted by different authors showed a negative correlation between growth and heat stress (Silanikove, 2000b; Marai *et al.*, 2007; Renaudeau *et al.*, 2012). It can be due to the accelerated tissue catabolism and dwindled anabolic activity during the stress period. Decrease in anabolic activity can be due to the decrease in the voluntary feed consumption of essential nutrients (Marai and Haeeb, 2010). Further, growth indices like ADG, BW, BCS, feed conversion efficiency, BMI, and allometric measurements are found to be reduced during the summer seasons (Marai *et al.*, 2000, Shelton, 2000; Sejian *et al.*, 2010a; Niyas *et al.*, 2015).

2.2.1. Body weight

The BW is the first and foremost growth parameter that is found to be affected due to the heat stress. Several studies reported drastic decline in BW of animals during the periods of heat stress (Nardone *et al.*, 2010; Hooda and Upadhyay, 2014; Popoola *et al.*, 2014). In a study conducted in 3 groups (G1- control, G2- exposed to sun from 8.00 A.M. to 13.00 P.M., G3- exposed to sun from 1.00 P.M. to 6.00 P.M) of West African dwarf goats, Okoruwa (2014) observed a significant ($P < 0.05$) decrease in the BW in G3 goats followed by G2 goats and they attribute this reduction in BW to decreased dry matter intake of heat stressed animals. Similarly Helal *et al.* (2010) also reported a reduction in the live BW in Damascus and Balady goats that are exposed to heat stress for a period of 12 hours. Likewise, Saab *et al.* (2011) also reported a reduction in live BW of Awassi rams after thermal exposure. In another study

conducted in Alpine x Beetle kids Hooda and Upadhyay (2014) observed 25% reduction in BW in heat exposed kids than the control group kids. Similarly Helal *et al.* (2010) reported a reduction in BW in heat stressed Damascus and Balady goats, with higher BW reduction in exotic Damascus goats than indigenous Balady goats.

2.2.2. Average daily gain

The ADG is the rate of weight gain per day over specific period of time. During the periods of high ambient temperature, ADG is reported to be severely affected. Mitloehner *et al.* (2001) reported a reduction in ADG for temperature above 30°C. West African dwarf goats exposed to summer heat stress also showed a reduction ADG, which can be due to the decrease in feed intake by the animal (Popoola *et al.*, 2014). Similarly, in another heat stress experiment conducted in uncastrated Santa Inês and F1 Santa Inês × Dorper sheep breeds Gesualdi Júnior *et al.* (2014) found a reduction in ADG with more impact on Santa Inês sheep. Further, Sun and Christopherson (2001) reported a significant ADG reduction in young sufflok lambs exposed to heat stress than cold stress. Likewise, Mahjoubi *et al.* (2014) reported 36% reduction in ADG in Afshari lambs exposed to heat stress than pair-fed Afshari lambs kept under thermo-neutral conditions. Further, Alpine x Beetle cross kids exposed to heat and nutrient stress showed significant reduction in ADG. They attributed this to the decline in dry matter intake and poor forage quality in summer season (Hooda and Upadhyay, 2014). However, no significant difference in ADG was found between the control and heat stress group in the study conducted in indigenous black bedwin goat kids (Al-Tamimi, 2005).

2.2.3. Feed conversion efficiency

Feed conversion efficiency is a major measure that affects the economic value of meat. Suffolk lambs exposed to a temperature of 30.5°C in the climatic chamber showed significant reduction in the feed conversion efficiency than the lambs kept under thermo-neutral conditions (19.3°C) in the shed (Padua *et al.*, 1997). Fattening

Dalagh lambs exposed to thermal stress showed significant reduction in feed conversion efficiency compared to the shorn lambs, and they suggested summer shearing of lambs as an ameliorative strategy to improve sheep production in the changing climate scenario (Moslemipur and Golzar-Adabi, 2017). Likewise, Alam *et al.* (2013) reported a significant reduction in feed conversion efficiency in Black Bengal goats kept under 30.5°C in climate chamber than goats kept in 19.3°C during spring season. Similar findings of reduced feed conversion ratio was also reported in Alpine x Beetle kids subjected to thermal stress and the others attributed this to decline in feed intake and poor quality of summer pasture (Hooda and Upadhyay, 2014). However, indigenous Black Bedwin goat kids exposed to heat stress were not reported to show any change in feed conversion efficiency than goat kids kept under thermo-neutral conditions (Al-Tamimi, 2005).

2.2.4. Allometric measurements

Allometric measurements refers to the growth of body parts at different rates, resulting in a change of body proportion in the animals. Studies conducted by several authors reported a decrease in allometric parameters such as wither height (WH), heart girth (HG), and body length (BL). Likewise, Ali and Hayder (2008) reported a significant decrease in chest depth (CD) in fat-tailed Farafra sheep subjected to thermal stress and they attributed this to the adaptive capability of fat-tailed Farafra sheep to dissipate excess body heat by increasing their body surface area. However, Rana *et al.* (2014a), reported a non- significant change in BL and HG in the indigenous sheep of Bangladesh. Likewise Hashem *et al.* (2013) also reported insignificant changes in HG and BL among different treatment groups (zero, four and eight hours of heat exposure) of Black Bengal goats.

Genetic and environmental factors are largely translated into hormonal signals affecting growth processes involving a complex sequence of interactions between different hormones. The somatotrophic (growth hormone, GH; growth hormone

receptor, GHR; insulin-like growth factor, IGF-1) axis is considered to be one of the most important among them, because of their broad range of effects and central role in growth governance (Jaquier et al., 2012). The IGF-1 gene is a key component of somatotrophic axis which plays a significant role in proliferation of cells, mitosis, myogenesis, meiosis, differentiation in foetal development and post natal growth. In addition, IGF-1 also plays key roles in cellular transformation, organ regeneration, immune function, development of the musculoskeletal system, and aging.

2.3. Impact of heat stress on the rumen functions

Rumen is a four chambered structure that helps the animals to ferment and digest the feed consumed by them to produce energy. Elevated ambient temperature during the summer months can have a significant influence on the basic physiology of rumen thereby affecting the nutritional status of the animals (Baumgard and Rhoads, 2012). Rumen volatile fatty acid (VFA) production are found to be altered during the conditions of extreme temperature. Feed digestibility was found to be positively increased with increasing ambient temperature because of reduction in the passage rate and thus giving more time to the microbes and enzymes to digest feed (Hirayama *et al.*, 2004).

2.3.1. Rumen fermentation pattern

Feed and forages consumed by the animals are fermented into micro energy sources with the aid of rumen micro flora and fauna. Pattern of rumen fermentation occasionally becomes dysfunctional due to various environmental and biological factors which results in acidosis, bloat, and stress (Owens and Basalan, 2016). Environmental factors such as temperature and RH can have significant role in the feed consumption of animals. Hence increase in these weather parameters can decrease the dry matter intake of the animals and rumination as a result of reduction in the amount of buffering agents entering to the rumen (Bernabucci *et al.*, 2010). Additionally, in animals the blood flow is deviated from gastro intestinal tract to periphery for heat

dissipation which further decreases the digestibility. Furthermore, increased respiration during summer season will increase the CO₂ intake and lead to decrease in pH and acidosis (Choubey and Kumar, 2012). Likewise, Shafie (1994) and his co-workers also observed a drastic increase in ammoniacal nitrogen (NH₃-N) concentration and rumen temperature in heat stressed rams exposed to a temperature of 35°C.

2.3.2. Volatile fatty acid production

Microbial population residing in the rumen are responsible for the production of volatile fatty acids (VFA). During the process of digestion, microbes ferment the fibers and produce energy in the form of VFAs. Acetate, butyrate, and propionate are the major VFAs produced as the end product of digestion in rumen (Allard, 2009). There are reports showing the decrease in VFA production during the periods of heat stress (Nonaka *et al.*, 2008; Tajima *et al.*, 2007; Salles *et al.*, 2010). Similarly, Tajima *et al.* (2007) reported a decrease in the acetate and acetate to propionate ratio and an increase in butyrate level in heat stressed animals and this can be due to the alterations in the number of rumen microbiota during the periods of heat stress. Likewise, Hirayama *et al.* (2004) also reported a reduction in the plasma acetate and total volatile fatty acid (TVFA) concentration in heat exposed (35°C) Saanen goats than Saanen goats kept at thermo neutral conditions (20°C). According to Kelley *et al.* (1967) reduced feed intake and rumen microbial diversity are the reasons for the reduction in VFA production. Further, Nonaka *et al.* (2008) reported a reduction in molar concentration of acetate as well as non-significant reduction in the propionate and butyrate concentrations. The reduction in the VFA concentration could be attributed to the increased rumen temperature during the heat stress periods. In contrary to the above statements, Shafie *et al.* (1994) reported an increase in the VFA production in rams exposed to a temperature of 35°C.

2.3.3. Rumen microbial population

The rumen in the stomach of ruminant animals, houses millions of microorganisms that include bacteria (up to 10¹¹ cells/g), protozoa (10⁴-10⁶/g), fungi (10²-10⁴/g), virus and archea. These microbes work in a unified manner to ferment and digest feed and fodder ingested by them (Christopher *et al.*, 2005). Efficiency of the rumen micro flora and fauna to convert feed determines the effectiveness of feed conversion ratio and growth of the animals (Tajima *et al.*, 2007). Methanobrevibacter and Methanomicrobium are the two important methanogens (group of archea) residing in the rumen that are responsible for the production of methane (CH₄) which causes dietary energy loss (Janssen and Kirs, 2008).

Heat stress induced rumen function impairment is mainly associated with the increase of *Streptococcus* genus bacteria and with a decrease in the bacteria of *Fibrobactor* genus (Freestone and Lyte, 2010). Further, Tajima *et al.* (2007) also reported the ruinous effects of heat stress in conjunction with altered rumen bacterial diversity with a decrease in uncultivated Cluster E group sequences. Similarly in another experiment Uyeno *et al.* (2010) observed a decrease in the *Streptococcus* genus and an increase in both *Streptococcus* spp. and *Clostridium coccoides*-*Eubacterium* genus in the rumen. Changes in the rumen microbial ecosystem due to heat exposure can influence feed digestibility and composition of the end products by altering the rumen fermentation pattern (Uyeno *et al.*, 2010). Further, Bernabucci and his co-workers (2009) observed a decline in the concentrations of amylolytic and cellulolytic bacteria in Sardinian ewes exposed to an atmosphere having THI 85. Reduction in the bacterial diversity can offset the positive effects of the increased digestibility due to decreased dry matter intake and passage rate and ultimately culminate in decreased diet digestibility (Bernabucci *et al.*, 2009).

2.3.4. Enteric methane emission

Methane (CH₄) is the second major greenhouse gas with 21 times more global warming potential than Carbon dioxide (CO₂; IPCC, 2007). Small ruminants such as goat and sheep produces CH₄ as a result of the microbial digestion of the feed materials. Changes in the feed intake and feed utilization efficiency will influence the production of enteric CH₄. Conversion of feed to CH₄ is mainly carried out by bacteria, fungi and protozoans (primary fermenters) and finally by methanogens (Moss *et al.*, 2000). These primary fermenters will produce VFAs such as acetate, propionate and butyrate as an end product of digestion. Acetate and butyrate production releases two hydrogen molecules that will add up the CH₄ production. Generally, enteric CH₄ is produced by slow growing and fast growing methanogens. Slow growing methanogens such as *Methanosarcina* produce CH₄ from acetate and fast growing methanogens produce CH₄ by reducing CO₂ with H₂. But in rumen CH₄ is produced generally by fast growing methanogens due to the lower retention time of digesta (Madan and Yadav, 2013).

Environmental temperature is a key factor which determines the CH₄ production since feed intake and digestibility differs with ambient temperature. Mbanzamihiyo *et al.* (2002) reported an increase in the enteric CH₄ emission in sheep during the late season (August-September) than early season (June-July). Similarly, in another experiment conducted in young wethers grazing at summer moist hilly island, perennial rye grass/ white clover dominant pasture and late summer pastures during late summer season showed a CH₄ yield of 4.1%, 3.9% and 5.3%, respectively. Increased CH₄ yield in wethers grazing in late summer season pasture is attributed to the quality deterioration (poor dry matter digestibility, protein and soluble carbohydrate content and increased cell wall content) of the pastures during the summer season (Ulyatt *et al.*, 2005). The study revealed the indirect effect of elevated ambient temperature on the CH₄ production. Further, in another experiment Ulyatt *et al.* (2002) reported an increase in CH₄ emission from Romney cross wether lambs grazing in summer grassland than Kikuyu grassland.

2.4. Impact of heat stress on the metabolic activity

Sheep and goats exposed to heat stress compromise their productive and reproductive functions by deviating energy from body metabolic activities to thermogenesis or thermolysis processes (Babinszky *et al.*, 2011). As a result of reduction in the allocated energy to metabolic process animals tend to reduce their metabolic activity as an adaptive process. In order to reduce the metabolic activity and heat production animals reduce their feed consumption during heat exposure periods.

2.4.1. Feed intake

Voluntary feed intake is the factor which determines the growth and production performance of the ruminant animals (Silanikove, 2000b). Increased ambient temperature can directly affect the appetite center of hypothalamus and decrease the feed intake of the animals (Das *et al.*, 2016). Decline in the feed consumption can cause negative energy balance in the animals resulting in their reduced performance. Several studies conducted in small ruminants established a reduction in feed intake during heat exposure (Bernabucci *et al.*, 2009; Rana *et al.*, 2014b; Shilja *et al.*, 2016). Chauhan *et al.* (2014) observed a decline in daily feed intake in heat stressed Merino × Poll Dorset crossbred ewes (718 g/d) when compared to control group (772 g/d). Similarly, Indu *et al.* (2014) also observed a decreased feed intake in Malpura ewes kept under simulated heat stress condition in the climate chamber than the ewes kept under thermo-neutral conditions. The reduction in feed intake between control and heat stress was 7.08 DMI g/kg W^{0.75}/day. And authors attributed this decrease in feed intake to the severity of heat stress. Heat exposure upregulates the production of adipokines like adiponectin and leptin (Bernabucci *et al.*, 2009; Morera *et al.*, 2012) which further excites the hypothalamic axis and forces the animal to reduce feed intake (Slimen *et al.*, 2015).

2.4.2. Thyroid hormone production

Thyroid hormones play a crucial role in adaptive and productive functions in livestock species (Todini, 2007). Generally, it is well established that thermal stress is associated with reduced thyroid hormone level and declined thyroid gland activity (Rasooli *et al.*, 2004; Indu *et al.*, 2015). According to Indu *et al.* (2015) there was a significant reduction in thyroid hormone concentration in Malpura ewes when they are subjected to thermal stress condition. They stated that the reduction in these metabolic hormones could be an adaptive mechanism to reduce the heat load since synthesis of these metabolic hormones during heat stress could add up additional heat load to the existing heat load. In another study conducted in Iranian fat-tailed sheep, Nazifi *et al.* (2003) observed a decrease in serum T₃ and T₄ concentration during heat stress exposure than cold stress exposure and normal condition. Sejian and Srivastava (2010) also found a decline in T₃ and T₄ concentration during heat stress in Marwari goats. This was a clear cut indicator of curbed pituitary thyroid axis during thermal stress condition. Similarly, Johnson and his co-workers also reported a decline in plasma T₃ concentration from 2.2ng/ml to 1.16ng/ml (Johnson *et al.*, 1967; Farooq *et al.*, 2010). Further, McManus *et al.* (2009) observed a significant decline in blood circulating levels of T₃ and T₄ in order to bring down metabolic heat production. Sivakumar *et al.* (2010) observed a significant ($p < 0.05$) reduction in T₃ and T₄ levels in heat stressed Black Bengal goats. During heat stress a decline in T₃ and T₄ production was observed in Balady and Damascus goats. In Balady goats the T₃ reduction was from 1.68 ng/ml to 0.94 ng/ml (44.05%) and in Damascus goats reduction was from 1.88 ng/ml to 1.06 ng/ml (43.62%) than normal condition, also 41.52% and 25.52% reduction in T₄ and was recorded in Balady and Damascus goats, respectively during the same heat stress condition (Abdel-Fattah, 2014). Likewise, Helal *et al.* (2010) recorded a significant T₃ and T₄ reduction in Damascus goats during short and long terms heat stress condition. Al-Samawi *et al.* (2014) also observed a decrease in T₃ and T₄ concentration during high ambient temperature in female Aardi goats. Reduced T₃ and T₄ production in

sheep during heat stress can be a mechanism to counteract excess metabolic heat production (Todini, 2007; Mader *et al.*, 2010). Additionally, Rathwa *et al.* (2017) also reported a significant reduction in T₃ and T₄ hormone concentration during summer season (1.46 ng/ml and 43.09 ng/ml) than winter (2.55 ng/ml and 54.93 ng/ml) season and they opined that this could be due to the direct effect of heat stress on hypothalamus. In an experiment conducted in male kids of Alpine x Beetle cross at different temperatures Hooda and Upadhyay (2014) observed significant T₃ and T₄ reduction at a temperature 40°C, but the reduction was not significant at temperatures 42 and 44°C. They also stated that the reduction in T₃ and T₄ could be a response to manage negative energy balance and to bring down metabolic heat production.

2.4.3. Metabolic enzymes

Metabolic enzymes drive the body functions by acting at the level of blood, tissues and organs. These enzymes are produced internally in the body and are considered vital for growth, maintenance and development of cells, tissues and organs. Several studies conducted in small ruminants reported a decline in the metabolic enzymes during the periods of heat stress (Sejian *et al.*, 2010b; Helal *et al.*, 2010). Alpine x Beetle cross kids exposed to heat stress showed a reduction in both acid phosphatase (ACP) and alkaline phosphatase (ALP) enzymes which could be attributed to the decrease in metabolic heat production and thyroid hormone concentrations (Hooda and Upadhyay, 2014). Similarly Sejian *et al.* (2010b) also observed a reduction in the ACP, ALP concentration in heat stressed Marwari goats. Further, Sevi *et al.* (2001) also observed significant decline in the ALP level in heat exposed Comisana ewes and they attributed this to the hypo-function of thyroid gland during heat stress hours. Likewise, Sejian and his co-workers found a reduction in ACP and ALP concentration in both thermal stress and combined stress (thermal and nutritional stress) groups of Malpura ewes (Sejian *et al.*, 2010b). All these findings show the metabolic shift in the animals in order to cope with the changing environmental conditions.

Climate change which emerges as the major threat to global food security was found to be negatively influencing global small ruminant production systems. Pernicious effects of climate change such as heat stress and frequent flood and drought events are suppressing the food and economic needs of present generation by affecting the productivity of their animals. Sufficient research evidences exist to validate the malignant effects of heat stress on the growth performance of small ruminants. There is a general agreement that heat stress can alter the growth and allometric attributes of small ruminants. Apart from the detrimental effect on growth, rumen physiology is also reported to be altered during the heat stress period by affecting abiotic and biotic components of the rumen. However, only paucity of information available on the effect of heat stress on rumen ecosystem. Further, many of the reported literatures established the conjunction between the heat stress and retarded metabolic activity. Selective breeding of indigenous thermo-tolerant breeds and dietary manipulations to improve rumen physiology can pave the way towards the sustainable production goals.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

The experiment was conducted in the experimental livestock unit of the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India located on latitude 77°36'25.3"E, longitude 12°57'04.3"N and altitude of 920 m above mean sea level. The mean annual maximum and minimum ambient temperature of this region ranges between 15 to 36°C respectively. The mean annual RH ranges from 20 to 85%. The average annual precipitation in this region varies between 200 to 970 mm with erratic distribution. The average annual minimum and maximum temperature ranges between 15-22°C and 27-34°C respectively. The study was conducted during the month of April to May. The temperature and RH variations during the study period (April-May) ranged between 26-40°C and 29-59% respectively under hot semi-arid environment. The THI values were calculated as per method described by McDowell (1972). Accordingly the formula used was $THI = 0.72(T_{db} + T_{wb}) + 40.6$ where, T_{db} - Dry bulb temperature in °C; T_{wb} - Wet bulb temperature in °C.

3.2 Animals

Three different indigenous goat breeds of southern peninsular India (Osmanabadi, Malabari, Salem black) were used in this experiment.

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

Coat color varies, but mostly it is black (73%) and the rest are white, brown or spotted. The average BW of adult male and female animals are 34 kg and 30 kg respectively.

Malabari also known as Tellicherry goat is the goat breed of Northern Kerala well known for its high prolificacy, meat, milk yield and adaptability to hot humid environments (Alex and Raghavan, 2012; Alex *et al.*, 2013). Malabari breed owed its name from the place of origin Thalassery and it is distributed over Kannur, Kozhikode and Malappuram districts of Kerala (Bindu and Raghavan, 2010). Generally Malabari goats are categorized under medium sized goat breeds known for their (Jimcy *et al.*, 2011). Coat colour varies greatly from pure white to black. Average BW of an adult male and female goats are 38.96 and 31.12 kg respectively (Acharya, 1982)

Salem black goats are one of the important meat breeds of India native to Salem district of Tamil Nadu. It is also distributed throughout Namakkal, Dharmapuri, Krishnagiri, Karur and Erode districts of the state. Name of this breed is derived from its place of origin and colour. These goats are characterized by lean body and long legs and well known for their adaptability to hot environment of Tamil Nadu (Thiruvankadan *et al.*, 2006). Average BW of an adult male and female Salem black goats are 47.86 and 33.63 respectively (Gopu, 2002).

The experiment was conducted in 8-12 months old Osmanabadi, Malabari and Salem black goats. Both Osmanabadi and Salem black goat had BW between 15-20 kg and Malabari with 10-15kg. The animals were housed in well-ventilated sheds made up of asbestos roofing at the height 2.4 m and open from side and maintained under proper hygienic conditions. Prophylactic measures against goat diseases like goat pox, peste des petits ruminants, enterotoxaemia; endo and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study

3.3 Technical program

The study was conducted for a period of 45 days between April-May 2017. Thirty six animals were used in this study. The animals were randomly allocated into six groups of six animals each, OC (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MC (n=6; Malabari control), MHS (n=6; Malabari heat

stress), SBC (n=6; Salem Black control) and SBHS (n=6; Salem Black heat stress). The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40 % concentrate (Maize 36kg, wheat bran 37kg, soybean meal 25kg, mineral mixture 1.5kg, common salt 0.5 kg/ 100kg). The OC, MC and SBC animals were maintained in the shed in thermo-neutral condition while OHS, MHS and SBHS animals were exposed outside to summer heat stress under direct sun between 10:00 h to 16:00 h during experimental period. The OC, MC and SBC animals were fed and watered inside the shed while OHS, MHS and SBHS animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily both inside and outside the shed throughout the study period. Rumen liquor and blood samples were collected at fortnightly intervals for the estimation of rumen metabolites, biochemical, enzyme and endocrine parameters. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to summer heat stress.



Plate 1: Goat breeds used for the study (Osmanabadi, Malabari, Salem Black)

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

Attribute	Concentrate mixture (kg/100 kg)	Napier hay (<i>Pennisetum purpureum</i>)
Ingredients		
Maize	36	-
Wheat bran	37	-
Soybean meal	25	-
Mineral mixture	1.5	-
Salt	0.5	-
Chemical composition (%)		
Dry matter	92.9±0.079	94.0±0.289
Organic matter	95.9±0.190	95.4±0.298
Crude protein	19.6±0.176	6.21±0.098
Ether extract	1.82±0.183	1.49±0.026
Total ash	4.10±0.190	4.64±0.298
Fibre fractions (%)		
Neutral detergent fibre	40.4±1.400	82.9±0.881
Acid detergent fibre	11.1±0.239	64.6±1.950
Acid detergent lignin	2.14±0.029	12.3±0.651
Nutritive value		
Total digestible nutrients %*	72.2	55.0
Digestible energy (kJ/kg)*	13.3	10.1

*Calculated values

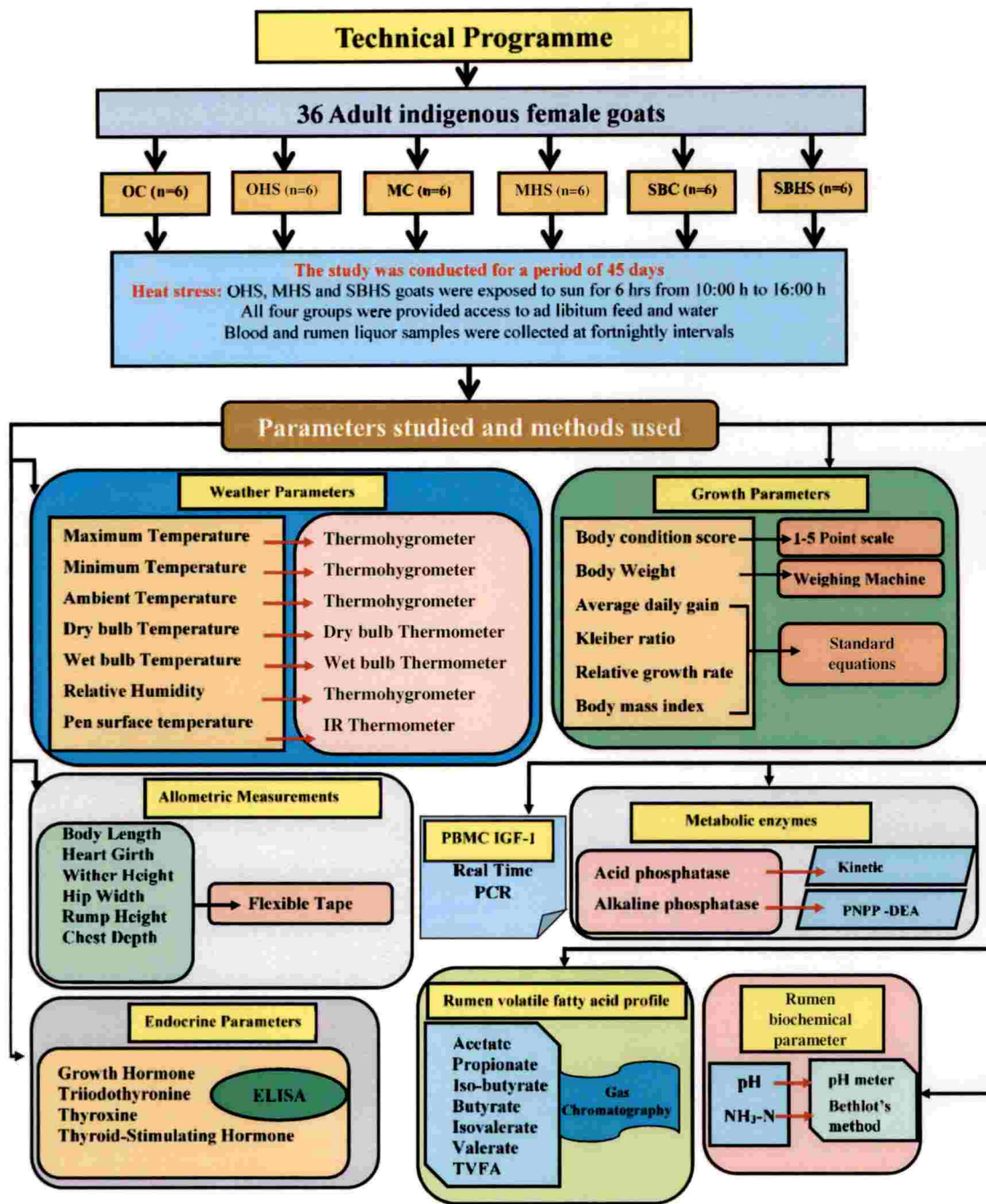


Fig. 2 Technical diagram of the present study

3.4 Weather parameter recording (procedure)

Inside and outside the shed weather parameters were measured twice daily (8.00 A.M. and 2.00 P.M.). Wet and dry bulb temperatures and pen surface temperature (PST) were measured using wet and dry bulb thermometer and thermal imager respectively. The maximum minimum ambient temperatures, RH were recorded manually using thermohygrometer.

3.5 Growth Variables (Procedure)

3.5.1 Body weight recording

The BW was measured using hanging scale weighing balance in kg.

3.5.2 Average daily gain

The ADG was calculated using the initial and final BW measurements and number of experimental days using the formula given below

$$ADG = (Final\ body\ weight - Initial\ body\ weight) / Number\ of\ days$$

3.5.3 Kleiber ratio

Kleiber ratio (KR) was calculated as a ratio between ADG and metabolic weight using the formula mentioned below

$$KR = Average\ daily\ gain / body\ weight^{0.75}$$

3.5.4 Relative growth rate

Relative growth rate (RGR) was calculated using the natural logarithms of both initial and final BW

$$RGR = \{(ln\ final\ body\ weight - ln\ Initial\ body\ weight) / 112\} * 1000$$

3.5.5 Body mass index

The BMI is calculated using BW, WH and BL using the formula given by Tanaka *et al.* (2003).

$$gBMI = \text{body weight(kg)} / \{ \text{withers height(m)} / \text{body length(m)} \times 10 \}$$

3.5.6 BCS

All the animals were condition scored using USA and Ireland system (1-5) of body condition scoring. Goats in BCS 1 indicates completely emaciated weak and unthrifty animals whereas BCS 5 animals will be obese with excess fat tissues. Goats were conditioned by careful palpation of the transverse and spinous process in loin region, immediately behind the last rib (Russel *et al.*, 1969). The condition scoring pattern followed for BCS measurement is depicted in table 2.

Table 2: BCS scores and description

Score	Assessment	Description
1	Emaciated	No fat tissue between skin and bones with sharp spinous processes
2	Thin	Goats with slight amount of fat tissue and relatively prominent spinous process
3	Average	Goats with average flesh and fat cover. Smooth and slightly rounded transverse process
4	Fat	Loin eye muscle with full fat cover. Spinous process are detectable only with pressure.
5	Obese	Goats with excess fat cover in brisket flank and tail head regions. Transverse process is not detectable.



Plate 2: Recording of body weight

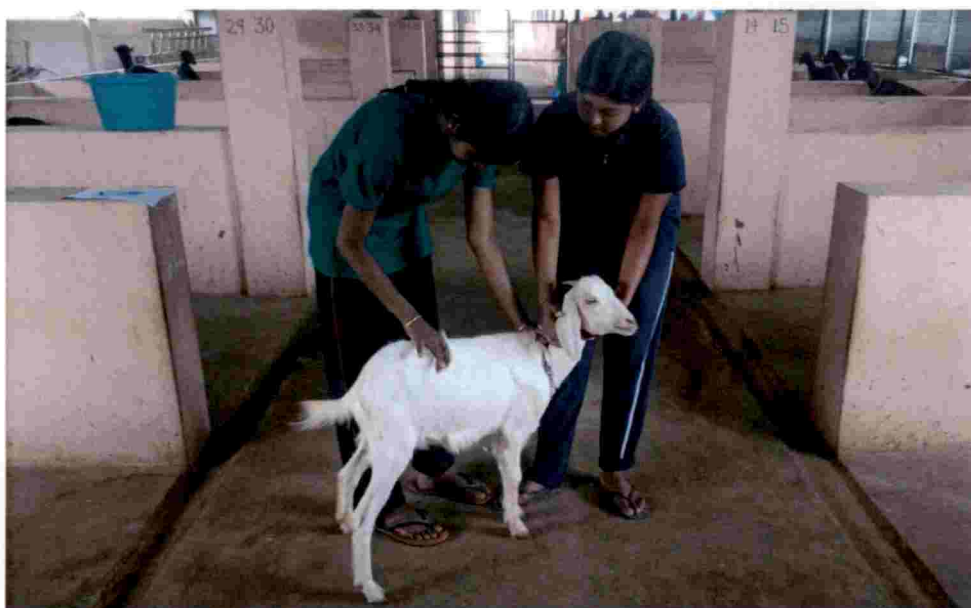


Plate 3: BCS recording

3.6 Allometric measurements

All allometric measurements were measured using a flexible tape in cm. This includes:

5.6.1 Body length: The BL refers to the distance from the point of shoulder to the pin bone point

5.6.2 Heart girth: This is a circumferential body measure taken around the chest immediately behind the fore limbs and withers perpendicular to the body axis.

5.6.3 Withers height: This was measured from tip of wither bone to the ground surface platform where the animal is standing.

5.6.4 Hip width: This measures the distance between the outer edges of the major hip bones on the right and left side.

5.6.5 Rump height: rump height (RuH) measures the distance from the rump to ground surface platform where the animal is standing.

5.6.6 Chest depth: It is the longest measurement of chest at rib level. This measures the distance from the backbone at the shoulder to the brisket between the fore limbs.

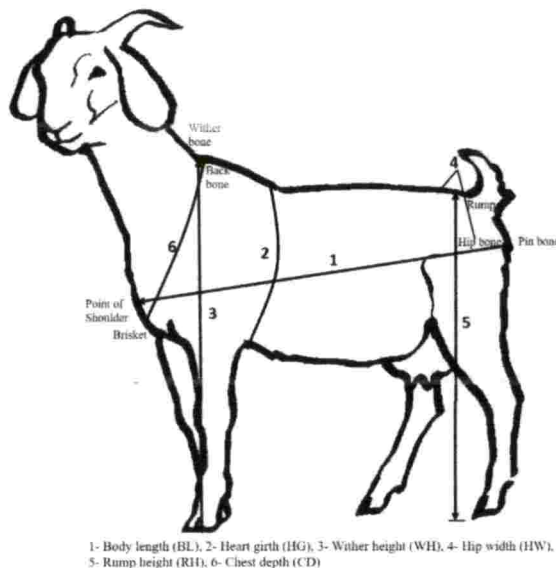


Fig. 3 Allometric measurements of Osmanabadi, Malabari and Salem Black breeds



Plate 4: Measuring the body length using flexible tape



Plate 5: Measuring the wither height using flexible tape



Plate 6: Measuring the heart girth using flexible tape



Plate 7: Measuring the rump height using flexible tape

3.7 Rumen associated parameters

Rumen liquor collection (procedure)

Rumen liquor collection Apparatus

- Hand held vacuum pump
- Conical flask with an inlet
- Perforated silicon tubes
- Hard plastic pipe (8 inches)

Procedure

1. A perforated silicon tube was inserted to the stomach (Rumen) of the animal through a plastic pipe kept at mouth.
2. Proper care was taken while inserting the silicon tube so that it should not enter trachea (animal should not cough).
3. Once the tube has reached the rumen tail end of the silicon tube was connected to a collection flask which is attached to a hand held vacuum pump.
4. Rumen liquor was drawn out from rumen by applying pressure into the collecting flask.
5. Rumen liquor was transferred into a plastic bottle and kept in ice box immediately after the collection.
6. After collection it was immediately transferred in a plastic bottle and stoppered and then placed in an ice box and brought to the laboratory.



Plate 8: Rumen liquor collection using stomach pump

3.7.1 pH determination

The rumen liquor pH was estimated using a digital pH meter (Eutech instruments, Cyberscan pH tutor, Made in Singapore).

Procedure

The pH meter was switched-on and calibrated at least 2 hrs before recording the pH of the rumen liquor. The buffers of pH 4 and 7 were used to calibrate the instrument. The pH of rumen liquor was measured by placing the pH meter electrode into sample bottle containing rumen liquor.

3.7.2 Strained rumen liquor (SRL)

The rumen liquor was filtered using four layered cheese/muslin cloth to get strained rumen liquor

3.7.3 Estimation of VFAs through gas chromatography (GC)

For the estimation of VFAs 0.2 ml of metaphosphoric acid (25%; w/v) was added to 0.8 ml of strained rumen liquor (SRL) in micro centrifuge tube and samples were centrifuged at 5000 rpm for twenty minutes. The supernatant was stored at -20°C

and later analyzed for VFA concentrations by GC (Agilent; Model 7890A GC System, Shanghai, China) using Flame Ionization Detector, programmable temperature vaporizer injector and capillary column (Agilent J&W DB-WAX GC Column 40 m × 0.18 mm × 0.18 μm). The analytical conditions for fractionation of VFA were injection port temperature 250°C, column temperature step up from 60 to 200°C in 7 min with a hold time of 10 min and the detector temperature was maintained at 300°C with GC grade air, hydrogen and nitrogen was used as carrier gas with flow rate of 1.0 ml/min. The injector was equipped with a glass liner of glass wool to separate particles of dirt from the sample. The 36 rumen liquor samples were injected by an automatic injector at an injection volume of 1 μL using the split method and a 30:1 splitting ratio. Standard solutions of appropriate concentration (mmol/L) were prepared from the individual substances namely acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid of analytical purity (Sigma-Aldrich) and the individual VFAs in the rumen liquor sample were identified based on the retention time of the standard. The concentrations of the individual VFA in the rumen liquor samples were determined by recording the area of both the VFA mixed standards and as well as the sample and expressed as mmol/L.

3.7.4 Ammoniacal Nitrogen estimation

Sampling of rumen liquor for NH₃-N estimation

2 ml of SRL was sampled for biochemical analysis. Few drops of saturated mercuric chloride (HgCl₂) was added in the SRL sample and stored at -20°C till analyzed.

NH₃-N in rumen liquor was estimated by Berthelot's method based on the color reaction between phenol/phenolic derivatives and ammonia.

Equipment's used

1. UV- Spectrophotometer
2. Water Bath

Reagents

Solution A: 1 g phenol and 5 mg sodium nitroprusside was dissolved in 100 ml of distilled water.

Solution B: 0.5 g NaOH and 0.84 ml sodium hypochlorite was dissolved in 100 ml distilled water.

Solution A and B were stored in amber colour bottles in refrigerator.

Stock standard solution of $(\text{NH}_4)_2\text{SO}_4$: 0.048 g $(\text{NH}_4)_2\text{SO}_4$ was dissolved in 100 ml distilled water to get a final concentration of 10 mg of $\text{NH}_3\text{-N}$ per 100 ml of solution.

Working standard solution of $(\text{NH}_4)_2\text{SO}_4$: 10 ml solution was taken and made up to 100 ml (The solution contains 0.01 mg $(\text{NH}_4)_2\text{SO}_4$ per ml).

Procedure

1. Working standard solution was dispensed in to all the test tubes with the concentration range of 0.5 to 10 μg
2. Distilled water was added to make up the volume to 1 ml
3. 2.5 ml solution B was dispensed into all test tubes
4. Immediately after adding solution A, solution B was added in all the tubes and the contents were mixed vigorously using vortex shaker.
5. All the tubes were incubated for 15 min at 39°C for colour development
6. The absorbance "A" was recorded at 625 nm against blank using (U-2900) UV-VIS Spectrophotometer double beam with inbuilt LCD Monitor, Hitachi, Kyoto, Japan.
7. The calibration curve was prepared by plotting "A" against standard $\text{NH}_3\text{-N}$ concentration.

The concentration of $\text{NH}_3\text{-N}$ in the sample was calculated by reading "A" on the calibration curve.

Protocol

Reagents	Test tube number								
	Blank	1	2	3	4	5	6	7	Unknown
Working standard	-	50	100	200	400	600	800	1000	-
Distilled water	1000	950	900	800	600	400	200	-	900
Unknown sample	-	-	-	-	-	-	-	-	100
Concentration of NH_3N	0	0.5	1.0	2.0	4.0	6.0	8.0	10.0	?
Solution A				2.5 ml in each tube					
Solution B				2.5 ml in each tube					

3.8 Blood collection

Five ml of blood samples were collected at fortnightly interval from all four groups simultaneously at 11:00 h using 20 gauge sterilized needles and plastic syringe from external jugular vein in tubes. Heparin (Sisco Research Laboratories pvt. Ltd, Bombay) was used as the anticoagulant at the rate of 10 IU per ml of blood.

3.8.1 Plasma separation

Plasma was separated from blood by centrifugation at 3500 rpm at room temperature for 20 min. The plasma was then divided into aliquots in micro centrifuge

tubes, and kept frozen at -20°C till further analysis. Plasma samples were used to estimate enzymes and endocrine parameters.

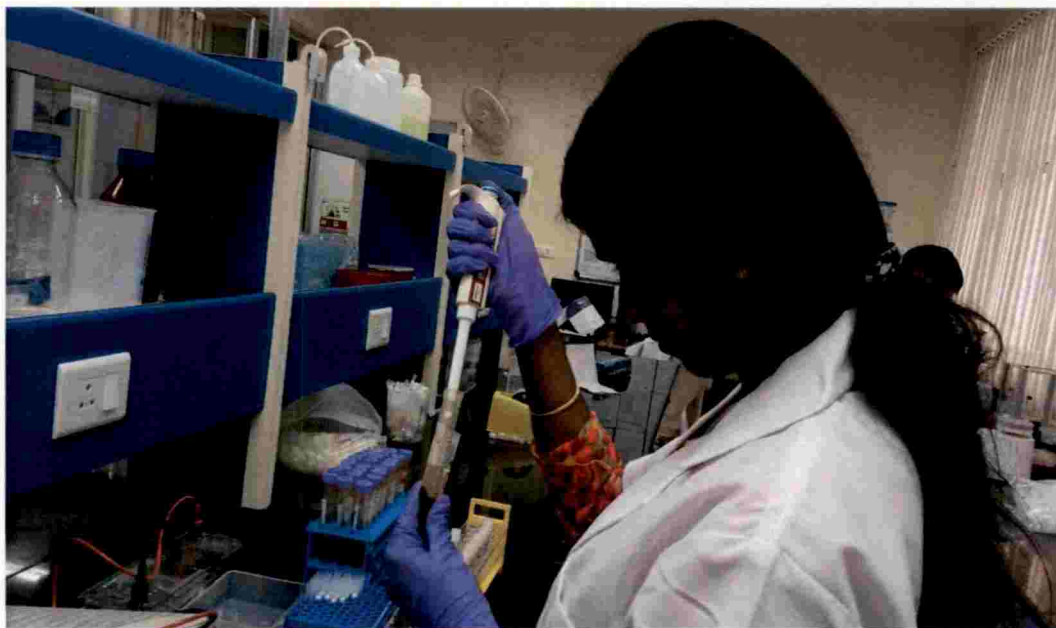


Plate 9: plasma separation after centrifugation

3.8.2 Estimation of metabolic enzymes

3.8.2.1 Acid phosphatase (ACP)

The plasma ACP was estimated using kinetic method using Spectrophotometer (ACCUREX, Mumbai, India).

Principle:

In acidic pH of buffer system, acid phosphatase hydrolyses α -naphthylphosphate to α -naphthol and phosphate. The α -naphthol is then coupled with Diazotised Fast red TR to form a Diazo Dye which has a strong absorbance at 405 nm. The increase in absorbance is directly proportional to the level of acid phosphatase in plasma.



Procedure

1. The required amount of working solution was pre warmed at 37°C before use.
2. 0.01 ml of specimen was added to 1 ml of total ACP working solution.
3. The contents were mixed thoroughly and the assay mixture was immediately transferred to the thermo stated cuvette. The stop watch was started simultaneously, the first reading was recorded at 300th sec and subsequently 3 more readings with 60 sec interval at 405 nm. The absorbance was recorded using Bio spectrophotometer Basic, Eppendorf, Hamburg, Germany.

Calculation

Concentration in IU/L = 743 * change in Abs/min.

3.8.2.2 Acid phosphatase (ALP)

The plasma ALP was estimated using PNPP-DEA method using Spectrophotometer (ASRITHA, Hyderabad, Telangana, India).

Principle:



Procedure:

1. Working solution was prepared by dissolving substrate reagent vial in equal volume of buffer reagent
2. 1.0 ml working solution was pipetted in to a test tube and incubate at 37°C for 1 min

3. 0.02 ml plasma sample was added to same test tube and vortexed vigorously
4. Initial absorbance A_0 was measured after 1 minute at 405 nm using Bio spectrophotometer Basic, Eppendorf, Hamburg, Germany.
5. Absorbance reading was repeated after every 1,2 and 3 minutes
6. Mean absorbance change per minute ($\Delta A/\text{min}$) was calculated

Calculations

ALP activity in IU/L = $\Delta A/\text{min} * 2754 * \text{tf}$

Temperature conversion factor (tf) for 37°C (assay temperature) is 1.00

3.8.3 Estimation of endocrine parameters

Endocrine parameters estimated in the study were GH, thyroid stimulating hormone (TSH), T_3 and T_4 . The parameters GH (LDN, Nordhorn, Germany), TSH (LDN, Nordhorn, Germany), T_3 (LDN, Nordhorn, Germany) and T_4 (LDN, Nordhorn, Germany) were estimated by Enzyme -linked immunosorbent assay (ELISA).

3.8.3.1 Growth hormone ELISA

Principle of the test

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for hGH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of hGH is conjugated to horse radish peroxidase (HRP). hGH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by

addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of hGH in the sample. A set of standards is used to plot a standard curve from which the amount of hGH in patient samples and controls can be directly read.

Test procedure

1. Working solutions of the anti-hGH-HRP - conjugate and wash buffer were prepared.
2. Required number of microwell strips were removed. Bag was resealed and unused strips were returned to the refrigerator.
3. 25 μL of each calibrator, control and specimen samples were pipetted into correspondingly labelled wells in duplicate.
4. 100 μL of the conjugate working solution was pipetted into each well.
5. Wells were incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wells were washed at least 3 times with wash buffer (300 μL /well for each wash) and taped the plate firmly against absorbent paper to ensure that it is dry.
7. 100 μL of TMB substrate was pipetted into each well at timed intervals.
8. Plate was incubated on a plate shaker at room temperature for 10-15 minutes.
9. 50 μl of stopping solution was pipetted into each well at the same timed intervals as in step 7

10. The plate was read on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution

Calculations

1. The average absorbance values were calculated for each set of standards, controls and samples.
2. Standard curve was plotted and the corresponding concentration for each sample was determined automatically using the 4 parameter curve fit method.

3.8.3.2 TSH ELISA

Principle of the test

The TSH ELISA kit is a solid phase enzyme - linked immunosorbent assay (ELISA), based on the sandwich principle. The micro titer wells are coated with a monoclonal [mouse] antibody directed a unique antigenic site of the THS molecule. An aliquot of samples containing endogenous THS is incubated in the coated well with enzyme conjugate, which is an anti THS antibody conjugated with horseradish peroxidase. After incubation of the unbounded conjugate is washed off. The amount of bound peroxidase conjugate is proportional to the concentration of THS in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of THS in the sample.

Test procedure

1. 25 μ L of each standard, control and samples were dispensed into appropriate wells.

2. Plate was incubated at room temperature for 10 minutes.
3. 10 μL enzyme conjugate was dispensed into each well and thoroughly mixed for 10 seconds.
4. Plate was incubated at room temperature for 90 minutes.
5. The wells were washed 5 times with diluted wash solution (300 μL per well) and the plate was taped firmly against absorbent paper to remove residual droplets.
6. 100 μL of substrate solution was pipetted into each well.
7. The plate was incubate at room temperature for 20 minutes.
8. 100 μL of stop solution was pipetted into each wells to stop the enzymatic reaction.
9. The plate was read on a microwell plate reader (Thermo Scientific, MULTISCAN GO, Finland) at 450 ± 10 nm within 5 minutes of addition of the stopping solution

Calculation of results

1. The average absorbance values were calculated for each set of standards, controls and samples.
2. Standard curve was plotted and the corresponding concentration for each sample was determined automatically using the 4 parameter curve fit method.

3.8.3.3 T₃ ELISA

Principle of the test

The T₃ ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal goat-anti-mouse antibody. Standards, controls and patient serum incubate together with Assay Reagent containing monoclonal anti-T₃ antibodies. In the following incubation with Conjugate the endogenous T₃ of a patient sample competes with the T₃-horseradish peroxidase conjugate for a limited number of insolubilized binding sites. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of T₃ in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of T₃ in the patient sample.

Test procedure

1. 50 µL of each standard, control and sample with new disposable tips were pipetted into appropriate wells.
2. 50 µL of Assay Reagent was added into each wells and thoroughly mixed for 10 seconds.
3. The plate was incubated at room temperature (20°C - 27°C) for 30 minutes.
4. 50 µL Enzyme Conjugate was dispensed into each wells and thoroughly mixed for 10 seconds.
5. The plate was incubated at room temperature (20°C - 27°C) for 30 minutes.

6. The wells were washed 5 times with diluted wash solution (300 ml per well) and the plate was taped firmly against absorbent paper to remove residual droplets.
7. 100 μ L of Substrate Solution was pipetted into each wells.
8. The plate was incubated at room temperature (20°C-27°C) for 10 minutes.
9. Enzymatic reaction was stopped by adding 100 μ L of Stop Solution to each well.
10. The absorbance (OD) of each well was determined at 450 \pm 10 nm with a microplate reader.

Calculation of results

1. The average absorbance values were calculated for each set of standards, controls and samples.
2. Standard curve was plotted and the corresponding concentration for each sample was determined automatically using the 4 parameter curve fit method.

3.8.3.4 T₄ ELISA

Principle of the test

The T₄ ELISA kit is a solid phase enzyme- linked immunosorbent assay (ELISA), based on the principle of competitive binding. The micro titer wells are coated with a monoclonal [mouse] antibody directed towards an antigenic site of the T₄ molecule. Endogenous T₄ of a sample competes with the T₄-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the conjugate was

washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of T₄ in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of T₄ in the sample.

Test procedure

1. 10 μ L of the each standard, control and samples were pipetted into appropriate wells.
2. Wells were incubated at room temperature for 5 minutes (18°C-25°C).
3. 100 μ L enzyme conjugate was dispensed into each well and thoroughly mixed.
4. Wells were incubated at room temperature for 80 minutes (18°C-25°C).
5. The wells were washed 5 times with diluted wash solution (400 ml per well) and the plate was taped firmly against absorbent paper to remove residual droplets.
6. 100 μ L substrate solution was pipetted into each well.
7. Wells were incubated at room temperature (18°C-25°C) for 10 minutes.
8. Wells were incubated at room temperature (26°C-29°C) for 7 minutes.
9. Wells were incubated at room temperature (more than 29°C) for 5 minutes.
10. Enzymatic reaction was stopped by adding 100 μ L of stop solution.
11. The absorbance (OD) of each well was determined at 450 \pm 10 nm with a microplate reader.

Calculation of results

1. The average absorbance values were calculated for each set of standards, controls and samples.
2. Standard curve was plotted and the corresponding concentration for each sample was determined automatically using the 4 parameter curve fit method.

3.9 Expression of IGF-1 mRNA in PBMC

Blood samples were collected from the external jugular vein of all the three breeds into the tubes coated with EDTA anticoagulant, 1 day prior to slaughter. Ice-cold 1× RBC lysis buffer was added to the blood at the ratio of 9:1, mixed gently and incubated at room temperature for 10–15 min. Centrifugation was done at 3500 rpm for 25 min at 4°C. Decant the supernatant and wash the peripheral blood mononuclear cell (PBMC) pellet with lysis buffer one more time, if needed.

The total RNA was isolated from the PMBC pellet using GeneJET Whole Blood RNA Purification Mini Kit (Thermo Scientific, Lithuania), and the procedure was done as per manufacturer's protocol with slight modifications as follows: The pellet was re-suspended in 600 µL of lysis buffer, mixed well by vortexing. Four hundred fifty microliters of ethanol (96-100%) was added and mixed by pipetting. The lysate was transferred to the given column and centrifuged at the rate of 12,000 rpm for 1 min, and the flow through was discarded. Then, 700 µL of wash buffer 1 was added to the purification column and centrifuged at the rate of 12 rpm for 1 minute, and the flow

through was discarded. Five hundred microliters of wash buffer 2 was added to the purification column and centrifuged at the rate of 12 rpm for 1 minute, and the flow through was discarded. The empty purification column was re-spinned at 13,000 rpm for 4 min. The preheated 50°C nuclease-free water of 35 μ L was added at the center of the column and incubated for 5 min and centrifuged at the rate of 12,000 rpm for 4-5 minute. The RNA eluted was collected in fresh nuclease free microfuge vials and processed immediately.

The total RNA was reverse transcribed into complementary DNA (cDNA) using Maxima first strand cDNA synthesis kit for real-time quantitative polymerase chain reaction (RTqPCR) (Thermo Scientific, Lithuania). The procedure was performed as per manufacturer's protocol with modifications are as follows: 4 μ L of 5 \times Reaction Mix, 2 μ L Maxima Enzyme Mix, 1 μ g of template RNA was used for PBMC samples, and 20 μ L of nuclease-free water were added into a sterile, RNAase-free tube. Then, the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 min at 25°C, followed by 20 min at 50°C, and the reaction was terminated by heating at 85°C for 5 min). The product of the first-strand cDNA synthesis was diluted to a final concentration of 25 ng/ μ L with nuclease-free water, and 2 μ L of diluted cDNA was used for each reaction in qPCR.

Sequences of the primer used for amplifying the target regions of IGF-1 and GAPDH have been published by Bagath *et al.* (2016) and Shaji *et al.* (2016) respectively. The primer sequences are described in table 3.

The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20- μ L reaction was carried out in duplicates using 50 ng of template and 0.5 μ M primer concentrations. The real-time qPCR 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). The melt curve analysis was performed to check the non-specific amplification. The GAPDH gene was used as an internal control, and the relative expression was analyzed using the formula, $2^{-\Delta\Delta CT}$ (Shaji *et al.*, 2016).

Table 3: Primers used for IGF-1 expression. GAPDH used as reference gene to normalize the gene expression of target genes

Gene ID	Primers	Primer sequence (5''-3'')	Primer length (bp)	Product size (bp)	Accession No.
IGF-1	F	CTTGAAGCAGGTGAAGATGCC	21	132	NM_001285697.1
	R	AGAGCATCCACCAACTCAGC	20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG	20		

IGF-1 - Insulin like growth factor 1, GAPDH - Glyceraldehyde 3-phosphate dehydrogenase, bp- base pair

3.10 Statistical Analysis

The experimental data were analysed using general linear model (GLM) repeated measurement analysis of variance (SPSS 18). Effect of fixed factors, namely breed (Osmanabadi, Malabari, Salem Black) and group (OC, OHS, MC, MHS, SBC

and SBHS) were taken as between subject factor and days (longitudinal time over which experiment was carried out; Day 0, Day 15, Day 30 and Day 45) were taken as within subject factor and also the interaction between breed, treatment and experimental days were analysed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests as described by Kramer (1957). The changes in relative expression of PBMC IGF-1 messenger RNA (mRNA) in relation to GAPDH (glyceraldehyde 3-phosphate dehydrogenase) as the house keeping gene were analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis to compare the means among the groups. The correlation association between THI and all the growth attributes, rumen metabolites, enzymes and endocrine variables were assessed based on Pearson's correlation coefficient test using SPSS (Version 18.00) software. The R^2 values were used to assess the correlation association. The results were expressed as mean and SE and the statistical significance level was set at $P < 0.05$.

RESULTS

CHAPTER 4

RESULTS

4.1 Weather parameters

The maximum, minimum, wet and dry bulb temperatures, RH and PST were recorded and the THI was calculated on an average at fortnightly interval. The THI values during morning and afternoon (outside and inside the shed) are described in table 4. The THI values outside the shed shows that during the morning hours the animals were in comfort zone while during afternoon the animals were under extreme distress condition. The THI values were calculated as per method described by McDowell (1972) and presented in fig. 4. The THI values 72 and less are considered comfortable; THI values between 75 and 78 are considered stressful and THI above 78 considered Extreme distress. The comparative assessment of average summer season THI in the native tract of Malabri (Kerala) and Salem Black (Tamil Nadu) breed to the current experimental location (Karnataka) clearly indicated the significantly ($p < 0.05$) lower average THI in the experimental location as compared to their native tract (fig. 5).

Table 4: Average weather parameters for the entire study period

	Time of Recording	DBT (°C)	WBT (°C)	MaxT (°C)	MinT (°C)	RH (%)	AT (°C)	PST (°C)	THI
Inside	Morning (8:00 h)	23.2± 0.11	17.5± 0.17	41.5± 1.40	22.5± 0.92	56.7± 1.76	26.6± 0.31	25.5± 0.20	69.9± 0.16
	Afternoon (14:00 h)	26.0± 0.16	21.6± 0.15	44.6± 0.94	24.1± 1.02	37.1± 1.62	34.2± 0.22	30.6± 0.46	74.9± 0.14
Outside	Morning (8:00 h)	24.5± 0.55	21.2± 0.30	44.0± 1.27	23.0± 0.93	58.6± 2.54	28.8± 0.61	29.5± 0.61	73.5± 0.56
	Afternoon (14:00 h)	34.6± 0.37	29.1± 0.43	44.9± 0.81	24.4± 1.27	29.1± 1.75	39.9± 0.63	47.4± 0.76	86.5± 0.39

DBT-Dry Bulb Temperature; Wet Bulb Temperature; MinT-Minimum Temperature; MaxT-Maximum Temperature; RH-Relative Humidity; AT-Ambient Temperature; PST-Pen Surface Temperature; THI-Temperature Humidity Index

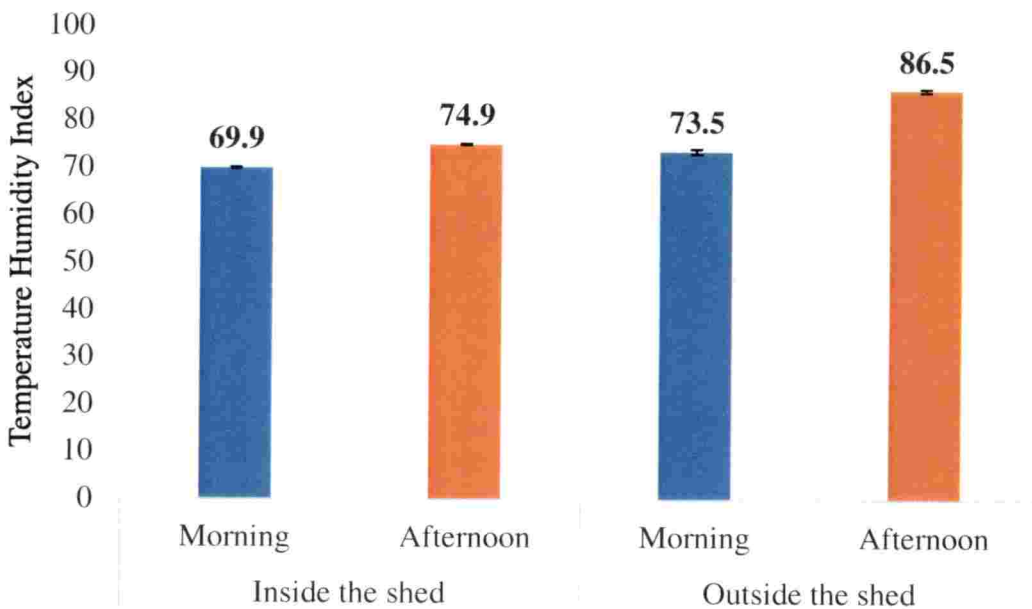


Fig. 4: Average temperature humidity index (THI) both inside and outside the shed

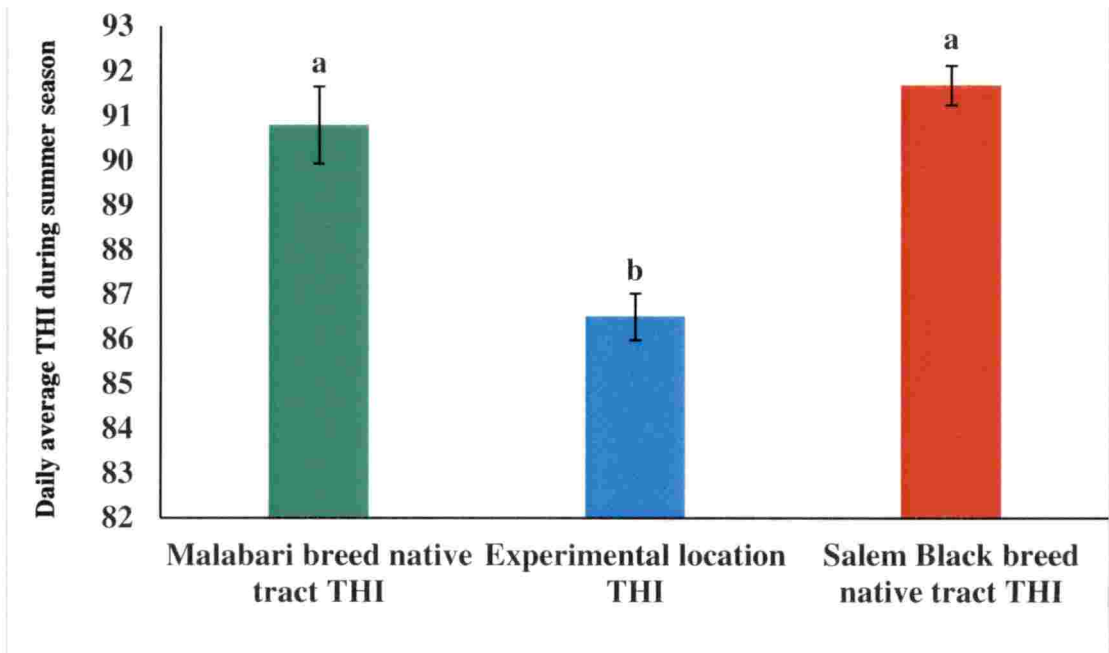


Fig. 5: Comparative average temperature humidity index (THI) during summer season in the native tract of Malabari and Salem black breed and the current experimental location

4.2 Growth variables

The effect of heat stress on growth parameters in Osmanabadi, Malabari and Salem Black goats are presented in Table 4. Body weight showed significant variation for both the breed ($P < 0.01$) and treatment ($P < 0.05$). Comparing the body weight changes between the breeds, control group of Malabari breed showed lower body weight than the control group of other two breeds. Further, the body weight did not differ between the control group of Osmanabadi and Salem Black breeds. Within the breeds, body weight did not differ between the control and heat stress groups in all three breeds. The rate of reduction in body weight at the end of study period were 3.4 kg, 2.685 kg and 2.65 kg in OHS, MHS and SBHS groups respectively. However, both the experimental days and interaction between breed, treatment and experimental days did not influence the body weights of all three indigenous goats. The ADG did not show any significant difference between the breeds. But heat stress treatment significantly ($P < 0.01$) reduced the ADG in all the heat stress groups as compared to

their respective control groups. KR showed similar trend as that of ADG for both the breed and treatment. The heat stress treatment significantly ($P<0.01$) reduced the KR in all the heat stress groups as compared to their respective control groups. Further, RGR also did not exhibit any significant variation between the breeds. However, the RGR was significantly ($P<0.01$) lower in both OHS and MHS groups as compared to their respective control groups. Further, the RGR did not differ between SBC and SBHS. In addition, the BMI showed significant ($P<0.05$) variation for the breed factor. However, the heat stress treatment did not influence the BMI in all three breeds. The BCS showed significant ($P<0.05$) variation for both breed as well as treatment. Further, the experimental days significantly influenced only BMI ($P<0.05$) and BCS ($P<0.05$) in the study. In addition, the interaction between breed, treatment and experimental days did not influence any of the growth variables. In addition, a strong negative correlation was established between THI and all growth variables (table 6).

Table 5: Comparative assessment of growth parameters in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	BREED* TRT*DAY
Body weight (kg)	0	16.78	17.07	13.43	13.45	16.85	16.53				
	7	17.13	16.07	13.45	12.88	17.08	17.50				
	15	17.57	15.77	13.43	12.86	17.21	17.42				
	21	17.53	15.73	13.72	13.17	17.52	17.17				
	30	18.10	15.03	13.73	12.50	17.72	15.88	**	*	NS	NS
	37	18.45	14.77	14.37	12.12	18.05	15.28				
	45	18.80	15.40	15.08	12.40	18.30	15.65				
	Pooled SE	±0.83	±0.83	±0.83	±0.83	±0.83	±0.83				
	7	50.00	-142.86	2.38	-80.95	33.33	138.10				
Average daily gain (g)	15	61.91	-42.86	-2.38	-3.57	17.86	-11.91				
	21	-4.76	-4.762	40.48	44.05	44.05	-35.71				
	30	80.95	-100.00	2.38	-95.24	28.57	-183.33				
	37	50.00	-38.10	90.48	-54.76	47.62	-85.71	NS	**	NS	NS
	45	50.00	90.48	102.38	40.48	35.72	52.38				
	Pooled SE	±16.68	±16.68	±16.68	±16.68	±16.68	±16.68				

	7	6.07	-18.39	-0.67	-13.03	3.76	15.85				
	15	6.54	-5.61	-0.40	-0.66	1.84	-1.60				
	21	-0.30	-0.75	6.15	6.72	5.08	-4.06				
Kleiber ratio	30	9.20	-13.36	0.21	-14.17	3.20	-24.05	NS	**	NS	NS
	37	5.62	-5.26	12.24	-9.38	5.56	-10.76				
	45	5.76	11.18	13.51	5.18	4.00	6.82				
	Pooled SE	±2.11	±2.11	±2.11	±2.11	±2.11	±2.11				
	7	0.19	-0.51	-0.01	-0.41	0.12	0.55				
	15	0.20	-0.17	-0.00	-0.02	0.06	-0.05				
	21	-0.01	-0.02	0.22	0.23	0.16	-0.12				
Relative growth rate	30	0.28	-0.41	0.03	-0.46	0.10	-0.73	NS	**	NS	NS
	37	0.17	-0.17	0.41	-0.31	0.17	-0.33				
	45	0.18	-0.36	0.44	0.17	0.12	0.22				
	Pooled SE	±0.07	±0.07	±0.07	±0.07	±0.07	±0.07				
	0	1.41	1.48	1.21	1.26	1.48	1.41				
	15	1.54	1.41	1.24	1.13	1.44	1.52				
Body mass index (g)	30	1.60	1.33	1.32	1.12	1.49	1.39	*	NS	*	NS
	45	1.71	1.37	1.49	1.14	1.64	1.30				
	Pooled SE	±0.09	±0.09	±0.09	±0.09	±0.09	±0.09				
	0	2.42	2.33	2.00	2.25	2.33	2.42				
	15	2.75	2.42	2.00	1.92	2.33	2.17				
Body condition score	30	2.92	2.33	2.08	2.00	2.00	2.00	*	*	**	NS
	45	3.08	2.42	2.67	2.00	2.92	2.17				
	Pooled SE	±0.16	±0.16	±0.16	±0.16	±0.16	±0.16				

OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS- Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- treatment, BREED*TRT*DAY- Breed, treatment and day interaction.

**P<0.01, *P<0.05, NS- Non-significant.

Table 6: Correlation Association between THI and growth variables

	THI	BW	ADG	KR	RGR	BMI	BCS
THI	1						
BW	-0.334**	1					
ADG	-0.361**	0.272**	1				
KR	-0.347**	0.259**	0.993**	1			
RGR	-0.339**	0.249**	0.988**	0.999**	1		
BMI	-0.356**	0.933**	0.312**	0.297**	0.288**	1	
BCS	-0.354**	0.656**	0.318**	0.309**	0.301**	0.649**	1

THI- Temperature Humidity Index, BW-Body Weight, ADG- Average Daily Gain, KR -Kleiber Ratio, RGR- Relative Growth Rate, BMI- Body Mass Index, BCS- Body Condition Score

**P<0.0

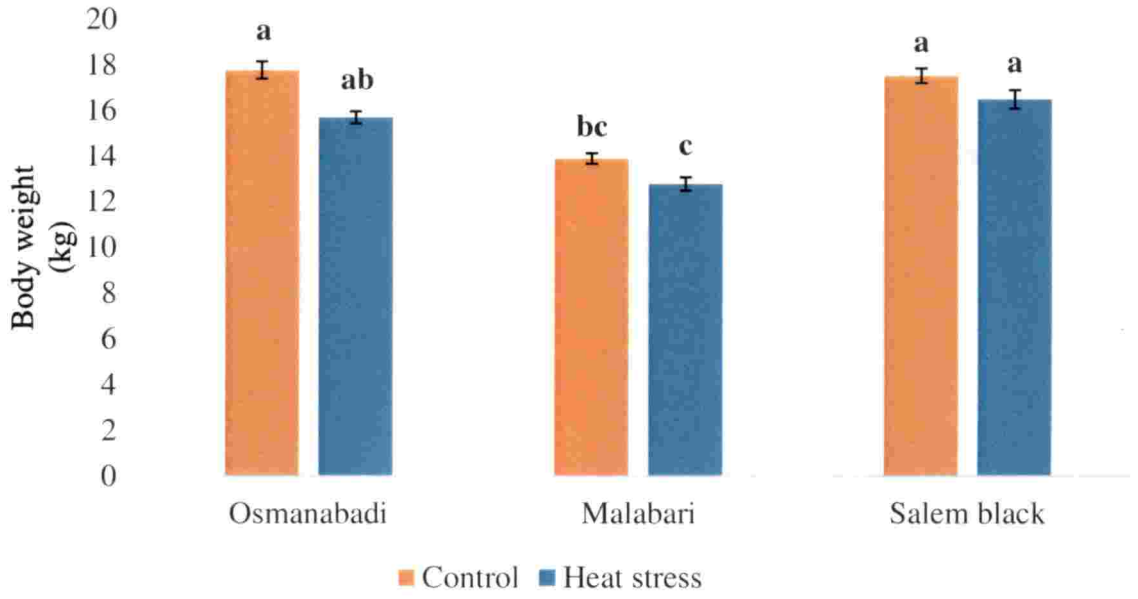


Fig 6: Impact of heat stress on body weight

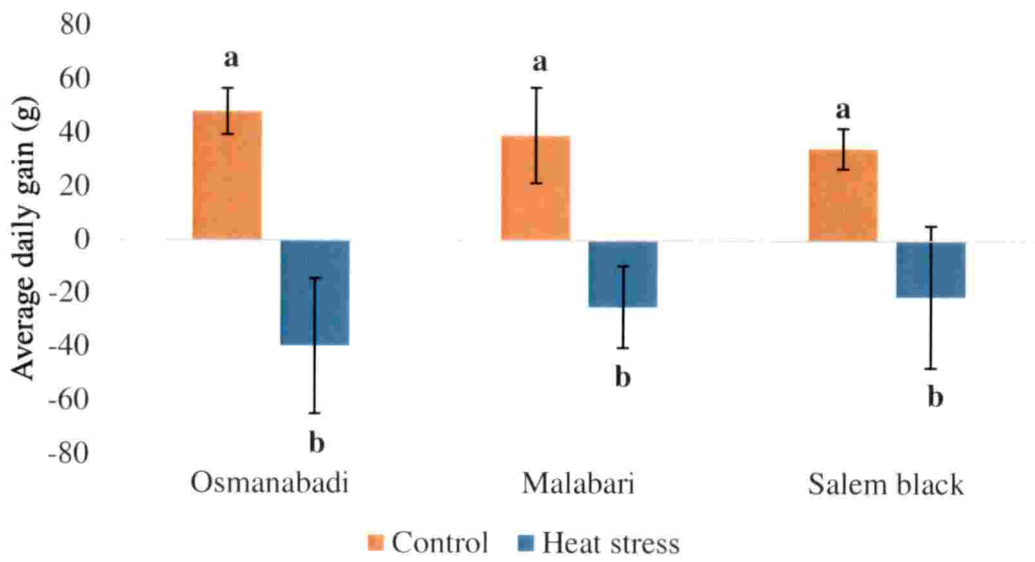


Fig 7: Impact of heat stress on average daily gain

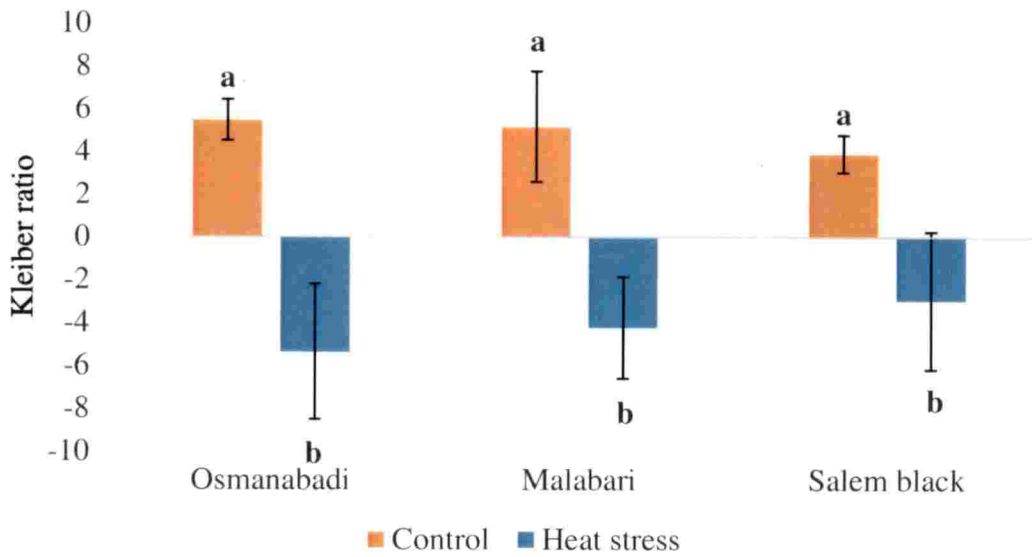


Fig 8: Impact of heat stress on kleiber ratio

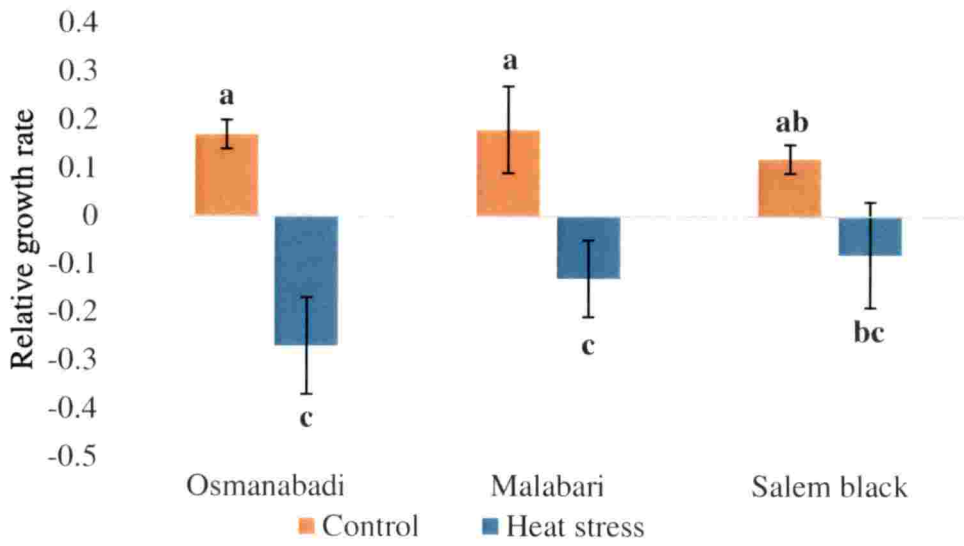


Fig 9: Impact of heat stress on relative growth rate

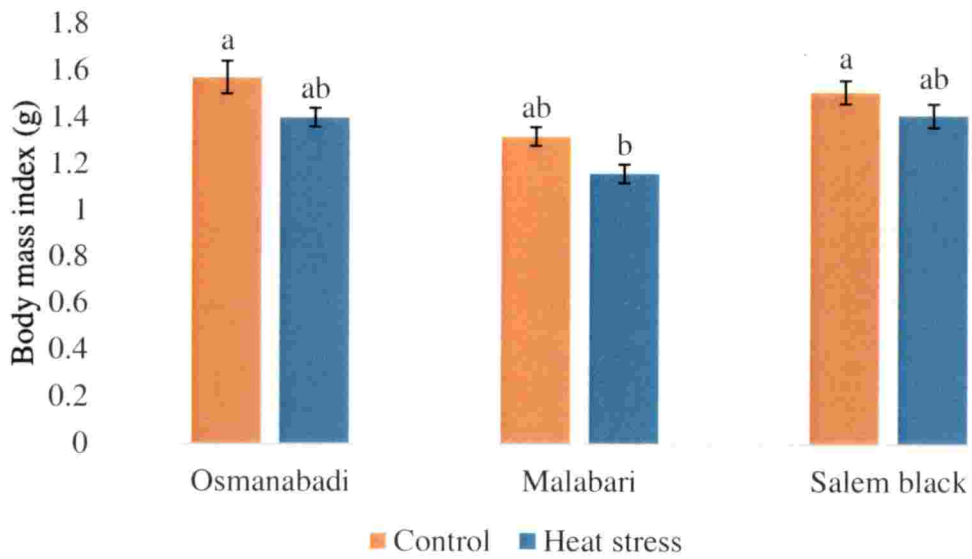


Fig 10: Impact of heat stress on body mass index

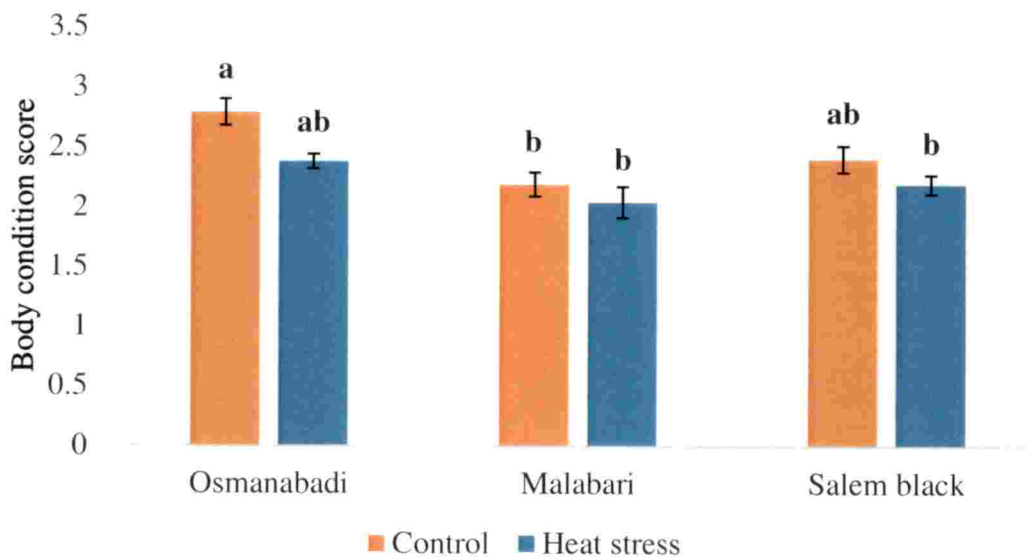


Fig 11: Impact of heat stress on body condition score

4.3 Allometric variables

The effects of heat stress on allometric measurements of three different indigenous breeds are presented in table 7. BL showed significant ($P < 0.01$) variation for the breed factor. However, the BL did not show any significant difference for the treatment, experimental days and interaction between breed, treatment and experimental days. The HG showed significant ($P < 0.05$) variation among all three indigenous breeds. However, treatment did not show any significant variation for HG. But experimental days significantly ($P < 0.05$) influenced the BW of all the experimental animals. However, interaction between the breed, treatment and experimental days did not show any significant variation for HG. The WH showed highly significant ($P < 0.01$) changes for the treatment. However, the treatment, experimental days and interaction between breed, treatment and experimental days did not influence the WH in the study. Both hip width (HW) and RuH showed similar trend as that of WH for the breed showing significant ($P < 0.01$) difference for these parameters while the treatment, experimental influencing these parameters. Additionally, both breed and treatment in

the current study significantly ($P<0.01$) influenced the CD of the goats. However, both experimental days and interaction between breed, treatment and experimental days did not show any significant variation for CD. Further among the allometric measurements, a negative correlation was established only between THI and HG ($P<0.05$), HW ($P<0.05$) and CD ($P<0.01$) (table 8)

Table 7: Comparative assessment of allometric variables in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	BREED* TRT *DAY
Body length (cm)	0	55.55	56.72	51.60	53.65	58.92	59.88				
	15	56.85	57.12	52.23	52.40	55.15	59.57				
	30	58.67	56.13	53.13	51.95	56.70	59.43	**	NS	NS	NS
	45	60.22	57.07	55.78	52.53	58.47	57.17				
	Pooled SE	±1.35	±1.35	±1.35	±1.35	±1.35	±1.35				
Heart girth (cm)	0	61.35	60.63	58.15	57.88	61.85	63.67				
	15	60.88	57.63	58.73	57.53	59.95	62.30				
	30	59.52	57.03	59.87	56.87	60.90	60.60	**	NS	*	NS
	45	59.25	57.98	60.48	57.23	60.43	61.17				
	Pooled SE	±1.13	±1.13	±1.13	±1.13	±1.13	±1.13				
Wither height (cm)	0	66.31	64.97	57.23	57.37	67.20	70.38				
	15	65.43	64.13	56.63	59.85	66.33	68.55				
	30	66.73	63.68	55.57	58.05	67.60	68.00	**	NS	NS	NS
	45	66.92	64.33	56.40	57.52	65.20	69.07				
	Pooled SE	±1.03	±1.03	±1.03	±1.03	±1.03	±1.03				
Hip width (cm)	0	19.13	17.83	17.67	16.85	18.10	18.87				
	15	18.50	17.73	17.00	17.05	18.37	18.85				
	30	19.63	17.43	17.12	16.27	18.85	18.30	**	NS	NS	NS
	45	18.30	17.87	17.58	16.55	19.10	18.83				
	Pooled SE	±0.45	±0.45	±0.45	±0.45	±0.45	±0.45				
Rump height (cm)	0	70.30	69.07	60.95	61.15	72.70	73.77				
	15	69.87	66.35	61.70	62.92	70.18	73.18				
	30	70.13	65.82	61.80	61.42	70.98	72.60	**	NS	NS	NS
	45	70.42	67.60	61.72	61.67	72.20	72.57				
	Pooled SE	±1.17	±1.17	±1.17	±1.17	±1.17	±1.17				

Chest depth (cm)	0	29.42	30.03	24.62	24.10	25.00	25.80				
	15	28.97	25.30	24.08	23.95	26.58	22.78				
	30	27.45	24.37	24.63	23.63	28.17	26.32	**	**	NS	NS
	45	27.07	26.48	23.88	23.43	28.83	26.65				
	Pooled SE	±0.61	±0.61	±0.61	±0.61	±0.61	±0.61				

OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS- Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- treatment, BREED*TRT*DAY- breed, treatment and day interaction.

**P<0.01, *P<0.05, NS- Non-significant.

Table 8. Correlation Association between THI and allometric variables

	THI	BL	WH	HG	HW	RuH	CD
THI	1						
BL	-0.050	1					
WH	0.068	0.618**	1				
HG	-0.200*	0.591**	0.459**	1			
HW	-0.207*	0.569**	0.624**	0.573**	1		
RuH	-0.052	0.622**	0.870**	0.573**	0.696**	1	
CD	-0.337**	0.395**	0.473**	0.321**	0.356**	0.514**	1

THI- Temperature Humidity Index, BL- Body Length, WH- Wither Height, HG- Heat Girth, HW- Hip Width, RuH- Rump Height, CD- Chest Depth

**P<0.01, *P<0.05

ES

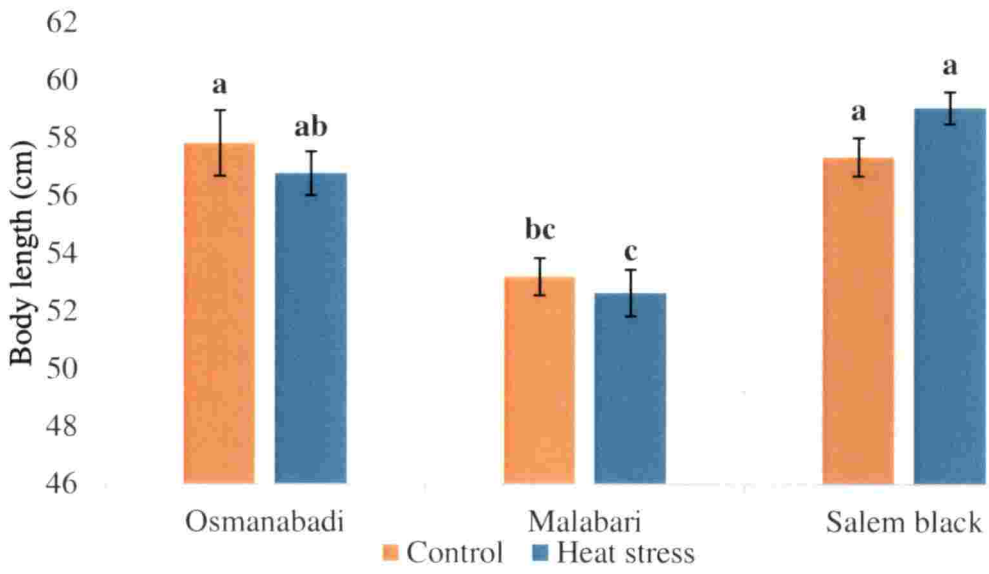


Fig 12: Impact of heat stress on body length

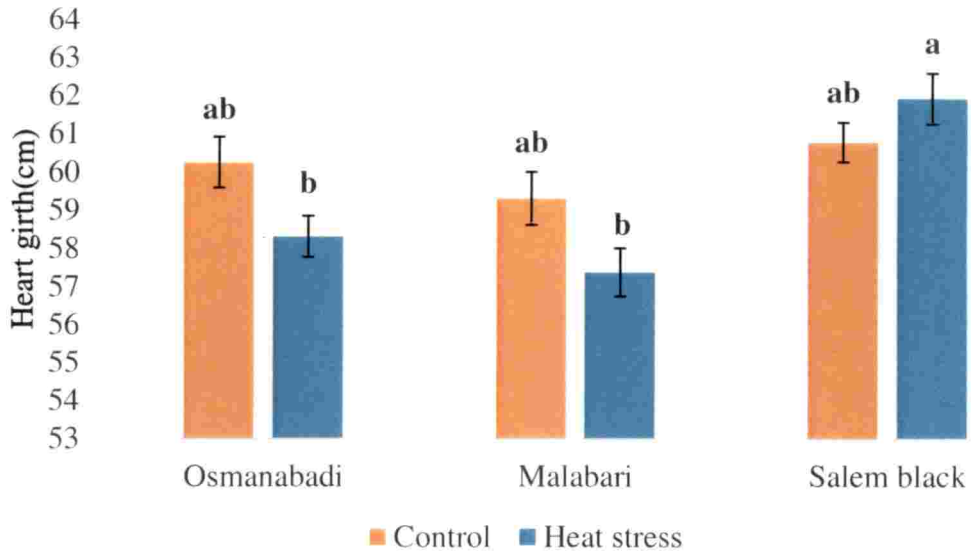


Fig 13: Impact of heat stress on heart girth

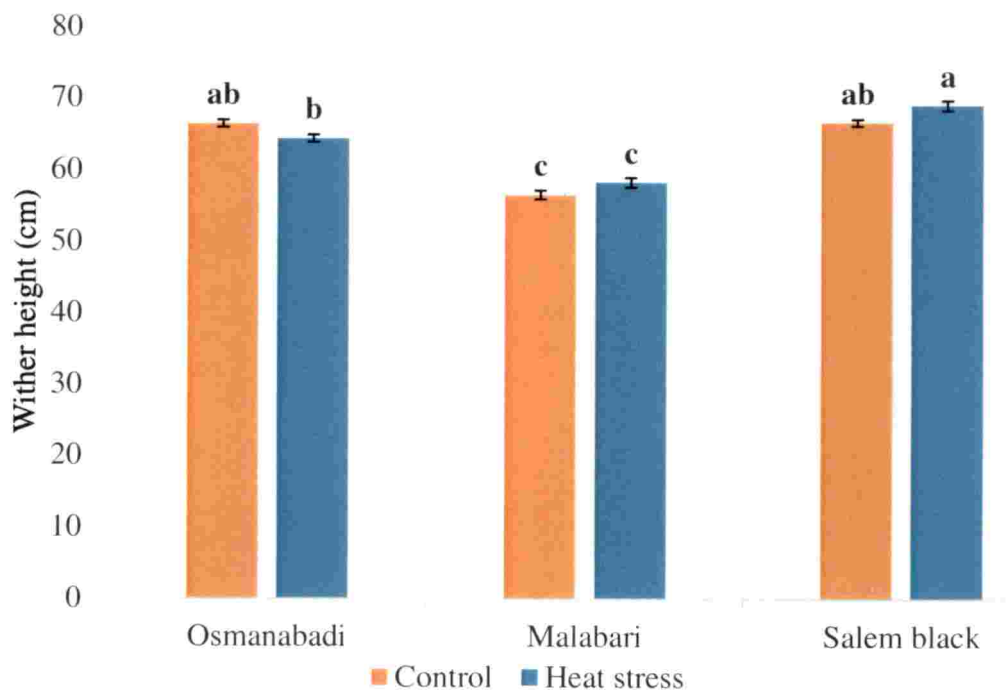


Fig 14: Impact of heat stress on wither height

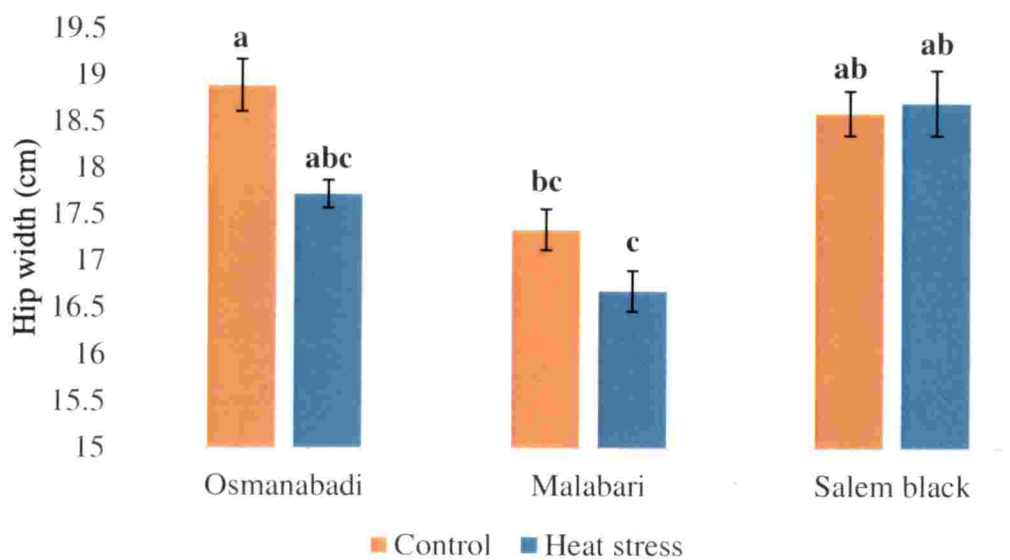


Fig 15: Impact of heat stress on hip width

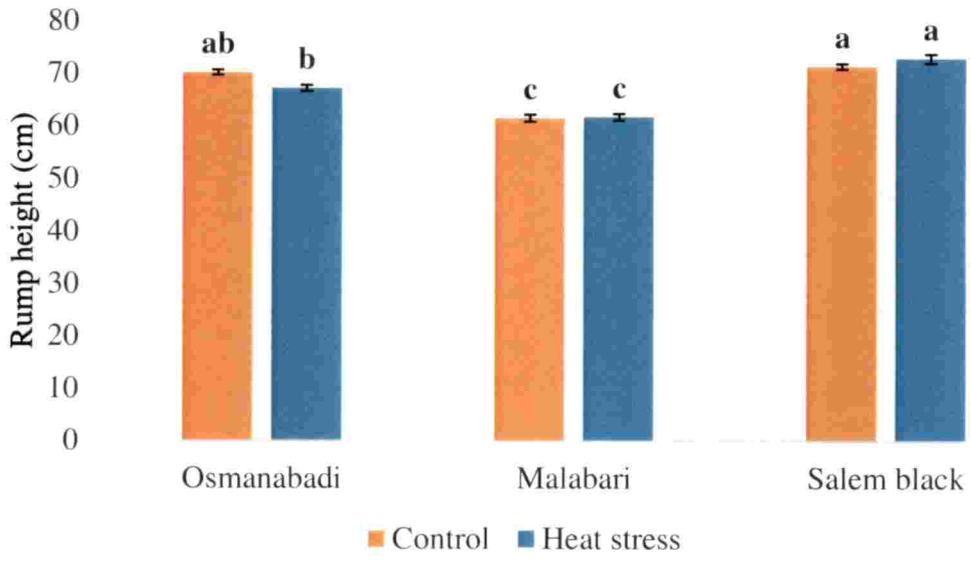


Fig 16: Impact of heat stress on rump height

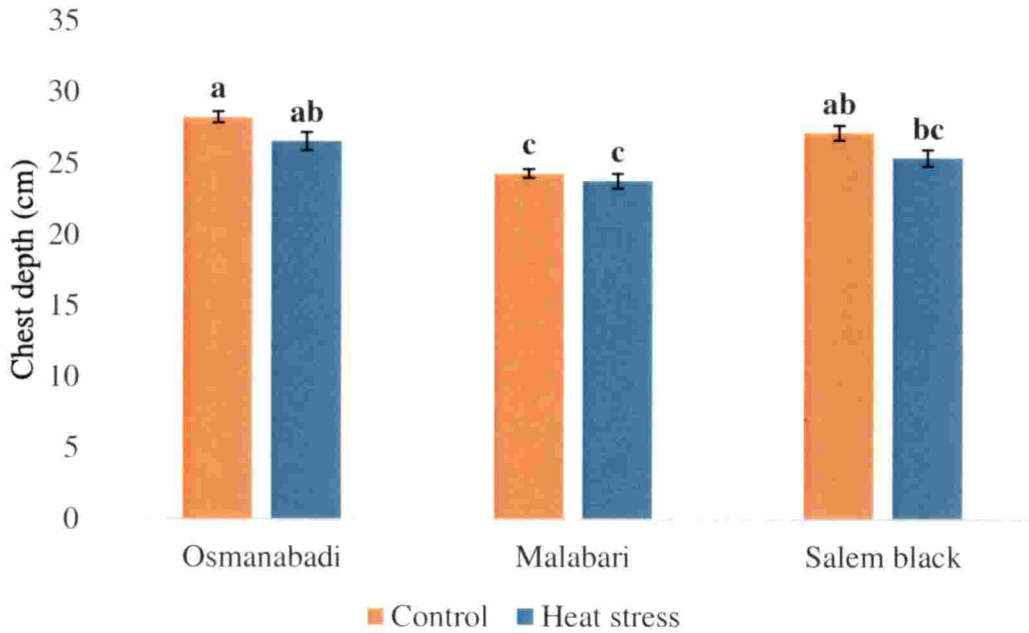


Fig 17: Impact of heat stress on chest depth

4.4 Feed intake

The effect of heat stress on the feed intake is depicted in table 9. Both the breed factor ($P < 0.01$) and experimental days significantly influenced the feed intake of the animals. However, the treatment did not cause significant variation among the groups. Further, the interaction between breed, treatment and experimental days did not influence the feed intake of the animals.

Table 9: Comparative assessment of feed intake in three different indigenous goat breeds subjected to heat stress

Attribute	Days	Treatments						Effects			
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	BREED*TRT*DAY
Feed intake (DMIg/kg ^{0.75} *Day)	0	51.03	48.21	46.64	42.04	50.97	48.86				
	15	48.48	38.71	39.72	39.07	37.06	42.76				
	30	43.90	31.25	31.83	25.99	44.32	33.47				
	45	40.84	40.47	47.59	34.55	45.62	31.61				
	Mean	46.06^a	39.66^{ab}	41.45^{ab}	35.41^b	44.49^a	39.18^{ab}	NS	**	**	NS
Pooled SE	±4.47	±4.47	±4.47	±4.47	±4.47	±4.47					

DMI- Dry matter intake, OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS- Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- Treatment, BREED*TRT*DAY- Breed, treatment and day interaction.

**P<0.01, *P<0.05, NS- Non-significant.

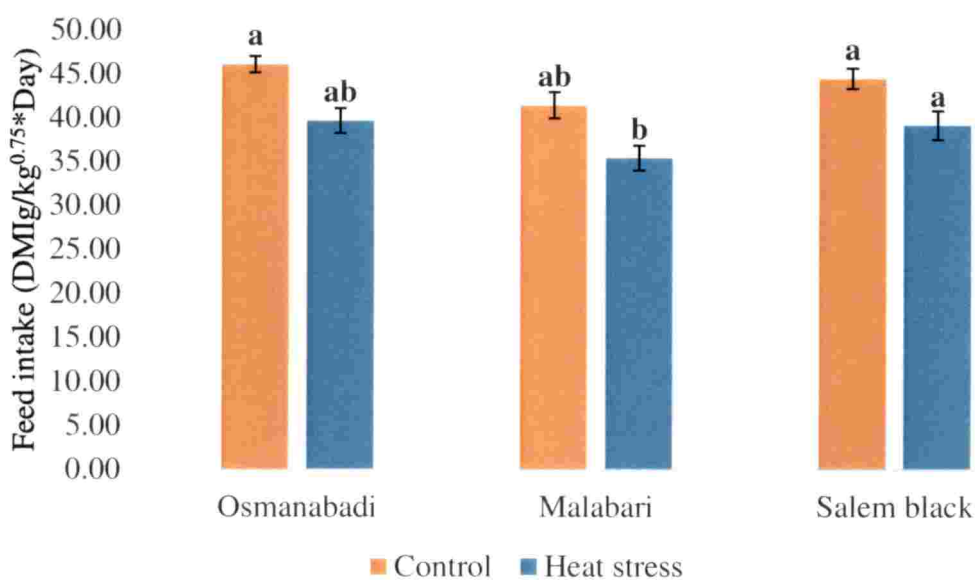


Fig 18: Impact of heat stress on feed intake

4.5 Rumen liquor biochemical parameters

The results for rumen liquor biochemical parameters are presented in table 10. Rumen liquor pH did not show any significant variation for breed factor. However, the treatment in the present study showed significantly ($P<0.01$) higher variation for the rumen liquor pH. Further, the experimental days, and interaction between breed, treatment and experimental days significantly ($P<0.05$) influenced the rumen liquor pH in the study. In addition, a strong ($P<0.01$) negative correlation was established between THI and rumen liquor pH (table 11).

Table 10: Comparative assessment of rumen liquor biochemical parameters in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	BREED* TRT*DAY
Rumen liquor pH	0	6.49	6.56	6.54	6.44	6.71	6.51				
	15	6.61	6.50	6.55	6.42	6.69	6.68				
	30	6.77	6.39	6.74	6.51	6.66	6.63				
	45	6.46	6.37	6.62	6.37	6.27	6.37	NS	**	*	*
	Mean	6.58^{ab}	6.46^{bc}	6.61^a	6.44^c	6.58^{ab}	6.55^{abc}				
	Pooled SE	±0.05	±0.05	±0.05	±0.05	±0.05	±0.05				
NH ₃ -N	0	30.12	29.73	20.70	22.15	20.96	22.71				
	45	22.34	16.85	20.49	15.74	19.90	14.27				
	Mean	26.23^a	23.29^{ab}	20.60^{bc}	18.95^c	20.43^{bc}	18.49^c	**	*	**	NS
	Pooled SE	±1.53	±1.53	±1.53	±1.53	±1.53	±1.53				

OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS- Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- Treatment, BREED*TRT*DAY- Breed, treatment and day interaction.

** $P<0.01$, * $P<0.05$, NS- Non-significant.

Breed differences was significantly ($P<0.01$) evident in the NH₃-N concentration. Osmanabadi breed showed significantly ($P<0.05$) higher NH₃-N concentration than other two indigenous goat breeds. Further, the treatment also showed significant variation among the control and heat stress group of each breed. In

addition the significant differences in the experimental days indicates the variation in the animal response for NH₃-N throughout the study period. However, the interaction between breed, treatment and experimental days did not influence the NH₃-N in the study. Further, a strong (P<0.01) negative correlation was established between THI and NH₃-N concentration (table 12).

Table 11. Correlation Association between THI and Rumen liquor pH

	THI	pH
THI	1	
pH	-0.317**	1

THI- Temperature Humidity Index

**P<0.01

Table 12. Correlation Association between THI and Ammoniacal Nitrogen

	THI	NH ₃ -N
THI	1	
NH ₃ -N	-0.796**	1

THI- Temperature Humidity Index, NH₃-N - Ammoniacal Nitrogen

**P<0.01

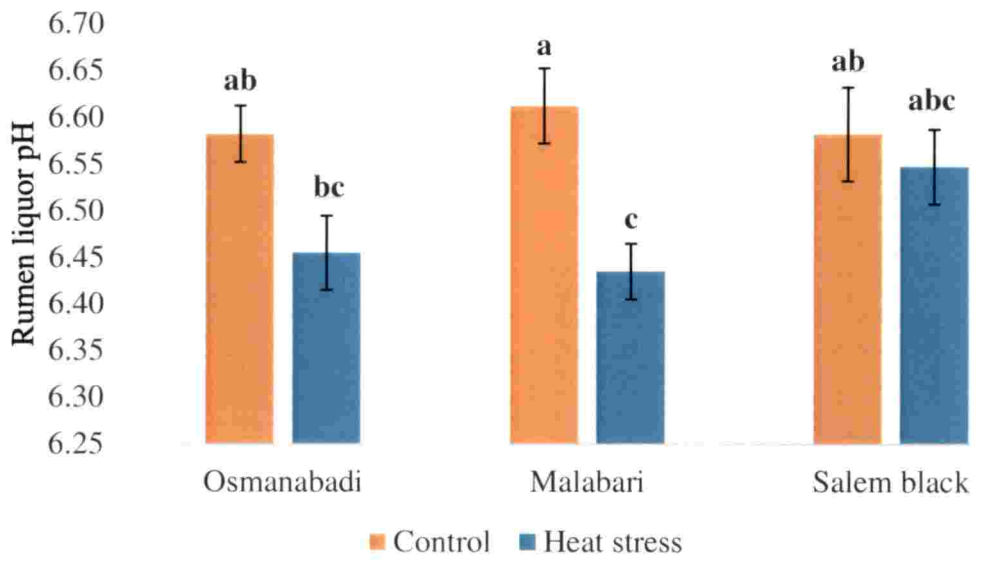


Fig 19: Impact of heat stress on rumen liquor pH

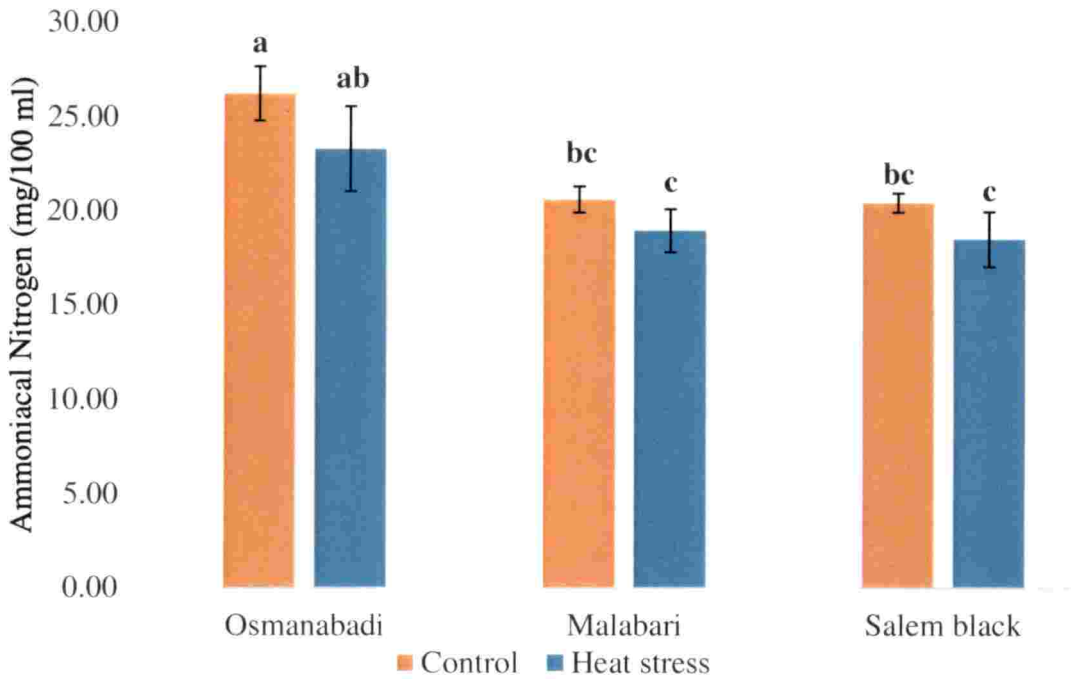


Fig 20: Impact of heat stress on ammoniacal nitrogen concentration

4.6 Rumen volatile fatty acid profile

Rumen volatile fatty acids play an important role in the digestive physiology of the animals, they are the end products of digestion. Table 13 depicts the impact of heat stress on volatile fatty acid production in three different indigenous goat breeds. In the present study breed factor significantly ($P < 0.01$) influenced the acetate concentration in the animals. Among all the three indigenous goat breeds Salem black animals showed significantly ($P < 0.01$) higher acetate concentration signifies the breed differences among the goats. Further treatment also showed significant ($P < 0.01$) variation for the acetate concentration with significantly higher values in heat stress group than control group except for Salem black goats. Similarly, experimental days also significantly ($P < 0.01$) influenced the acetate concentration of goats. However the interaction between breed, treatment and experimental days did not show any significant variation for acetate. Propionate concentration showed similar trend as that of acetate for the breed factor and experimental days. Further, the treatment also showed significant ($P < 0.05$) variation among control and heat stress group goats for the propionate production. Further, the interaction between breed, treatment and experimental days did not influence the propionate concentration significantly. Iso-butyrate did not show any significant variation for the breed, treatment and experimental day effects. However, the interaction between breed, treatment and experimental days significantly ($P < 0.01$) influenced the Iso-butyrate concentration in the goat rumen. Butyrate concentration significantly varied for ($P < 0.01$) both breed and treatment effect. However both experimental days and interaction between breed, treatment and experimental days did not show any significant variation for butyrate concentration. Iso-valerate did not show any significant variation for the breed, treatment and breed*treatment *experimental day interaction. But the experimental days significantly ($P < 0.01$) influenced the iso-valerate concentration in the present experiment. Valerate concentration did not show any significant influence for the breed, treatment, experimental days and interaction between breed, treatment and

experimental days. The TVFA concentration showed significantly ($P<0.01$) higher variation for the breed, treatment, experimental days. Further, interaction between breed, treatment and experimental days significantly ($P<0.05$) influenced the TVFA concentration in goats. In addition, among rumen volatile fatty acids, a strong negative correlation ($P<0.01$) was established only between THI and TVFA concentration (table 14).

Table 13: Comparative assessment of rumen volatile fatty acid profile in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			BREED* TRT*DAY
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	
Acetate (%)	0	61.40	63.87	61.82	63.88	64.68	68.06				
	30	61.15	62.76	61.08	66.75	72.88	72.51				
	45	65.82	69.34	63.87	68.23	70.44	67.81				
	Mean	62.79^c	65.32^b	62.26^c	66.29^b	69.33^a	69.46^a	**	**	**	NS
	Pooled SE	±1.24	±1.24	±1.24	±1.24	±1.24	±1.24				
Propionate (%)	0	27.21	24.90	21.73	20.78	20.41	18.32				
	30	23.08	21.56	23.06	19.75	15.02	16.02				
	45	21.86	19.35	18.99	18.60	15.65	17.41				
	Mean	24.05^a	21.94^b	21.26^{bc}	19.71^c	17.03^d	17.25^d	**	*	**	NS
	Pooled SE	±0.98	±0.98	±0.98	±0.98	±0.98	±0.98				
Acetate: Propionate	0	2.28	2.57	2.85	3.09	3.17	3.72				
	30	2.65	2.91	2.65	3.41	4.94	4.56				
	45	3.02	3.60	3.38	3.75	4.58	3.91				
	Mean	2.65^c	3.03^{bc}	2.96^c	3.42^b	4.23^a	4.06^a	**	NS	**	NS
	Pooled SE	±0.23	±0.23	±0.23	±0.23	±0.23	±0.23				
Iso-butyrate (%)	0	0.94	1.01	1.12	1.20	1.39	1.04				
	30	1.43	1.36	1.18	1.13	1.00	1.08				
	45	0.87	0.91	1.30	0.93	1.15	1.14				
	Mean	1.08^a	1.09^a	1.20^a	1.09^a	1.18^a	1.09^a	NS	NS	NS	**
	Pooled SE	±0.09	±0.09	±0.09	±0.09	±0.09	±0.09				
Butyrate (%)	0	8.47	8.17	13.00	11.39	10.85	10.34				
	30	12.36	11.68	12.22	10.24	8.80	8.52				
	45	9.66	8.30	13.77	10.38	10.71	11.60				
	Mean	10.16^b	9.38^b	13.00^a	10.67^b	10.12^b	10.15^b	**	**	NS	NS
	Pooled SE	±0.81	±0.81	±0.81	±0.81	±0.81	±0.81				

Iso-valerate (%)	0	1.09	1.25	1.18	1.46	1.52	1.16	NS	NS	**	NS
	30	1.13	1.75	1.55	1.32	1.76	1.20				
	45	0.88	1.13	0.63	0.92	0.97	1.04				
	Mean	1.03^c	1.38^{ab}	1.12^{bc}	1.23^{abc}	1.42^a	1.13^{abc}				
	Pooled SE	±0.13	±0.13	±0.13	±0.13	±0.13	±0.13				
Valerate (%)	0	0.89	0.80	1.15	1.29	1.15	1.07	NS	NS	NS	NS
	30	0.86	0.89	0.91	0.82	0.55	0.68				
	45	0.91	0.97	1.45	0.94	1.09	1.00				
	Mean	0.89^a	0.89^a	1.17^a	1.02^a	0.93^a	0.92^a				
	Pooled SE	±0.13	±0.13	±0.13	±0.13	±0.13	±0.13				
TVFA (mmol/L)	0	90.08	91.12	67.51	63.91	72.16	76.68	**	**	**	*
	30	86.06	60.45	64.18	42.74	82.46	74.03				
	45	76.20	62.37	50.77	44.73	66.72	49.71				
	Mean	84.11^a	71.31^b	60.82^d	50.46^e	73.78^b	66.81^c				
	Pooled SE	±2.69	±2.69	±2.69	±2.69	±2.69	±2.69				

OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS, Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- Treatment; BREED*TRT*DAY- Breed, Treatment and Experimental day interaction

**P<0.01, *P<0.05, NS- Non-significant

Table 14: Correlation Association between THI and Rumen volatile fatty acids

	THI	Acetate	Propionate	Acetate : Propionate	Iso- butyrate	Butyrate	Iso- valerate	Valerate	TVFA
THI	1								
Acetate	0.230	1							
Propionate	-0.136	-0.864**	1						
Acetate : Propionate	0.091	0.921**	-0.973**	1					
Iso-Butyrate	-0.141	-0.603**	0.315	-0.412*	1				
Butyrate	-0.282	-0.740**	0.315	-0.458**	0.646**	1			
Iso-Valerate	0.097	-0.122	0.073	-0.026	0.237	-0.016	1		
Valerate	-0.127	-0.409*	0.159	-0.296	0.302	0.526**	-0.286	1	
TVFA	-0.529**	0.123	-0.004	0.127	-0.066	-0.239	0.167	-0.332*	1

THI- Temperature Humidity Index, TVFA- Total Volatile Fatty Acids

**P<0.01, *P<0.05

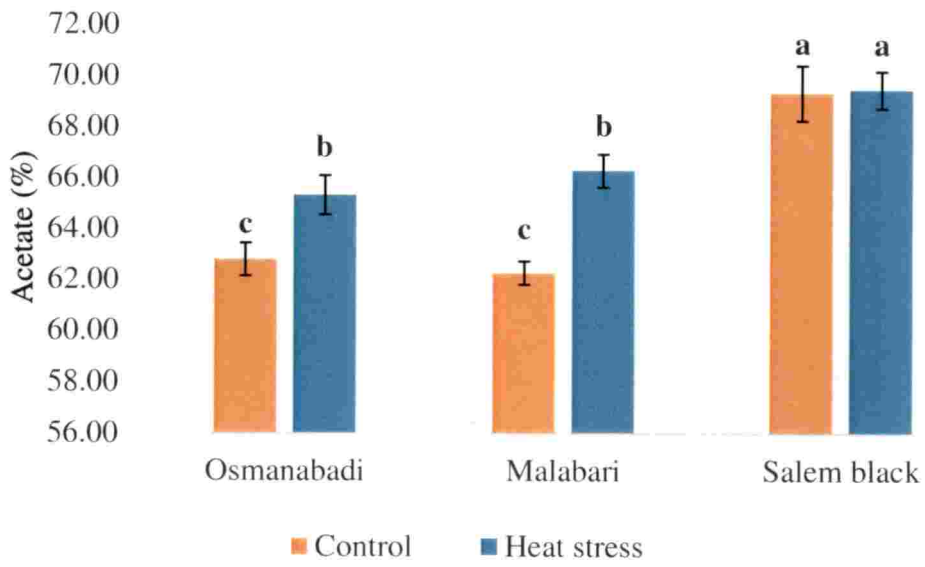


Fig 21: Impact of heat stress on acetate concentration

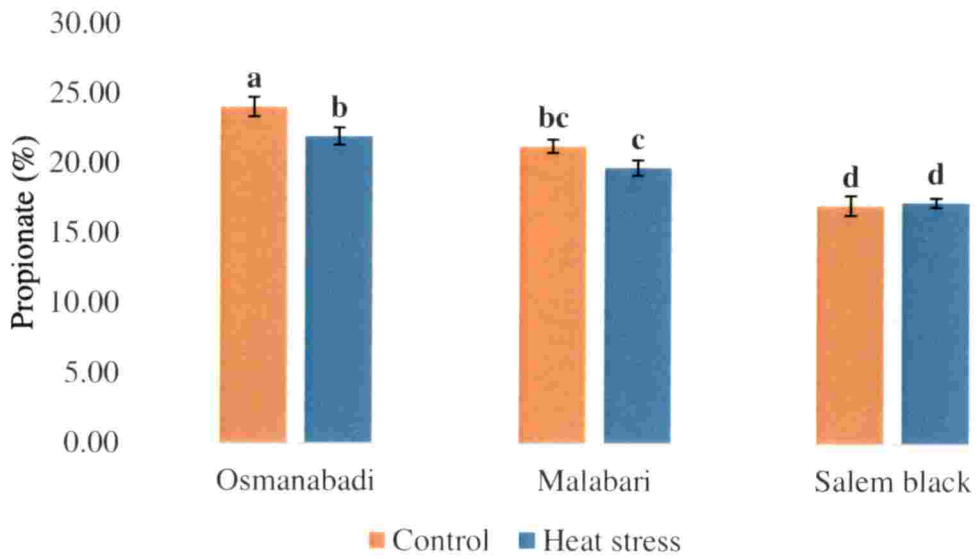


Fig 22: Impact of heat stress on propionate concentration

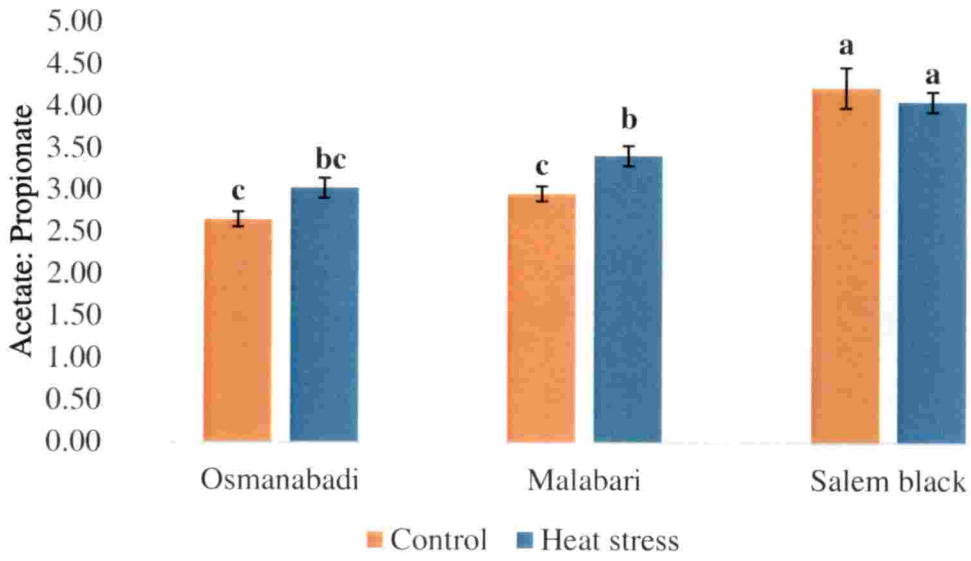


Fig 23: Impact of heat stress on acetate to propionate ratio

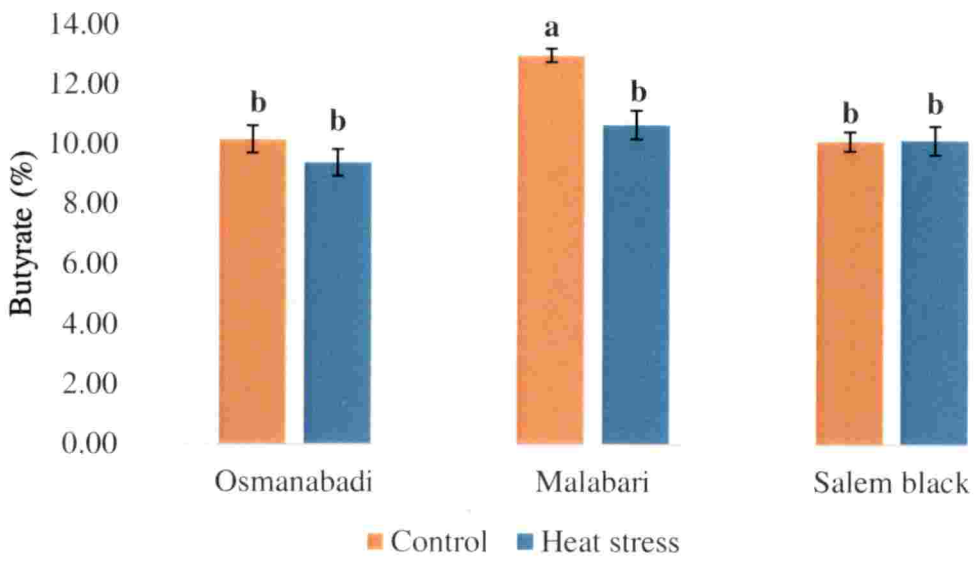


Fig 24: Impact of heat stress on butyrate concentration

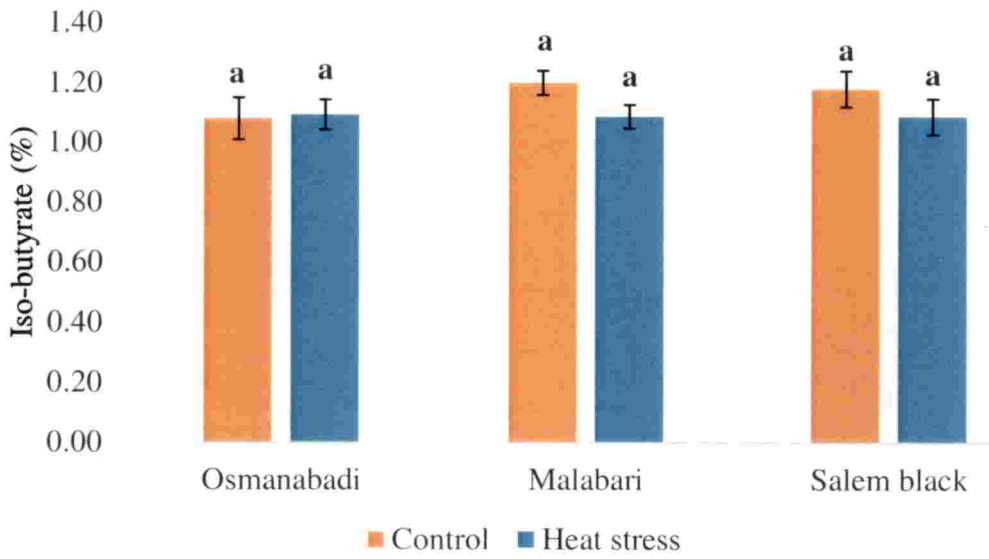


Fig 25: Impact of heat stress on iso-butyrate concentration

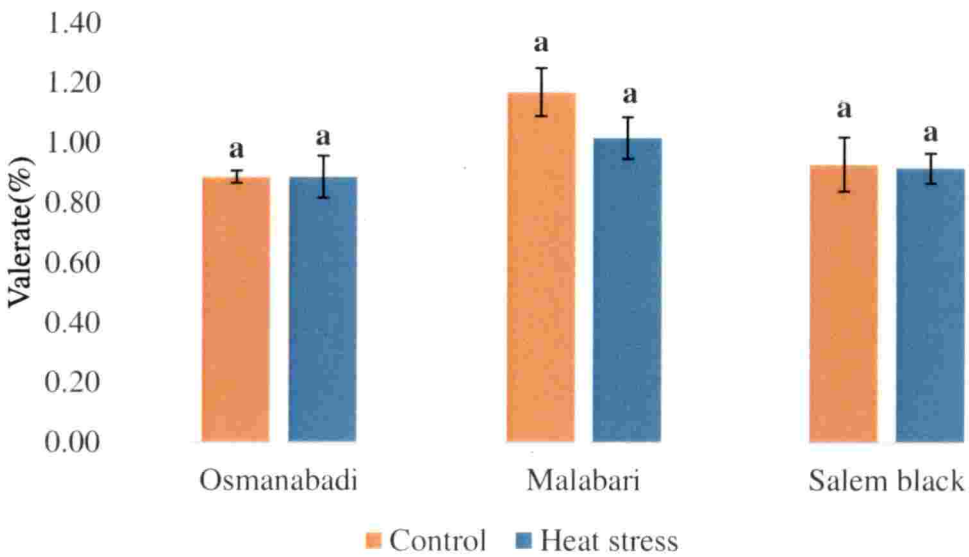


Fig 26: Impact of heat stress on valerate concentration

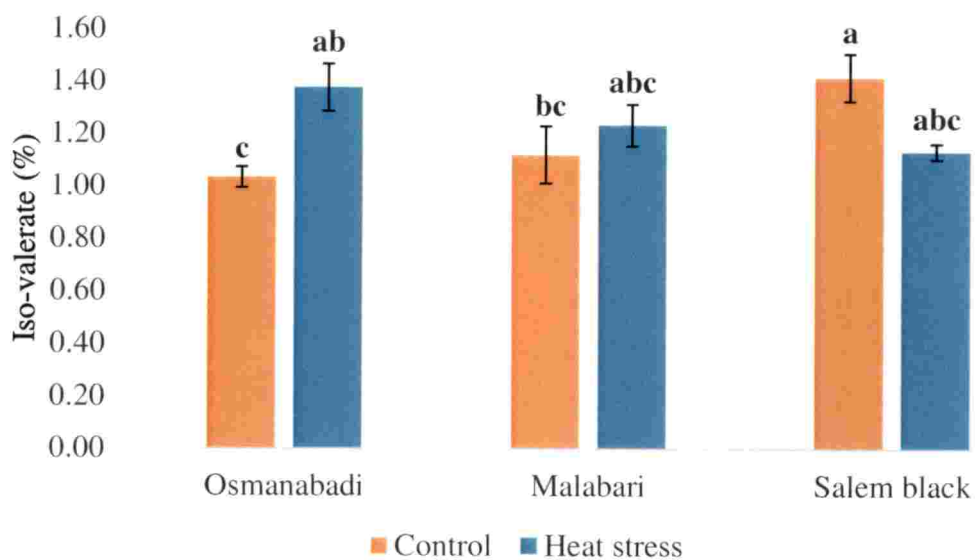


Fig 27: Impact of heat stress on iso-valerate concentration

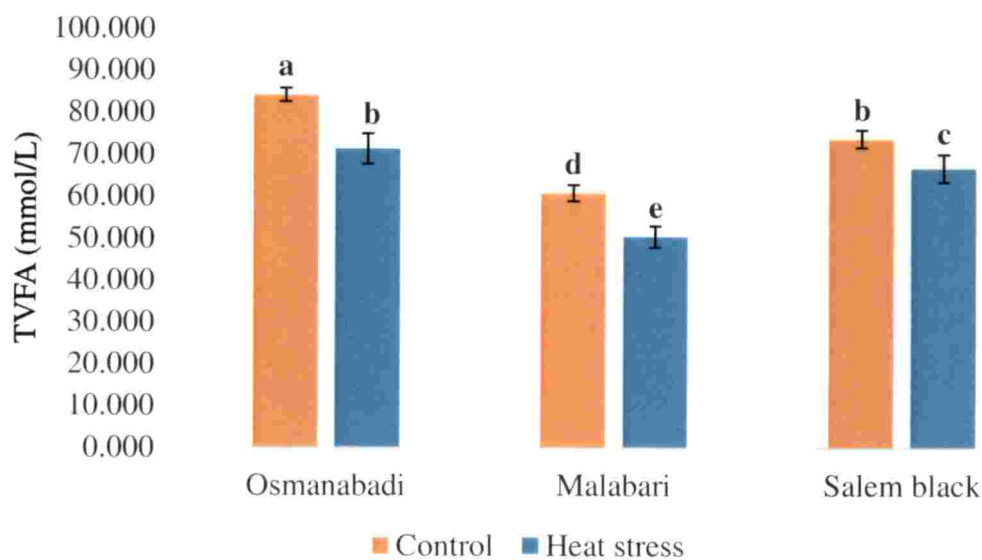


Fig 28: Impact of heat stress on total volatile fatty acid concentration

4.7 Enzyme parameters

The effect of heat stress on enzyme parameters in Osmanabadi, Malabari and Salem Black goats are presented in table 15. The plasma ACP enzyme showed significant ($P<0.05$) variation for the breed factor. Comparing the plasma ACP enzyme concentration between the breeds, Salem black breed showed significantly ($P<0.05$) lower plasma ACP level than the other two breeds. However, both the treatment and interaction between breed, treatment and experimental days did not influence the plasma ACP concentration. But experimental days showed significant ($P<0.01$) variation for the plasma ACP concentration. The plasma ALP concentration did not show any significant variation for the breed, treatment, experimental days and interaction between these factors. Further, THI was found to be negatively correlated with both ACP ($P<0.05$) and ALP ($P<0.01$) in the study (table 16).

Table 15: Comparative assessment of enzyme parameters in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	BREED*TRT*DAY
ACP	0	2.27	2.35	2.31	2.42	1.93	1.87				
	45	2.28	1.55	1.95	1.69	1.53	0.90				
	Mean	2.28^a	1.95^{ab}	2.13^a	2.06^a	1.73^{ab}	1.39^b	*	NS	**	NS
	Pooled SE	±0.29	±0.29	±0.29	±0.29	±0.29	±0.29				
ALP	0	0.15	0.14	0.13	0.14	0.16	0.14				
	45	0.15	0.11	0.22	0.09	0.14	0.05				
	Mean	0.15^a	0.13^{ab}	0.18^{ab}	0.12^{ab}	0.15^b	0.10^b	NS	NS	NS	NS
	Pooled SE	±0.03	±0.03	±0.03	±0.03	±0.03	±0.03				

ACP- Acid Phosphatase, ALP- Alkaline Phosphatase, OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS, Malabari heat stress, SBC- Salem Black control, SBHS- Salem Black heat stress, SE- Standard error, TRT- Treatment, BREED*TRT*DAY- Breed, Treatment and Experimental day interaction
 ** $P<0.01$, * $P<0.05$, NS- Non-significant

Table 16. Correlation Association between THI and Metabolic enzymes

	THI	ACP	ALP
THI	1		
ACP	-0.407*	1	
ALP	-0.520**	0.257	1

THI- Temperature Humidity Index, ACP- Acid Phosphatase, ALP- Alkaline Phosphatase

**P<0.01, *P<0.05

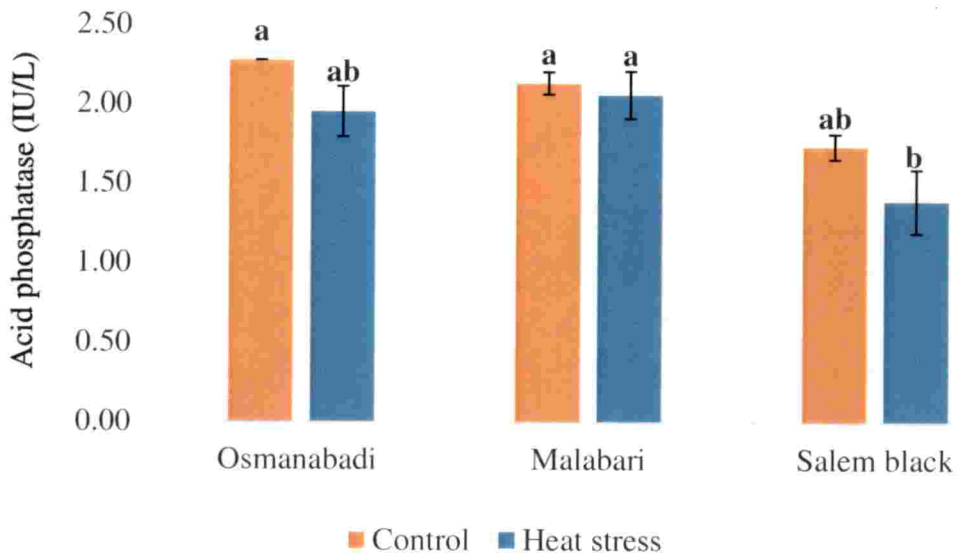


Fig 29: Impact of heat stress on plasma acid phosphatase concentration

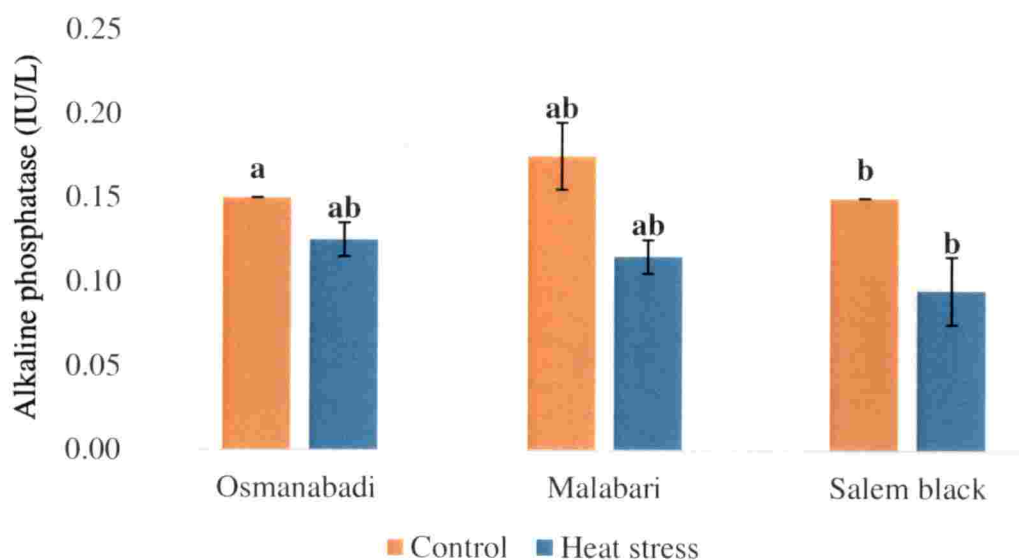


Fig 30: Impact of heat stress on plasma alkaline phosphatase concentration

4.8 Endocrine variables

The effect of heat stress on endocrine parameters of Osmanabadi, Malabari and Salem Black goats are presented in table 17. Breed factor did not influence the plasma GH level in the experimental goats. However, heat stress treatment significantly ($P < 0.01$) influenced the plasma GH level. Heat stress groups of all the breeds showed significantly ($P < 0.01$) higher plasma GH concentration as compared to their respective control groups. Among the heat stress groups, Osmanabadi breed showed significantly ($P < 0.01$) higher plasma GH concentration. However, the experimental days also did not influence the plasma GH level. Similar to that of GH, the breed factor did not influence plasma TSH concentration. However, both the heat stress treatment ($P < 0.01$) and experimental days ($P < 0.01$) significantly influenced plasma TSH level. The plasma TSH was significantly lower in SBHS group as compared to OHS and MHS groups. Both breed and heat stress treatment significantly ($P < 0.05$) influenced the plasma T_3 concentration. Among the breeds, heat stress significantly reduced ($P < 0.05$) plasma T_3 only in Malabari breed while it did not influence the T_3 concentration both in Osmanabadi and Salem Black goats. Furthermore, experimental days also significantly

($P < 0.01$) influenced the plasma T_3 hormone concentration. Plasma T_4 showed reverse trend to that of plasma T_3 concentration in that breed, heat stress treatment and experimental days did not influence the plasma T_4 concentration in the study. Plasma T_4 did not differ between the control and heat stress group of any of the three breeds. In addition, the interaction between breed, treatment and experimental days did not influence any of the endocrine parameters included in the study. However, a strong positive correlation ($P < 0.01$) was established between THI and plasma GH concentration (table 18).

Table 17: Comparative assessment of endocrine parameters in three different indigenous goat breeds subjected to heat stress

Attributes	Days	Treatments						Effects			Breed* TRT *DAY
		OC	OHS	MC	MHS	SBC	SBHS	BREED	TRT	DAY	
Growth hormone (GH)	0	0.47	0.48	0.75	0.72	0.31	0.28				
	15	0.24	0.49	0.27	0.54	0.26	0.39				
	30	0.34	0.72	0.28	0.48	0.30	0.46				
	45	0.37	0.57	0.22	0.54	0.29	0.44	NS	**	NS	NS
	PSE	0.36^b	0.57^a	0.38^{ab}	0.57^a	0.29^b	0.39^{ab}				
Thyroid-Stimulating Hormone (TSH)	0	±0.11	±0.11	±0.11	±0.11	±0.11	±0.11				
	15	0.15	0.14	0.13	0.14	0.16	0.14				
	30	0.15	0.11	0.22	0.09	0.14	0.05				
	45	0.17	0.10	0.21	0.09	0.14	0.05	NS	**	**	NS
	PSE	0.15^{ab}	0.11^{bc}	0.18^a	0.09^{bc}	0.15^{ab}	0.07^c				
Triiodothyronine (T_3)	0	±0.03	±0.03	±0.03	±0.03	±0.03	±0.03				
	15	1.89	1.87	2.87	2.80	2.83	2.95				
	30	1.69	1.16	4.61	2.64	2.35	2.99				
	45	1.30	0.90	3.58	0.96	1.25	1.54	*	*	**	NS
	PSE	1.86^b	1.30^b	3.32^a	1.19^b	2.29^{ab}	2.40^{ab}				
Thyroxine (T_4)	0	±0.56	±0.56	±0.56	±0.56	±0.56	±0.56				
	15	105.34	107.72	113.04	108.94	114.17	116.35				
	30	91.37	85.10	106.91	79.25	95.64	93.37				
	45	90.39	85.71	115.39	85.71	102.38	105.80	NS	NS	NS	NS
	PSE	95.64^a	91.55^a	110.28^a	87.25^a	107.63^a	108.19^a				
	PSE	±12.06	±12.06	±12.06	±12.06	±12.06	±12.06				

OC- Osmanabadi control, OHS- Osmanabadi heat stress, MC- Malabari control, MHS- Malabari heat stress, SBC- Salem black control, SBHS- Salem black heat stress, TRT- treatment, TRT x DAY- treatment and day interaction.

** $P < 0.01$, * $P < 0.05$, NS- Non-significant

Table 18: Correlation Association between THI and endocrine parameters

	THI	GH	TSH	T ₃	T ₄
THI	1				
GH	0.486**	1			
TSH	-0.532**	-0.334**	1		
T ₃	-0.261**	-0.267**	0.266**	1	
T ₄	-0.190*	-0.155	0.062	0.368**	1

THI- Temperature Humidity Index, GH-Growth Hormone, TSH- Thyroid-Stimulating Hormone, T₃- Triiodothyronine, T₄- Thyroxine

**P<0.01, *P<0.05

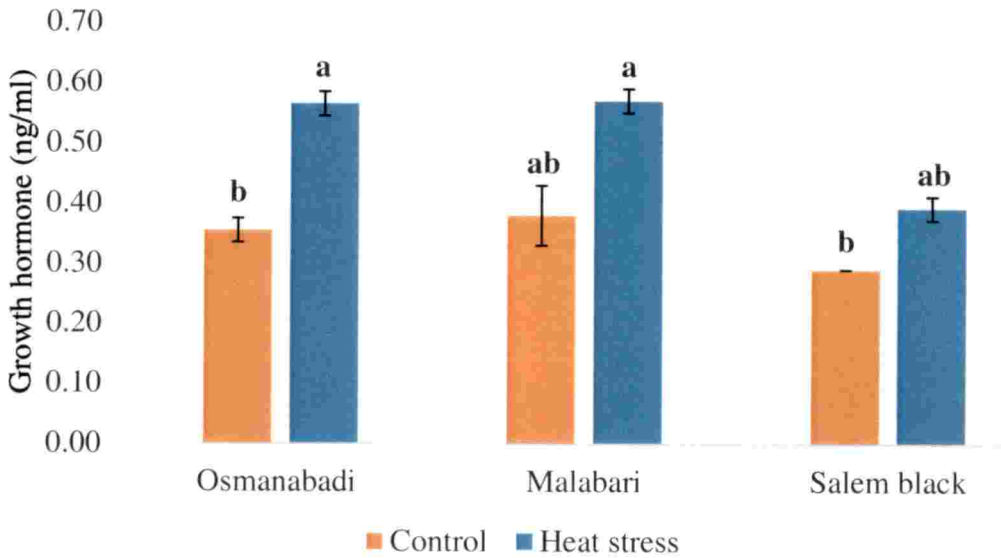


Fig 31: Impact of heat stress on plasma growth hormone concentration

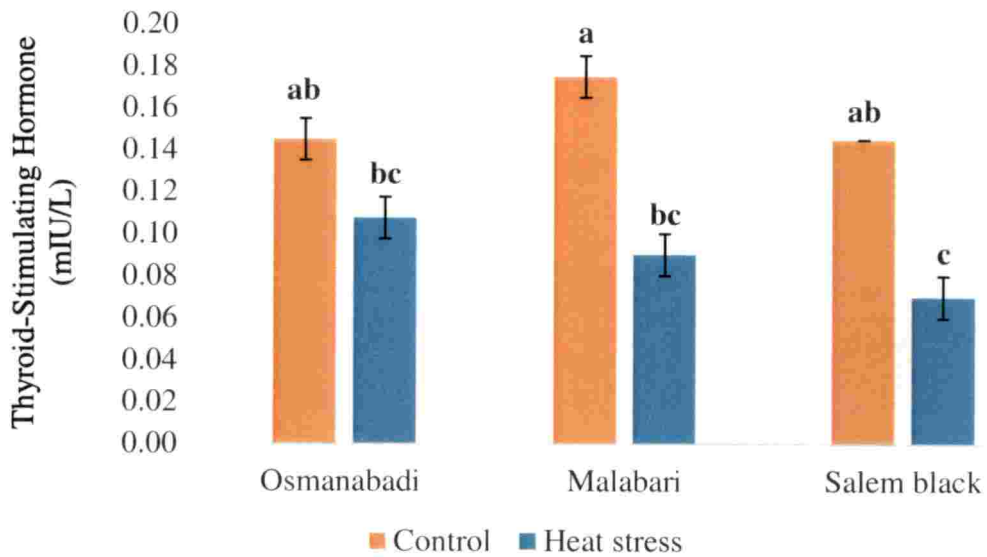


Fig 32: Impact of heat stress on plasma thyroid stimulating hormone concentration

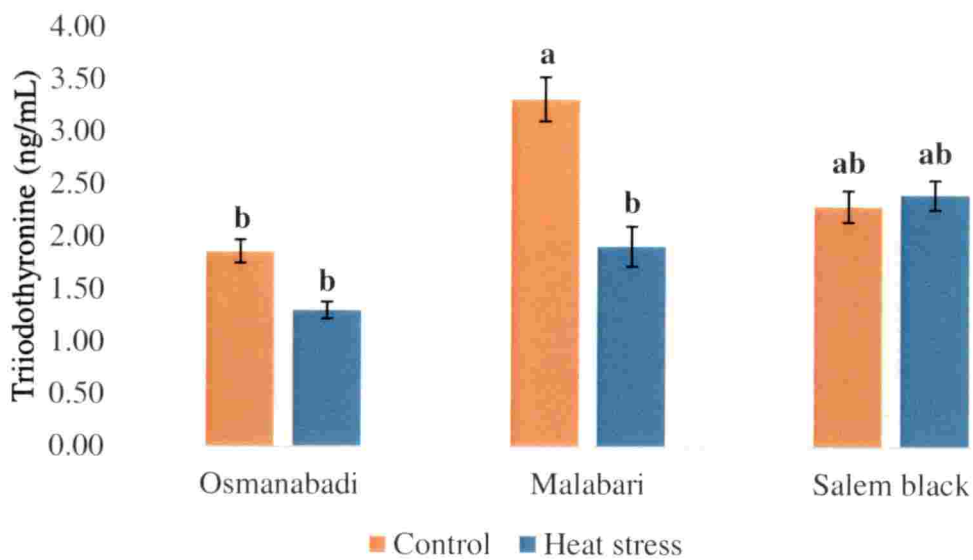


Fig 33: Impact of heat stress on plasma triiodothyronine concentration

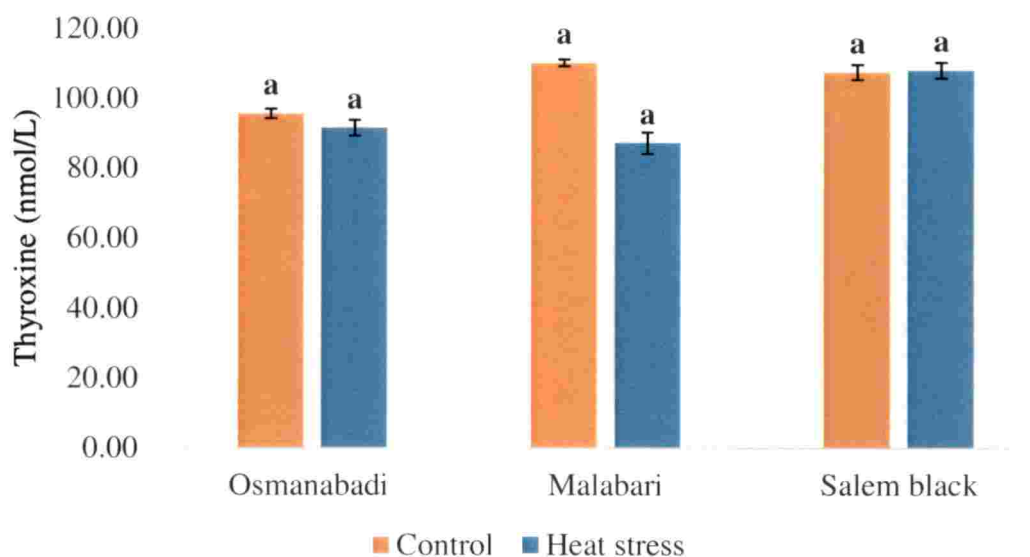


Fig 34: Impact of heat stress on plasma thyroxine concentration

4.9 PBMC IGF-1 mRNA expression

Expression of IGF-1 mRNA transcript in the PBMC between the OC, OHS, MC, MHS, SBC and SBHS groups of goats are shown in fig 35. IGF-1 did not show any significant variation between the breeds. However, heat stress significantly ($P < 0.05$) influenced the expression pattern of PBMC IGF-1 mRNA among the breeds. Among the stress groups, the lower ($P < 0.05$) IGF-I mRNA expression was recorded in OHS, while the higher ($P < 0.05$) expression was observed in SBHS. The SBHS group behaved in contrast to OHS and MHS for IGF-I mRNA expression with significantly ($P < 0.05$) higher value in heat stress as compared to its control. Further only in Malabari breed, heat stress did not influenced the expression pattern of IGF-I mRNA between control and heat stress groups.

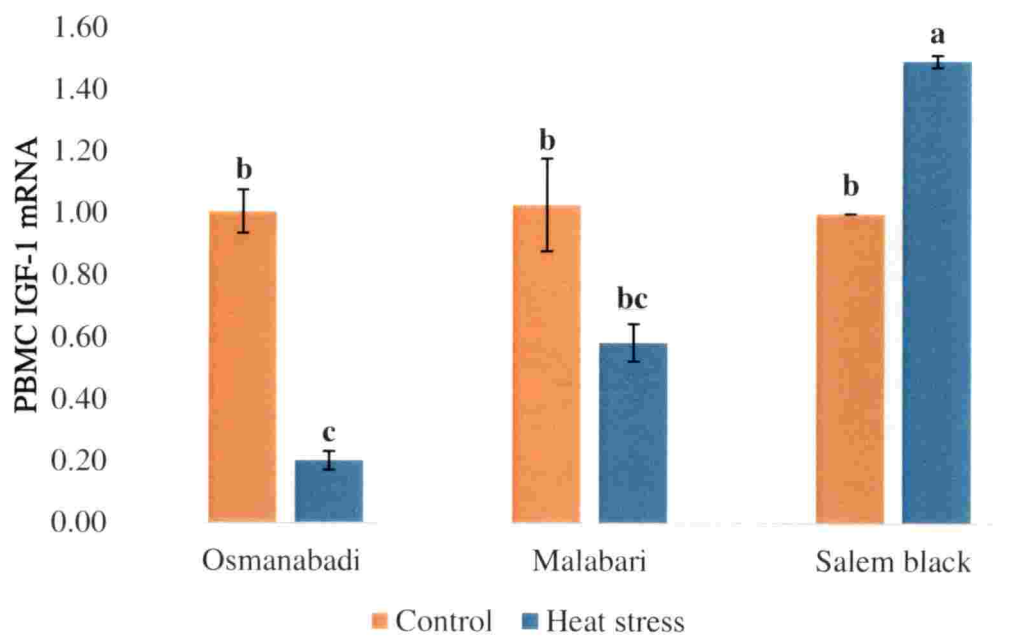


Fig 35: Impact of heat stress on PBMC IGF-1 mRNA expression

DISCUSSION

CHAPTER 5

DISCUSSION

5.1 Growth variables

The BW is an important parameter for explaining the adaptive capability of goats. Breed difference was significantly evident in the BW of goats. Further, the significantly reduced BW of Malabari breed as compared to that of other two breeds could be attributed to the breed differences in BW as generally it is believed that Malabari is categorized under small sized breed whereas Salem black and Osmanabadi are considered as medium sized breeds (Shettar, 2011). Further, significant differences in the BW among the treatment groups shows the influence of heat stress on the growth performance in goats. This BW reduction could be attributed to the deviation of energy for adaptation mechanisms rather than for production pathways (Hamzaoui *et al.*, 2013). Similarly Indu *et al.* (2014) also observed a significant BW reduction in Malpura ewes subjected to thermal stress and they attributed this to the collective effects of increased energy allocation for altered gut physiology, metabolic activity and thermoregulation. However, in the current study rate of BW reduction was higher in Osmanabadi breed compared to other two breeds. This relatively low rate of BW reduction in Malabari and Salem back goats could be attributed to their superior adaptability to heat stress as these breeds are brought from the humid tropical agro-climatic zones and therefore the stress level could have been less severe for these breeds in the current locality. Similarly, Helal *et al.* (2010) also reported significant BW reduction in heat exposed Balady and Damascus goats with higher rate of reduction in Damascus goats as Balady goats are indigenous breed while Damascus is an exotic breed. These literature clearly shows the superior adaptive capability of indigenous goat breeds from exotic and crossbred goats.

The ADG did not show any significant variation for the breed factor indicating all the three breeds behaved in the similar pattern for gaining BW in this study. However, heat stress significantly reduced the ADG in all three breeds indicating that

the production potential is compromised even in indigenous breeds while trying to adapt to the harsh climatic condition. This results coincides with the findings of Popoola *et al.* (2014) who reported significant ADG reduction in West African dwarf goats subjected to high ambient temperature and they attributed this reduction to the decreased feed intake in heat stressed goats. Similar results are also observed in sufflok, Santa Inês and Afshari (Sun and Christopherson, 2001; Gesualdi Júnior *et al.*, 2014; Mahjoubi *et al.*, 2014) breeds of sheep and Bedouin and Alpine x Beetle cross goats (Abdel-Same, 1996; Hooda and Upadhyay, 2014).

The KR is a type of growth parameter to measure the growth efficiency in animals irrespective of their BW (Kleiber, 1947). Several researchers studied the KR in goats to relate it to their growth performance (Supakorn and Pralomkarn, 2012; Thepparat *et al.*, 2012; Shettar, 2011). However, there is no report explaining the impact of heat stress on KR in goats. Therefore, this study is the first to establish the impact of heat stress on KR in three different indigenous goats. Breed factor did not influence the KR of all three indigenous breeds. However, the treatment significantly influenced the KR in goats. Significantly lower KR in heat stressed goats may serve as an indicator for reduction in growth efficiency especially during prolonged heat exposure in these breeds. RGR also showed similar trend as that of KR indicating its significance in assessing the growth performance in indigenous goat breeds. Similarly, Sharma and Pathodiya (2006) indicated that RGR may be a good indicator for assessing the growth performance in Sirohi goats. Further, they observed that RGR was determined by genetic difference and this explanation could be the reason for treatment differences among the breeds for RGR in the current study. In addition, Singh *et al.* (2002) and Karna *et al.* (2002) also reported seasonal influence on RGR with significantly lower values recorded during summer season in Black Bengal and Cheghu goats respectively. This was in agreement in the current study with significantly lower RGR recorded in all heat stress groups as compared to that of respective controls.

The BMI was also considered to be a reliable growth indicator in animals (Habibu *et al.*, 2016). Further, the nutritional status of the animals are indicated based on relative changes in the BMI (Riis and Madsen, 1985; Estrada-Cortes *et al.*, 2009). Breed factor significantly influenced BMI in the current study. Similar report of genotype influencing BMI was established in Red Sokoto and Sahel kids by Habibu *et al.* (2016). However, heat stress did not influence BMI in the current study. This was in contrast to the findings of (Cooper and Washburn, 1998; Goor *et al.*, 2016) who reported negative correlation between growth and heat tolerance in goats. The significant influence of the experimental days on the BMI in the current study indicates the responses of the breeds to heat stress changed. However, the severity of heat stress could be relatively to low/ moderate to have its influence in BMI.

The BCS is an important growth parameter that influences the productivity and health of the animals (Maurya *et al.*, 2010; Niyas, 2015). In the present study treatment showed significant influence on the BCS of all three (Osmanabadi, Malabari and Salem black) breeds. According to Sejian *et al.* (2010b) this decline in BCS in the heat stressed animals could be due to higher rate of mobilization of fat storage for energy synthesis to support life sustaining activities. Further, the significant reduction in BCS of heat stressed Osmanabadi goats as compared to that of heat stressed Malabari and Salem black goats could be attributed to their lesser adaptability as compared to that of other two indigenous breeds.

The non-significant influence of the interaction between breed, treatment and experimental days on all growth parameters indicates that all the three breed animals more or less behaved in similar fashion in influencing the growth parameters throughout the study period. Further, the strong negative correlation for all the growth variables with THI indicates the severity of heat stress in negatively influencing growth performance in the study.

5.2 Allometric measurements

Allometric measurements generally reflect the growth potential of animals. In the present study breed effect showed significant influence on the BL of all indigenous goats. The highly significant effect of breed on all the allometric measurements in the current study establishes the genotype influences on these measurements (Cam *et al.*, 2010; Hagan *et al.*, 2012). Further, these body measurements are typical to a particular goat breed of indigenous nature in a specific agro-ecological zone (Hagan *et al.*, 2012). This could be the reason for wide variation for the allometric measurements among the three breeds in the current study, as these animals are indigenous breeds and well known for their survival in three different agro-ecological zones of southern India. These differences in the allometric measurements among the Osmanabadi, Malabari and Salem black breeds in this study indicates that these animals possess typical morphologically adaptable characteristics which might help them to survive in their specific agro-ecological zone.

Further, BL did not show significant variation between both heat stress and control group. Our results coincided with the reports of Rana *et al.* (2014a) who established a non-significant influence of heat stress on the BL of indigenous Bangladesh sheep. However, contrary reports of increased and decreased BL are also established in pigs and Osmanabadi bucks respectively (Wiegert, 2015; Niyas, 2015). It is generally believed that the increased BL in response to heat stress may represent a mechanism through which the animal alters their surface area to body mass ratio in an effort to conserve or dissipate body heat (Stahly and Cromwell, 1979).

Breed factor significantly influenced the HG of all three indigenous breeds. This could be attributed to their body confirmation changes between the breeds. However, HG did not vary significantly among both the control and heat stress groups. Similar results of non-significant influence of heat stress on HG was also established in Bengal goats (Hashem *et al.*, 2013) and native Bangladesh sheep (Rana *et al.*, 2014a). This non-significant difference in HG could be attributed to the adaptive

capability of indigenous goats to heat stress conditions. This is evident from the significant influence of experimental days on HG indicating the varied animal response to cope with the heat stress condition.

The significant variations in WH among all three indigenous goat breeds suggests that there could be breed differences to influence this particular parameter. There are reports suggesting the differences in WH among different breeds of livestock and they opined that factor such as climate, feed resources and management practices determines this breed differences in their respective agro-ecological zones (Cam *et al.*, 2010; Hagan *et al.*, 2012). However, WH did not vary significantly among control and heat stress groups in this study. This indicates that the magnitudes of heat stress these animals were subjected did not influence the body confirmation changes as these breeds are well known for their thermo-tolerant capability. However, Nardone *et al.* (2006) reported negative influence of thermal environment on the WH in Holstein Friesian calves. Generally the BL and WH reflect the animal's skeletal size and BCS (Hagan *et al.*, 2012). The non-significant effect of HS on BL and WH in the current study indicates the extreme adaptive capability of the three indigenous breeds in the current study. Further, heat stress also did not influence the HW of all three indigenous goats in the current study. In contrary to our findings Nardone *et al.* (2006) reported significant reduction in HW of Holstein Friesian calves subjected to thermal stress than calves kept under thermo-neutral conditions.

Among the three indigenous goat breeds Salem black breed showed higher RuH. Increased RuH and limb length could be an adaptive mechanism to survive in the hot tropical climatic condition (Daramola and Adeloye, 2009; Shettar, 2011; Laden, 2012). Further, it was reported by Chacón *et al.* (2011) that goats in hot arid conditions generally possess longer limbs to reduce the heat from the surface radiation. These authors explained this as an evolutionary adaptation in these animals by keeping their body at an elevated position to avoid the reflected solar radiation from the ground. The higher RuH in Salem black goats could be attributed to thin and longer legs of this

particular breeds to avoid the ground radiation during grazing condition in hot humid tropics.

The CD significantly varied among control and heat stress groups. The CD is the only allometric measurement that was influenced by heat stress treatment in the study. Similar results of diminished CD have also been established in the Bali cattle (Nuriyasa *et al.*, 2017) and fat-tailed Farafra sheep (Ali and Hayder, 2008) exposed to heat stress. However, Hsia and Lu. (2004) Contradicted the above findings reporting increased CD in three-way cross [Landrace \times (Yorkshire \times uroc)] pigs exposed to an atmospheric temperature of 30°C than pigs kept at 20°C and this could be an attempt to dissipate excess body heat by increasing their body surface area. Further, the negative correlation for the HG, HW and CD with THI indicates the significance of these allometric variables to assess the severity of heat stress on growth performance in indigenous goats.

5.3 Feed intake

The feed intake did not alter among the breeds indicating the potential of these breeds being indigenous in nature they can thrive well on any feeding patterns. However, heat stress reduced the feed intake in the Malabari breed. It is known that heat exposed animals will reduce their feed intake as an adaptive mechanism in order to reduce the metabolic heat production by transmitting suppressive nerve impulses to appetite center of the hypothalamus (Marai *et al.*, 2007; Sejian *et al.*, 2010a). The significantly lower feed in Malabari breed on exposure to heat stress indicates the severity of heat stress in inducing reduced feed intake probably in an effort to reduce the metabolic heat production to cope with external hot environment.

5.4 Rumen biochemical parameters

Rumen liquor pH is an important parameter that controls the physiology of digestion (Terry *et al.*, 1969). In the present study breed effect did not show any significant variation among all three indigenous breeds. However, the heat stress significantly reduced the rumen liquor pH of all three indigenous goats as compared to

that of control group goats. This could be attributed to the increased production of carbonic acid in the rumen as a result of reduced feed intake and increased respiratory rate (Kadzere *et al.*, 2002). Similar findings of reduced rumen liquor pH are also observed in Israeli mix-breed Saanen dairy goats (Arieli *et al.*, 2005), Sardinian ewes (Bernabucci *et al.*, 2009) and Holstein heifers (Tajima *et al.*, 2007) after exposing these animals to heat stress. In contrary to our findings Chaidanya *et al.* (2017) reported non-significant change in rumen liquor pH in Osmanabadi goats exposed to heat stress. Further, the significant changes in the interaction between breed, treatment and experimental days showed that the responses of these breeds to heat stress on rumen liquor pH differed at certain time points which indicates the superior adaptive capability of these indigenous goats to heat stress. Further, the strong negative correlation between THI and rumen liquor pH indicates the alteration in the rumen functions during the heat stress condition in goats.

The $\text{NH}_3\text{-N}$ concentration is an important factor that determines the diet digestibility of the animal particularly the fibre digestion (Islam *et al.*, 2000). Breed difference was established in the current study for the concentration of $\text{NH}_3\text{-N}$. Among all three indigenous goat breeds, Salem black group showed significantly lower $\text{NH}_3\text{-N}$ concentration and this could be attributed to the lower magnitude of heat stress experienced by this particular breed in the current study as this breed was well known to adapt to much harsher climatic conditions in its origin locality. Further, heat stress significantly reduced the $\text{NH}_3\text{-N}$ concentration in all three breeds as compared to that of the control group animals. In contrary to our findings Salles *et al.* (2010) reported a significant increase in $\text{NH}_3\text{-N}$ concentration in heat stressed Holstein calves. Further, the significant variation in $\text{NH}_3\text{-N}$ for the experimental days indicates the variation in the animal responses. Further, the non-significant changes in the interaction between breed, treatment and experimental days showed that the severity of heat stress on $\text{NH}_3\text{-N}$ persisted throughout the study. Further, the strong negative correlation between THI

and NH₃-N indicates that this changes in the level of this variable could reflect the magnitude of heat stress altering rumen functions in the goats.

5.5 Rumen volatile fatty acids

Rumen volatile fatty acids are an important source of energy and nutrition that helps the animals in their growth, production and maintenance (Soren *et al.*, 2017). Like all the VFAs, acetate also plays an important role in the rumen fermentation pattern. In the present study breed effect significantly influenced the acetate concentration. Further, the heat stress also significantly influenced the acetate concentration in the rumen liquor. Both Osmanabadi and Malabari breed showed significant increase in acetate concentration during heat exposure and this could be attributed to the differences in the variations in the rumen microbes and differences in the diet digestibility among these breeds (O'Mara, 2004). However, Tajima *et al.* (2007) reported contradictory result of significantly lower acetate concentration in Holstein cattle after exposing them to heat stress. Similar reports of decreased acetate concentration are also established in heat exposed Saanen goats (Hirayama *et al.*, 2004) and Holstein heifers (Nonaka *et al.*, 2008). Increased acetate production in both Osmanabadi and Malabari goats as compared to Salem black indicates the breed differences in acetate production in response to acetate production.

Breed differences was evident in the production of propionate in the present study this could be due to the differences in their diet digestibility and intrinsic characteristics of rumen microbes. Further, the heat stress significantly altered the propionate production with significant increase in Salem black goats and decrease in Osmanabadi goats as compared to their respective control groups. Significantly increased propionate production in Salem black goats could be attributed to ability to produce less methane as usually the targeted end product of digestion to propionate leads to less methane production in ruminants (Moss *et al.*, 2000; Janssen, 2010; Bodas *et al.*, 2012). Similarly, Nonaka *et al.* (2008) also reported an increase in the propionate

in Holstein heifers subjected to thermal stress. Results from the current study highlights the significance of rearing Salem black goats as the suitable animal in coming climate change era. Decreased production of acetate and increased propionate production in the Salem black animals indicates their lesser CH₄ production potential.

Breed difference was significantly evident among all three indigenous goats for the acetate propionate ratio. This could be attributed to the differences in their digestion ability and the functional differences in their rumen fermentation pattern (Lima *et al.*, 2016). However, heat stress did not influence acetate: propionate ratio in the current study. This was in contrast to the findings of (Tajima *et al.*, 2007; Nonaka *et al.*, 2008) who reported negative correlation between acetate: propionate ratio and heat stress in cows.

The significant variations in butyrate concentration among all three indigenous goat breeds indicates the breed differences to influence this particular parameter. Further, butyrate concentration varied significantly among control and heat stress groups in this study. Similar to our findings Nonaka *et al.* (2008) also reported a significant decline in butyrate concentration in Holstein heifers kept at 33°C than heifers kept at 20°C or 28°C. However, Tajima *et al.* (2007) contradicted our finding and reported that the concentration of butyrate decreased during heat stress.

Both breed effect and heat stress did not influenced the concentrations of the iso-butyrate, iso-valerate and valerate in the rumen liquor. Similarly to our findings Nonaka *et al.* (2008) also reported non-significant changes for other most of volatile fatty acid production in prepubertal Holstein heifers subjected to high environmental temperatures. In our study also the animals used were between 8-12 months old and therefore, it may be inferred that heat stress was not able to induce changes in the VFA composition in young animals.

Breed factor significantly influenced the TVFA concentration in all three indigenous breeds. This could be attributed to the differences in the feed preferences

and ruminal bacterial composition. The TVFAs production was significantly lower only in all stress groups as compared to their respective control groups. There are reports suggesting HS reduced VFA production (Tajima *et al.*, 2007; Nonaka *et al.*, 2008). Similarly Salles *et al.* (2010) reported that the higher ruminal temperature significantly decreased the TVFAS concentration in cattle. However, there are also reports indicating that ruminal temperature did not affect the proportion of VFA in cattle (Salles *et al.*, 2010; King *et al.*, 2011; Yadav *et al.*, 2013). This difference in TVFA concentration between the groups could be attributed to the type of microbial population residing in rumen and heat stress brings about reduction by reducing the activity of particular microbes (Uyeno *et al.*, 2010). Further, few authors have reported that changes in rumen fermentation pattern also may be brought about by changes in DMI (Smith *et al.*, 2013; Yadav *et al.*, 2013). The experimental days significantly influenced the production of TVFAs. Further, the interaction between treatment and experimental days significantly influenced TVFAs production acid. This showed that the relationship between the groups for different VFAs production changed over time indicating that the animals are trying to adapt to the existing conditions. Further among the VFA profile, the only negative correlation between THI and TVFA indicates the significance of this variable in assessing the digestive inefficiency during heat stress condition in indigenous goats.

5.6 Metabolic enzymes

The ACP and ALP are the two major enzymes that plays an important role in the metabolic adaptation of animals. In the present experiment, significant variation in plasma ACP enzyme concentration among three breeds could be attributed to the variation in the body temperature and thermoregulatory ability (Williams *et al.*, 2004). The non-significant change in the plasma ACP and ALP concentration for the heat stress treatment can be correlated with the non-significant changes in the plasma T₄ hormone concentration. Lack of metabolic shift indicates their superior adaptive nature as they all are indigenous breeds of different agro-ecological zones. Similar to our

findings Abdel-Samee. (1996) also reported a non-significant change in ACP and ALP level in heat exposed Bedouin goats. However, contrary reports of reduced ACP and ALP concentrations were also established in Malpura sheep (Sejian *et al.*, 2010b) and Osmanabadi goats (Chaidanya, 2015) exposed to heat stress. And they attributed this to the metabolic shift happening in these heat stressed animals in order to cope with the high ambient temperature conditions. The differences between the studies could be correlated to the heat stress magnitude as well as the breed differences as generally it was observed that the breeds in the current study did not relied much on controlling their metabolic activity indicating their ability to cope up to the heat stress challenges. Therefore, there were no changes observed for the heat stress treatment on both plasma ACP and ALP concentration. Further, the negative correlation obtained for both ACP and ALP with THI establishes the fact that these variables could serve as reliable indicators reflecting the metabolic status during heat stress condition in goats.

5.7 Endocrine variables

Growth hormone is a major peptide hormone that promotes the growth, cell multiplication and cell regeneration. It has been observed in goats that the balance between the rate of energy production and utilization determines the concentration of plasma GH (Hirayama and Katoh, 2004). In the current study, significantly increased GH concentration was observed in the heat stress group of all the three breeds indicating the severity of heat stress on growth performance as generally the level of plasma GH was found to be significantly increased in heat stressed animals in ruminant species (Pulina *et al.*, 2012; Bagath *et al.*, 2016). Similar heat stress induced increase in GH concentration was established by Sejian *et al.* (2014) in sheep and they attributed the increased GH secretion to the reduced feed intake of the heat stressed animals. Similar observation was made by Pulina *et al.* (2012) in dairy sheep and they attributed the heat stressed induced increase in plasma GH to the reduced binding of GH to its receptor as well as to the marked reduction in GH receptor synthesis. Further, in a study conducted by Bagath *et al.* (2016) in the Osmanabadi bucks during summer season to

assess the different level of feed intake on GH concentration established significantly higher plasma GH concentration in the nutritional stress group animals. This indicates the importance of optimum nutrition during hot summer conditions to maintain growth performance. In contrast to our findings, Mitra *et al.* (1972) reported a significant decrease in GH secretion in heat exposed Jersey cow and they attributed this to the attempt of the animal in reducing the body metabolic heat through reduced calorogenic activity. Based on the above findings, it could be inferred that circulating GH level can serve as a good indicator of nutritional status during heat stress exposure in different breeds of goats. Therefore, GH can be used as an important biological marker to quantify heat stress impact on growth performance in indigenous goat breeds. In addition a strong positive correlation between THI and plasma GH level supports the above inference of GH being reliable indicator to quantify heat stress impact on the growth performance in goats. However, more studies are required to elucidate the GHR dynamics before reaching a convincing conclusion.

The TSH is an important pituitary hormone that triggers the production of thyroid hormones. TSH concentration in the blood is regulated by the negative feedback mechanisms of thyroid hormones (Lalsangpuii *et al.*, 2015). In the present study, TSH hormone concentration was lower in the heat stress group as compared to that of the control group. This could be attributed to the direct effect of heat stress on hypothalamic-pituitary-thyroid axis in an effort to produce less thyroid hormones to prevent more metabolic heat production to cope with elevated ambient temperature (Aleena *et al.*, 2016). Similar results of reduced TSH concentration was also reported in heat stressed Holstein steers (Kahl *et al.*, 2015) where they had attributed this to the hypo-functioning of pituitary thyroid axis. However, contrary findings of increased TSH concentration were also reported in Angora goats (Pehlivan and Dellal, 2017) and Butana x Friesian crossbred cows (Omer, 2010) exposed to summer heat stress.

Thyroid hormones plays an important role in the metabolic adaptation and growth performance of the animals (Haque *et al.*, 2012). Thyroid hormones increases

basal metabolic rate by increasing the availability of glucose to the cells for production, cardiac and neuronal functions and for stimulating protein synthesis (McNabb, 1995). In the present experiment, significant variation in T₃ hormone concentration among three breeds could be attributed to the variation in the body size, body temperature and thermoregulatory ability (Williams *et al.*, 2004; Dwyer and Morgan, 2006). Further, the heat stress groups of all the three breeds showed significant reduction in plasma T₃ concentration whereas, T₄ did not vary significantly among the control and heat stress groups. This emphasizes that during thermal stress conditions, variations in T₃ is a reliable indicator for reflecting metabolic activity in indigenous breeds of goats compared to T₄. Similar results of decreased T₃ concentration and non-significant variations in T₄ were also established in Holstein heifers exposed to heat stress (Nonaka *et al.*, 2008), Alentejana, Limousine and Mertolenga heifers (Pereira *et al.*, 2008). Further, there are also reports suggesting predominant role for T₃ in controlling the thermogenesis (Habbeeb *et al.*, 1992; Alnaimy *et al.*, 1992). The significantly lower T₃ concentration in all three heat stress groups as compared to their control group could be due to the direct effect of heat stress on hypothalamic pituitary axis to reduce the thyrotropin releasing hormone production in order to control the basal metabolism. Further, heat stress induced restricted feed intake can also decrease the T₃ production (Rhoads *et al.*, 2009). Additionally, energy balance can also play a major role in decreasing thyroid hormone level in ruminants (Kong *et al.*, 2004). Therefore, the T₃ may serve as one of the most important biological marker to quantify the severity of heat stress in indigenous goat breeds. Further, the strong negative correlation of THI for TSH, T₃ and T₄ establishes the fact that these variables could serve as reliable indicators of nutritional status in indigenous goats during heat stress condition.

5.8 PBMC IGF-1 mRNA expression

The IGF-1 is an endocrine, autocrine and paracrine growth factor that plays a major role in the cell growth, development and differentiation (Delafontaine *et al.*, 2004). Heat stress significantly influenced the PBMC IGF-1 mRNA expression in both Osmanabadi and Salem black goats. Significantly reduced PBMC IGF-1 mRNA expression in Osmanabadi heat stress group could be attributed to the reduction in protein synthesis and higher level of protein breakdown in these animals (Gasparino *et al.*, 2014). Lower rates of IGF-1 mRNA expression in Osmanabadi heat stress animals indicate the compromised growth performance in Osmanabadi breed (Scanes, 2009). However, Bagath *et al.*, (2016) in a study conducted in Osmanabadi bucks to assess the different level of feed intake on IGF-1 mRNA expression pattern, did not observe any variation between the groups. These authors attributed this non-existing difference between the groups to the limited number of animals in each group rather than the nutritional status. Nutritional deprivation has been shown to decrease hepatic IGF-1 production by diminishing IGF-1 gene expression in cells (Jaquier *et al.*, 2012). The heat stress induced compromised DMI might have brought down the expression pattern of IGF-1 mRNA in OHS group in the current study. Bossis *et al.* (2000) attributed the reduced IGF-1 mRNA expression to the decreased GH binding to hepatocellular membranes. Reduction in the IGF-1 mRNA expression can also be due to the decrease in the GHR level in the liver as a consequence of reduced DMI in heat stressed animals (Jaquier *et al.*, 2012; Del Vesco *et al.*, 2014). Similar results of decreased IGF-1 mRNA expression were also established in heat exposed Karan Fries and Sahiwal cows (Somal, 2013). The significantly high IGF-1 mRNA expression in Salem black heat stress group could be attributed to the ability of this breed to maintain growth performance even in adverse environmental conditions. This establishes the superior ability of Salem Black breed over other two breeds for maintaining growth during heat stress exposure. Thus, IGF-1 gene expression may act as an important indicator for growth performance of the different indigenous breeds of goats when they are exposed

to high ambient temperature. Therefore, IGF-1 may act as one of the important biomarker to quantify heat stress impact on growth performance in Indigenous goat breeds. Further, based on the differences in the expression pattern of IGF1 mRNA expression between the three breeds in the current study proves the superior ability of Salem Black breed to maintain their growth performance during exposure to adverse environmental condition.

SUMMARY AND CONCLUSION



CHAPTER 6

SUMMARY AND CONCLUSION

Among the various environmental stressors, heat stress one of the major stressor that affects the production performance of livestock species, particularly in the tropical region. Generally the heat stressed animals reduce their growth, production and reproductive performances in order to adapt to elevated ambient temperature. Reduced growth performance associated with summer heat stress is a common phenomenon in the tropical and sub-tropical regions of the globe. Decreased anabolic activity due to the reduced feed intake and enhanced tissue catabolism are the major reasons for the growth retardation in livestock. Exposure of goats to high ambient temperature switches the thermoregulation by reducing the production of metabolic enzymes and hormones. Generally, heat stressed animals reduce their feed intake and slow down their basal metabolism causing hypo-function of thyroid gland in order to prevent the additional metabolic heat production. Further, the animals reduce the production of T_3 and T_4 during both short and long term exposure to high ambient temperatures.

Despite the general agreement that indigenous breeds are more adaptable to testing environmental conditions, not much attention has been focused on how different indigenous breeds respond to heat stress of different magnitude while shifting and exposing them to different agro-ecological zones. This approach is very crucial as the scientific community seeks solution to sustain livestock production in the changing climate scenario. Therefore, the present study was conducted to evaluate the effect of shifting two extremely adapted indigenous breeds in their native tract to another agro-ecological zone and assess their growth performance in the new locality in comparison to the local breed. For this purpose, Malabari and Salem Black breeds are the two breeds well known for their ability to survive in extremely hot and humid environment were shifted to a new locality where the heat stress was of much lower magnitude.

Their growth performance was compared to the local Osmanabadi breed well known for its survival in the current experimental location. The primary objectives of the study are to compare the productive performances in terms of changes associated with various growth variables between these breeds. The study was conducted for a period of 45 days between April-May 2017. Thirty six animals were used in this study. The animals were randomly allocated into six groups of six animals each, OC (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MC (n=6; Malabari control), MHS (n=6; Malabari heat stress), SBC (n=6; Salem Black control) and SBHS (n=6; Salem Black heat stress). The differences in the growth performance, allometric measurements, growth and metabolic activity related endocrine variables and IGF-1 expression patterns among the breeds were studied.

Breed factor did not influence the feed intake in present study. However, the treatment significantly ($P<0.01$) influenced the feed intake of the animals. Among the breeds, heat stress significantly reduced the feed intake only in MHS group. But the interaction between breed, treatment and experimental days did not influence the feed intake of the animals. The BW showed significant variation for both the breed ($P<0.01$) and treatment ($P<0.05$). Comparing the BW changes between the breeds, control group of Malabari breed showed lower BW than the control group of other two breeds. Further, the BW did not differ between the control group of Osmanabadi and Salem Black breeds. Within the breeds, BW did not differ between the control and heat stress groups in all three breeds. The rates of reduction in BW at the end of study period were 3.4 kg, 2.685 kg and 2.65 kg in OHS, MHS and SBHS groups respectively.

The ADG did not show any significant difference between the breeds. But heat stress treatment significantly ($P<0.01$) reduced the ADG in all the heat stress groups as compared to their respective control groups. KR showed similar trend as that of ADG for both the breed and treatment. The heat stress treatment significantly ($P<0.01$) reduced the KR in all the heat stress groups as compared to their respective control groups. Further, RGR also did not exhibit any significant variation between the breeds.

However, the RGR was significantly ($P<0.01$) lower in both OHS and MHS groups as compared to their respective control groups. Further, the RGR did not differ between SBC and SBHS. In addition, the BMI showed significant ($P<0.05$) variation for the breed factor. However, the heat stress treatment did not influence the BMI in all three breeds. The BCS showed significant ($P<0.05$) variation for both breed as well as treatment. Further, the breed factor significantly ($P<0.01$) influenced all the allometric measurements. However, the heat stress treatment did not influence most of the allometric variables except CD. The heat stress treatment negatively ($P<0.01$) influenced the CD in all the three breeds.

The plasma ACP enzyme showed significant ($P<0.05$) variation for the breed factor. Comparing the plasma ACP enzyme concentration between the breeds, Salem Black breed showed significantly ($P<0.05$) lower plasma ACP level than the other two breeds. Among all the three indigenous goat breeds Salem Black animals showed significantly ($P<0.01$) higher rumen acetate concentration signifies the breed differences among the goats. Further heat stress treatment also significantly increased ($P<0.01$) the rumen acetate concentration in both Osmanabadi and Malabari goats. Further, the treatment also showed significant ($P<0.05$) variation among control and heat stress group goats for the propionate production. In addition, butyrate concentration significantly varied for ($P<0.01$) both breed and treatment effect. The total volatile fatty acid (TVFA) concentration also showed significantly ($P<0.01$) higher variation for the breed and heat stress treatment.

Breed factor did not influence the plasma GH level in the experimental goats. However, heat stress treatment significantly ($P<0.01$) increased the plasma GH level only in OHS group. But the GH level did not differ between the control and heat stress groups of both Osmanabadi and Salem Black breed. Breed factor did not influence plasma TSH concentration. However, both the heat stress treatment ($P<0.01$) and experimental days ($P<0.01$) significantly influenced the plasma TSH level. The plasma TSH was significantly lower in the SBHS group as compared to OHS and MHS groups.

Both breed and heat stress treatment significantly ($P<0.05$) influenced the plasma T_3 concentration. Among the breeds, heat stress significantly reduced ($P<0.05$) plasma T_3 only in Malabari breed while it did not influence the T_3 concentration both in Osmanabadi and Salem Black goats. Plasma T_4 did not differ between the control and heat stress group of any of the three breeds.

The PBMC IGF-I expression pattern did not differ between the OC, MC and SBC groups. However, among the stress groups PBMC IGF-I expression differed significantly ($P<0.05$) only between OHS and SBHS groups. Among the stress groups, the lower ($P<0.05$) IGF-I mRNA expression was recorded in OHS, while the higher ($P<0.05$) expression was observed in SBHS. The SBHS group behaved in contrast to OHS and MHS for IGF-I mRNA expression with significantly ($P<0.05$) higher value in SBHS as compared to SBC. However, only in Malabari breed, heat stress did not influence the expression pattern of IGF-I mRNA between MC and MHS groups.

The current study provided an insight into the impact of heat stress on growth performance in indigenous goats. The results also indicated that plasma GH and IGF-1 gene expression may act as ideal biomarkers for assessing the heat stress impact on growth performance in indigenous goats. Further, the results indicated that summer season-related heat stress influenced the rhythmic pattern of metabolic activities and rumen fermentation profiles in all the three indigenous breeds. However, these changes were of different magnitude among the breeds. In addition, the study also indicated that plasma T_3 may act as an ideal biological marker associated with metabolic heat production which may aid in assessing the impact of heat stress on the adaptive capabilities in goats. The study also proved that alterations in the metabolic activities may be an important mechanism by which indigenous goat breeds cope to heat stress during summer season.

Based on the changes observed on various growth and metabolic variables during heat stress exposure, it was observed that the Salem Black breed performed much better compared to both Osmanabadi and Malabari breeds indicating the superior

ability of this breed to survive in different location. Therefore, shifting of Salem Black from a very harsh climatic condition to a locality with much lower magnitude of heat stress proved beneficial in terms of maintaining its growth potential. However, shifting of Malabari breed in a similar fashion did not yield rich dividends in terms of maintaining the growth performance. These findings are of higher significance as the scientific community battles in its effort to identify suitable agro-ecological zone specific breeds for sustaining livestock production in the changing climate scenario.

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**ASSESSING THE DIFFERENCES IN BODY WEIGHT
CHANGES, RUMEN FERMENTATION PROFILE AND
METABOLIC ACTIVITY BETWEEN DIFFERENT INDIGENOUS
BREEDS OF GOATS SUBJECTED TO SUMMER HEAT STRESS**

by

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to evaluate the differences in the growth performance and adaptive capabilities of three indigenous goat breeds (Osmanabadi, Malabari and Salem Black) to heat stress challenges. The primary objective of the study was to compare the growth performance and adaptive capacity of two indigenous goat breeds (Malabari and Salem Black) when they were shifted from their native tract to a new agro-ecological zone with the local breed (Osmanabadi) during heat stress exposure. The growth performance of these breeds were assessed based on BW changes, allometric measurements, GH level and peripheral blood mononuclear cell (PBMC) Insulin like growth factor-I (IGF-I) gene expression patterns while their adaptive capabilities were assessed based on the changes in their metabolic and rumen fermentation profiles. Thirty six ten months to one year old female goats of Osmanabadi, Malabari and Salem Black breeds were randomly divided into six groups, OC (n=6; Osmanabadi control), OHS (n=6; Osmanabadi heat stress), MC (n=6; Malabari control), MHS (n=6; Malabari heat stress), SBC (n=6; Salem Black control) and SBHS (n=6; Salem Black heat stress). The study was conducted for a period of 45 days. All group goats had access to *ad libitum* feed and water throughout the study period. Heat stress group goats were exposed to summer heat stress for six hours from 10:00 h to 16:00 h while control group animals were kept in the shed protected from heat stress. Breed factor significantly ($P<0.05$) influenced only few growth variables such as BW, BMI, BCS. However, heat stress treatment significantly ($P<0.05$) reduced all growth parameters except BMI. Further, the heat stress significantly ($P<0.01$) increased plasma GH concentration in goats with significantly higher ($P<0.05$) concentration recorded in OHS. Among the stress groups, the lower ($P<0.05$) PBMC IGF-I mRNA expression was recorded in OHS, while the higher ($P<0.05$) expression was observed in SBHS. Significantly higher PBMC IGF-1 mRNA expression in Salem black goats revealed the extreme adaptive capability of this breed as compared to other breeds to heat stress conditions. Among the metabolic activity controlling hormones,

the breed factor significantly ($P < 0.05$) influenced only plasma tri-iodo-thyronine (T_3). However, heat stress significantly ($P < 0.05$) decreased thyroid stimulating hormone (TSH) in both MHS and SHS groups while significantly ($P < 0.05$) decreased the plasma T_3 in MHS. The rumen metabolites such as acetate, propionate, butyrate and total volatile fatty acids (TVFAs) showed significant ($P < 0.05$) variation for both breed and treatment effect. The Salem Black breed did not show any significant variation for most of the rumen metabolites as compared to both Osmanabadi and Malabari breeds for the heat stress treatment. The results indicated that on comparative basis, Salem Black breed adapted better to the heat stress challenges as evident from the non-significant difference in circulating thyroid hormone levels and for most of the rumen metabolites between the control and heat stress group in this breed. Further, it has been observed that shifting of Malabari goats to the new location did not proved beneficial in terms of improving their growth performance. In addition based on the alterations in rhythmic metabolic activities, Salem Black goat breed exhibited higher adaptive capability to heat stress than the other two breeds. Thus, it can be concluded from the study that Salem Black breed was found to be superior in terms of both maintaining its productive function as well as adapting to the adverse environmental condition by altering its metabolic activities during heat stress exposure.

Keywords: BMI, BCS, heat stress, goat, growth, IGF-I, Kleiber ratio, T_3 , TVFA

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