EVALUATING THE DIFFERENCES IN MEAT CHARACTERISTICS BETWEEN DIFFERENT INDIGENOUS BREED GOATS SUBJECTED TO SUMMER HEAT STRESS

by

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(2012-20-107)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Evaluating the differences in meat characteristics between different indigenous breed goats subjected to summer heat stress"** is a bonafide record of research work done by me during the research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Dedicated to Sejian sir

&

my beloved family....

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SYMBOLS AND ABBREVIATIONS

AOAC	- Association of Official Analytical Chemists
a*	- Redness
b*	- Yellowness
BLAST	- Basic Local Alignment Search Tool
BW	- body weight
C	- Control
cDNA	- Complementary DNA
CL	- Cooking loss
CO_2	- Carbondioxide
DNA	- Deoxyribo nucleic acid
DP	- Dressing percentage
ELISA	– Enzyme-linked immunosorbent assay
EE	- Ether extract
TE buffer	– Tris- Ethylenediaminetetraacetic acid buffer
FAO	– Food and agriculture organisation
FAOSTAT	– Food and Agriculture Organization Corporate Statistical Database
GAPDH	- Glyceraldehyde-3-phosphate dehydrogenase
GDP	- Gross domestic product
GHG	- Greenhouse gas
HCW	- Hot carcass weight
Hr	- Hour
H_2SO_4	- Sulphuric acid
HPA	- Hypothalamus pituitary axis
HRP	- Horseradish peroxidase
HS	- Heat stress
HSP70	- Heat shock protein
IPCC	– Intergovernmental panel on climate change
L*	- Lightness
LEA	- Loin eye area

m	-Meter
M	- Malabari
mg	- Milligram
min	- Minute
ml	- Milliliter
mmol/L	- millimole per liter
Mrna	- Messenger ribonucleic acid
ns	- Non significant
OD	- Optical density
OS	- Osmanabadi
OSC	- Osmanabadi Control
OSHS	- Osmanabadi Heat stress
PBS	- Phosphate buffered saline
Pmol/litre	- Picomole/litre
PO_4	- Phosphate buffer
PSW	- Pre-slaughter weights
RNA	-Ribonucleic acid
RH	- Relative humidity
RT-qPCR	- Real time quantitative polymerase chain reaction
SB	- Salem Black
SBC	- Salem Black Control
SBHS	- Salem Black Heat stress
SEM	- Standard Error Mean
SOD	- Superoxide dismutase
SPSS	- Statistical package for the social sciences
T_{db}	- Dry bulb temperature
T_{wb}	- Wet bulb temperature
TE	- Tris Ethylenediaminetetraacetic Acid
THI	-Temperature humidity index
TMB	- Tetra methyl benzidine
Trt	-Treatment

°	- Degree
E	– East
N	– North
°C	– Degree centigrade
kg	– Kilogram
hrs	- Hours
w/v	- weight/volume
gm	- Gram
N	- Normal
µg	- Microgram
nm	- Nanometer
NaCl	- Sodium chloride
µL	- Microlitre
IU	- International Unit
Abs	- Absorbance
µM	- Micrometer
g	- Gravity
ng	- nanogram/millilitre
Fig.	- Figure
H_2O	- Water
WHC	- Water holding capacity
Wt.	- Weight

INTRODUCTION

INTRODUCTION

Goats are believed to be associated with man in a symbiotic relation from over 10,000 years and are amongst the earliest farm animals to be domesticated (Aziz, 2010). Goat is recognised as the poor man's cow because of its immense contribution in securing the livelihood of poor and marginal farmers. Rural poor farmers including women find it difficult to procure and manage large ruminants and hence practice rearing of small ruminants which include sheep and goat. Further, goat rearing involves low initial investment but give maximal output primarily because of its small size, less housing requirements, prolific breeding, less feed requirements and management care. The role played by goats in providing food as well as revenue to humans is well recognised (Aziz, 2010). Goats are reared for their milk, meat and hide. Goats are usually reared for their meat and that is the reason why major portion of goat production system is comprised of meat goats. Goat meat (chevon) has huge importance in the international meat market because of their high nutritional value and lean content. The low cholesterol and saturated fat content in chevon and medicinal value in goat milk and easy digestibility makes the importance of goat rearing immense from human health perceptive (Anaeto *et al.*, 2010).

Goats are especially important from the current climate change perspective compared to other livestock species. They have the ability to sustain in any testing environmental condition due to their small size and capability to conserve water (Hamzaoui *et al.*, 2012). Goat possess the exemplary capability to survive in any agro-ecological zone because of their skilful grazing behaviour, extreme disease resistance, drought tolerance, and high feed conversion efficiency (Shilja *et al.*, 2016; Debele *et al.*, 2013). They can effectively tackle the feed scarcity and water shortage which arise from climatic extremes, which would be especially useful in tropical countries like

India. However, the potential of this genetic resource is not fully exploited by human, which resulted in several breed that are non-descript, especially in the developing countries (Aziz, 2010). The extent of their contribution in meeting the food demands of rural poor is often underestimated. However, in the coming years they are going to be the most admirable animal from the perspectives of climate change, rural livelihood security and especially for physiological and biomedical research pertaining to study the impacts of climate change on livestock (Shilja *et al.*, 2016).

According to FAOSTAT (2013), porcine meat is the most consumed meat globally (15.8 kg/capita/year), followed by poultry (13.6 kg/capita/year), beef (9.6 kg/capita/year), mutton and chevon (1.9 kg/capita/year).In developing countries, small ruminants play a vital role in the economic sustenance of the weaker sections of the society (Agarwal *et al.*, 2014). Developing countries constitute 80% of the goat population and hence these animals are the major backbone of the rural economy in these countries (Shilja *et al.*, 2016). The largest goat population is in Asia (545 million) followed by Africa (245 million), both of which together accounted for about 93% of the global goat population (Devendra and Solaiman, 2010). Sheep are important meat animals globally. However, in tropical countries, like India chevon is more preferred over mutton and mutton is often used as an adulterant in chevon (Sen *et al.*, 2004). Further, chevon is universally accepted meat and is devoid of religious and cultural taboos, unlike beef and pork (Ozung *et al.*, 2011). China, India and Pakistan are the top rankers in chevon production. Developing countries are home to many poor and landless farmers who are unable to procure and manage large ruminants and hence have to depend on goat rearing. Taken together the confined population of goats in developing countries and the economic status of the rural household, goat meat is going to be of utmost importance in these countries particularly. According to Food and Agricultural Organization (FAO, 2016) world meat production is projected to double by 2050, particularly in the developing

countries, mainly because of the rising human population which is projected to surpass 9.6 billion by 2050, and also for the changing economic situation and living standards. Goats constitute the major source of animal protein in many North African and Middle Eastern countries. These animals also have immense role in Southeast Asia, the Caribbean, and other tropical regions (FAO, 2016).

Meat is a concentrated nutrient source which is considered to be intricately involved in the human evolution (Pereira and Vicente, 2013). The nutritious richness and high value protein content of meat make its role indispensable in a healthy and well balanced diet, and is considered optimal for human growth and development. However, there is a changing trend in the human diet pattern and moreover, people are more cautioned about the consumption of ruminant meat as it contains high saturated fat which is a pre-disposing factor for many chronic diseases (Adeyemi *et al.*, 2015). Red meat is high in saturated fat and contributes to the boosting level of cholesterol in blood, which would lead to certain cardiovascular diseases. Goat meat is however, concordant with the present day consumer demands and expectations due its lean meat and high nutritional value. Chevon has become increasingly important for human health management because of its nutritious and healthy attributes. Further, lean meat is an excellent source of several minerals. Chevon has low calorie content, total fat, saturated fat and cholesterol when compared to other red meats. Further, it has high bioavailability of iron (3.2mg), compared to other traditional meats such as beef (2.9 mg), pork (2.7 mg), lamb (1.4 mg), and chicken (1.5 mg). Amino acid composition of chevon is almost equal to that of beef and lamb. The potassium content is high and sodium content is low in chevon on comparative assessment with other meats (Anaeto *et al.*, 2010). Chevon provides high value protein and constitute of low saturated fat and cholesterol which is a healthy alternative to a balanced diet for human compared to other red meats (Anaeto *et al.*, 2010). Further, the sensory attributes of goat meat

and palatability is also accepted by the consumers globally (Webb *et al.*, 2005). In the overall red meat consumption, chevon comprise 63% worldwide. Goat is distributed worldwide and chevon is considered as a staple red meat in human diet which is believed to be consumed by people since the very beginning of human civilization and is believed to be the best alternative red meat from the current climate change and food security perspectives.

The meat and carcass quality characteristics are governed by several factors categorised as intrinsic and extrinsic factors. The intrinsic factors that affect the ruminant meat quality includes species, breed, age and weight at slaughter, gender (male, female, castrated), whereas extrinsic factors include stress agents (environmental effect, transportation and handling), diet and weaning (Guerrero *et al.*, 2013). Breed is an important factor influencing the morphology of the carcass, in terms of the meat quality and fat quantity, and is the basis for comparing and identifying the choicest breed for goat production. However, research on this area pertaining to the influence of breed on carcass characteristics is inadequate. According to Ivanovic *et al.* (2014), breed has significant influence on chemical composition (amino acid, moisture, total fats, proteins, ash), cooking loss and in sensory traits of chevon. Similarly, Kadim *et al.* (2004) had suggested that breed has significant effect on meat pH and cooking loss, in a study wherein they showed the remarkably different carcass traits of Batina, Dhofari and Jabal Akdhar goat breeds. In a comparative study conducted in three Indian goat breeds (Marwari, Barbari and Jamnapari), it was identified that Barbari is comparatively the choicest breed with overall acceptability (Das and Rajkumar, 2010). However, they concluded that breed had no much influence on meat quality. Oman *et al.* (1999) had opined that cross-bred goats are more advantageous than pure bred, primarily due to its large body size and higher capacity for growth, which he had proved through study in Boer x Spanish and Spanish meat goats (Oman *et al.*, 1999).

Osmanabadi, Malabari and Salem Black are the important indigenous goat meat breeds originated in the tropical India. Osmanabadi is dual purpose breed used for both milk and meat. It is a large sized breed. They give an average daily yield of 0.5 to 1.5 kg for a lactation length of about 4 months. The does will breed regularly twice a year. Twinning is also common in this breed (Deokar *et al.*, 2006). Malabari is a small size breed, known for their good quality skin and palatable meat. Further, this breed has good heritability for reproductive trait, which is the most important production aspect of small ruminants. Salem black is an important meat breed in Southern tropical India, with tall body and predominantly black in colour. The body weight of adult goats ranges between 38.5±1.0 kg in males and 29.5±0.6 kg in females. The current study is an attempt to investigate the possible impacts of climate change induced heat stress on the meat quality of these indigenous breeds and the reasons underpinning the alterations in meat quality.

The emerging food safety risks due to climate change may pose a serious challenge to the meat industry. Therefore, a better understanding of anticipated climate change impact on meat quality would be of the utmost importance for governments to ensure preparedness. In this line, the proposed project would delineate the underlying biological mechanisms by which heat stress influences the meat characteristics of three different indigenous goat breeds. The study would help in identification of appropriate goat breed to be promoted for ensuring the livelihood securities of poor and marginal farmers who relies on the quality meat production ability of these precious animals. The findings from this study may pave way for developing suitable strategies to counter the adverse impact of climate change over meat industry.

The objectives of the present study are:

1. To evaluate the differences in carcass characteristics between different indigenous breed goats exposed to summer heat stress

2. To compare differences in whole sale cuts and edible and inedible offals between different indigenous breed goats exposed to summer heat stress

3. To determine the differences in expression pattern of selected genes in skeletal muscle between different indigenous breed goats exposed to summer heat stress

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Meat production in the changing climatic scenario

Climate change is considered to be crisis of the present era (Scholtz *et al.* 2013). The Inter-governmental panel on climate change (IPCC) has predicted earth surface temperature to rise in the range of 2.6° C to 4.8° C by 2100 creating alarming situation to the planet, population and economies (IPCC, 2013). According to Food and Agricultural Organisation (FAO), food security issue is the unprecedented challenge faced by the world's growing human population in view of global warming. Elevated atmospheric temperature causes heat stress in animals and is considered the most concerned issue emerging from the vagaries of climate change which would exacerbate the food security issue (FAO, 2016). Heat stress is the condition wherein the animals fail to regulate the thermal balance in their body and the condition becomes severe when the high ambient temperature couples with high relative humidity. Heat stress hampers both the productive and reproductive efficiency in animals and this is brought about both through direct and indirect effects of changing climate (Sejian, 2013).

Meat production is one of the major agricultural enterprises contributing substantially to the global economy. Meat is a valuable livestock product which plays an important role in meeting the global food requirement (Pereira and Vicente, 2013). The high nutritional value of animal meat is widely been recognised. Meat is considered to have high biological value as it serves as an unequivocal protein source with an average of almost 22% protein content. In addition, meat also comprises of all the essential amino acids and acts as the source of several micro-nutrients (iron, selenium, zinc, phosphorus, Vit-B_{12}) and possess high digestibility (Pereira and Vicente, 2013). Moreover, offal meats like liver are

crucial sources of vitamin A and folic acid (Biesalski, 2005). The major meat animals include pig, poultry, cattle, sheep and goats.

Environmental temperature is considered an important ante-mortem stress affecting the post-mortem meat quality (Nardone *et al.*, 2010). The impact of heat stress is expected to be reflected on the overall meat yield, quality and composition (Gregory 2010). During heat stress, the animals deploy several adaptive mechanisms as part of thermoregulation during which the production potential is compromised in the animal (Sejian *et al.*, 2016). Unsurprisingly, as part of jeopardizing the productive functions, meat production was also established to be impaired in several livestock species (Gregory, 2010; Nardone *et al.*, 2010). These findings clearly suggest that climate change associated heat stress seems to be the major factor which negatively influences the livestock meat production. Although detailed review reports are available on the subject of heat stress impacting livestock production, reproduction, adaptation and immune responses, literatures pertaining to heat stress impact on livestock meat production are very scanty (Kumar *et al.*, 2017). In a recent report on sheep, Gowane *et al.* (2017) addressed the impact of climate change on meat attributes but without detailed information on meat quality variables and genes associated with heat stress and meat production. Therefore, efforts are needed to gather this information to project the adversities associated with heat stress on meat industries. Given the significance of livestock meat production in meeting the global food security, this review aims to address in detail the climate change impact in terms of heat stress hampering both the meat production and quality variables in livestock. Efforts were made to cover in detail the consequences of heat stress on carcass characteristics, meat quality attributes, proximate composition and alterations in expression patterns of different genetic traits associated with meat production. Such an effort may pave way for identifying the intervention points to reverse these adversities which may help to sustain meat production in the changing climate scenario.

2.2 Meat industry as future solution for food crisis: economical and nutritional aspect

Currently, food insecurity is a growing concern with almost 1 billion people struggling globally due to hunger and undernourishment (Tendall *et al.*, 2015). The growing human population is projected to surpass 9.6 billion by 2050 and global climate change is considered as the major threat for food security (FAO, 2016). Given the fact of agrarian crisis from the global food security perspectives, livestock production especially the meat production is considered to have a radiant future in the changing climate scenario. World meat production is projected to double by 2050 and is suggested as the possible solution to feed the growing human population (FAO, 2016).

2.3 Meat production statistics from major livestock species

Important livestock species used for meat purposes around the globe are pig, cattle, poultry, sheep and goat, among which pork is the most widely, consumed meat followed by poultry, beef and mutton. Meat consumption especially in the developed countries is on the rise and has almost doubled compared to developing countries (FAOSTAT, 2013). World meat production is projected to continue to rise mainly to meet the needs of growing population, changing life patterns and affluence. In 2017, it is anticipated that world meat production would be doubled with 262.8 metric tons production value compared to 1986 value (OECD/FAO, 2011). In meat industry, poultry sector has emerged to be the fastest growing enterprise over the years. In pork production, South and East Asia topped during the years 2013-15 with 67.13 million tonne, followed by Western Europe with 23.25 million tonne. Latin America has the highest beef production, followed by South and East Asia. In poultry production, South and East Asia ranks first with 32.74 million tonne, followed by Latin America and North America. In mutton production, South and East Asia ranks first with 6.39 million tonnes (OECD/FAO, 2016).

2.4 Heat stress: Unprecedented challenge for meat production

Increasing environmental temperature is considered a real challenge for sustainable meat production from livestock industry (Nardone *et al.,* 2010). Especially in tropical countries such as India and Australia, heat stress would be a major concern to meat producers as stress response is inversely linked to meat quality (Wan *et al.,* 2016). The impacts of heat stress are reflected on the meat quantity, quality and composition (Gregory, 2010). During heat stress, the animals deploy several thermoregulatory mechanisms during which the production functions are compromised in the animal (Sejian *et al.,* 2016). Unsurprisingly, because of the jeopardizing of the production functions, meat production show discernible impacts. The HPA axis is stimulated during thermal stress in animals that leads to increased secretion of catecholamines and cortisol as a homeostatic response. During this circumstance, the energy reserves are depleted along with dehydration and catabolism of protein takes place and all of which culminates in deterioration of meat quality (Nikbin *et al.,* 2016). Literatures suggest that proper protection of animals from pre-slaughter stresses such as heat stress, handling and transportation during high temperature would not only ensure animal welfare, but also increase profitability from the sector through increased productive performance (Kadim *et al.,* 2006). Studies have been conducted on most of the livestock species including cattle, goat, sheep and poultry during heat stress condition and among this, pig meat was found to be most susceptible to heat stress (Gregory, 2010; Kadim *et al.,* 2008; Hashem *et al.*, 2013).

2.5 Heat stress impact on the growth performance of the animals

The most important factor determining the meat output from the animals is their body weight at slaughter (Guerrero *et al.*, 2013). The negative impact of high ambient temperature on the body weight changes in livestock are well established by several researchers (Lu *et al.*, 2007; Ma *et al.*, 2015). Results of decreased body weight during high ambient temperatures had been established in cattle (Mader and Davis, 2004), sheep (Rana *et al.*, 2014), pig (Ma *et al.*, 2015) and poultry (Lu *et al.*,

2007). Interestingly, in pigs it has been studied by Ma *et al.* (2015), that more the body weight of the animal, greater will be the effect of heat stress and more the body weight decline as reflected by their average daily gain (ADG). Yusuf *et al.* (2014) had studied in rabbits that the growth determining elements such as feed intake, daily weight gain and weight to gain ratio are parallel to the hot and cold carcass weights as well as the overall meat quality and composition. Heat stress effects on growth rate can have a significant effect on the weight at slaughter and/or the age at slaughter and the age or weight at slaughter can have major effects on a range of meat quality characteristics as well as carcass composition. Hence, taking into account that all these growth performance factors are hampered during high ambient temperatures, the influence of heat stress on the growth efficiency and meat production and quality in livestock species are obvious.

2.6 Heat stress impacts on carcass characteristics

2.6.1 Dressing percentage, carcass weight and proportion of fat and muscle tissues

Carcass evaluation is vital for understanding the relative efficiency of the animal to convert the consumed feed to animal muscle mass (Karim *et al.*, 2007). During carcass evaluation, dressing percentage is considered as a potential indicator of the overall meat yield from an animal. Reduced dressing percentage and carcass weight loss have huge economic significance, especially in small ruminant meat (Kadim *et al.*, 2006). At elevated ambient temperature, dressing percentage has been reported to be enhanced in broiler chicken (Lu *et al.*, 2007). In accordance to this, Rajkumar *et al.* (2011) had conducted study on naked neck chicken and reported better dressing percentage in heat stressed group compared to the control group chicken. However, contrary report has also been established, stating the reduced dressing percentage and associated carcass weight loss (Tankson *et al.* 2001) in broiler chicken exposed to high ambient temperature of above 30°C. Further, Gu *et al.* (2008) also reported reduction in live weight and carcass and meat cuts in heat stressed broilers. In addition, Lu *et al.* (2007) conducted experiments on broiler chickens and reported a significant decrease in the breast muscle

proportion indicating reduced meat yield during heat stress. Furthermore, there are also reports suggesting significant reduction in subcutaneous, intramuscular and abdominal fat percentage in heat stressed broiler chicken (Lu *et al.*, 2007).

Another deleterious effect of heat stress in chicken is the weakened skin which may ultimately result in tearing and muscle damage during plucking (Gregory, 2010). In pigs exposed to warmer summer temperature, the fat deposition in the muscle was found to be significantly reduced and this was attributed to the reduced feed intake and energy consumption in order to reduce the metabolic heat load (Ma *et al.*, 2015). In finishing pigs reared under hot environments (33°C), reduced intramuscular fat in the *longissimus* muscle, accompanied with impaired ability of fatty acids to synthesise de novo have been reported due to the suppressed activity of acetyl co-enzyme A carboxylase enzyme (Wu *et al.*, 2015; Ma *et al.*, 2015). However, this is not the case with grower pigs as generally the intramuscular fat content is very less in them (Ma *et al.*, 2015). However, a study also revealed that in growing pigs, lipid metabolism was enhanced in liver and adipose tissue during heat stress resulting in increased fatness in muscle (Lu *et al.*, 2007). During heat stress, adiposity in pigs was reported to be enhanced both due to the increased adipose tissue deposition and reduced mobilization of energy reserves compromising the lean muscle accretion (Johnson *et al.*, 2015). These authors had stipulated the mechanism underlying the increased adipose: lean ratio as an adaptation mechanism of the animal to reduce heat load*et al.* (2010) had previously shown that the increased adiposity in pigs during heat stress would result in better dressing percentage. This was in agreement with the study conducted by Wiegert (2016), in which the author showed that offspring from heat stressed dam showed increased dressing percentage that is related to the enhanced adiposity in those pigs. Increased back fat thickness and reduced lean% was also reported in the same study, further confirming the enhanced adiposity during heat stress in pigs. However contrarily, reports have also emerged stating the reduction of back fat thickness and suppressed functioning of certain enzymes (malate dehydrogenase and glucose-6-phosphate dehydrogenase in back fat and periphery kidney fat by 60% (Ma *et al.*,

2015). Further, it was reported that heat stressed swine possess an increased carcass length, probably as an adaptation mechanism to adjust the body surface area to the body mass ratio for better heat dissipation (Wiegert, 2016). In pigs, re-allocation of subcutaneous fat to the internal sites leading to increased flare fat weight is a usual mechanism occurring during heat stress condition, primarily as an adaptive mechanism to cope up the heat stress (Nardone *et al.*, 2010).

Heat stress driven carcass weight loss, dressing percentage decrease, reduced fat thickness and leaner carcass have also been established in beef cattle raised under elevated ambient temperature (Nardone *et al.*, 2010). In cattle, during elevated environmental temperatures, instead of subcutaneous fat, internal fat depots will be enhanced like pigs, accompanied by enhanced muscle marbling which is a preferred trait in some beef markets (Gregory, 2010). However, higher temperatures could also result in dark cutting beef which has been a major concern in US and Oman beef markets (Kadim *et al.,* 2004). The reduced marbling also occurs in beef cattle when the farmers adopt heat-tolerant cattle sires such as Brahman crosses as a management strategy against heat stress (Gregory, 2010). In sheep, dressing percentage was found to be reduced because of the enhanced muscular activities leading to hypertrophy and increased size in majority of the vital offals such as liver, kidney, heart, blood and also head and feet (Rana *et al.*, 2014). Carcass weight loss in goats transported during peak summer hours had resulted in live weight and carcass weight loss, reduction in intramuscular fat content or marbling mainly because of the moisture loss and tissue shrinkage (Kadim *et al.*, 2006). Body fluid losses from both muscle and non-muscle tissues are involved during ante-mortem related heat stress during transportation, resulting in carcass weight losses and reduced dressing percentage (Kadim *et al.,* 2006). The pH decline in muscles of stressed goats was found to be associated with deterioration of the myofibrillar structure and integrity, degradation of muscle proteins, and affecting the solubility of the proteins (Nikbin *et al.,* 2016). Kadim *et al.* (2008) also reported similar effect of heat stress on myofibrillar protein degradation which was indicated

by higher myofibrillar fragmentation index and they attributed this to decreased meat pH in heat stressed goats and sheep.

2.7 Heat Stress impact on meat quality: sensory attributes, organoleptic quality and meat safety as causes for market failure

Turn over from the meat industry commensurate with the supply of safe, nutritious, and good quality meat to the consumers (Warner *et al.*, 2010). Market failure is mainly linked with the consumer preference and acceptability of meat. Further, the preference by consumers and processors depends mainly on the meat quality, which is determined by several factors (Grunert, 2006). Meat quality is assessed by two main aspects: nutritional value, which is merely determined by meat composition is an objective measure while eating quality that is a subjective assessment of meat by consumers and processors which includes the meat colour, tenderness, juiciness and flavour (Bender, 1992). Heat stress has its effect on various organoleptic quality factors which are determined by sensory and visual perception of the consumers such as colour, shear force, tenderness, juiciness, flavour and palatability of meat.

2.7.1 Meat pH and colour

Ultimate pH of meat is a primary determinant of meat quality that is interrelated to several other quality attributes such as colour, water holding capacity and tenderness. The meat purchasing decisions are largely dependent on the meat pH. The post-mortem changes that take place in the meat have discernible effects on the ultimate meat quality. Depending on the duration of heat stress, there would be changes in the meat pH during the post-mortem period. During conversion of muscle to meat, there would be decline of pH from 7.0-7.2 to pH 5.5-5.7 during 24 h post slaughter. However, if the animal encounters any kind of stress such as heat stress immediately prior to slaughter, glycogen will be released to blood and converted into lactic acid. This sudden increase in acidity may result in a condition termed as pale soft exudative meat, which is very common especially in pork. This PSE is the major cause for the rejection of swine and poultry meat in the market

both by the consumers and the meat processors and is more pronounced during the summer months (Webb and Casey, 2010).

Meat pH is intricately related to several other attributes, leading to reduced water holding capacity (WHC), accelerated rigor mortis and paler colour (Ma *et al.*, 2015). Reduced WHC would in turn result in increased cooking loss, shearing force, drip loss and reduced flavour (Ma *et al.*, 2015). Rana *et al.* (2014) in an experiment on heat stressed sheep, reported that the increased ultimate pH of meat during heat stress may affect the cooking loss and keeping quality of meat. Similarly in a study on Black Bengal goats, Hashem *et al.* (2013) also established increased meat pH during heat stress exposure and they interrelated this to the cooking loss of meat. However, in the breast muscles of broilers, chronic heat stress significantly decreased the meat pH, increased L* and drip loss (Lu *et al.*, 2007; Feng *et al.*, 2008). Further, during acute heat stress in broilers, increased L* and decreased a* and b* have also been reported (Zhang *et al.*, 2012). These authors had attributed such alterations to the denaturation of sarcoplasmic proteins and scattering of light. However unlike as in PSE, if the animals are exposed to heat stress for longer duration, all glycogen reserves will be fully depleted. This would result in insufficient amount of lactic acid after slaughter, thereby increasing the meat pH and resulting in a condition termed as dark, firm and dry in pigs and dark-cutting in beef cattle (Miller, 2007). High pH would be accompanied with higher WHC and increased light absorbance, thereby imparting dark colour to the meat. Dark cutting beef or DFD meat is a serious concern among the beef producers because of the consumer rejection for their undesired appearance.

It has also been reported that heat stress severely enhances formation of DFD in beef carcass (Miller, 2007). DFD meat besides having unappealing appearance is highly prone to meat contamination and spoilage (Miller, 2007). Further, a report also states that increased respiration rate during the heat stress condition reduce the oxygen availability, thereby increasing the deoxygenated myoglobin concentration resulting in darkening of meat (Liu *et al.*, 2012). Dark cutting in beef is considered a negative issue which has serious economic

consequences in the meat markets of Oman and US (Kadim *et al.*, 2004; Gregory, 2010).

Colour of meat can be evaluated by visual appearance and also as instrumental colour (lightness L*, redness a* and yellowness b*). As the consumers usually relate the colour of meat, fat content and visual appearance to the freshness, quality and safety of meat, color is considered a vital meat parameter (Nikbin *et al.*, 2016). It was estimated that there was 15% loss of commercial value for beef due to the meat discoloration (Falomir-Lockhart *et al.*, 2015). Meat colour is essentially affected by the pre-slaughter stressors, among which temperature stress has been identified as a critical factor (Weglarz, 2010). Castrated sheep during heat stress showed increased values of colour parameters (L^*, a^*, b^*) of meat along with augmented activity of creatin kinase which is related to the muscular damage and mutton quality (Chulayo and Muchenje, 2013). However, reduced colour (L^*, a^*, b^*) has also been reported in heat stressed sheep (Kadim *et al.*, 2008). In contrast, higher lightness (L*) and lower redness (a*) have also been reported in turkeys exposed to heat stress (Sandercock *et al.*, 2001). Further, in both the thigh and breast muscles exposed to heat stress, higher L^*, b^* and lower a^* were established in broilers (Zhang *et al.*, 2012).

2.7.2 Meat tenderness and juiciness

Eating quality of meat is primarily determined by the meat tenderness and juiciness (Listrat *et al.*, 2016). At high ambient temperatures, the usual rigor mortis development after slaughter will be more exacerbated, leading to enhanced stiffening of meat. Further with the accompanying alterations in meat pH and moisture losses during heat stress, meat gets firmer and drier, resulting in reduced tenderness and eating quality (Nikbin *et al.*, 2016). Feng *et al.* (2008) and Gu *et al.* (2008) also reported declined meat tenderness in heat stressed broilers and they attributed this to the increased shear force value of the breast muscle. Literatures suggest that the enhanced dehydration in animals exposed to heat stress due to severe moisture losses through the process of respiration and evaporation reduces

the meat tenderness and quality (Nikbin *et al.*, 2016). In goats, it has been studied that ante-mortem stress related generation of lactate is another factor reducing the tenderness of the meat (Nikbin *et al.*, 2016). Hao *et al.* (2016) reported up regulation of desmin (DES) gene associated with meat tenderness in heat stressed pigs reflecting poor meat quality. Further, juiciness of meat also was found to be compromised in goats exposed to extreme environmental temperature (Casey and Webb, 2010). Also Spehar *et al.* (2009) studied various factors affecting sensory attributes in beef and reported temperature stress to be one of the important factor influencing fat content of meat and established an association between marbling score of the carcass and meat tenderness. Enhanced marbling score or intramuscular fat deposition in beef during heat stress results in, toughening of meat (Gregory, 2010). Further, the sensory parameters such as tenderness and juiciness are interrelated and the tougher meat was found to be associated with less juice content during heat stress condition (Gregory, 2010). Similarly Kadim *et al.* (2008) also reported that in sheep and goats, meat tenderness was declined through exposure to increased ambient temperature. These authors also documented reduced juiciness and colour (L^*, a^*, b^*) in sheep meat during heat stress.

2.7.3 Meat safety

It is generally assumed that meat safety could be affected by the changing climatic conditions (Gregory, 2010). In poultry, retail meat contamination with pathogens such as *Salmonella* or *Campyllobacter* showed peak occurrence in summer. In fact, one of the primary causes for foodborne illness in humans through consumption of poultry products is attributed to the seasonal up shoot of the food-borne pathogens such as *Salmonella* and *Campyllobacter* (Domingues *et al.*, 2012). Such bacterial pathogens have the potential to alter the neuroendocrine responses during stress response in the host, thereby enhancing their proliferation and pathogenicity. This direct effect of microbial pathogens on the stress hormones and mediators is the emerging concept of 'microbial endocrinology' which affects the safety of meat and meat products (Lara and Rostagno, 2013). In a study by Moro *et al.* (2000), pig carcasses showed increased numbers of *Escherichia coli*, resistant

of ampicillin and tetracycline during elevated ambient temperatures. However, the mechanism underpinning the increased prevalence of these pathogens during summer could not be established. However, studies pertaining to the impacts of climate change on meat spoilage and safety are inadequate, particularly in red meat and this warrants further research investigations (Gregory, 2010).

2.7.4 Heat stress impact on proximate composition

During heat stress in broilers, higher moisture content and fat content have been reported along with reduced protein content in thigh and breast muscle (Zhang *et al.*, 2012). These authors suggested the reason for reduced protein deposition, to the decreased ribosomal activity and increased fat deposition, especially the abdominal fat, to the reduced basal metabolism and physical activities during heat stress. Gu *et al.* (2008) reported significant decrease in content of crude protein and increase in crude fat in broiler meat exposed to high ambient temperatures combined with high relative humidity (33°C). Further, these authors also reported increase of moisture content in the breast meat, but not in the thigh muscle. Similar result of reduced crude protein was reported in the meat samples of sheep exposed to heat stress (Rana *et al.*, 2014). However in the same study, no significant alterations were reported in dry matter, ash and ether extract in meat samples of control and heat stressed sheep. Likewise, another study in black Bengal goats concluded that heat stress caused no variations on dry matter, crude protein and ash, but ether extract varied significantly in heat stressed group (Hashem *et al.*, 2013). Like meat safety, reports pertaining to impact of heat stress on meat proximate composition are very scanty and therefore future research efforts are needed to fully understand the mechanisms associated with heat stress compromised proximate composition of meat.

2.7.5 Heat stress impact on meat quality related blood biochemical parameters

Stress response is a vital adaptive process and involves release of enzymes and hormones which controls the basic life sustaining activities such as glycogenolysis and gluconeogenesis (Wan *et al.*, 2016). There are several reports

pertaining to the identification of suitable biological markers for heat stress associated meat deterioration. In a study conducted by Tang *et al.* (2013), it was identified that plasma creatine kinase and glutamic-pyruvic transaminase acts as reliable biomarkers to assess heat stress in broilers meat. These authors identified that increased concentration of creatine kinase was negatively correlated to pH of muscle causing skeletal muscle damage. The increased activity of creatine kinase in serum could be correlated to the degree of injury to the muscle and therefore it can serve as a reliable indicator for heat stress associated meat quality in broilers (Wan *et al.*, 2016). Further, cortisol level in heat stressed livestock species was established to cause detrimental effects on meat quality (Yoshioka *et al.*, 2005; Zhang *et al.*, 2012). In pigs, it has been studied that the animals subjected to pre-slaughter heat stress showed increased blood, cortisol level which was found to affect the meat pH, color and drip loss (Yoshioka *et al.*, 2005). There are also similar reports in pigs establishing the increased blood levels of cortisol, lactate and creatine kinase in heat stressed pigs enhancing the rigor mortis development imparting higher degree of toughness to the meat (Dokmanovic *et al.*, 2015). Further, it was reported in the same study that the increased cortisol released in response to those stresses was responsible for the increased muscular damage. Further, these authors observed that the increased apoptotic activity and muscle injury after heat stress exposure was closely associated to the meat color and WHC. Furthermore, it was established that meat quality attributes are impacted due to changes in the levels of cytoskeletal proteins, cellular elements and shrinking of muscle cells during stressful conditions (Ashino *et al.*, 2016; Sciorati *et al.*, 2016). In addition, in goat also it has been established that the increased level of lactate during ante-mortem stress exposure resulted in increased toughness of meat (Nikbin *et al.*, 2016).

2.7.6 Heat stress impact on expression pattern of some important skeletal muscle genes

Physiological and functional mechanisms governing the skeletal muscle growth and development during heat stress has been widely studied in animals (Yoshihara *et al.*, 2013). However, to date there are no conclusive reports on the molecular mechanisms regulating the muscle development and meat quality in animals exposed to heat stress.

Heat shock proteins (HSPs) are the widely studied genes whose expression gets altered during heat stress in animals (Dangi *et al.*, 2012). However, it has become evident in the recent years that over 50 genes other than HSPs are altered during heat stress (Gupta *et al.*, 2013). Chauhan *et al.* (2014) had reported the up regulation of HSPs namely, HSP70, HSP90 and HSF-1 in the skeletal muscle of heat stressed sheep. Further, regulation of HSP27, CRYAB and DNAJC5 in the skeletal muscle of pig was found to be influenced in response to heat stress in these animals (Hao *et al.*, 2016).

It has been established that heat stress decreased the expression patterns of myogenesis related genes such as MyoD, myogenin and myostatin in chicken embryos and the down regulation of these genes were found to affect the development of skeletal muscles in chicken (Gabriel *et al.*, 2003). However, in pigs during heat stress, desmin gene was found to be up regulated, which has been identified as an important factor deteriorating meat tenderness and quality (Hao *et al.*, 2016).

Oxidative stress usually associated with heat stress in livestock animals increases the activity of antioxidant enzymes in skeletal muscles, namely superoxide dismutase, catalase and glutathione peroxidase (Slimen *et al.* 2015). However, in a study by Chauhan *et al.* (2014) in heat stressed sheep the genes glutathione peroxidase and superoxide dismutase were found to be down regulated in skeletal muscles during chronic heat stress, while catalase and manganese superoxide dismutase showed up regulation.

Studies on the regulatory mechanisms of muscle development and meat quality at the epigenetic level are inadequate. A recent epigenetic study based on changes in the DNA methylation pattern in heat stressed pigs had established the differential expression pattern of several candidate genes responsible for muscle development and meat quality (Hao *et al.* 2016). Up regulation of genes such as small muscle protein X-linked, myosin heavy chain 11, collagen type XVI alpha 1, and collagen, type IV and alpha 3 was identified following the constant heat stress that would affect the phenotypic characteristics involving muscle fibre size and meat tenderness. Further, the study also identified the differential expression pattern of genes related to energy metabolism, lipid metabolism carnitine palmitoyltransferase 1B, carnitine palmitoyltransferase 1A, leptin receptor and calcium signalling pathways (ryanodine receptor gene) and some transcription factors, all of which are associated with poor meat quality.

Genes regulating fatty acid oxidation namely, mitochondrial acyl-CoA dehydrogenase, very long chain and acyl-CoA dehydrogenase significantly reduced in heat stressed cows at late gestation (Koch *et al.* 2016). Further, the same report also revealed the significant up-regulation of proteolysis regulating genes in the skeletal muscle in cows exposed to heat stress, namely the forkhead boxO3.

2.7.7 Significance of small ruminant meat industry from global food security perspective

Demand for meat and meat products are growing rapidly in the developing countries. Population increase and changing economic situation are the primary drivers for the growing demand of the meat sector (OECD/FAO, 2011). The small ruminant production draws increasing attention especially from the meat production perspective. They constitute 56% of the total ruminant population globally with 1178 million sheep and 1000 million goats (FAOSTAT, 2013). Global sheep production is expected to show 60% increase by 2050. Further, sheep and goats are considered as excellent animals for meat production with a radiant future in the current scenario, especially from the view that they are free from religious taboos

unlike beef and pig and also for the shorter period of generation for these small ruminants (Ozung *et al.*, 2011). The meat and milk production from the world flock of goats and sheep was estimated to be more than 13 million tons and 28 million tons respectively in 2013 (FAOSTAT, 2013).

Small ruminant meat production seeks huge relevance especially in countries of Asia, Africa and Middle East, where the tradition of mutton and chevon consumption exists (Devi *et al.*, 2014). Goat meat is consumed in more than 40 countries, with China holding the first position in chevon consumption, followed by India and Pakistan (FAO, 2009). The growth pattern in goat production clearly emphasize the relevance of concentrating future research efforts on this animal and it has been projected worldwide as the ideal meat animal foreseeing the future food crisis worldwide (Webb, 2014).

The potential of goats to thrive in a wide array of environmental conditions and ability to survive on low quality feed resources makes goat the most adequate animal for meat production from both food security and climate change perspectives (Mahgoub *et al.*, 2012). High nutritious content and lean meat in chevon is concordant with the present day consumer demands (Webb *et al.*, 2005). In fact, higher levels of amino acids such as arginine, leucine and isoleucine are present in chevon compared to mutton and it has been revealed that goat meat is not inferior to sheep and other red meats based on their protein contents (Dhanda *et al.*, 2003). Taking into account the nutritive and adaptive aspects, goat meat industry offers huge scope for future investments and may play a huge role in meeting the increased food demand. Hence, both the economics and growing concern and interest on the welfare of animals warrants concrete efforts to be taken to investigate the heat stress impacts and the mechanisms governing in these animals.

2.7.8 Closing remarks

Stress responses of the animals are closely linked to meat quality. Heat stress increases the risk of PSE and DFD in livestock meat which is of major concern for meat industry. Majority of the carcass parameters: meat pH, water

holding capacity, shear force, myofibrillar fragmentation index and sensory attributes (tenderness, juiciness, colour and appearance) which are the determinants of the consumer preference and acceptability of meat are negatively influenced by heat stress. Such undesirable alterations in the sensory and quality attributes of meat reduce the consumer acceptance and thereby hamper the meat industry. The heat stress driven carcass weight losses have significant economic implication in meat industry. Further to the adverse consequences on meat quality and safety, heat stress also brings about significant alterations in the profile of blood biochemistry in animals which may reflect the drop in meat quality. In addition, heat stress also alters the expression patterns of several genes regulating muscle development and meat quality. From this literature review it may be concluded that the current understanding of the intricate mechanisms underlying the meat quality deterioration is inadequate and needs further investigation. The future research efforts also must target identifying ideal biological markers reflecting the meat quality under different testing environmental condition which may help to identify species/breed which may survive and produce optimally in the adverse environmental condition.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

The experiment was conducted at the National Institute of Animal Nutrition and Physiology experimental livestock farm (NIANP), Bengaluru, India which is located at the longitude 77° 38´E and the latitude of 12° 58´N and at altitude of 920 m above mean sea level. The average annual maximum and minimum ambient temperature in this geographical region ranges between 15 to 36°C. The mean annual RH ranges between 20 and 85%. The experiment was conducted during the summer months of April-May, 2017. The temperature and RH variations during the study period (April-May) ranged between 28-40 °C and 29-58 % respectively under hot semi-arid climatic condition. The rainfall pattern in this region is erratic throughout the year and the annual rainfall in this area ranges between 200 to 970 mm. The THI values were calculated according to formula described by McDowell (1972) as per follows: $THI = 0.72(T_{db} + T_{wb}) + 40.6$ where, T_{db} = Dry bulb temperature in °C; T_{wb} = Wet bulb temperature in °C. The THI values equals 72 and less are considered as comfortable for the animals; THI values between75-78 are considered stressful and THI above 78 considered extreme distress for the animals.

3.2 Animals

Three indigenous female goat breeds of India were used in the present study; Osmanabadi (OS), Malabari (M) and Salem black (SB). All the goats procured were in the age group of 10 months to 1 year. The animals were maintained in galvalume roofed sheds with proper ventilation. Shed roofing was at a height 2.4 m and open.

3.2.1. Osmanabadi (OS)

Osmanabadi are large sized goat breed native to the semi-arid regions of central tropical India. This breed originated in the Osmanabadi district of Maharashtra. They are distributed in Osmanabadi in Ahmednagar, Solapur and Osmanabad districts in Maharashtra; Karnataka and Andhra Pradesh (Deokar *et al.*, 2006). It is a hardy breed used for milk as well as meat. Goats of this breed are predominantly (73%) black in colour. However, they are also found as white, brown and spotted. The average body weights of adult male and female goats are 34 kg and 30 kg respectively. They give an average daily yield of 0.5 to 1.5 kg for a lactation length of about 4 months. The does will breed regularly twice a year. Twinning is also common in this breed. The OS goats used in the study had an average body weight of 12-19 kg.

3.2.2 Malabari (M)

Malabari (also known as Telicherry) is the native breed of Kerala which is reared mainly for meat. Although their size is small, this breed is known for their palatable meat. Further, this breed has good heritability for reproductive trait, which is the most important production aspect of small ruminants (Thiruvenkadan *et al.*, 2008). Milk yield of this breed ranges from 1-2 kg/day. These goats are mostly seen in white, purple and black colour. Body weight of this breed goats used in the study ranged between 12-19 kg.

3.2.3. Salem black (SB)

The Salem black breed is an important meat breed originated in the north-western state of Tamil Nadu in India. The breed has derived its name from the place of origin and characteristic black colour of coat. This breed is prevalent in Salem, Dharmapuri, Krishnagiri, Erode, Karur and Namakkal districts of Tamil Nadu. This breed is characterized by tall body and black colour (Thiruvenkadan and Karunanithi, 2006). The goats used in the present study had an average body weight of 12-19 kg. The body weight of adult goats ranges between 38.5±1.0 kg in males and 29.5±0.6 kg in females.

3.3 Technical program

The study was conducted for a period of 45 days between April-May 2017. Thirty six animals in the age group of 10 months to 1 year old were used in the study. The animals were randomly allocated into six groups of six animals each, GI (n=6; OSC), GII (n=6; OSHS), GIII (n=6; MC), GIV (n=6; MHS), GV (n=6; SBC) and GV1 (n=6; SBHS). 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). Table1 describes the feed ingredients and the chemical composition of the feed offered to the animals. The GI, GIII and GV animals were maintained in the shed in thermo-neutral condition while GII, GIV and GVI animals were exposed outside to summer heat stress between 10:00 h to 16:00 h to expose to heat stress during experimental period. The GI, GIII and GV animals were fed and watered inside the shed while GII, GIV and GVI animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily for the entire duration of the study. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to heat stress. Blood was collected at fortnightly intervals for hormone estimation. The animals were slaughtered at the end of the study to assess the meat and carcass characteristics differences between the breeds and representative skeletal muscle muscles were collected for gene expression study. Fig.1 depicts the technical programme of the current study.

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

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

*Calculated values

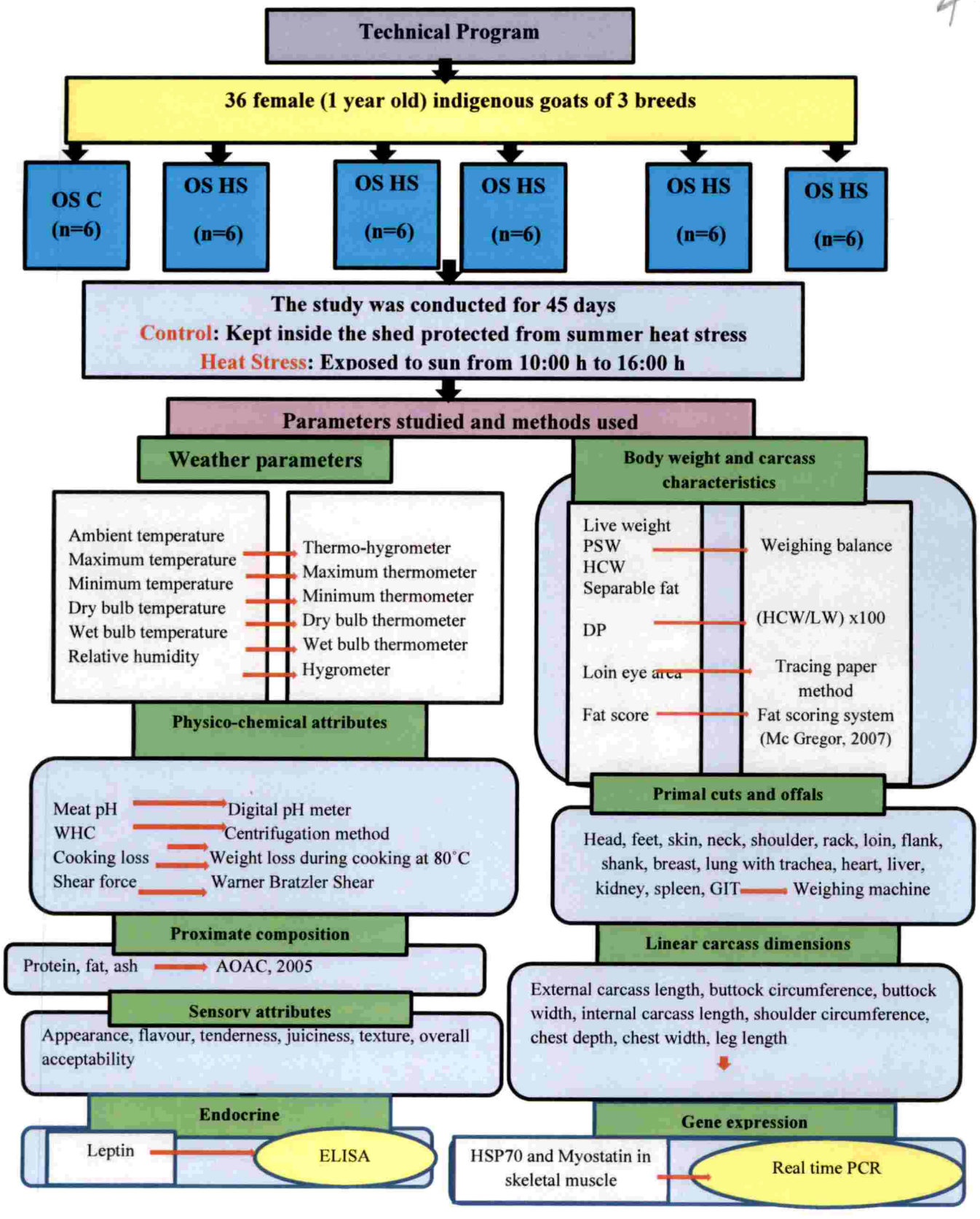

Fig 1: Represents overall technical programme for the entire study

3.4 Weather parameters recording

Weather parameters were recorded twice daily (8:00 h and 14:00 h) for the entire study period. Maximum temperature, minimum temperature, ambient temperature and relative humidity were recorded using thermo-hygrometer. Dry bulb and wet bulb thermometers were used for recording dry bulb and wet bulb temperatures respectively. Infrared thermometer was used for measuring pen surface temperature.

3.5 Slaughter procedure and carcass evaluation

All the animals were fasted for 12h overnight with *ad libiltum* access to water before slaughter. The animals were slaughtered with all the hygienic measures in the slaughter house at environmental livestock farm unit, National Institute of Animal Nutrition and Physiology (NIANP), Bangalore. The animals were slaughtered by the traditional Muslim halal method by severing the jugular vein and carotid artery of the goats. After slaughter, the head was removed at the atlanto-occipital joint and fore and hind feet were removed at the carpal and tarsal joints, respectively. The animals were partially skinned lying on their back on the floor and then suspended to gambrel by the hind leg (Achilles tendon) for further skinning. Sticking, legging, dressing and evisceration were performed as per the procedure described by Gerrard (1964). Fat scoring of the animals were done following method of Mc Gregor, 2007. The animals were made to stand in a relaxed state. The balls of fingers were used to feel the body over the 12^{th} long rib and score was given according to the following system.

Fat scoring system

Fat Score	1	2	3	4	5
What you feel over the ribs	Ribs very easily felt. No tissue over the ribs.	Ribs very easily felt. Slight amount of tissue over the ribs.	Ribs easily felt. Some tissue over ribs.	Ribs can be felt. Lots of tissue present.	Ribs only felt with pressure. Tissue very prominent and may be fluid.

3.5.1 Body weight and carcass characteristics

Body weight and pre-slaughter weights (PSW) was recorded using weighing machine (Essae-Teraoka Limited, India) in kg. Immediately after slaughter, blood was collected in a trough and weighed in a weighing machine. The edible offals (liver, blood, heart, liver, kidney, intestine) and inedible offals (skin, head, feet, spleen, lung with trachea) were separately weighed. The weight of total internal fats (fats from the kidney, scrotal, pelvic, and heart) was recorded using sensitive balance. Dressing percentage (DP) was calculated on the basis of hot carcass weight (HCW) and live weight (LW) using the formula HCW/LW * 100.

Plate 1: Recording of pre-slaughter weight of the animal

Plate 2: Recording of hot carcass weight of the animal

Plate 3: Measuring buttock circumference

Plate 4: Measuring chest circumference

Plate 5: Recording offal (GIT) weight

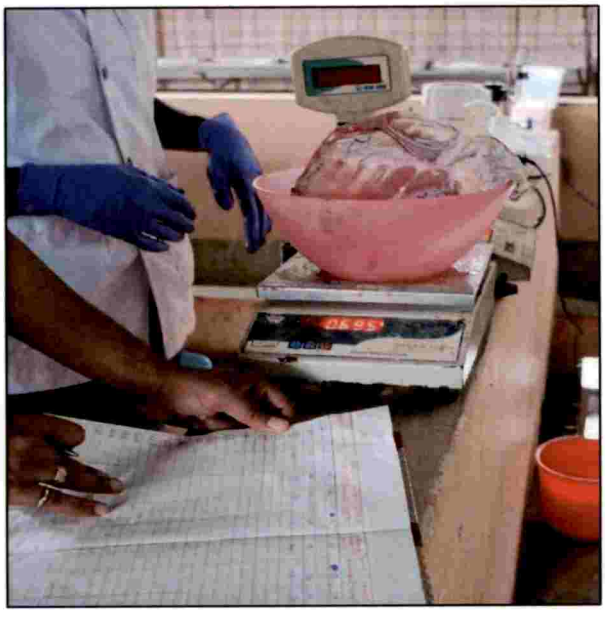

Plate 6: Recording primal cut weight

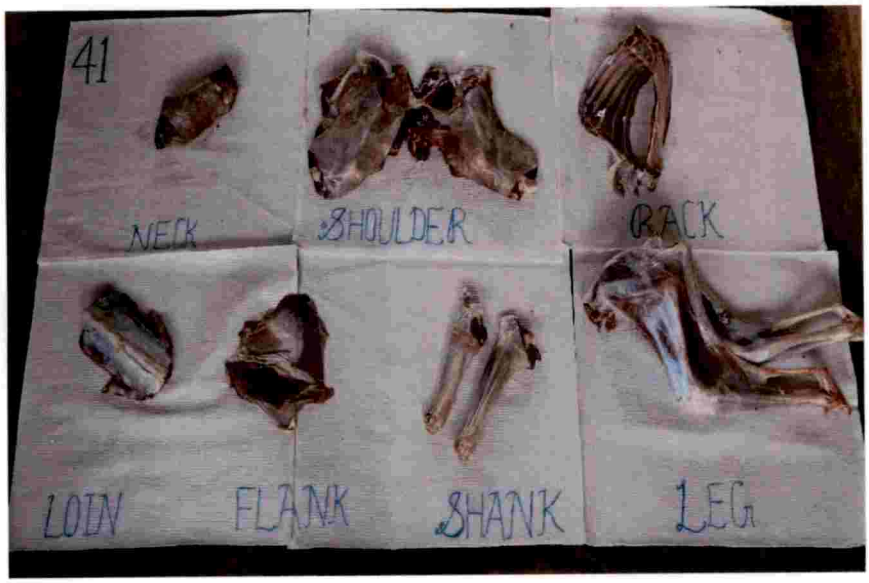

Plate 7: Primal cuts in chevon

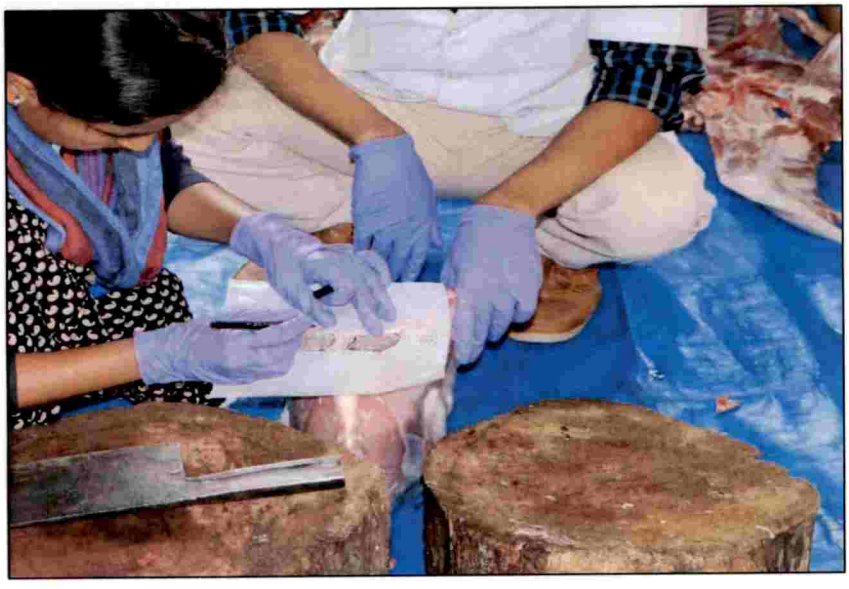

Plate 8: Tracing the loin eye area (LEA)

3.6.2 Water holding capacity (WHC)

Water holding capacity (WHC) was determined according to the protocol of Wardlaw *et al.* (1973). The 20 g of minced meat sample was placed in a centrifuge tube containing 30 ml NaCl (0.6 M) and was stirred with glass rod for 1 minute. The tube was then kept at refrigeration temperature (4±1 °C) for 15 minute, stirred again and centrifuged at 5000 rpm using refrigerated centrifuge (Sorvall Biofuge Stratos, Thermo electron LED GmbH, D-37520, Osterode, Germany) for 15 minute. The supernatant was measured and amount of water retained by samples were measured and WHC was expressed in percentage.

3.6.3 Cooking loss %

Cooking loss % was determined by weight loss after cooking of meat for 1 h in water bath at 80°C (Babikerm *et al.*, 1990). The weights of samples were recorded before (raw weight) and after cooking. Cooked weight was divided by raw weight and the result was multiplied by 100 to get per cent cooking yield.

Cooking loss (%) = (Weight of cooked meat/Weight of raw meat)* 100

3.6.4 Shear force

The shear force values were estimated using the protocol described by Wheeler *et al.* (1996) with certain modifications. The meat samples were sealed in a polythene bag and cooked at 100°C for 30 minutes in a water bath. The samples were then blotted dry and kept in unsealed bag in refrigerator for 12h. Six cores of meat samples with 1.27cm diameter were taken by cutting parallel to the muscle fibres. Theses cores were then sheared using Warner Bratzler Shear (G. R. Electric Manufacturing Company, Manhattan, USA). The mean shear force needed to shear through the core was assessed by taking the average of the six readings.

3.6.5 Colour Analysis

Colour of the meat sample sample was measured using Hunter lab Mini scan XE plus Spectro- colorimeter (Model No. 45/O-L, Reston Virginia, USA) with geometry of diffuse/80 (sphere - 8mm view) and an illuminant of D65/10 deg (Bindu *et al.*, 2007).

Principle

Colorimetry measures colour with quantitative physical methods and can define them within well-established numerical values. They are expressed using the standard Hunter L* a* b* system. L*, a*, b* values (non-dimensional units) which refer to the three axes of the system: a lightness axis (white – black, L*); and two axes representing both hue and Chroma, one red - green (a*) and other blue – yellow (b*). An unambiguous description of colour is given by the system. The instrument has the advantage that colour variations between samples can be estimated using simple and user-friendly computer programs.

Calibration of the instrument was done using a black and white tile. (L* = 94, a* = 1.10 and b* = 0.6) every time before the colour reading was recorded. The colour was expressed as L (brightness), a*(redness) and b* (yellowness). Average values were taken for each colour parameter of each sample by keeping the meat sample in three different positions.

3.7 Proximate composition

Proximate compositions such as crude protein, ether extract and ash were estimated using the method by (Association of Official Analytical Chemists) AOAC, 2005.

3.7.1 Crude protein

Total nitrogen was measured by Kjeldahl method. A known quantity of sample (about 0.5-1 g) was taken in Kjeldahl flask and digested with 20-30 ml concentrated H_2SO_4 and 2-3 g of digestion mixture till the solution became colourless. After digestion, the contents were cooled and volume was made to 100 ml. 10 ml of aliquot was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer) after adding 10-15 ml of 40% NaOH solution. About 60-75 ml of distillate (light green colour) was collected into an erlenmeyer flask containing 10 ml of 2% boric acid indicator solution. The distillate was then titrated against N/100 H_2SO_4 solution and the end point was recorded when colour changed to

slight pinkish. Volume of N/100 H_2SO_4 solution used in titration was measured and recorded.

Calculation:

$$N\ (\%) = \frac{0.014 \times 0.01 \times \text{Volume of N/100 } H_2SO_4 \text{ used} \times \text{Volume made (ml)} \times 100}{\text{Aliquot taken (ml)} \times \text{Sample taken (g)}}$$

The crude protein (%) of sample was calculated by multiplying the N content with factor 6.25. This was based on the principle that protein contains 16% nitrogen.

3.7.2 Ether extract

A known quantity of ground sample (about 3 g) was taken in a cellulose thimble and extracted for 8 hours with petroleum ether (40-60°C) in Soxhlet's extraction apparatus attached to a pre weighed oil flask. The oil flask was removed and after evaporating the excess of ether, it was dried overnight in a hot air oven (temp. 100±5°C). The flask was cooled in a desiccator and weighed to a constant value. The ether extract was estimated as the difference in the weight of oil flask with and without oil.

Calculation:

$$\text{Ether extract (\%)} = \frac{(\text{Wt. of oil flask with ether extract} - \text{Wt. of oil flask}) \times 100}{\text{Wt. of sample}}$$

3.7.3 Total ash

A known quantity of sample (about 2.5-5 g) was taken in pre-weighed silica crucible. After charring the sample on heater (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550°C for 2-3 h. The crucible was removed on cooling and kept in a desiccator and weighed again to find out weight of ash. The ash content was calculated as given below:

Calculation:

$$\text{Total ash (\%)} = \frac{\text{(Wt. of crucible + ash after cooling – Wt. of oil flask) X 100}}{\text{Wt. of fresh sample}}$$

3.8 Sensory Evaluation

Appearance, flavour, juiciness, texture and overall acceptability of the cooked meat samples were evaluated by semi trained panellists using an 8-point descriptive scale (where, 8= extremely desirable, 1= extremely undesirable) (Keeton, 1983).

3.9 Estimation of endocrine parameters

Endocrine parameter estimated in the study was Leptin. The parameters Leptin (LDN, Nordhorn, Germany) was estimated by Enzyme-linked immunosorbent assay (ELISA).

3.9.1 Leptin

Principle of the test

The principle of the enzyme immunoassay test follows a typical two step capture or sandwich type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilised antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and

unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin- HRP is added, which binds specially to any bound biotinylated antibody. Again, unbound streptavidin- HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

Assay procedure:

1. The working solutions of the streptavidin- HRP- conjugate and wash buffer were prepared.
2. 20 µL of each standard, control and specimen samples were pipetted into the corresponding wells in duplicate.
3. 80 µL of the monoclonal anti- leptin- biotin conjugate was pipetted into each well.
4. The plate was incubated on a plate shaker (approximately 200 rpm) for 1 h at room temperature.
5. The wells were washed 3 times with prepared wash buffer (300 µL/ well for each wash) and the plate was taped firmly against absorbent paper to ensure that it is dry.
6. 100 µL of prepared streptavidin- HRP-conjugate was pipetted into each well.
7. The plate was incubated on a plate shaker (approximately 200 rpm) for 30 min at room temperature,
8. The wells were washed 3 times with prepared wash buffer (300 µL/well for each wash) and the plate was taped firmly against absorbent paper to ensure that it is dry.
9. 100 µL of TMB substrate was pipetted into each well at timed intervals.

10. The plate was incubated on a plate shaker for 10-15 min at room temperature.
11. 50 µL of stopping solution was pipetted into each well at the same timed interval as in the above step.
12. The plate was read on a microwell plate reader (Thermo Scientific, MULTISCAN GO, Finland) at 450 nm within 20 min after addition of the stopping solution.

Calculation

1. The mean optical density of each calibrator duplicate was calculated.
2. A calibrator curve was drawn on a semi- log paper with the mean optical densities on the Y- axis and the calibrator concentrations on the X-axis.
3. The mean optical density of each unknown duplicate was calculated.
4. The values of the unknowns were read directly off the standard curve.

Plate 9: Recording of meat pH

Plate 10: Recording of shear force

Plate 11: Preparation for proximate analysis

Plate 12: Muscle sample collection for gene expression study

3.10 Gene expression

3.10.1 Expression patterns of Myostatin and HSP70 in skeletal muscle

Principle

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

3.10.1.1 Sample collection and storage

The muscle (*longissimus dorsi*) samples were collected from all the animals in each group immediately after slaughter. The samples were cut into small pieces, washed in phosphate buffered saline (PBS) and immersed in RNALater® Stabilization Solution (Life Technologies GmbH, Darmstadt, Germany). All the samples were stored at -80°C till further use.

3.10.1.2 Sample preparation for RNA isolation

After thawing, the tissues were removed from RNALater® Stabilization Solution (Life Technologies GmbH, Darmstadt, Germany) and immediately processed for RNA isolation. The total RNA was isolated from tissues using GeneJET RNA Purification Kit (Thermo Scientific, Lithuania) and the procedure was done as per manufacturer's protocol with slight modifications as follows:

About 30 mg of tissue was homogenized by grinding in Liquid Nitrogen (LN_2) (-196°C) in RNAase ZAP (Ambion, USA) treated mortar and pestle. After homogenization, 300 µL of lysis buffer supplemented with β-mercaptoethanol (10 µL/ml) was added and the content was transferred to 1.5 ml microcentrifuge tube.

The lysate was vortexed for 10 sec. To the lysate, 10 µL of proteinase K in 590 µL of Tris Ethylenediaminetetraacetic Acid (TE) buffer was added, then vortexed and incubated at 15-25°C for 10 min. Then, the contents were centrifuged for 8 min at 12000 g and the supernatant was transferred into a new RNase-free micro centrifuge tube. 450 µL of ethanol was added and mixed well by pipette. Then 700 µL of was transferred to a spin column with a 2 ml collection tube and centrifuged for 1 min at 12000 g. After discarding the flow through, 700 µL of wash buffer 1 was added and centrifuged for 1 min at 12000 g followed by two time washing with 600 µL and 250 µL of wash buffer 2 followed by centrifugation at 12000 g for 1 min and 2 min respectively. About 40 µL of warm nuclease free water was added to the membrane, and centrifuged at 10000 g for 1 min to elute RNA. The purified RNA samples were stored at -80°C until complementary DNA (cDNA) synthesis.

3.10.1.3 DNase treatment

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

3.10.1.4 cDNA Synthesis

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

4 µL of 5X Reaction Mix, 2 µL Maxima Enzyme Mix, 1µg of Template RNA and 20 µl of nuclease free water were added into a sterile, RNAase-free tube. Then the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 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/μL with nuclease free water and 2 μL of diluted cDNA was used for each reaction qPCR.

3.10.1.5 Primer design and synthesis

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

3.10.1.6 Quantitative RT-PCR analysis

The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20 μL reaction was carried out in duplicates using 50 ng of template and 0.5 μM primer concentrations. The real time qPCR reaction conditions were: enzyme activation at 95^{0}C for 10 min and amplification cycle (40 cycles; initial denaturation at 95^{0}C for 15 sec, annealing at 60°C for 30 sec and extension at 72^{0}C for 30 sec). The melt curve analysis was done to check the non-specific amplification. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control and the relative expression was analyzed using the formula, $2^{\Delta\Delta CT}$ (Shilja *et al.*, 2016). The results were expressed in fold change as compared to untreated control (control=1fold).

3.11 Statistical analysis

The data was analysed by one- way analysis of variance (ANOVA) SPSS (version 18.0) software. 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 skeletal muscle myostatin (mRNA) and PBMC HSP70

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 means among the groups. Further, the correlation coefficient between the THI and all carcass traits were established by Pearson's correlation coefficient test using SPSS (version 18.0) software. The R^2 values were used to establish the correlation association between THI and various carcass traits. Results are shown as mean ± standard error (SE) and the significance level was set at $P < 0.05$.

Table 2: Primers used for studying Myostatin and HSP70 expression in skeletal muscle. 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
Myostatin	F	ACCAAGCAAACCCAAAGGT	20	201.1	BA00728946
	R	CACCCACAGCGATCTACTACC	21	193.5	BA00728947
HSP70	F	TGGCTTTCACCGATACCGAG	20	167	NM_00128 5703.1
	R	GTCGTTGATCACGCGGAAAG			
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG			

bp- base pair; HSP 70 - Heat Shock Protein 70; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase

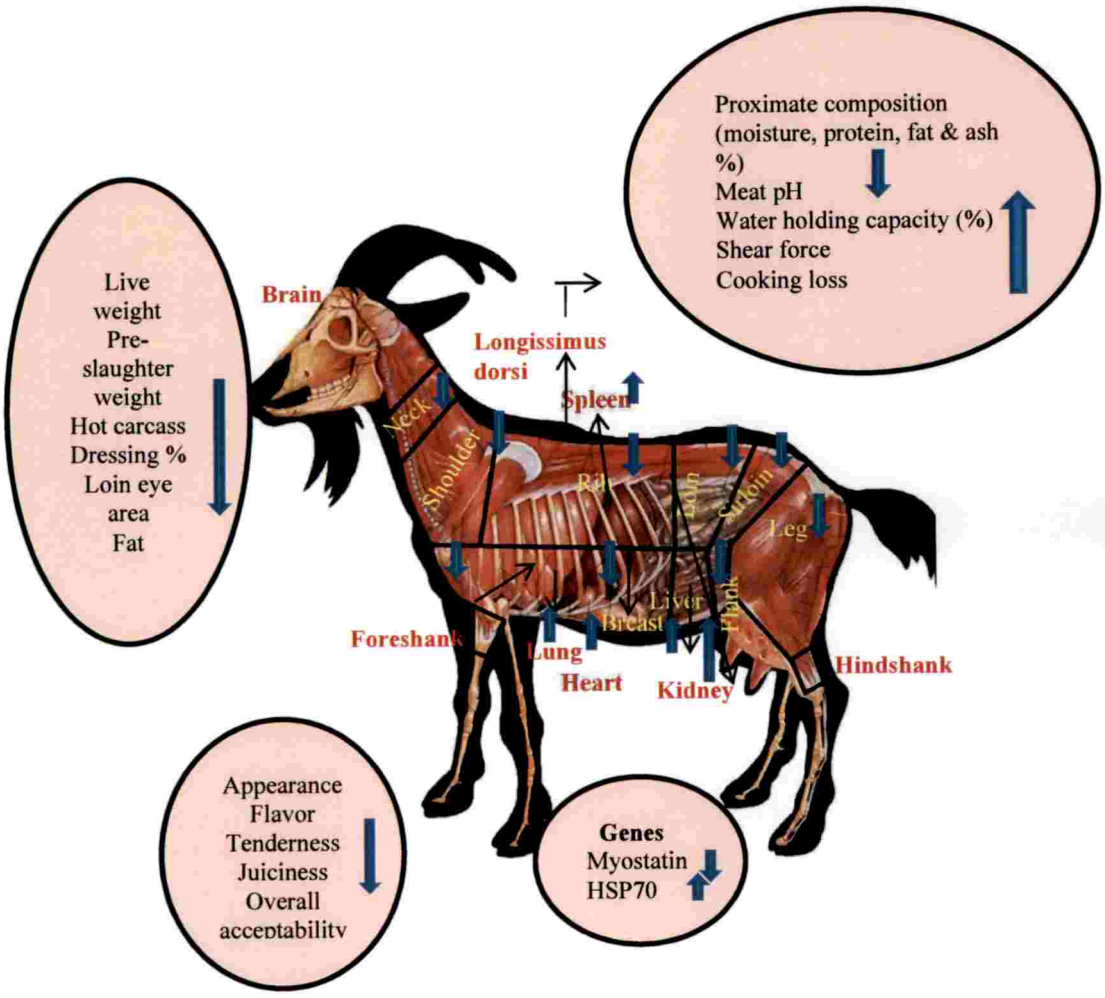

Fig.2: Concept figure of the current study

RESULTS

CHAPTER 4

RESULTS

4.1 Weather parameters

The maximum, minimum, wet and dry bulb temperatures and RH were recorded and the THI was calculated on an average at fortnightly interval. The obtained THI inside during both morning and afternoon are described in table 3 and depicted in Fig. 3. The THI values inside the shed shows that the animals were in comfort zone both during morning and afternoon.

The THI values outside the shed shows that during the morning hours the animals were in comfort zone while during afternoon the animals were under extreme distress. The THI values were calculated as per method described by McDowell (1972). Accordingly the formula used was THI = 0.72(Tdb + Twb) + 40.6 where, Tdb = Dry bulb temperature in °C; Twb = Wet bulb temperature in °C. 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.

Table 3: 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 the shed	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 the shed	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; WBT- Wet Bulb Temperature; MinT-Minimum Temperature; MaxT-Maximum Temperature; RH-Relative Humidity; AT-Ambient Temperature; PST-Pen Surface Temperature; THI-Temperature Humidity Index

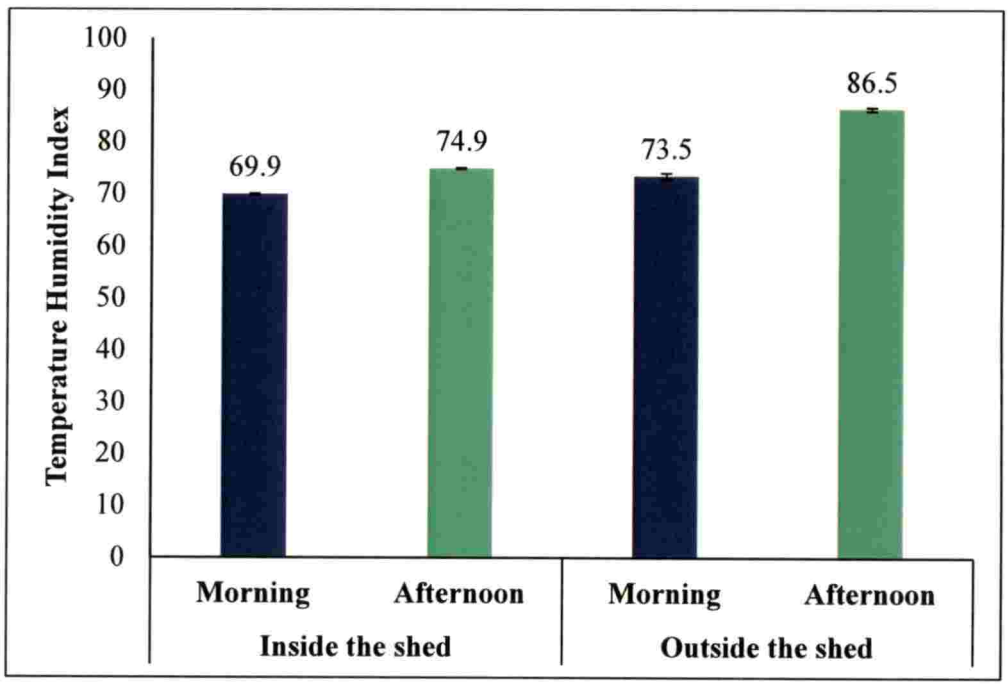

Fig. 3. Average temperature humidity index (THI) both inside and outside the shed

THI values ≤ 70= Comfortable; THI values between 75 and 78=stressful; THI >78= extreme distress

4.2 Body weight and carcass characteristics

4.2.1 Live weight (LW)

The live body weights of three different indigenous breed goats are presented in table 4. There was significant ($P<0.01$) influence of genotype observed on the live body weights of the goats. Malabari breed had significantly lower live weight in comparison to other two breeds. Significant variation ($P<0.01$) was also observed in the live body weights between the control and heat stress goats in all the three breeds. Significantly lower live body weights were recorded in all the heat stress goats compared to their respective control group. However, the magnitude of the effect of heat stress was observed to be the most intense in Malabari breed compared to the other two breeds. Among all the groups, live body weight was lowest ($P<0.01$) for the heat stress group of Malabari breed, whereas it was highest among the control group animals of both Osmanabadi and Salem Black breed. Further, the interaction between the breed and treatment influenced live weight significantly ($P<0.01$). In addition, a strong negative correlation ($P<0.01$) was established between THI and live weight (table 5).

4.2.2 Pre-slaughter weight (PSW)

Pre-slaughter weight showed similar trend of live weight. Breed effect was significantly ($P<0.01$) evident on the PSW. Osmanabadi and Salem black showed comparable PSW, and were significantly higher over the Malabari breed. The effect of heat stress was significantly ($P<0.01$) high in all the three breeds, with heat stress groups showing lower pre-slaughter weight compared to their respective control animals (table 4). Similar trend of LW was observed in PSW with the lowest PSW observed in the heat stress group of Malabari goats and highest in the control group of both Osmanabadi and Salem Black goats. However, the intensity of variation in PSW due to heat stress was found to be more in Malabari breed. Further, the interaction ($P<0.01$) also showed significant effect on PSW. In addition, a strong negative correlation ($P<0.01$) was established between THI and PSW (table 5).

4.2.3 Hot carcass weight (HCW)

Breed influence was significant ($P<0.01$) on the HCW of the animals. Among the different breeds, the Malabari breed showed lower HCW as compared to the other two breeds. The effect of heat stress on the hot carcass weight showed significant ($P<0.01$) difference only in the Salem black goats, with the heat stressed Salem Black goats showing lower HCW compared to the respective control group. Overall in all six groups, the highest ($P<0.01$) HCW was recorded in the control group of both Osmanabadi and Salem Black breeds. In addition, a strong negative correlation ($P<0.01$) was established between THI and HCW (table 5).

4.2.4 Dressing percentage (DP)

Dressing percentage of the three indigenous breed goats are shown in table 4. Breed difference was significantly ($P<0.01$) evident on the dressing percentage of the goats. The dressing percentage values showed no significant variation between control and heat stress groups within the breeds. There was significant influence of group on DP with the highest value being recorded in the control group of Salem black breed. In addition, interaction between breed and treatment also influenced the DP significantly ($P<0.01$). In addition, a strong negative correlation ($P<0.01$) was established between THI and DP (table 5).

Table 4: Effect of genotype and heat stress on the body weight changes and major carcass traits in Osmanabadi, Malabari and Salem black goats

Traits	Osmanabadi Breed		Malabari Breed		Salem Black Breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Live weight (kg)	18.80±1.05^{a}	15.40±0.79^{b}	15.08±0.39^{b}	12.40±0.99^{c}	18.30±0.92^{a}	15.65±1.00^{b}	**	**	**
PSW (kg)	18.47±1.03^{a}	15.50±0.84^{b}	14.68±0.35^{b}	11.35±0.79^{c}	17.75±0.96^{a}	14.85±0.89^{b}	**	**	**
HCW (kg)	8.05±0.85^{ab}	6.73±0.32^{bc}	5.98±0.10^{cd}	5.06±0.39^{d}	8.38±0.42^{a}	6.65±0.33^{bc}	**	**	**
DP	42.27±2.18^{bc}	43.79±0.78^{ab}	39.71±0.39^{c}	40.92±0.71^{bc}	45.81±0.29^{a}	42.75±1.06^{abc}	**	NS	**
LEA (cm^2)	7.74±0.34^{a}	4.48±0.28^{c}	4.58±0.17^{c}	3.21±0.17^{d}	5.51±0.18^{b}	4.01±0.22^{c}	**	**	**
Separable fat (kg)	0.29±0.06^{a}	0.03±0.00^{b}	0.12±0.03^{b}	0.04±0.01^{b}	0.06±0.01^{b}	0.03±0.01^{b}	*	**	**
Fat score	3.00±0.22^{a}	2.08±0.08^{bc}	2.42±0.24^{ab}	1.33±0.17^{d}	2.67±0.25^{ab}	1.75±0.17^{cd}	NS	**	**

Table 5. Correlation association between THI and body weight and carcass characteristics

	THI	LW	PSW	HCW	DP	LEA	Separable fat	Fat score
THI	1							
LW	-.500**	1						
PSW	-.491**	.992**	1					
HCW	-.430**	.944**	.942**	1				
DP	-.018	.330*	.343*	.618**	1			
LEA	-.669**	.502**	.513**	.434**	.059	1		
Separable fat	-.545**	.477**	.486**	.435**	.066	.572**	1	
Fat score	-.684**	.761**	.756**	.690**	.167	.583**	.629**	1

THI- Temperature Humidity Index, LW- Live Weight, PSW- Pre-slaughter Weight, HCW-Hot Carcass Weight, DP-Dressing Percentage, LEA-Loin Eye Area

**P<0.01; *P<0.05

4.2.5 Loin eye area (LEA)

Loin eye area has been significantly ($P<0.01$) influenced by genotype, breed and interaction. The heat stressed goats in all the breeds showed significantly lower LEA compared to the control goats. The Osmanabadi breed showed the highest LEA, whereas the Malabari breed had the lowest LEA. In addition, a strong negative correlation ($P<0.01$) was established between THI and LEA (table 5).

4.2.6 Separable fat and fat score

The mean values of separable fat and fat scores for the three breeds of goats are presented in table 4. In terms of separable fat, Osmanabadi goats showed significant ($P<0.01$) variation within the breed with heat stress animals bearing lower values. However, Salem Black and Malabari breeds showed no variation in separable fat within the respective breeds. Among all the groups, the highest ($P<0.01$) separable fat was recorded in Osmanabadi control group. The separable fat obtained from the Osmanabadi goats were nearly two fold more compared to other two breeds in the control condition. Unlike the separable fat, fat score showed high significant ($P<0.01$) difference between the control and heat stress group in all the breeds. Further, the severity of heat stress on fat score was more in the Malabari breed, whereas the other two breeds had comparable effect on fat score due to heat stress. However, there was no breed difference observed in fat score. The interaction effect was significant ($P<0.01$) on both separable fat as well as fat score. In addition, a strong negative correlation ($P<0.01$) was established between THI and both separable fat and fat score (table 5).

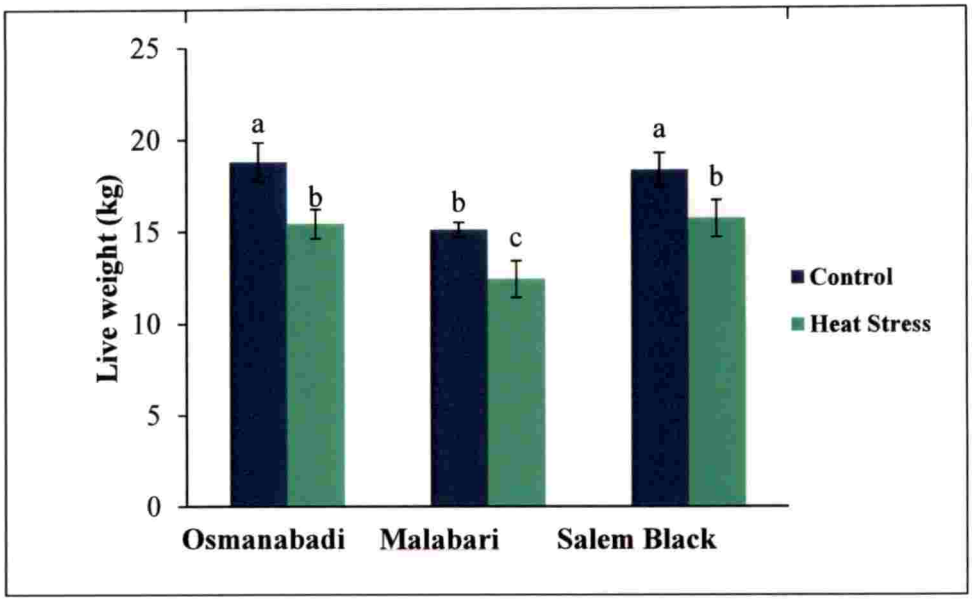

Fig. 4: Live weight

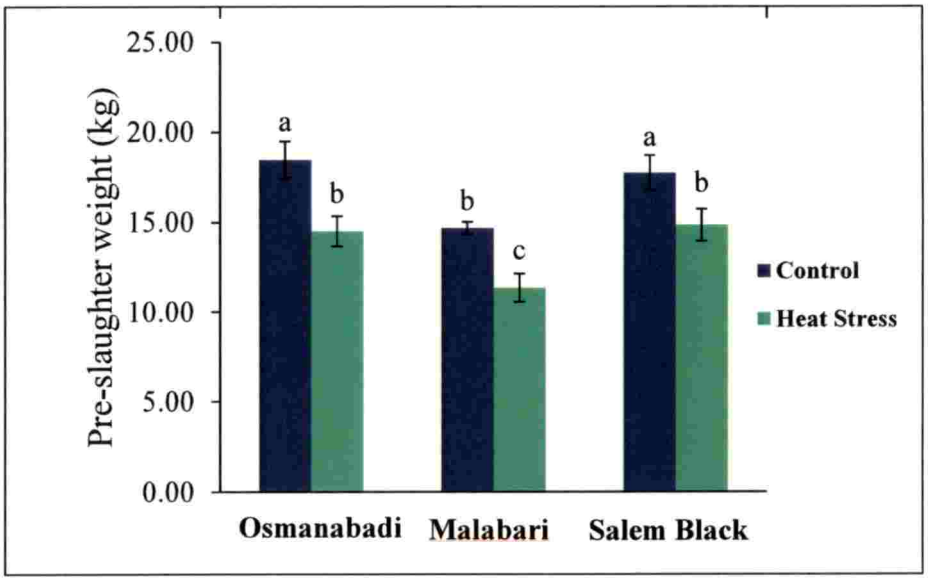

Fig. 5: Pre-slaughter weight

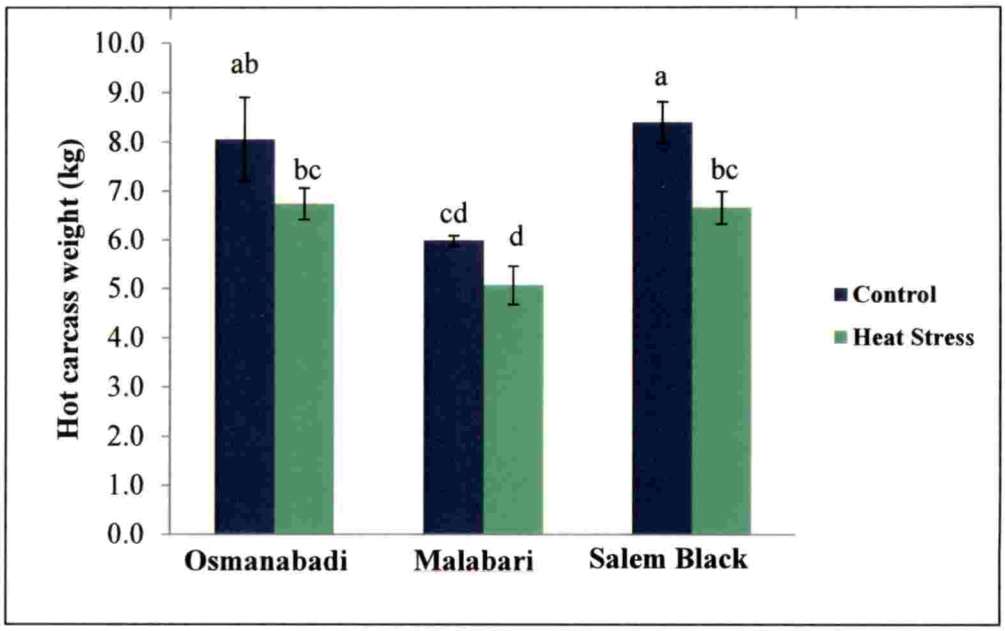

Fig. 6: Hot carcass weight

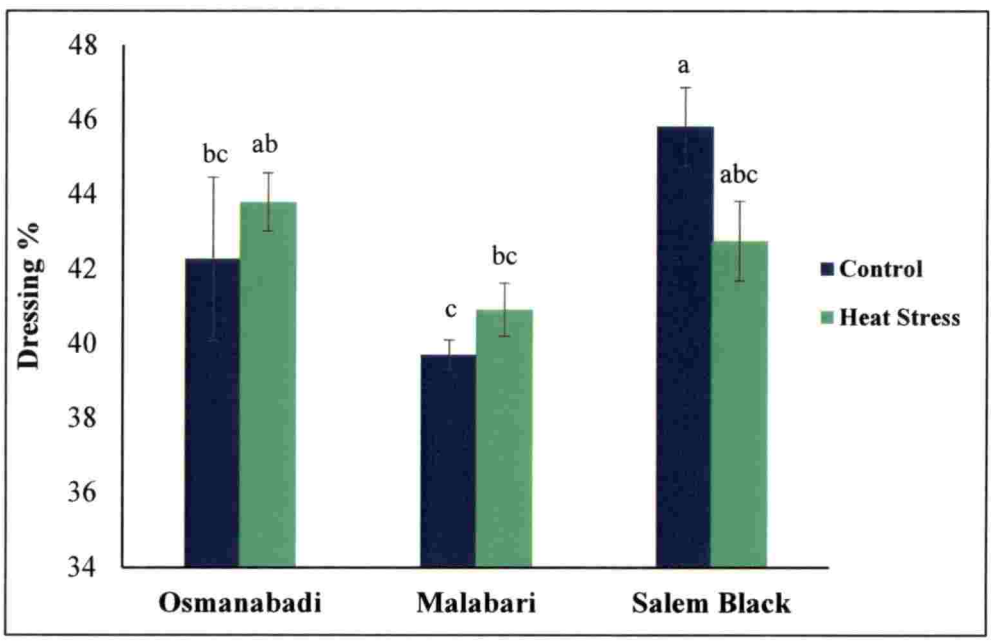

Fig. 7: Dressing percentage

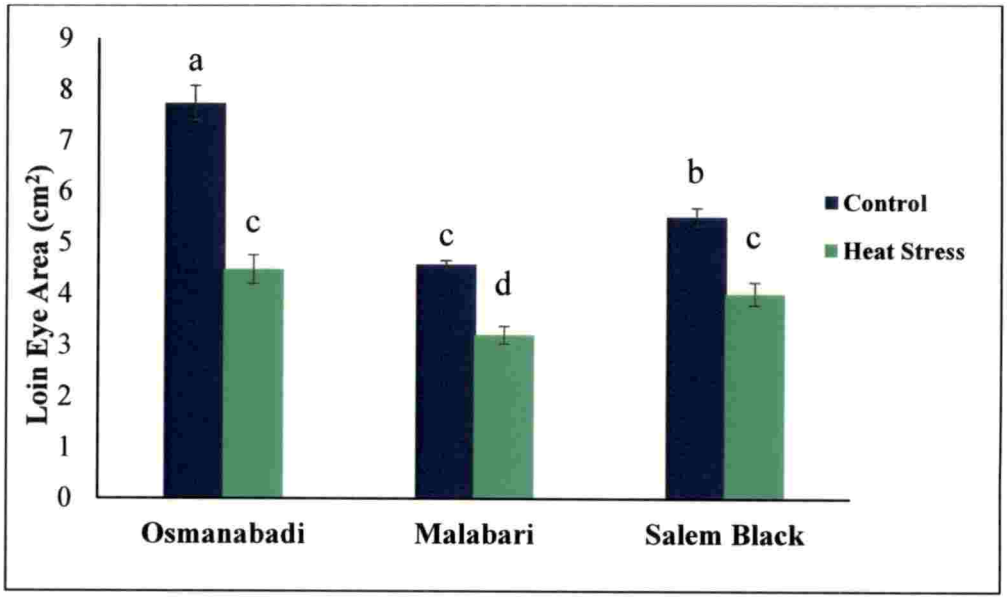

Fig. 8: Loin eye area

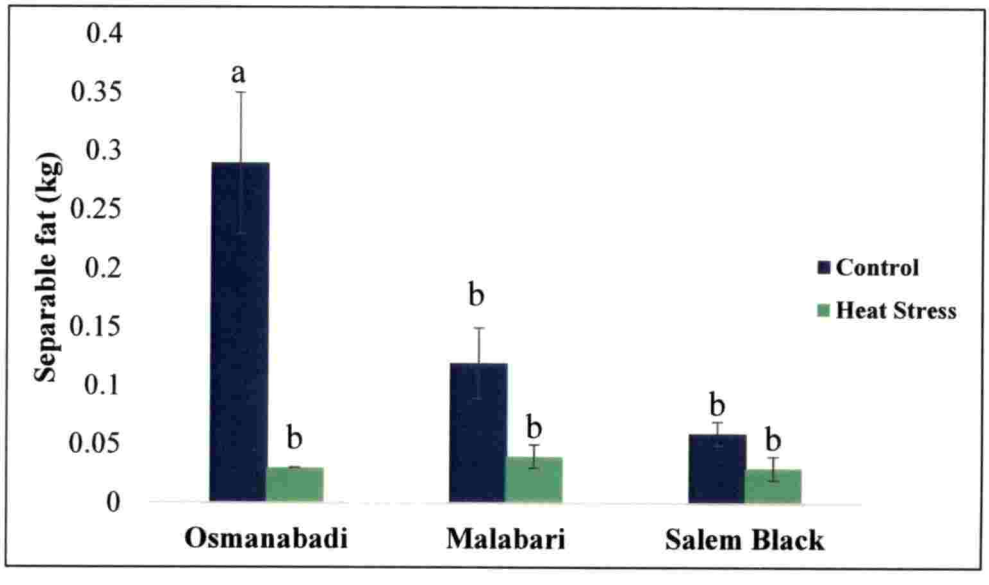

Fig. 9: Separable fat

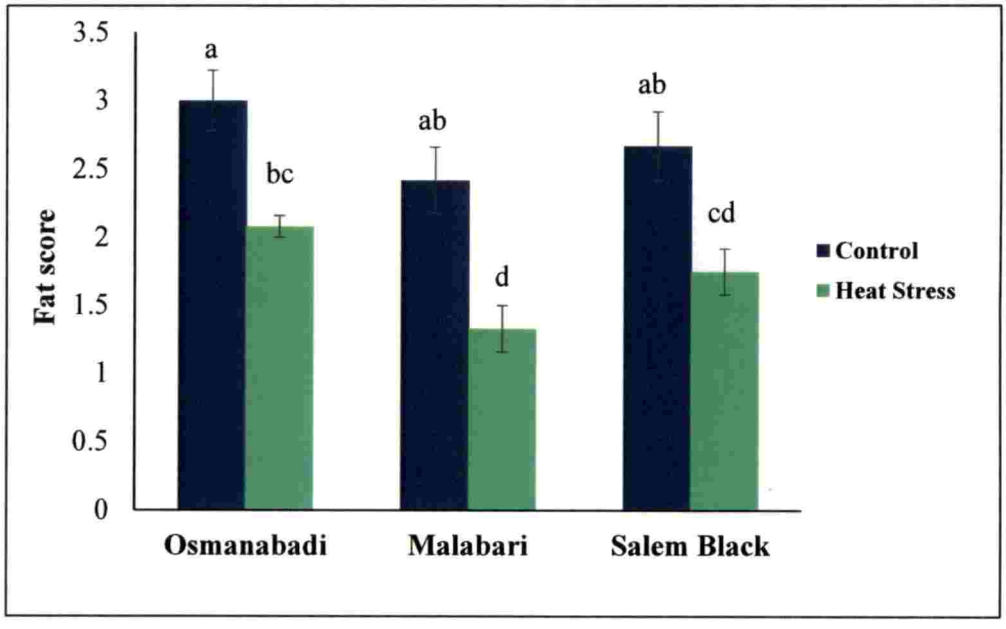

Fig. 10: Fat score

Plate 13: Effect of heat stress on the fat score of Malabari goats

4.2.7 Primal cuts

The primal cut yields of all three breed goats are presented in table 6. Among the control groups, the significantly lower fore saddle was recorded in Malabari breed. On comparison between the control and heat stress goats, significant ($P<0.01$) variations were observed on the fore saddle in the Salem black breed. On comparative basis among the control groups, the hind saddle was found to be significantly lower ($P<0.01$) in Malabari breed. However, the control and heat stress goats in each breed showed no significant variation between them. Neck and shoulder cuts showed similar pattern within the control group animals, with Salem black and Osmanabadi goats showing significantly higher ($P<0.01$) values compared to Malabari breed. Rack cut of the control group animals from Osmanabadi breed goats showed highly significant value ($P<0.01$) on comparison with rest all groups. Among all the groups, loin showed the highest value in the control and heat stress groups of Osmanabadi breed with high significance ($P<0.01$). However, none of the group showed significant variation for loin cut between the control and heat stress goats. The control group animals of the Osmanabadi breed showed the significantly higher ($P<0.01$) value for flank when compared with the control and heat stress goats of the other two breeds. However no significant variation was observed for flank cut between the control and heat stressed animals within the same breed. Shank was recorded to have significantly lower ($P< 0.01$) value in the Malabari breed goats among all the control groups. Significantly lower ($P<0.01$) shank was observed in the heat stressed group of Osmanabadi breed when compared to the control goats. Both the Osmanabadi and Salem black breeds showed to have significantly higher values for leg cut among the control group goats. Further, between the control and heat stress goats, significant ($P<0.01$) variation in leg cut was observed only in the Salem black breeds. The heat stress animals of the Malabari goats showed significantly lower ($P<0.01$) values for leg compared to the heat stressed animals of the Salem black breed. In breast cut, significantly lower ($P<0.01$) values were observed in the heat stressed animals of both Osmanabadi and Salem black breeds when compared with the control animals in the respective breeds. In addition, a

strong negative correlation (P<0.01) was also established between THI and both fore-saddle and breast. Furthermore, a mild negative correlation (P<0.05) was established between hind saddle and rack (table 7).

4.2.8 Linear carcass measurements

The effect of genotype and heat stress on the non-carcass components and offals are presented in table 8. Heat stress had no significant effect on majority of the carcass measurements recorded. However, external carcass length varied significantly (P<0.01) varied between the control and heat stress animals in the Salem black breed. However, no significant result was observed in the other two breeds for all the carcass dimensions recorded. Internal carcass length recorded was observed to have significantly higher (P<0.05) values in control group animals of both Osmanabadi and Salem black breeds compared to the Malabari breed. Due to heat stress, no significant effect was observed in internal carcass length between the control and heat stress animals in any of the breed. The leg length in Salem Black control group was found to be significantly (P<0.01) higher than Osmanabadi heat stress and both control and heat stress groups of Malabari breed. Carcass measurements such as buttock circumference, buttock width, chest circumference, shoulder circumference, and chest width showed no significant variations between the groups. Further among the linear carcass dimensions, mild negative correlation (P<0.05) was only established between THI and chest depth (table 9).

Table 6: Effect of genotype and heat stress on the primal cuts in Osmanabadi, Malabari and Salem black goats

	Osmanabadi Breed		Malabari breed		Salem Black Breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Fore saddle (kg)	4.30±0.47^{ab}	3.73±0.20^{bc}	3.42±0.12^{cd}	2.85±0.22^{d}	4.72±0.29^{a}	3.65±0.14^{bcd}	**	**	**
Hind saddle (kg)	3.75±0.40^{a}	3.00±0.14^{ab}	2.57±0.18^{b}	2.21±0.17^{b}	3.67±0.35^{a}	3.00±0.22^{ab}	**	*	**
Neck (kg)	0.52±0.03^{a}	0.44±0.04^{ab}	0.41±0.03^{b}	0.42±0.02^{b}	0.52±0.03^{a}	0.46±0.02^{ab}	*	NS	*
Shoulder (kg)	1.87±0.12^{a}	1.55±0.11^{ab}	1.29±0.09^{b}	1.24±0.11^{b}	1.84±0.11^{a}	1.75±0.10^{a}	**	NS	**
Rack (kg)	1.00±0.10^{a}	0.77±0.07^{b}	0.63±0.06^{b}	0.59±0.06^{b}	0.77±0.06^{b}	0.63±0.04^{b}	**	*	**
Loin (kg)	0.54±0.04^{a}	0.54±0.01^{a}	0.34±0.03^{cd}	0.29±0.02^{d}	0.46±0.05^{b}	0.43±0.02^{bc}	**	NS	**
Flank (kg)	0.28±0.03^{a}	0.23±0.02^{ab}	0.16±0.01^{b}	0.17±0.03^{b}	0.18±0.01^{b}	0.17±0.02^{b}	**	NS	**
Shank (kg)	0.50±0.04^{a}	0.38±0.04^{bc}	0.34±0.03^{c}	0.36±0.02^{c}	0.48±0.04^{ab}	0.43±0.04^{abc}	*	NS	**
Leg (kg)	2.49±0.23^{ab}	2.25±0.10^{bcd}	1.93±0.13^{cd}	1.80±0.13^{d}	2.88±0.10^{a}	2.33±0.13^{bc}	**	NS	**
Breast (kg)	0.27±0.03^{ab}	0.19±0.03^{c}	0.20±0.02^{bc}	0.18±0.02^{c}	0.33±0.04^{a}	0.22±0.02^{bc}	*	**	**

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; Trt- Treatment.
**P < 0.01; *P < 0.05; NS-Non-Significant

Table 7. Correlation association between THI and primal cuts

	THI	Fore saddle	Hind saddle	Neck	Shoulder	Rack	Loin	Flank	Shank	Leg	Breast
THI	1										
Fore saddle	.434**	1									
Hind saddle	.367*	.739**	1								
Neck	.289	.519**	-.803**	1							
Shoulder	-.224	.782**	.798**	.708**	1						
Rack	-.339*	.602**	.741**	.658**	.690**	1					
Loin	-.120	.560**	.759**	.605**	.659**	.652**	1				
Flank	-.152	.422*	.507**	.552**	.546**	.712**	.625**	1			
Shank	-.253	.613**	.815**	.762**	.761**	.675**	.561**	.492**	1		
Leg	-.299	.747**	.926**	.765**	.771**	.601**	.650**	.341*	.740**	1	
Breast	-.449**	.533**	.772**	.794**	.584**	.508**	.447**	.261	.764**	.775**	1

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; Trt- Treatment.

**P < 0.01; *P < 0.05; NS-Non-Significant

Table 8: Effect of genotype and heat stress on the linear carcass measurements in Osmanabadi, Malabari and Salem black goats

Traits	Osmanabadi Breed		Malabari breed		Salem Black Breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
ECL (cm)	61.33±1.23^{bc}	60.83±1.11^{bc}	58.17±1.38^{bc}	57.83±0.47^{c}	61.17±2.09^{a}	62.00±0.89^{b}	**	NS	**
BC (cm)	28.50±0.99^{a}	29.00±0.58^{a}	26.33±0.72^{a}	26.67±1.28^{a}	30.17±1.89^{a}	29.00±1.29^{a}	*	NS	NS
BW (cm)	10.33±0.42^{a}	10.33±0.33^{a}	10.00±0.58^{a}	10.00±0.52^{a}	11.33±0.42^{a}	10.17±0.54^{a}	NS	NS	NS
ICL (cm)	46.50±1.33^{a}	43.83±0.48^{ab}	42.67±1.17^{b}	42.50±0.95^{b}	47.00±1.10^{a}	44.17±1.64^{ab}	*	NS	*
CC (cm)	47.83±1.52^{a}	47.17±1.64^{a}	46.83±0.87^{a}	45.00±1.32^{a}	47.17±1.28^{a}	46.33±1.91^{a}	NS	NS	NS
CD (cm)	17.5±0.56^{ab}	16.83±0.31^{b}	17.50±0.34^{ab}	16.83±0.31^{b}	18.50±0.56^{a}	17.50±0.56^{ab}	NS	*	NS
SC (cm)	30.33±0.67^{a}	30.50±1.12^{a}	29.50±1.34^{a}	28.33±1.05^{a}	30.00±0.73^{a}	30.33±1.41^{a}	NS	NS	NS
CW (cm)	7.00±0.26^{a}	7.00±0.26^{a}	7.33±0.21^{a}	7.17±0.17^{a}	7.67±0.42^{a}	7.50±0.22^{a}	NS	NS	NS
LL (cm)	36.50±0.67^{ab}	33.50±0.72^{bc}	34.00±0.82^{bc}	31.83±1.30^{c}	38.33±1.26^{a}	36.67±1.45^{ab}	**	*	**

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; Trt- Treatment

**P < 0.01; *P < 0.05; NS-Non-Significant

Table 9. Correlation association between THI and linear carcass dimensions

	THI	ECL	BC	BW	CC	CW	CD	SC	ICL	LL
THI	1									
ECL	-.237	1								
BC	-.018	.499**	1							
BW	-.168	.233	.329*	1						
CC	-.164	.275	.062	.189	1					
CW	-.085	.423*	.287	.051	.313	1				
CD	-.350*	.481**	.429*	.280	.432**	.280	1			
SC	-.044	.179	.176	.275	.344*	.313	.415*	1		
ICL	-.301	.198	.295	.468**	.051	.293	.376*	.221	1	
LL	-.127	.490**	.373*	.170	.112	.099	.348*	.203	.323	1

THI- Temperature Humidity Index, ECL-External Carcass Length, BC- Buttock Circumference, BW- Buttock width, CC- Chest Circumference, CW- Chest Width, CD- Chest Depth, SC- Shoulder Circumference, ICL- Internal Carcass Length, LL- Leg Length

**P<0.01; *P<0.05

4.2.9 Non-carcass components and offals

Table 10 presents the non-carcass components of the three goat genotypes. The head weight was found to be significantly high for the control group animals of the Salem black breed when compared with the control and heat stressed animals of the other two breeds. However, no significant variation was observed for head weight between the control and heat stressed animals in all three breeds. Further, both Osmanabadi and Salem black breeds showed significantly higher values ($P<0.01$) for feet weight compared to the Malabari breed. However, feet weight did not differ between control and heat stress groups within each breed. Both the control and heat stress groups of Osmanabadi breed showed significantly higher ($P<0.01$) values for skin weight compared to the Malabari breed. There was no significant difference recorded between the control and heat stressed animals for skin weight in all the three breeds. The effects of heat stress were significant ($P<0.05$) on some of the offals such as lung with trachea, liver and GIT. Lung with trachea was observed to have significantly ($P<0.05$) higher value in the heat stressed group of the Salem black breed compared to the control animals. However, in the same breed liver showed contrasting result to this, with control group goats showing significantly higher ($P<0.05$) value than the heat stressed animals. Control group animals of the Salem black goats showed the highest values for liver when compared with the heat stressed animals of all the breeds. Spleen showed significantly lower ($P<0.05$) values in the heat stressed groups of both Malabari and Salem black breeds, when compared with the Osmanabadi heat stress group. However, no significant effect was observed on spleen between the control and heat stress groups of all three breeds or within the control group animals. Further, in Osmanabadi goats, GIT weight showed significantly higher ($P<0.05$) value in the heat stress group compared to the control group animals. Among all the groups, heat stressed goats of Osmanabadi breed showed the highest value for GIT. Further, among the stress groups, blood weight showed significantly ($P<0.05$) higher value in Osmanabadi breed as compared to the other two breed. However, blood weight did not differ between the control and heat stress group of all the three breeds. In addition, a strong negative correlation ($P<0.01$)

was also established between THI and liver weight. Furthermore, a mild negative correlation ($P<0.05$) was established between head and heart weight (table 11).

4.3 Physico-chemical attributes

The pH of *longissimus dorsi* muscle samples taken from the three breeds of goats, post slaughter, 45 min post-mortem and 1h post-mortem are presented in table 12. There was significant $P<0.01$) variation of muscle. There was significant $P<0.01$) variation of muscle pH_{45min} between the control and heat stress groups only in the Osmanabadi goats. The ultimate muscle pH_{24} was significantly higher in the heat stressed animals of Malabari breed compared to the heat stressed animals of other two breeds. The ultimate pH_{24} of muscles from the control and heat stress groups of both Osmanabadi and Malabari breed goats had significant ($P<0.01$) variation between them. The heat stress groups in both the breeds showed significantly higher ($P<0.01$) value for mean ultimate pH compared to control group animals. However, no significant difference on muscle pH_{24} was observed between the control and heat stressed animals from the Salem black goats due to heat stress. Water holding capacity had increased significantly ($P<0.05$) only in the Malabari breed goats during heat stress. Cooking loss showed no significant variation between any of the groups. Shear force significantly ($P<0.01$) increased in the heat stress group of all three breeds. Color values (L*, a* and b*) of LD muscle obtained from the three indigenous breed goats are presented in table 12. It was observed that there was significant influence of breed ($P<0.01$) and interaction of breed and treatment ($P<0.05$) on the lightness (L*) values of meat. However, no significant variations were recorded in the redness (a*) and yellowness (b*) values of the meat in all breeds. In addition, a strong positive correlation ($P<0.01$) was also established between THI and pH_{24} and shear force (table 13).

Table 10: Effect of genotype and heat stress on the non-carcass components and offals in Osmanabadi, Malabari and Salem black goats

Traits	Osmanabadi Breed		Malabari Breed		Salem Black Breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Head (kg)	1.13±0.05^{b}	1.08±0.03^{b}	1.13±0.05^{b}	0.93±0.18^{b}	1.44±0.10^{a}	1.18±0.03^{ab}	*	*	**
Feet (kg)	0.57±0.02^{a}	0.57±0.02^{a}	0.44±0.01^{b}	0.46±0.02^{b}	0.55±0.02^{a}	0.61±0.03^{a}	**	NS	**
Skin (kg)	1.37±0.09^{a}	1.36±0.06^{a}	1.01±0.07^{b}	1.06±0.08^{b}	1.21±0.07^{ab}	1.43±0.08^{a}	**	NS	**
Offals									
Lung with trachea (kg)	0.39±0.02^{ab}	0.42±0.02^{a}	0.32±0.32^{b}	0.35±0.32^{ab}	0.33±0.03^{b}	0.42±0.02^{a}	NS	*	*
Heart (kg)	0.08±0.01^{a}	0.07±0.00^{ab}	0.06±0.00^{ab}	0.06±.00^{b}	0.07±0.01^{ab}	0.07±0.01^{ab}	NS	NS	NS
Liver (kg)	0.31±0.02^{ab}	0.26±0.01^{bc}	0.27±0.01^{abc}	0.24±0.02^{c}	0.32±0.02^{a}	0.25±0.02^{c}	NS	**	*
Kidney (kg)	0.06±0.00^{ab}	0.06±0.00^{ab}	0.05±0.01^{ab}	0.05±0.00^{b}	0.07±0.00^{a}	0.06±0.00^{ab}	*	NS	NS
Spleen (kg)	0.04±0.00^{a}	0.04±0.01^{a}	0.03++±0.00^{ab}	0.02±0.00^{b}	0.03±0.00^{ab}	0.02±0.00^{b}	*	NS	*
GIT (kg)	1.97±0.21^{b}	2.63±0.33^{a}	1.74±0.04^{b}	1.79±0.12^{b}	1.82±0.17^{b}	2.06±0.15^{b}	*	NS	*
Blood (kg)	0.38±0.05^{ab}	0.43±0.03^{a}	0.33±0.42^{b}	0.30±0.38^{b}	0.30±0.03^{b}	0.29±0.03^{b}	**	NS	*

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; GIT- Gastro-intestinal tract; Trt- Treatment **P < 0.01; *P < 0.05; NS-Non-Significant

Table 11. Correlation association between THI and non-carcass components and offals

	THI	Head	Feet	Skin	LwT	Heart	Liver	Kidney	Spleen	GIT	Blood
THI	1										
Head	-.331*	1									
Feet	.140	.363*	1								
Skin	.191	.299	.774**	1							
LwT	.359	.263	.530**	.827**	1						
Heart	-.236*	.316	.561**	.503**	.403*	1					
Liver	-.485**	.375*	.353*	.331*	.262	.582**	1				
Kidney	-.198	.522**	.291	.353*	.400*	.365*	.466**	1			
Spleen	-.202	.262	.275	.499**	.475**	.420*	.540**	.534**	1		
GIT	.306	.144	.427**	.542**	.643**	.323	.326	.274	.501**	1	
Blood	.007	.180	.337*	.415*	.570**	.270	.420*	.3	.502**	.705**	1

THI- Temperature Humidity Index, LwT-Lung with Trachea; GIT-Gastro-intestinal tract

**P<0.01; *P<0.05

Table 12. Effect of genotype and heat stress on the physico-chemical attributes in Osmanabadi, Malabari and Salem black goats

Traits	Osmanabadi Breed		Malabari Breed		Salem Black Breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Initial pH	6.81±0.03 [a]	6.75±0.05[a]	6.81±0.03 [a]	6.79±0.03 [a]	6.83±0.04 [a]	6.80±0.02[a]	NS	NS	NS
pH_{45}	6.57±0.04[a]	6.38±0.04[b]	6.41±0.06[ab]	6.44±0.03[ab]	6.48±0.08[ab]	6.43±0.06[ab]	NS	NS	NS
pH_{24}	5.74±0.07[c]	6.03±0.07[b]	5.88±0.07[bc]	6.44±0.03[a]	5.85±0.10[bc]	5.93±0.09[bc]	*	**	**
WHC (%)	63.21±8.10[a]	63.65±2.85[a]	42.27±3.49[b]	66.58±5.05[a]	57.51±2.14[ab]	58.62±7.75[ab]	NS	NS	*
CL (%)	33.95±3.02[ab]	31.47±2.04[ab]	34.46±3.09[ab]	26.05±3.82[b]	25.01±5.12[b]	39.72±4.26[a]	NS	NS	NS
Shear force (kg/cm^2)	5.15±0.05[d]	8.42±0.35[b]	5.47±0.04[cd]	8.92±0.20[a]	5.74±0.05[c]	8.43±0.12 [b]	NS	**	**
L*	43.52±3.37[b]	41.74±1.99[b]	45.31±2.46[b]	53.36±3.87[a]	42.73±1.23[b]	40.11±1.62[b]	**	NS	*
a*	14.79±1.79 [a]	15.58±1.14 [a]	14.38±1.25[a]	9.89±2.58[b]	13.59±0.44[ab]	15.21±0.32[a]	NS	NS	NS
b*	14.02±1.43 [a]	15.79±1.06 [a]	16.31±0.54[a]	13.07±1.46[a]	14.43±0.57[a]	15.23±0.63[a]	NS	NS	NS

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; WHC- Water holding capacity; CL- Cooking loss; Trt- Treatment; L* Lightness; a* Redness; b* Yellowness. **P < 0.01; *P < 0.05; NS-Non-Significant

Table 13. Correlation association between THI and physico-chemical attributes

	THI	Initial pH	pH_{45}	pH_{24}	WHC	CL	Shear force	L*	a*	b*
THI	1									
Initial pH	-.083	1								
pH_{45}	-.254	.438**	1							
pH_{24}	.556**	-.104	-.128	1						
WHC	.298	-.170	.191	.114	1					
CL	.068	-.008	-.096	-.097	.044	1				
Shear force	.965**	-.056	-.247	.581**	.317	-.003	1			
L*	.084	.003	-.110	.310	-.109	.252	.151	1		
a*	-.092	-.053	.066	-.267	.079	.346*	-.226	-.742**	1	
b*	-.044	-.078	-.137	-.134	.043	.281	-.145	-.354*	.730**	1

THI- Temperature Humidity Index, WHC-Water holding capacity, CL-Cooking loss, L*-Lightness, a*-redness, b*-yellowness

**P<0.01; *P<0.05

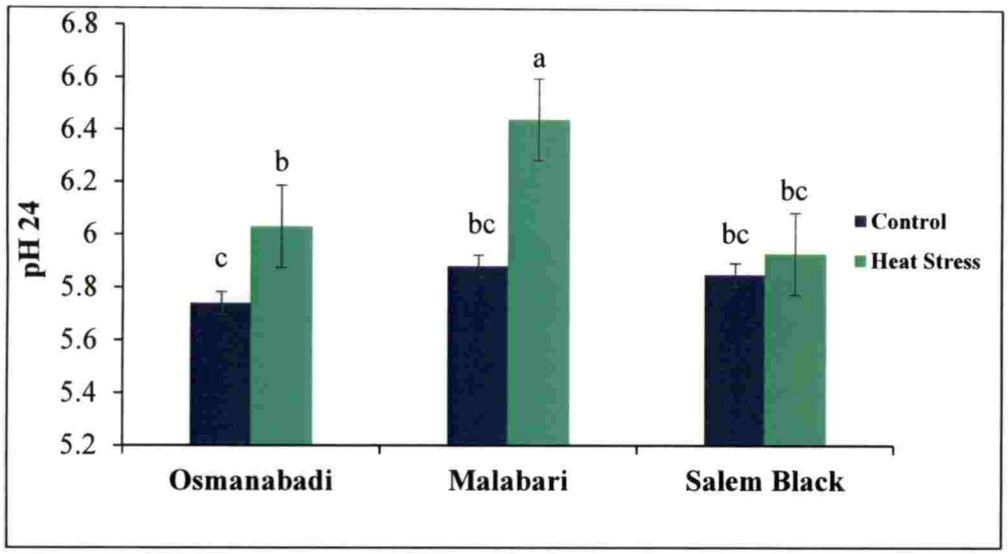

Fig.11: pH_{24}

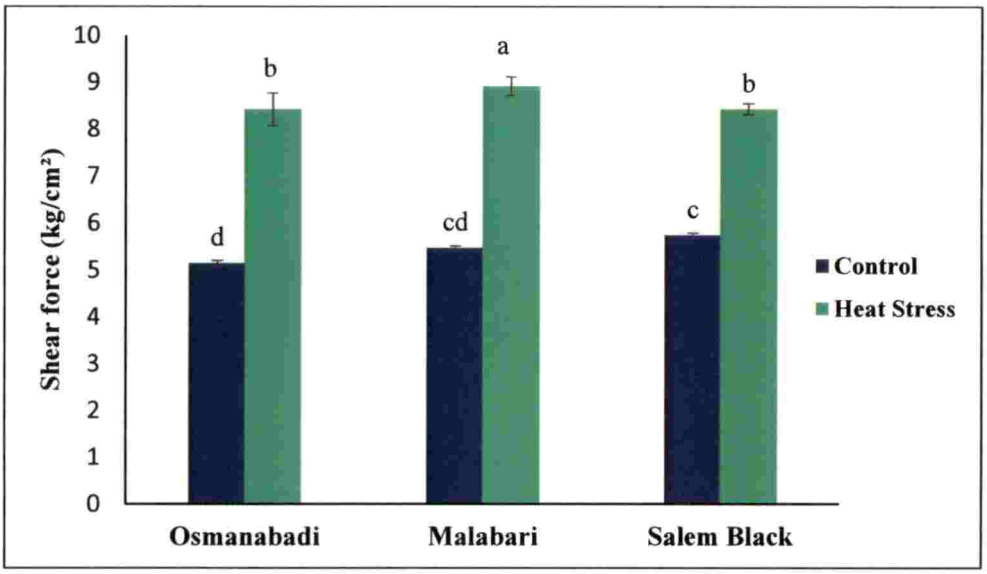

Fig.12: Shear force

4.4 Proximate composition

The effect of genotype and heat stress on the proximate composition of three breeds of goats are presented in table 14. Breed significantly influenced the protein ($P<0.05$) and fat ($P<0.01$) content in the goats. However, treatment did not influence proximate meat composition of the goats. Interaction significantly ($P<0.05$) influenced the fat content in the goats. Further, there was no any correlation established between THI and proximate composition parameters (table 15).

4.5 Organoleptic quality of the three breeds of goat

The effect of genotype and heat stress on the organoleptic attributes of three breeds of goats are presented in table 16. In appearance, Salem black goat meat was significantly ($P<0.01$) better than the Osmanabadi goat meat in the control group. However, there was no significant difference in appearance between the meat of Salem black and Malabari breed in control groups. Among the heat stress groups, Salem black goats showed significantly higher ($P<0.01$) score for appearance compared with other two breeds. Between the control and heat stress groups, there was significant ($P<0.01$) difference in appearance in both Osmanabadi and Malabari breeds. But there was no effect of heat stress on the Salem black goats for the appearance trait. Heat stress had significant effect ($P<0.01$) on the flavour of the Malabari and Salem black goat meat and this is evident from the significant differences in flavour between control and heat stress group of these two breeds. Among all groups, meat from Salem black control goats was significantly ($P<0.01$) superior in terms of flavour. The texture in Osmanabadi breed differed between the control and heat stress group with significantly ($P<0.01$) lower texture value recorded in control group. Further, the texture in Osmanabadi control group differed significantly ($P<0.01$) from the rest of the groups. Lastly, in the overall acceptability score, meat of Salem black goat was rated to be the most superior with highly significant ($P<0.01$) score when compared with all groups. Further, the differences between control and heat stress group for overall acceptability was significantly ($P<0.01$) different only in Salem Black breed. In addition, both strong and mild

Table 14. Effect of genotype and heat stress on the proximate composition in Osmanabadi, Malabari and Salem black goats

Trait	Osmanabadi breed		Malabari breed		Salem Black breed		Effects		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Protein	24.35±2.01[a]	27.27±1.07[ab]	23.06±1.23[b]	23.83±1.37[ab]	22.05±0.70[b]	22.60±1.5[b]	*	NS	NS
Ether	5.16±1.10[a]	6.47±1.44[a]	3.77±0.11[b]	5.08±0.76[ab]	3.42±0.55[ab]	2.25±0.36[b]	**	NS	*
Ash	4.79±0.31[a]	4.93±0.24[ab]	5.18±0.31[a]	5.64±0.30[ab]	4.72±0.11[ab]	5.19±0.24[b]	NS	NS	NS

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem black Control; SBHS- Salem black Heat stress; Trt- Treatment

**P < 0.01; *P < 0.05; NS-Non-Significant

Table 15. Correlation table between THI and proximate composition

	THI	Protein	Ether	Ash
THI	1			
Protein	-.102	1		
Ether	.104	.025	1	
Ash	.272	-.046	.164	1

THI- Temperature Humidity Index

Table 16: Effect of genotype and heat stress on the organoleptic attributes of goat meat in Osmanabadi, Malabari and Salem black goats

Traits	Osmanabadi Breed		Malabari Breed		Salem Black Breed		Effect		
	OSC	OSHS	MC	MHS	SBC	SBHS	Breed	Trt	Breed*Trt
Appearance	4.33±0.21^{b}	3.33±0.21^{c}	4.83±0.40^{ab}	3.33±0.21^{c}	5.50±0.34^{a}	4.83±0.48^{ab}	**	**	**
Flavor	4.33±0.21^{bc}	4.67±0.21^{b}	5.00±0.37^{b}	3.83±0.17^{c}	5.50±0.34^{a}	4.33±0.21^{bc}	NS	**	**
Juiciness	4.00±0.26^{a}	5.17±0.60^{a}	4.83±0.17^{a}	4.33±0.21^{a}	5.17±0.31^{a}	4.83±0.54^{a}	NS	NS	NS
Texture	4.17±0.40^{a}	4.33±0.21^{b}	4.67±0.21^{b}	4.00±0.26^{b}	5.50±0.34^{b}	4.50±0.22^{b}	*	NS	**
Overall acceptability	4.17±0.17^{bc}	4.17±0.17^{bc}	4.33±0.21^{bc}	4.00±0.00^{c}	5.33±0.33^{a}	4.67±0.21^{b}	**	NS	**

OSC- Osmanabadi Control; OSHS- Osmanabadi Heat stress; MC-Malabari Control; MHS- Malabari Heat Stress; SBC- Salem Black Control; SBHS- Salem BBlack Heat stress; Trt- Treatment

**P < 0.01; *P < 0.05; NS-Non-Significant

Table 17. Correlation table between THI and organoleptic attributes

	THI	Appearance	Flavour	Juiciness	Texture	Overall Acceptability
THI	1					
Appearance	-.487**	1				
Flavour	-.421*	.682**	1			
Juiciness	.058	.283	.369*	1		
Texture	-.313	.582**	.588**	.372*	1	
Overall Acceptability	-.259	.726**	.612**	.470**	.733**	1

THI- Temperature Humidity Index

**P<0.01; *P<0.05

correlation were established between THI and appearance ($P<0.01$) and flavour ($P<0.05$) respectively (table 17).

4.6 Plasma leptin concentration

The effect of heat stress on the plasma leptin levels is presented in Fig. 18. Heat stress treatment significantly ($P<0.01$) influenced plasma leptin levels in both Malabari and Salem Black breeds. In both these breeds, the leptin level was significantly ($P<0.01$) higher in heat stress group as compared to their respective control group. However, plasma leptin level did not differ between the conrol groups of all three breeds. But the plasma leptin level differed ($P<0.01$) between the OHS and MHS groups. Further, both experimental days, breed and interaction between breed, treatment and experimental days did not influence the plasma leptin level. In addition, a strong positive correlation ($P<0.01$) was established between THI and plasma leptin concentration in the study with $R^2 = .376$.

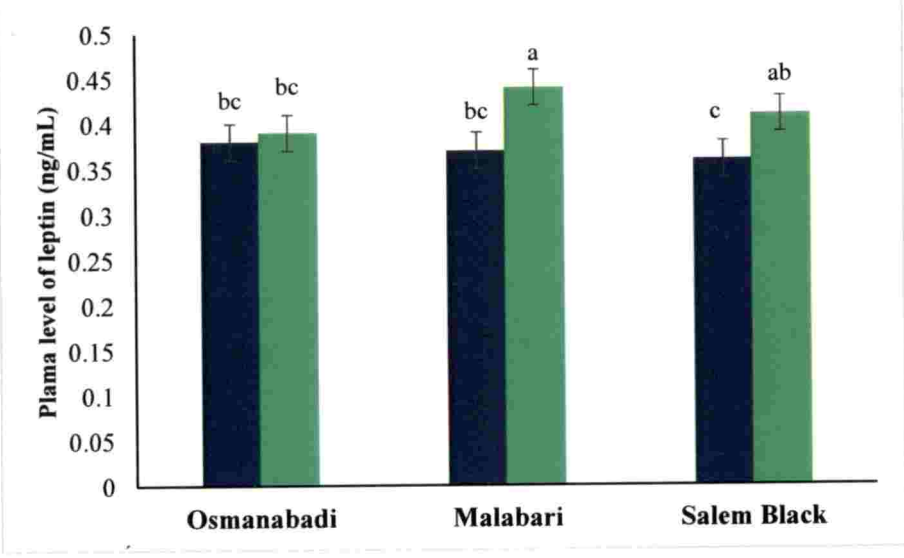

Fig. 13: Plasma leptin concentration

4.7 Gene Expression

4.7.1 Myostatin gene mRNA Expression in muscle

The effect of heat stress on the skeletal muscle myostatin gene expression is presented in Fig. 14. The expression of myostatin gene in the skeletal muscle of the goats showed significant ($P<0.05$) breed effect. Further, heat stress treatment also had significant effect on both Osmanabadi and Malabari breed. The heat stressed goats of both these breeds showed reduced mRNA abundance of myostatin gene as compared to their respective control goats. However, no significant variations in mRNA abundance of this gene were observed between the control and heat stress group of Salem black goats.

4.7.2 HSP70 mRNA Expression in muscle

The effect of heat stress on the skeletal muscle HSP70 gene expression is presented in Fig. 15. Significantly ($P<0.05$) higher mRNA abundance of HSP70 gene was observed in all the heat stress groups compared to their respective control group goats. Further, breed effect was also evident on the gene expression. Osmanabadi breed showed the maximum ($P<0.05$) up regulation of HSP70gene, followed by Malabari and Salem black breed.

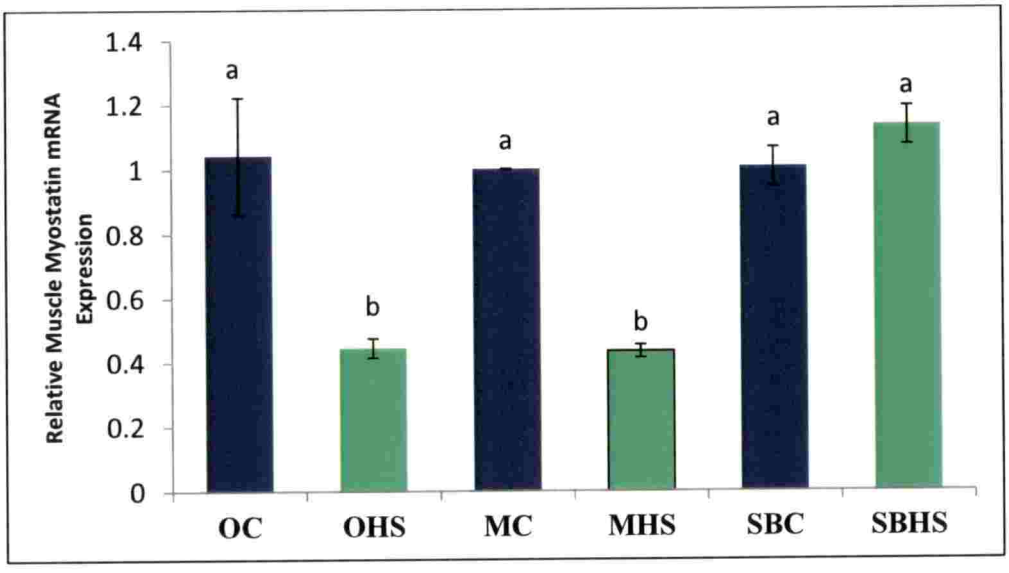

Fig. 14: Skeletal muscle myostatin mRNA transcript expression between the control and heat stress groups of three indigenous breeds of goats

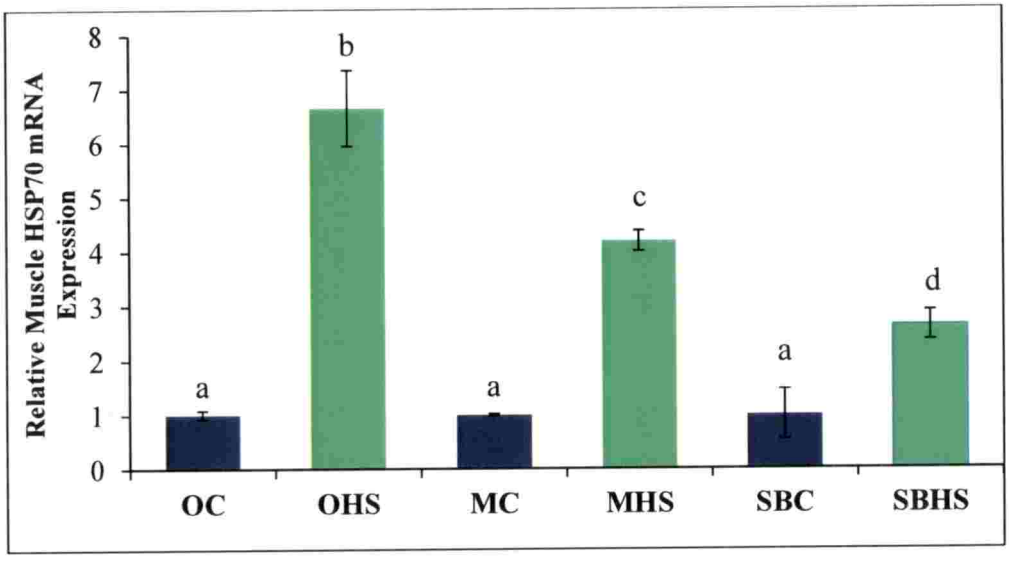

Fig. 15: Skeletal muscle HSP70 mRNA transcript expression between the control and heat stress groups of three indigenous breeds of goats

DISCUSSION

CHAPTER 5

DISCUSSION

5.1 Body weight and carcass characteristics

The body weight of the animal is one of the crucial factors determining the meat output. The significant influence of heat stress on the live body weights of all the three indigenous breeds in the current study indicates that heat stress had discernible effect on these breeds. During heat stress condition, all the energy reserves are depleted for maintaining the homeothermy of the animals through various thermoregulatory activities and thereby helping the animal to thrive in the stressed condition. However, during the process of energy partitioning, the energy deviated for production aspects appear negligible (Shilja *et al.*, 2016). This explains clearly the reason for the reduction of live body weights in the animals during heat stress condition. There are several reports published on the reduced body weight during heat stress condition (Sejian *et al.*, 2016; Okoruwa, 2014). In addition, the reason for decreased live weights of heat stressed goats could be directly attributed to the reduced feeding behaviour in goats. Reduced feed intake is a primary behavioural response observed in ruminants during heat stress condition as an adaptive mechanism to reduce the metabolic heat load (Okoruwa, 2014). In such situations of reduced feed consumption, the animal experience negative energy balance (NEB), which consequently hampers the growth performance and result in body weight decline. The reduced live body weight in heat stressed goats in the current study coincided with the findings of Hamzaoui *et al.* (2012) in dairy goats. Further, the intensified magnitude of heat stress in Malabari breed indicate the increased susceptibility of this breed to heat stress compared to Osmanabadi and Salem black goats. In addition, a strong negative correlation between THI and live weight establishes the severity of heat stress on this important carcass trait in the study.

5.1.1 Pre-slaughter weight

The depletion of energy reserves during heat stress condition in goats was also evident from the reduced pre-slaughter weights of the heat stressed goats in all three breeds as compared to their respective control group animals. Kadim *et al.* (2006) also reported similar finding of reduced PSW in different breed goats subjected to heat stress. These authors had reported that the ante-mortem stressors are often associated with reduced live weight, pre-slaughter weight and carcass weight. The reduced PSW during heat stress condition could be attributed to the higher energy demands of these animals for thermoregulatory activities which ultimately culminate in depletion of muscle glycogen, intramuscular fat thereby leading to weight loss. In addition, the increased moisture losses from the body through sweating and panting could also pave way for the weight losses (Kadim *et al.*, 2006). However, there are contrary reports showing non-significant influence of heat stress on both body and carcass weight in goats (Hashem *et al.*, 2013) as well as in sheep (Rana *et al.*, 2014; Liu *et al.*, 2012). Further, the intensified impact of heat stress on the PSW of the Malabari goats as compared to the other two breeds in the current study indicate the increased susceptibility of this breed to heat stress. In addition, a strong negative correlation between THI and PSW establishes the severity of heat stress on this important carcass trait in the study.

5.1.2 Hot carcass weight

The effect of heat stress was clearly evident on the HCW of only the SB breed. The significantly lower HCW of goats as compared to their control groups could be attributed to the translation of the heat stress induced live weight losses to the carcass weight losses (Kadim *et al.*, 2006; Blaine and Nsahlai, 2011). Similar result of diminished HCW has been also established in pigs (Cruzen *et al.*, 2015), heifers (Mitlöhner *et al.*, 2001), broilers (Mello *et al.*, 2015) and rabbits (Zeferino *et al.*, 2013). Contrary findings stating non-significant influence of heat stress on carcass weights are also reported in sheep (Liu *et al.*, 2012) and goats (Hashem *et*

al., 2013). In addition, a strong negative correlation between THI and HCW establishes the severity of heat stress on this important carcass trait in the study.

5.1.3 Dressing percentage

Dressing percentage is a potential indicator of both yield and value of the carcass which can serve as an ideal tool for assessing the potential and profitability of meat animals (Assan, 2015). In most species the fat storage usually mislead the researchers in assessing the carcass quality and the animals with fatter carcasses are often over estimated as having better carcass quality. However, in goats most of the fat depots are accumulated internally and is dropped along with the internal organs and GIT during slaughter and as a result dressing % may serve as a better indicator for assessing carcass quality in goat as compared to other species (Assan, 2015).

The non-significant variations in dressing percentage between the control and heat stressed goats implies that heat stress had no significant influence on the dressing percentage of the indigenous goats. This could be directly attributed to the inherent capacity of the native tract breed goats to survive the stress condition without compromising the carcass yield. However, findings from the current study revealed the influence of genotype on DP. There are several studies pertaining to the influence of goat genotype on the DP (Alexandre *et al.*, 2010; Assan, 2015). This could be directly associated to the differences in their body size as well as body weight between the breeds. The Salem black breed goats were the tallest amongst the three breeds studied and this could be seen as the reason for these goats to have the highest DP. Hence it could be elucidated that Salem black breeds have better carcass proportion among all breeds. The non-significant variation of DP between the Osmanabadi and Malabari breed goats despite the breeds having significant difference in their live weights. This finding was in line with the report of Mahgoub and Lu (1998), wherein it was reported that small sized breeds have better carcass muscularity than large body sized ones. This could be the reason for the comparable dressing percentage among these two breeds although the Malabari goats were much smaller sized than Osmanabadi goats. In addition, a strong negative

correlation between THI and DP establishes the severity of heat stress on this important carcass trait in the study.

5.1.4 Loin eye area

The significantly higher loin eye area in the Osmanabadi breed goats could be attributed to the higher muscular development as well as the pre-slaughter weight of these goats compared to the other two breeds. Similar reason was suggested by Sen *et al.* (2004) in sheep and goats. Further, the significant effect of heat stress evident on the loin eye area of all three breeds during heat stress could be indicative of the severity of the heat stress and could be associated with the body weight losses induced by the stress. In addition, a strong negative correlation between THI and LEA establishes the severity of heat stress on this important carcass trait in the study.

5.1.5 Separable fat

The body fat differed only between Osmanabadi control and heat stress group. Further, rest all the groups possessed significantly lower body separable fat than Osmanabadi control group. This shows there are breed differences for internal fat deposition in the current study. Similar hypothesis were also established by other researchers indicating fat deposition in hyperthermic animals depends on the breed (Lu *et al.*, 2007; Slimen *et al.*, 2015). The significantly lower separable fat in Osmanabadi heat stress group as compared to its control group could be attributed to the depletion of fat storage in heat stressed animals in an effort to supply sufficient energy to favour the life sustaining activities through the gluconeogenesis pathway. The significantly lower fat score in all three breed heat stress groups than their respective control groups in the current study further supports fat storage depletion hypothesis to support body vital functions. Similar findings were reported by Cruzen *et al.* (2015) in pigs and these authors observed lesser carcass separable fat from pigs subjected to heat stress condition compared to those animals maintained in controlled environment. However it is a general finding in hyperthermic animals is that there is increased fat deposition in these heat stressed

animals (Lu *et al.*, 2007). Further, ambient temperature-induced heat stress was shown to reduce fat oxidation in different species (Pearce *et al.*, 2011). The probable reason for this difference in the fat deposition in heat stressed animals could be attributed to the breed differences. This explanation was supported by the findings of Lu *et al.* (2007) who reported lesser fat deposition in thermo-tolerant breeds. This could be the reason in the current study for lower fat deposition in all the three heat stressed breeds as these three breeds are indigenous types and well known for their thermo-tolerant ability. In addition, a strong negative correlation between THI and both separable fat and fat score establishes the severity of heat stress on these important carcass traits in the study.

5.1.6 Primal cut yield

Studies pertaining to the effect of heat stress on the individual primal cuts of goats are inadequate. However, there are several studies in this line in broiler poultry. The study clearly indicated the breed differences for almost all the primal cuts in the study. The reason for this could be the size the breeds used in the current study as usually Malabari is categorized under the medium sized while both Osmanabadi and Salem Black goats are categorized under large sized breeds. Further, heat stress has compromised the yield of different primal cuts including fore saddle in Salem black goats, shank, breast in Osmanabadi and Salem black breed and leg in Salem black breed. Few primal cut yield reduction observed in Salem black goats as compared to other two breeds implies that meat yield is jeopardized to an extent in this breed during exposure to heat stress condition. To the best of our knowledge this is the first study reporting effect of heat stress on the primal cuts in goats and therefore we were not able to compare the findings with other studies especially in ruminants. However, there are few reports in poultry reporting negative influence of heat stress on the proportion of cuts such as breast and thigh are remarkably reduced (Zhang *et al.*, 2012; Lu *et al.*, 2007). However, Mello *et al.* (2015) have reported that the cuts yield is not compromised during heat stress in broilers. The higher proportion of neck and shoulder cuts in the Salem black and Osmanabadi goats over Malabari breed could be observed as a breed

difference or could be attributed to their posture while grazing (Sen *et al.*, 2004). It could be also seen as an evolutionary adaptation in these animals for grazing the sparse vegetation, shrubs and trees in arid and semi-arid regions (Sen *et al.*, 2004). Similar suggestion was opined by Bhatta *et al.* (2001) in goats. Further, the significant influence of breed and treatment interaction on all primal cuts in the study indicated that different breeds are responding in different ways and this evident from the few more primal cuts differences between control and heat stress Salem black goats than the other two breeds. In addition, a negative correlation between THI and fore saddle, hind saddle, rack and breast establishes the severity of heat stress on these important carcass traits in the study.

5.1.7 Linear carcass measurements

External carcass length in the Salem black goats subjected to heat stress has been shown to increase significantly compared to the respective control group animals. This highlight the exemplary capability of the Salem black breed goats to resist the heat stress condition. To our knowledge, such a report on the effect of high ambient temperature on the carcass length of goats has not yet been established. However, influence of heat stress on carcass length of heat stressed pigs has been reported in a study conducted by Stahly and Cromwell (1979). In the fore-mentioned study, it was concluded that there was a positive linear effect of rising ambient temperature on the carcass length of the pigs. This was explained as an adaptive mechanism of these animals to alter the surface area of the body relative to their body mass ratio as an attempt to dissipate more body heat. Internal carcass length was found to be significantly lower in the Malabari goats and this could be attributed to the lower average body weight of this breed. Similar suggestion was made by Vargas *et al.* (2007) stating that body linear dimensions are closely associated to the body weight in goats. Further, the difference in linear measurements due to breed characteristics could be seen in the leg length of the goats. The Salem black goats had significantly higher leg length in comparison to other two breeds. Salem black goats are naturally taller than the other two breed which could be probably the reason for the longer leg in this breed and this could

be attributed to the morphological adaptive characteristics of this breed to avoid the shifting ground radiation.

5.1.8 Non-carcass components and offals

Breed differences were established for head, feet and skin. Mostly the Osmanabadi and Salem black goats had similar measurements for these parameters as compared to Malabari. This could be attributed to the breed differences and their body conformation. However, the heat stress did not influence any of these three non-carcass parameters among the breeds indicating the morphological adaptation characteristics of these indigenous breeds. Similarly Sen *et al.* (2004) established non-significant effect on non-carcass characteristics in goats and they attributed to this to the superior adaptive capacity of these animals to the hot environment. The significantly higher weight of lung with trachea in the heat stressed Salem black goat indicates the hypertrophy of this organ due to the enhanced functioning of this organ to maintain the homeothermy through various thermoregulatory mechanisms. Muscle hypertrophy or increased muscle mass is often considered as a protective response in stressed conditions against the overload work (Kapoor and Singh, 2013). Increased respiration rate or panting in ruminants is a well- established adaptive mechanism exhibited during heat stress condition in an attempt to increase the respiratory heat loss (Shaji *et al.*, 2016). Hence this could be speculated as a reason for the higher weight of this organ in heat stressed goats. Further, the increased heart rate associated with heat stress (Panda *et al.*, 2016) is linked with the increased activity of lungs in order to maintain the pulmonary circulation and thereby the homeostasis of the lung cells (Corrin, 1981). This could be speculated as yet another factor influencing the higher lung weight during heat stress. Rana *et al.* (2014) had reported similar finding in heat stressed sheep. However, Hashem *et al.* (2013) had concluded that heat stress had no significant influence on the lung weight. Liver showed opposite trend through reduced weight in heat stressed condition which could be ascribed to the reduced feed intake and increased hepatic gluconeogenesis. During hyperthermia animals exhibit vasodilation, a process by which blood is redistributed from the internal organs to skin to enhance the body

heat loss. During this mechanism, blood circulation and oxygen supply to liver cells (hepatocytes) would be restricted resulting in reduced functioning of this organ. This could be another reason behind the lesser weight of this organ (Fowler, 2013). Such variations in organ weights in response to heat stress were observed only in the Salem black breed which could be ascribed to the superior adaptive performance of this breed to thrive in the harsh conditions. However, GIT showed significantly higher values in the heat stressed Osmanabadi goats indicating the hypertrophy of this organ during heat stress. This hypertrophy of GIT in Osmanabadi goats could be the overloaded activities in an attempt to synthesize energy and absorption into blood stream for supporting life sustaining activities in this breed. Similar finding was reported in heat stressed sheep by Moniruzzaman *et al.* (2000). However, Rana *et al.* (2014) did not observe any difference for GIT weight in heat stressed sheep. However, there are also report stating the reverse trend of GIT weight during heat stress (Fowler, 2013). They attributed this reduced weight of GIT to the reduced feed intake and hampered digestive functions due to reduced blood flow to internal organs. Similar to head, feet and skin, the significant influence of breed and treatment interaction on majority of edible offals indicated that the breeds were trying to adapt to the heat stress condition. In addition, a negative correlation between THI and head, heart, liver establishes the severity of heat stress on these important carcass traits in the study.

5.1.9 Physico-chemical attributes

5.1.9.1 Meat pH

Meat pH is one of the key factors influencing the quality of the meat which governs several physico-chemical properties of meat such as water holding capacity, color, shear force and tenderness (Ma *et al.*, 2015). In the current study, ultimate meat pH_{24h} of heat stressed goats of both Malabari and Osmanabadi goats showed significantly higher values compared to their respective control group goats. Such an increase of meat pH_{24} reflects the depleted glycogen reserves in the heat stressed goats. This finding of our study corroborated with that of Kadim *et al.*

(2008), Hashem *et al.* (2013) in goats and sheep and Kadim *et al.* (2004) in beef where they had assigned the reason for heat stress induced ultimate meat pH rise to the increased glycogenolysis in skeletal muscle which decreases the lactic acid formation. Liu *et al.* (2012) and Chulayo and Muchenje (2013) also have recorded similar results of increased meat pH in sheep exposed to high ambient temperatures. When the animals are subjected to chronic heat stress, all the glycogen and its precursors in the body including muscle glycogen reserves get utilized. During continued exposure to heat stress as in our study, due to the depleted glycogen reserves, the decline in normal pH of meat that was expected during rigor mortis would be affected which ultimately leads to higher pH, thereby affecting the meat quality (Miller, 2007). However, no significant variation of meat pH was observed in the current study in Salem black goats and this could be due to the better adaptability of the breed to hot and humid climatic condition in India. Similar observation was made by Shaw and Tume (1992) that while comparing different treatment groups, the one which shows minimal alterations in their responses to heat stress would be the least stressed. This is further evident from the significantly higher meat pH in both Malabari and Osmanabadi goats which on comparative basis was found to be more affected than Salem black breed for the adversities of heat stress. Therefore, the increased pH in Malabari and Osmanabadi breeds could be attributed to their high sensitivity to heat stress than Salem black breed. However, there are several contradictory reports stating the meat pH decline during chronic heat stress condition, where they had pointed the excessive lactic acid formation in muscle fibres during anaerobic glycolysis as the reason for meat pH drop in pigs (Parkunan *et al.*, 2017; Ma *et al.*, 2015) as well as in poultry (Zhang *et al.*, 2012). In addition, a strong positive correlation between THI and pH_{24} establishes the severity of heat stress on this important carcass trait in the study.

5.1.9.2 Water holding capacity

The significantly higher water holding capacity of the heat stressed Malabari goats compared to their respective control group animals could be correlated with their higher ultimate meat pH arising from the depleted energy reserves. According to Kreikemeier *et al.* (1998), there is a positive linear effect of increased ultimate pH of meat with the WHC resulting in a sticky texture. In addition, this high WHC could be related with the Dark-Firm-Dry (DFD) condition in beef where the high meat pH makes the lean surfaces to act like a sponge, binding all the water within the muscle. Suggesting the same, Abril *et al.* (2001) had explained that the physical state of proteins would be above their isoelectric point at higher meat pH which ultimately results in more water to be attracted and tightly held within the muscle fibres. However, there are also contradictory results of reduced WHC during heat stress established in species such as goats, sheep (Kadim *et al.*, 2008) and pig (Santos *et al.*, 1994). Such variations were not observed in both Osmanabadi and Salem black goats, from which it could be deduced that these indigenous goats had better adaptability to such harsh climate than Malabari breed.

5.1.9.3 Cooking loss

The significantly higher cooking loss in Salem Black goats as compared to both Osmanabadi and Malabari goats indicates strong breed influence on cooking loss in the current study. Similar finding of breed influence on cooking loss was reported in sheep by Chulayo and Muchenje (2013). The CL, pH_{24} and shear force are generally correlated to determine the quality of the meat produced. It has been observed in South African Mutton Merino sheep that CL is more when both the pH and toughness of the meat are very high (Chulayo and Muchenje, 2013). Similarly, the increased toughness as indicated by the higher shear force in Salem Black heat stressed animals could be the reason for very high CL in this breed. However, few authors have also correlated the CL with higher tenderness and pH of the meat (Miranda-de la Lama, 2011), and this could be attributed to the fact that higher pH_{24} may not always associated with toughness of meat (Muchenje *et al.*, 2008).

5.1.9.4 Shear force

Shear force is a direct indicator of the meat tenderness and eating quality of meat (Miranda-de la Lama, 2011; Chulayo and Muchenje, 2013). Significantly higher values of shear force were observed in the heat stressed groups of all three indigenous breeds. This could be associated with the increased WHC during exposure to high ambient temperatures. However, in the current study only Malabari goats showed higher WHC than the other two breeds. The increased toughness of meat in all three breed heat stressed group could be attributed to the severe protein denaturation which ultimately affects the proteins ability to hold the water content (Deng *et al.*, 2002). Further, Miller (2007) had suggested that higher WHC of muscle makes the meat compact, firm and the surface would be dry as the water is tightly bound within the muscle. Similar results were reported by Zhang *et al.* (2012) and Gu *et al.* (2008) in broilers exposed to heat stress. In addition, calcium is a vital element responsible for meat tenderization. During stressful condition, plasma calcium is transferred to the fatty tissue which declines the plasma calcium level, which ultimately affects the calcium availability to the muscle. Therefore, low calcium availability in heat stressed animals may also be a reason for increased toughness of meat (Moseley and Axford, 1973). However in contrast, reports stating the reduced shear force in muscles of animals exposed to heat stress have also been reported (Grandin, 1996; Kreikemeier *et al.*, 1998). The significantly higher shear force of heat stressed Malabari goats indicates that this breed were the most affected during the study period. Furthermore, shear force value beyond 5.5 is often considered as tougher and less palatable (Webb *et al.*, 2005; Santos *et al.*, 2008). Therefore, the increased shear force of over 5.5 in all the three breed heat stress groups reflects the severity of the heat stress during the study period. In addition, a strong positive correlation between THI and shear force establishes the severity of heat stress on this important carcass trait in the study.

5.1.9.5 Color

Among the instrumental color, only lightness (L*) varied significantly for both breed and interaction. Meat colour is essentially affected by the pre-slaughter stressors, among which temperature stress has been identified as a critical factor (Weglarz, 2010). The post-mortem changes that take place in the meat have discernible effects on the ultimate meat color. The most noteworthy effect is the significantly higher lightness in heat stressed Malabari goats as compared to its control group animals. Although both redness and yellowness did not show any variation between the breeds and for the treatment, still the redness was significantly lower in heat stressed Malabari goats. Similar to the finding in Malabari breed in the current study, in heat stressed broilers, higher lightness (L*) and lower redness (a*) have been reported in turkeys exposed to heat stress (Mckee and Sams, 1997; Sandercock *et al.*, 2001). In a report on breast muscles of broilers, chronic heat stress has been identified to significantly increase the L* (Lu *et al.*, 2007; Feng *et al.*, 2008). Further, during acute heat stress in broilers, increased L* and decreased a* and b* have been reported (Aksit *et al.*, 2006; Zhang *et al.*, 2012). These authors had attributed such alterations to the denaturation of sarcoplasmic proteins and scattering of light. However, reduced colour (L^*, a^*, b^*) has also been reported in heat stressed sheep (Kadim *et al.*, 2008).

5.1.9.6 Proximate composition

Breed differences were observed for meat proximate parameters protein and ether. However the treatment did not influence any of the proximate parameters. Although OS and SB animals are considered similar body sized breeds, still the meat protein content was significantly higher in heat stressed Osmanabadi breed as compared to heat stressed Salem Black breed. This shows the clear breed differences on meat protein content. It is the general observation that the body protein contents are reduced when the animals are subjected to heat stress of both acute and chronic magnitude (Zhang *et al.*, 2012). The significantly lower protein content in Salem Black could be attributed to the increased hepatic gluconeogenesis

mechanisms to regularly supply energy for vital body functions. Further, Yunianto *et al.* (1997) reported significantly lower meat protein in heat stressed broiler poultry. These authors postulated that the reduced protein content could be both due to decreased protein synthesis as well as increased protein breakdown. Further, Zhang *et al.* (2012) attributed the reduced protein content in heat stressed broilers to the lower ribosomal gene transcription which ultimately culminates in reduced the rate of protein synthesis. Similar to protein for ether extract also breed difference was established in the study. Lower level of ether extract was observed in Salem black breed as compared to Osmanabadi and Malabari breeds. The similar protein, ash and ether extract between the control and heat stress groups of each breed in the current study indicates that heat stress did not influence much these variables and the nutritional composition of both the normal and heat stressed animals remained the same.

5.2 Organoleptic attributes

Breed differences were established on different organoleptic parameters such as appearance, texture and overall acceptability. Among the organoleptic parameters, the effect of heat stress was evident only on the appearance and flavour. Visual appearance of meat was considered to be a vital parameter as the consumers usually relates it to the freshness, quality and safety of meat (Nikbin *et al.*, 2016). According to Zhang *et al.* (2012), appearance of meat is altered during heat stress condition in animals arising from the water imbalances occurring in muscle and disturbances in the various physico-chemical properties of meat. There are previous reports in this line stating the negative influence of heat stress on the sensory characteristics in beef and poultry (Ma *et al.*, 2015; Spehar *et al.*, 2009; Osman *et al.*, 1989). The heat stress did not influence visual appearance only in Salem Black breed as compared to both Osmanabadi and Malabari breeds. This indicates that the Salem black goats were able to sustain the effects of heat stress without compromising the appearance. Taking into account the lower effect of heat stress on the visual appearance in Salem black goats, and given its significance in representing the meat quality, we could deduce the ability of this indigenous breed

to combat such harsh climates without jeopardizing the meat quality. However, flavour was found to be better in Osmanabadi breed. Further, juiciness did not vary during the heat stress condition. Despite the clear impact of high temperature exposure on WHC and shear force of meat from heat stressed goats, juiciness which is related to WHC showed no significant variation among the breeds as well as for the heat stress treatment. The highly significant influence of breed and treatment interaction on the organoleptic parameters indicates that the breeds were responding differently to the treatment highlighting their adaptive potential in maintaining the quality of the meat. In addition, a negative correlation between THI and appearance and flavour establishes the severity of heat stress on these important carcass traits in the study.

5.3 Plasma leptin concentration

Leptin is a versatile protein hormone having crucial role in regulating the feed intake, body weight, metabolism, reproduction and adaptation in animals (Ma *et al.*, 2015; Maitra *et al.*, 2014; Bagath *et al.*, 2016). The influence of leptin on important carcass traits such as live and hot carcass weight, back fat depth and marbling have been reported in cattle (Cheong *et al.*, 2006), goat (Maitra *et al.*, 2014), sheep (Barzehkar *et al.*, 2009) and swine (Suzuki *et al.*, 2009). Leptin directly reflects the nutritional status of the animals and its level varies with the body fat mass of the animals and it was established to be a key hormone in governing the meat and carcass quality traits (Maitra *et al.*, 2014). There are also reports suggesting its role in regulating the energy homeostasis in animals during heat stress condition by regulating glucose metabolism and insulin sensitivity (Morera *et al.*, 2012; Min *et al.*, 2016). In the current study, the plasma leptin level was higher in the heat stress group of both Malabari and Salem Black breeds as compared to their control groups. This was similar to the findings in dairy cows, where increased level of plasma leptin was reported when the animals were subjected to chronic heat stress (Min *et al.*, 2015). In a similar study in Osmanabadi goats Bagath *et al.* (2016) reported significantly higher leptin concentration in stress group and they attributed this to the nutritional status of the animals. The higher

leptin level in Malabari and Salem Black heat stress groups could be due to the compromised nutritional status of these stressed animals. This compromised nutritional status was evident from the significantly lower values for most of the carcass traits in the heat stressed groups. The increased plasma leptin in heat stressed goats could be to provide signal to the somatotrophic axis to stimulate feed intake in these animals (Agarwal *et al.*, 2009). These results indicate that the plasma leptin could be used as a biomarker for stress determination in both Malabari and Salem Black heat stressed goats. The non-significant variation in leptin level between Osmanabadi control and heat stress groups could be attributed to the indigenous nature of this breed in addition to the fact that the study area was their native tract. In addition, a strong positive correlation between THI and leptin establishes the severity of heat stress on the nutritional status of the animals in the study.

5.4 Gene Expression

5.4.1 Myostatin mRNA Expression in muscle

In vertebrates, myostatin has been identified as a key peptide in the regulation of myogenesis or skeletal muscle formation (Gabriel *et al.*, 2011). Further, it is well known that the skeletal muscle transforms into the major edible portion in animal meat (Listrat *et al.*, 2016). In the current experiment, after the heat exposure there was significantly lower mRNA abundance of myostatin gene in both Osmanabadi and Malabari breed goats. This could be an attempt by the heat stressed goats of the two breed to prevent further deterioration of their growth performance. This was evident from the fact that both Osmanabadi and Malabari breed goats compromised majority of their growth variables during heat stress such as hampered body weight, loin eye area, fat score and some important physio-chemical properties of meat. Hence, myostatin being a growth inhibiting factor (Lee *et al.*, 2012) if up regulated may aggravate the already compromised growth of these two breeds. Similar finding of reduced expression of myostatin gene has been reported in chicken embryos subjected to heat stress (Gabriel *et al.*, 2003). However, this

trend of myostatin gene down regulation was observed only in Osmanabadi and Malabari goats while not in the Salem black breed. The same level of myostatin gene expression between Salem Black control and heat stress groups indicates the better ability of this breed to regulate growth performance during heat stress condition as generally myostatin gene was found to be an important regulatory factor for growth performance in ruminant species (Lee *et al.*, 2012; Gabriel *et al.*, 2011). This also shows the superior adaptive capability of this meat breed compared to the other two breeds to cope up to the testing climatic condition.

5.4.2 HSP70 mRNA Expression in muscle

The heat shock proteins (HSPs) play a crucial role in cellular protection and thermo-tolerance during heat stress in animals through their chaperonic activity. During stress conditions, these proteins have shown to prevent the unfolding and misaggregation of proteins, mediate the transport of proteins for degradation and help the proteins to maintain their native conformation and assist in their repair (Gupta *et al.*, 2013). As expected, HSP70 mRNA abundance was significantly higher in the longissimus dorsi muscle of heat stressed goats compared to the respective control group animals in all the three indigenous breeds. This could be attributed to the adaptive cellular response of these indigenous tract goats to withstand the high ambient temperatures by up regulating the HSP expression, thereby preventing the cellular damage. Similar reports have been established in goat (Dangi *et al.*, 2015), pig (Parkunan *et al.*, 2017), sheep (Chauhan *et al.*, 2014), cattle (Manjari *et al.*, 2015) and poultry (Xie *et al.*, 2014). Further, there was significant influence of breed on the expression pattern of HSP70. The differences in HSP expression pattern reflect the variations in adaptability of different breeds to sudden changes in the environment. On comparative basis, Salem black goats showed the highest mRNA abundance of HSP70. This indicates the high adaptability of this indigenous meat breed to the harsh climatic condition as it is able to combat the heat stress with the minimal up regulation of HSP70. Further, the maximum up regulation of HSP70 in Osmanabadi goats could be attributed to

the high susceptibility of this breed to heat stress that the animal had to produce more HSP to survive in the stress condition.

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Small ruminants serve as a vital source of income generation and livelihood for poor and marginal farmers, mostly in the developing countries. Goat production in particular has acquired huge relevance in the recent decades for their exemplary nature to adapt to harsh climates and because of their wide ecological adaptation. It is recognized as the ideal climate resilient animal most suitable for sustaining the global food security as well as for maintaining revenue from this sector. Further, sheep and goats are considered as excellent animals for meat production with a radiant future in the current scenario, especially from the view that they are free from religious taboos unlike beef and pig and also for the shorter period of generation for these small ruminants.

The emerging food safety risks due to climate change may pose a serious challenge to the meat industry. Therefore, a better understanding of anticipated climate change impact on meat quality would be of utmost importance for the policy makers to ensure preparedness. Further, research reports addressing the impact of shifting an adapted breed from a very harsh climatic condition to a location with relatively less magnitude of heat stress on the meat production potential is negligible. Such efforts are very crucial as the scientific community attempt to identify the most suitable breed to a specific agro-ecological zone in an effort to sustain meat production in the changing climate scenario. In this line, the study was designed to delineate the underlying biological mechanisms by which heat stress influences the meat characteristics of three indigenous Osmanabadi, Malabari and Salem Black goat breeds. Both Malabari and Salem Black breeds are well known for their survival in the hot humid tropical environment. Therefore, this study was an attempt to assess the impact of heat stress on the meat quality in the three indigenous goat breeds in a comparative mode with both the Malabari and Salem black breeds brought to the native zone of Osmanabadi goats. The primary objective of the study was to assess the impact of heat stress on the meat production

characteristics of all three breeds goats based on the changes in carcass characteristics, meat quality attributes, plasma leptin profile and skeletal muscle myostatin and HSP70 gene expression patterns.

The study was conducted for a period of 45 days in thirty six 10 months to one year old female goats (12 animals in each breed). The goats were randomly allocated into six groups: OSC (n=6; Osmanabadi Control), OSHS (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). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. All animals had access to *ad-libitum* feed and water and they were fed and watered individually. The OSC, MC and SBC goats were placed in the shaded pens while OSHS, MHS and SBHS goats were exposed to heat stress in outside environment between 10.00 h to 16.00 h. The animals were slaughtered at the end of the study and their meat characteristics were assessed. All cardinal weather parameters were recorded twice daily for the entire duration of the study. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to heat stress. Blood was collected at fortnightly intervals and plasma was separated for estimation of leptin level in these animals. The animals were slaughtered at the end of the study to assess the meat and carcass characteristics and representative skeletal muscle samples were collected for gene expression study.

A significant ($P<0.01$) influence of genotype was observed on the live body weights of the goats. Significantly lower live body weights were recorded in all the heat stress goats compared to their respective control group. However, the magnitude of the effect of heat stress was observed to be the most intense in Malabari breed compared to the other two breeds. Similar trend of live weight was observed in PSW with the lowest PSW observed in the heat stress group of Malabari goats. Among the different breeds, the Malabari breed showed lower HCW as compared to the other two breeds. The effect of heat stress on the hot carcass weight showed significant ($P<0.01$) difference only in the Salem black goats, with the heat

stressed Salem Black goats showing lower HCW compared to the respective control group. The dressing percentage values showed no significant variation between control and heat stress groups within the breeds. There was significant influence of treatment on DP with the highest value being recorded in the control group of Salem black breed. The heat stressed goats in all the breeds showed significantly lower LEA compared to the control goats. The Osmanabadi breed showed the highest LEA, whereas the Malabari breed had the lowest LEA. Among all the groups, the highest ($P<0.01$) separable fat was recorded in Osmanabadi control group. The severity of heat stress on fat score was more in the Malabari breed, whereas the other two breeds had comparable effect on fat score due to heat stress. Further, a strong negative correlation ($P<0.01$) was established between THI and body weight changes and other carcass characteristics.

Heat stress significantly influenced only the fore saddle ($P<0.01$), hind saddle ($P<0.05$), rack ($P<0.05$), and breast ($P<0.01$), weights of the primal cuts. Heat stress had no significant effect on majority of the carcass measurements recorded. However, external carcass length varied significantly ($P<0.01$) varied between the control and heat stress animals in the Salem black breed. The internal carcass length recorded was observed to have significantly higher ($P<0.05$) values in control group animals of both Osmanabadi and Salem black breeds compared to the Malabari breed. The leg length in Salem Black control group was found to be significantly ($P<0.01$) higher than Osmanabadi heat stress and both control and heat stress groups of Malabari breed. Carcass measurements such as buttock circumference, buttock width, chest circumference, shoulder circumference, and chest width showed no significant variations between the groups. Further among the offals, heat stress significantly reduced the liver ($P<0.01$) weight while significantly ($P<0.01$) increased the lung with trachea ($P<0.01$) weights in only SBHS group. In addition, heat stress increased ($P<0.01$) the ultimate meat pH in both OSHS and MHS groups. Among the sensory characteristics, appearance reduced significantly ($P<0.01$) in both OSHS and MHS groups and flavour reduced ($P<0.01$) in both MHS and SBHS groups. Further, texture was found to be

significantly ($P<0.05$) lower in OSHS while the overall acceptability was significantly ($P<0.01$) lower in SBHS group.

Heat stress treatment significantly ($P<0.01$) influenced plasma leptin levels in both Malabari and Salem Black breeds. In both these breeds, the leptin level was significantly ($P<0.01$) higher in heat stress group as compared to their respective control group. Further, the plasma leptin level differed ($P<0.01$) between the OHS and MHS groups. In addition, a strong positive correlation ($P<0.01$) was established between THI and plasma leptin concentration in the study. The expression of myostatin gene in the skeletal muscle of the goats showed significant ($P<0.05$) breed effect. Further, heat stress treatment also had significant effect on both Osmanabadi and Malabari breed. The heat stressed goats of both these breeds showed reduced mRNA abundance of myostatin gene as compared to their respective control goats. However, no significant variations in mRNA abundance of this gene were observed between the control and heat stress group of Salem black goats.

The study is the first of its kind to establish the impacts of heat stress on wide varieties of meat quantity and quality characteristics in three indigenous goat breeds on a comparative mode. The findings from the study indicated that all the three breeds had compromised some of their meat production variables during their exposure to high ambient temperature. On comparative basis, Malabari breed was found to more sensitive for the heat stress impact on meat characteristics followed by Osmanabadi and Salem black breeds. Further, the study identified that plasma leptin, myostatin and HSP70 genes in skeletal muscles could serve as ideal biological markers for assessing the impact of heat stress on meat quality in indigenous goats.

Moreover, the results from the study provided some crucial evidence for better resilience capacity of Salem Black breed as compared to both Osmanabadi and Malabari goats in maintaining the meat production during heat stress. This was evident from the non-significant influence of heat stress on vital meat quality

parameter such as separable fat, meat pH, appearance, and myostatin gene expression in Salem Black breed as compared to the other two breeds. Further, the comparatively lower expression of HSP70 gene in skeletal muscle of Salem Black breed as compared to Osmanabadi and Malabari breeds proves the superiority of this breed in maintaining the meat quality. Therefore, it could be concluded that shifting of Salem Black breed from a much harsher climatic condition to the lower magnitude heat stress location proved beneficial in terms of maintaining the meat production performance. However, when shifted from its native location to the current experimental location did not prove beneficial for Malabari breed. This finding has greater significance given the fact that the scientific community are looking to identify appropriate breed to sustain the livestock production in the changing climate scenario.

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EVALUATING THE DIFFERENCES IN MEAT CHARACTERISTICS BETWEEN DIFFERENT INDIGENOUS BREED GOATS SUBJECTED TO SUMMER HEAT STRESS

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to evaluate the differences in the meat production characteristics of three indigenous goat breeds (Osmanabadi, Malabari and Salem Black) to heat stress challenges. The primary objective of the study was to compare the meat production potential 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 impact of heat stress on meat production were assessed based on the changes in their carcass characteristics, primal cuts, carcass dimensions, physico-chemical attributes, proximate analysis, organoleptic variables, plasma leptin level and skeletal muscle myostatin and heat shock protein 70 (HSP70) gene expression patterns. The study was conducted for a period of 45 days in thirty six 10 months to one year old female goats (12 animals in each breed). The goats were randomly allocated into six groups: OSC (n=6; Osmanabadi control), OSHS (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). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. All animals had access to *ad-libitum* feed and water and they were fed and watered individually. The OSC, MC and SBC goats were placed in the shaded pens while OSHS, MHS and SBHS goats were exposed to heat stress in outside environment between 10.00 h to 16.00 h. The animals were slaughtered at the end of the study and their meat characteristics were assessed. The findings from the study revealed that heat stress caused significant reduction in live weight ($P<0.01$), pre-slaughter weight (PSW) ($P<0.01$), loin eye area (LEA) ($P<0.01$), and fat score ($P<0.01$) in all three breeds but with different magnitude. However, heat stress reduced ($P<0.01$) only the hot carcass weight and separable fat in SBHS and OSHS goats respectively. Further, heat stress significantly influenced only the fore saddle ($P<0.01$), hind saddle ($P<0.05$), rack ($P<0.05$),

and breast (P<0.01), weights of the primal cuts. Among the linear carcass dimensions, heat stress significantly (P<0.05) reduced only the chest depth and leg length in the goats. Further among the offals, heat stress significantly reduced the liver (P<0.01) weight while significantly (P<0.01) increased the lung with trachea (P<0.01) weights in only SBHS group. In addition, heat stress increased (P<0.01) the ultimate meat pH in both OSHS and MHS groups. Among the sensory characteristics, appearance reduced significantly (P<0.01) in both OSHS and MHS groups and flavour reduced (P<0.01) in both MHS and SBHS groups. Further, texture was found to be significantly (P<0.05) lower in OSHS while the overall acceptability was significantly (P<0.01) lower in SBHS group. Heat stress also was found to significantly (P<0.01) increase the plasma leptin level both in MHS and SBHS goats. In addition, heat stress reduced (P<0.05) the skeletal muscle myostatin mRNA expression both in OSHS and MHS goats. Furthermore, heat stress increased (P<0.05) the HSP70 mRNA expression in all the stress groups. However, the magnitude of difference pertaining to increased HSP70 expression was significantly (P<0.05) higher in OSHS while significantly (P<0.05) lower in SBHS group. Thus, it was concluded that heat stress induced deteriorating changes in the meat characteristics of all the three breeds. However, the magnitude of these changes was comparatively less severe in Salem Black breed. Further, the study also revealed the scope of using plasma leptin, myostatin and HSP70 genes as biomarkers to assess the impact of heat stress on meat production characteristics in indigenous goats.

Keywords: Carcass traits; Climate change; Goats; Heat stress; HSP70; Leptin; Myostatin