HEAT SHOCK PROTEIN 70 EXPRESSION IN DIFFERENT VITAL ORGANS OF HEAT STRESSED MALABARI GOATS

By

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(2013-20-102)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled "**Heat Shock Protein 70 Expression in different vital organs of Heat Stressed Malabari Goats**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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EXTERNAL EXAMINAR

Every Challenging Research works needs self-efforts as well as guidance from an expert, especially those who are close to my hearts…!!

Dedicated To

My Family

And

My Dear Mentor

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Afsal. A

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SYMBOLS AND ABBREVIATIONS

µL- Micro Liter

ACTH- Adrenocorticotropic Hormone

ALP- Alkaline Phosphate

ACP- Acid Phosphate

ANOVA- One-Way Analysis Of Variance

CDNA- Complementary DNA

Cm- Centimeter

CO_2- Carbon Dioxide

CRH- Corticotrophin-Releasing Hormone

DNA- Deoxyribonucleic Acid

ELISA- Enzyme Linked Immuno Sorbent Assay

FAO- Food and Agriculture Organization

Fig. - Figure

g- Gram

g/dl- Gram/ Deciliter

GAPDH- Glyceraldehyde 3-Phosphate Dehydrogenase

GDP- Gross Domestic Product

h- Hour

H_2O- Water

HR- Heart Rate

HSF 1- Heat Shock Factor 1

HSP70- Heat Shock Protein 70

IL- Interleukins

IPCC- Intergovernmental Panel on Climate Change

IU- International Unit

Kg- Kilogram

Km- Kilometre

LT- Lying Time

MAS- Marker Assisted Selection

MC- Malabari Control

MHS- Malabari Heat Stress

Nm- Nanometer

O2- Oxygen

OC – Degree Celsius

PBMC- Peripheral Blood Mononuclear Cell

PCR- Polymerase Chain Reaction

PCV- Packed Cell Volume

PR- Pulse Rate

PUN- Plasma Urea Nitrogen

RNA- Ribonucleic Acid

ROS- Reactive Oxygen Species

Rpm- Revolutions per Minute

RR- Respiration Rate

RT PCR- Real Time Polymerase Chain Reaction

RT- Rectal Temperature

S- Standard

SNP- Single- Nucleotide Polymorphism

SOD- Superoxide Dismutase

ST- Skin Temperature

ST- Standing Time

T3- Tri-Ido Thyronine

T4- Thyroxine

TCZ- Thermal Comfort Zone

THI- Temperature-Humidity Index

TLR- Toll like Receptor

TNZ- Thermal Neutral Zone

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INTRODUCTION

CHAPTER 1
INTRODUCTION

Climate Change is emerging as a serious threat to all living ecosystems on the earth. Increasing level of greenhouse gases (GHG) due to anthropogenic contribution in the atmosphere was widely believed to be the culprit contributing immensely to the changing climatic conditions (IPCC, 2013). The increasing human population is projected to surpass 9.6 billion by the end of 2050, which is qui*et al*arming from both the global food security and natural resources conservation perspectives (FAO, 2013). Ensuring proper food security to people worldwide is an unprecedented challenge in this changing climatic scenario with the limited natural resources. Agriculture is one of the important sectors which play a crucial role in supporting the global economy as well as the food production. In agriculture system, livestock sector is a major contributor to the global economy (FAO, 2013). However, the various environmental stresses associated with climate change affect the animal productivity, reproductive efficiency and disease occurrence which ultimately leads to severe economic losses for the livestock farmers.

Among different environmental stresses, heat stress is the major concern for the livestock sector. In a tropical country like India, temperature in the summer rises around 37-45°C which affect the thermal comfort zone of the animals. Further, the productive performances of animals including the milk yield, growth and reproductive efficiency are negatively impacted due to thermal stress (Pragna *et al*., 2017). Among domestic animals, goats especially possess the most adaptive capability to different environment stressors, however, while trying to adapt their productive performances are compromised. Further within a species, the indigenous breeds possess the superior adaptive capacity to the local climatic stressors and harsh environments (Silva *et al*., 2016). Goats have huge economic value especially in providing livelihood for the poor and marginal farmers. Goats are highly adaptable to heat stress due to its specific morphological, anatomical and physiological characteristic (Silanikove *et al.*, 2010). In addition the short hair, increased number of sweat glands and subcutaneous fat content also helps the goats to regulate their body temperature efficiently.

Biological markers are measurable indicators for certain physiological and cellular mechanisms associated with adaptation processes in animals. These markers could be of both phenotypic and genotypic origin. These markers could play a significant role in quantifying the stress response in the farm animals (Shilja *et al.*, 2016). Among the different markers, the genetic or molecular markers are considered to be the full proof indicators of stress level. Further, the molecular markers can be used as reference point in breeding programme to identify the best genetic character for producing animals with both superior adaptive and productive characteristics. Thus, the overall improvement in production aspects of livestock species was found to be aided by the application of genetic markers substantially.

When the cells are exposed to heat stress, it may show changes in most of its original functions, such as translation, transcription and protein synthesis (Negri *et al.*, 2013). There are specific proteins which show altered expression under the adaptive cellular mechanism such as slick hair gene and heat shock proteins (HSPs) (Bhat *et al.*, 2016). Among these proteins, HSP are well-established stress proteins which acts as molecular chaperones which prevents the misfolding and misappropriation of proteins within the cells (Shiber *et al.*, 2014). Further, HSPs are also identified to be the confirmatory biomarker for assessing the thermo-tolerance of the livestock species (Sejian *et al.*, 2013; Archana *et al.*, 2017). These identified biomarkers may pave way for development of thermo-tolerant breeds through marker assisted selection breeding program which can adapt and produce optimally in the current climate change scenario (Sejian *et al.*, 2017).

Extensive research findings have revealed that HSP70 can act as ideal molecular markers which protect the cells against exposures to heat shock in different livestock species (Vahid *et al.*, 2017; Bhat *et al.*, 2016; Manjari *et al.*, 2015; Sejian *et al.*, 2016). However, research efforts are lacking in establishing the functional significance of HSP70 in different organs of the animals. These warrants further research efforts in this line to establish the role HSPs in both adaptation and production associated vital organs in animals. Since goat has been projected as ideal animal model for coping to climate change, such efforts in this species would be very crucial as the scientific community battles against identifying the agro ecological zone specific breeds.

Understanding the regulation of heat stress at the molecular level and assessing the expression pattern of HSP70 gene in vital organs could pave way for establishing the underlying biological mechanisms by which the productive responses are compromised to support life sustaining activities in goats. Further, these attempts should target indigenous breeds as they play vital role in determining the livelihood securities of poor and marginal farmers in developing countries in particular. Malabari breed of goats are meat breed well known for their ability to survive and produce optimally in the hot humid tropics of Southern India and it plays a significant contribution to the economy of the local farmers. However, there are negligible efforts in establishing their adaptive potential and how they maintain production in their native track. Therefore, an attempt has been in this study to establish the expression patterns of HSP70 in some vital organs of indigenous Malabari goats in an effort to establish its role in maintaining the superiority of this breed to survive in harsh climatic condition

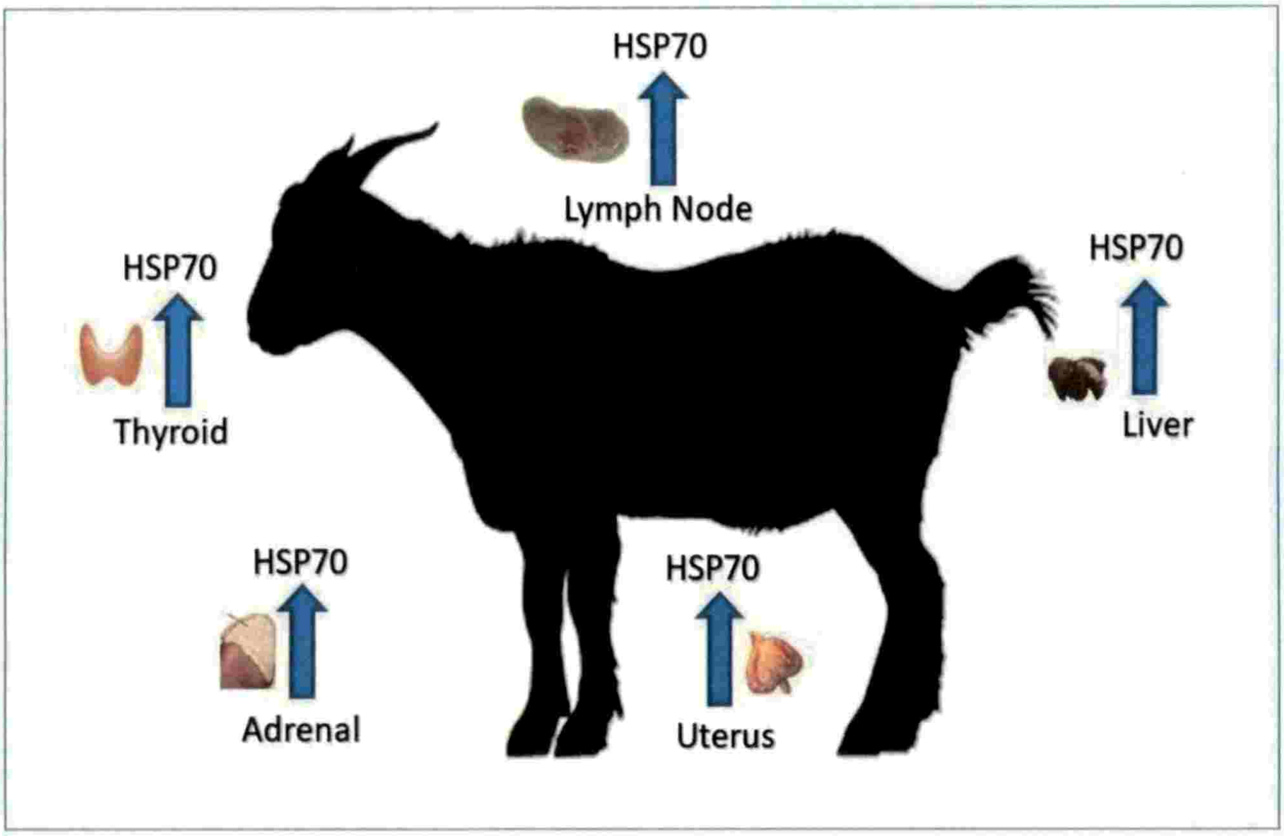
The primary objectives for the study are,

a) To assess the expression patterns of HSP70 gene in stress related endocrine organs in heat stressed Malabari goats.

b) To assess the expression patterns of HSP70 gene in reproduction and immune system related organs in heat stressed Malabari goats.

c) To establish the correlation between THI index and different organ HSP70 expression patterns in heat stressed Malabari goats.

Fig1.1. Hypothetical figure describing the expression pattern of different tissues of farm animals



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REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 General overview of thesis title

Livestock was considered as the oldest wealth resource of mankind and it also plays a vital role in providing nutritive value to humans all over the world. It is the major sector that accounts for 40% of the world's agriculture Gross Domestic Product (GDP) (Sejian *et al.* 2015). Goats are multi-purpose animal that can produce meat, milk, hide, fiber and also manure. Goats give more production per unit of investment. These animals are tolerant to hot climate and can well adapt to different agro-ecological zones where the climatic conditions can vary from dry to cold arid compared to other farm animals (Sejian *et al.* 2011; Shaji *et al.* 2017). These animals can survive in harsh hilly tracts as well as sandy zones. Unlike other farm animals, the goats can thrive well, even by consuming shrubs, herbs and other poor quality roughages, because of the increased digestibility of crude fiber. In the changing climatic scenario, goat is the most admirable animal for physiological and biomedical research in establishing the impact of climate change on livestock production.

Livestock can experience heat stress during hot weather, particularly if the heat is prolonged or temperatures are extremely high. The thermal environment is a major factor that can negatively affect goat performance. Increased body temperature and respiration rate are the most important signs for heat stress in goat (Alam *et al.*, 2013). Excessive heat stress may cause hyperthermia and potentially have several physiological side effects and economic impacts on the livestock industry (Ecklund *et al.*, 2005). When the environmental temperature increases, animals can maintain a core body temperature within the thermo-neutral zone by seeking shade, drinking water, changing posture, and/or reducing feed intake. When animals are subjected to heat stress, they react either by physiological or a behavioral responses, but most often a combination of both. According to Grandin (1997), to accurately assess an animal's reaction, a combination of behavioral and physiological measurements will provide the best overall measurements of animal discomfort.

Use of improved technologies in molecular analysis had permitted the improvement in the accuracy and intensity of use of genetic markers. Heat Shock Proteins (HSPs) are widely used to gauge stress tolerance, and it has become new approach to counter stress and also improve the stress tolerance in farm animals (Mayer and Bukaku, 2005). Many researchers have found that the heat shock proteins, especially the HSP70 gene expression can be used as a marker for thermal adaptation in different species, and information generated will have significant implications in the future for the development of strategies to cope up with the challenges of climate change (Fehrenbach *et al.*, 2000). Higher expression of HSP70 in caprine peripheral blood mononuclear cells (PBMCs) during heat stress, suggest a possible involvement of HSP70 in ameliorating the deleterious effect of thermal stress so as to maintain cellular integrity and homeostasis in goats (Mishra and Palai, 2014).

HSP70 is one of the most abundant and best characterized HSP family that consists of highly conserved stress proteins, expressed in response to stress, and plays crucial roles in environmental stress tolerance and adaptation in goat (Gupta *et al.*, 2013; Banergee *et al.*, 2014). Further, it is an established fact that HSP70 expression increases during heat stress in goat (Dangi *et al.*, 2012; Banergee *et al.*, 2014). In a study conducted on Osmanabadi goats, Chaidanya *et al.* (2017) reported higher expresseion of HSP70 during heat stress exposure. There are reports suggesting expression of HSP in the rumen (Cronje, 2005). Cronje (2005) attributed this HSP expression in the rumen to redistribution of blood to the periphery and compensatory reduction in the blood supply to the gut, which damages cells lining the gut, permitting endotoxin to enter the body. These results indicates that the expression pattern of

HSP70 differs based on the functions of different cell from which these expression patterns are studied (Archana *et al.*, 2017).

2.2 Global Climate Change

Climate worldwide has been changing and the fluctuations are predicted to be highly dynamic in the near future (IPCC, 2013). The world foremost scientific community governing climate change related issues concluded that "warming of the climate system is unequivocal" and the significant rise in global average temperatures is immensely due to the extreme contribution of anthropogenic GHG emissions (IPCC, 2007). Further, the various climate models predicted that, the increase in the average global surface temperature significantly rises between 2.6 and 4.8^{0}C by 2100.

Over the last century, atmospheric concentrations of carbon dioxide increased from a pre industrial value of 278 ppm to 379 ppm in 2005, and the average global temperature has increased by 0.74° C. Furthermore, the current observed CO2 level in the geological record analyzed from the ice core data was approximately 400 ppm. According to the IPCC projections, the global warming will continue to accelerate in 21st century qui*et al*armingly (IPCC, 2013). The global warming of the past 50 years is due to the extreme anthropogenic activities. Also, the potential contribution of human fingerprints have been identified in many aspects of the climate system, including changes in the ocean, earth heat budget and precipitation.

In addition, the tangible scientific evidences shown that global warming would result in certain types of extreme weather events, including heat stress, flooding, extreme precipitation and droughts (IPCC, 2013). Moreover, the temperatures are rising in the Polar Regions of the earth, particularly in the Arctic and the vast majority of the ice glaciers are melting faster and cause extreme flooding in several regions. Climate change may also result in the limited availability of fresh water and is likely to increase the drought risk in certain tropical regions (Carlton *et al.*, 2016). As the alarming increase in temperature, the prevalence and duration of drought has increased in the western part of America and climate projections are predicted to increase the drought in several countries. Further, climate change associated drought can hamper the agricultural and industrial production due to the limited availability of water (FAO, 2013).

Climate change is seen as a major threat to agricultural production systems globally. The existing literatures indicates the foreseen effects of climate change on the stability of the world food security (FAO, 2013). Global warming has the potential to seriously disrupt the sustainable food production in world, which have a significant reduction in crop and livestock production (Sejian *et al.*, 2017). In addition, the agricultural productivity might change at a rate of 10-20% by 2050 particularly in tropical countries ultimately may diminish the food availability. The developing countries are expected to face the pressure more and in fact these countries are already facing severe starvation and malnutrition and are expected to increase over time. The scientific community further suggests that the impacts of global warming are likely to be more severe and impose greater damage to environment and all economic sectors around the world.

2.3 Climate change consequences in India

India is one of the most vulnerable countries for the adverse impacts of climate change (COP 23). India accounts for 4.5 percent of the global greenhouse gases (GHG) concentration and therefore it plays a crucial role in combating the catastrophic climate change and global warming (IPCC, 2013). India is also recognized as the 6th most vulnerable nation among south Asian countries (United Nations Climate Change Conference, 2017). The devastating effects of climate change includes heat waves, drought, heavy monsoon, and storm which are common occurrences in India.

In 2016, the country reported the highest number of mortality due to extreme weather events (Murari *et al.*, 2015). Further, the effect of heat waves persisted in different regions of South Asia and recorded a highest temperature of 51^0C in

Rajasthan, India. The severity of heat waves have turn out to be a regular concern in India having adverse impact on thousands of people in various regions (O Brien *et al.*, 2004). In addition, there are reports which indicate that more than 18000 mortalities were reported due to the effect of hyperthermia. Drought has cause severe impact on the agricultural production and a rash of farmer suicides due to severe economic burden (Billett, 2010). The environmental extremities are continue to rise in the near future and as a tropical country like India feel the limits and importance of natural resources being overextended.

India has major reasons to be concerned about the impacts of climate change. Furthermore, it is one of the largest populated countries, depending mainly on climate sensitive sectors like agriculture, livestock and tourism for livelihoods. Agriculture is facing environmental and economic challenges, particularly livestock production in tropical countries (Aggarwal *et al.*, 2008). Sustainable development within the context of climate change urge for modern approaches which have to be implemented globally. In addition, climate resilient technologies are the need of hour to, mitigate the adversities associated with climate change.

2.4 Animal Agriculture and Climate Change

The world is facing major challenges such as over exploitation of population and extreme environmental crises including ecological degradation and catastrophic climate change. The effects pertaining to climate change would cause alterations in temperature, precipitation, humidity, atmospheric CO2 levels and reduces the water availability and finally result in the reduction of agricultural production (FAO, 2013). Livestock sector is one of the important sectors which contributes immensely to the global economy. In addition, our natural resources are very limited and not sufficient for satisfying the needs of future human population. In livestock sector, small ruminants play an important role in the economy of millions of poor and marginal farmers who earn their livelihood by rearing these animals in different agro ecological zones (Sejian *et al.*, 2013). Furthermore, livestock sector has a significant role in global food supply for satisfying the needs of growing human population. It is a key asset for the poor farmers (FAO, 2013). In agro pastoral systems, the livestock farmers from under developed and developing countries primarily choose extensive system of rearing for feeding the animals (Brito *et al.*, 2017). The impact of climate change is expected to intensify the vulnerability of livestock systems and reinforce the current environmental factors which are negatively correlated with animal production (Silanikove *et al.*, 2015). Further, the emerging climate extremities are of major concern to the livestock farmers as the sector emerges as one of the significant contributors to the phenomenon (FAO, 2011).

Heat stress is one of the most intriguing factors associated with climate change which hampers the productivity of the animal. Every animal possess a thermo-neutral zone within which their biological functions would be optimum. Depending on the environmental conditions like high temperature and humidity, the animal alters their physiological functions to retain the homeostasis back to their original state. In an effort to cope with different environmental stressors, animals have to deviate the productive energy for their adaptation purpose resulting in drastic reduction in productive performance (Sejian *et al.*, 2013). Furthermore, the effects of heat stress are evident in feed consumption, production efficiency in terms of milk and meat production (Collier *et al.*, 2017). Heat stress is one of the predominant factors which negatively influence animal production in the tropical countries. The effects of heat stress may aggravate with prospects of global warming to livestock farming caused by the accelerated effect of global emission of GHG. Indirectly climate change can impact the quality of pasture availability and forage, water availability and various livestock disease outbreaks all of which threatens the livestock production resulting in significant financial burden to poor livestock farmers.

2.5 Economic consequences of heat stress impact on livestock production

India holds the largest livestock population in the world, and among agriculture, livestock is the major subsector that has a great significance to the Indian economy and in particular for the welfare of the rural farmers in India (FAO, 2013). Recent years, the livestock sector has emerged as an important part of an expanding and diversifying agricultural subsector in the Indian economy. Also, the abnormal increase in temperature and humidity negatively affects the animal production resulting in significant economic burden to dairy industry to the tune of about $900 million per year. Similarly, the extreme level of heat stress would cause decline in milk and meat production particularly in tropical countries. Furthermore approximately 6.3% reduction in milk production is expected in US by 2080 (Fischer *et al.*, 2005).Also, there were reports found which established the impact of heat stress on dairy cattle and estimated the annual economic loss of 897 to 1,500 million dollars in US dairy industry (Walsh *et al.*, 2011). In US dairy industry a total annual loss of $1.5 billion or $167 per dairy cow per year was reported (St-Pierre *et al.*, 2003). Mauger *et al.* (2015) calculated the economic loss based on the costs of reduction in DMI, milk sales and increase in days open. In Orlando, the dairy cows were under heat stress for 51% of the time in natural field condition (St-Pierre *et al.*, 2003). The authors observed a loss of $687per cow per year in dairy industry as a result of heat stress. Sejian *et al.* (2013) indicated that, heat stress is the most severe environmental factor which negatively correlated with livestock production. These evidences indicated the severe impact on intensive livestock production in the changing climate scenario and cause significant financial burden to the farmers.

2.6 Different adaptive mechanisms of livestock

Adaptive natures of the animals are mostly evaluated by their ability to produce and reproduce optimally apart from possessing the superior ability to survive in extreme environmental conditions (Aleena *et al., 2016*). Animal welfare is one of the most discussed concerns among livestock farmers and eradicating chronic stress is one of its prerequisites. (Sejian *et al.*, 2013). The animal responds to various environmental extremities by deviating the energy expenditure for coping to extreme stress conditions. In order to maintain the homeostasis, the animal have to control their thermoregulatory mechanisms by implementing certain adaptive mechanisms such as morphological, behavioral, physiological, blood biochemical, neuro-endocrine and cellular responses (Sejian *et al.*, 2010).

2.6.1 Morphological Adaptive Mechanism

Morphological adaptive response is primarily the external body features of the livestock which imparts survival ability to the animals. There are morphological features of the animal which imparts thermoregulatory adaptive mechanisms involving coat, hair type, hair density, fur depth, subcutaneous fat, skin color and body size (Niyas *et al.*, 2015). Likewise, there are certain features of animals that affects the efficiency of evaporative heat loss from the skin surface which includes hair coat density, color and sweat gland density (Lytle *et al.*, 2004). The animals having light and shiny coats was found to absorb lesser heat in comparison with those having dark colored and woolly coats (Gizaw *et al.*, 2007). Further, the skin color is one of the major factors that influence the absorption of heat radiations by the animal (Niyas *et al.*, 2015). Also, the hair coat regulates the heat transfer from the skin to the adjacent environment resulting in effectively controlling the body temperature (Niyas *et al.*, 2015). Moreover, coat depth is an important factor in thermoregulation and the increase in coat depth reduces the sensible heat loss in an effective manner (Wolf *et al.*, 2000). Therefore, the possibility of developing heat tolerance in farm animals would help to improve the sweat gland function and further regulating the thermoregulatory mechanisms in an efficient manner (Sejian *et al.*, 2013). In addition, some animals consist of short and thin hair, small ears with tiny hair, movable and slender tail which helps in moderating the thermogenesis in diary animals in a comfortable manner (Niyas *et al.*, 2015). Moreover, the reduced resistance to flow of heat from the core to the periphery of the animal body and the salient features of the hair coat safeguarded the thermoregulatory mechanisms in cattle breeds (Al Tamimi *et al.*, 2007). Furthermore, there are research findings indicating crossbred cattle having larger and more sweat glands per unit area of the skin as well as greater sweat production than pure bred one (Hansen *et al.*, 2004). In addition, the buffaloes possess poor thermo tolerant capacity as compared to other domestic ruminants (Ganaie *et al.*, 2013). Sweat glands were described as the potential source for thermoregulation in different animals through which the heat is being dissipated (Collier *et al.*, 2008). Also, the increase in blood flow to the sweat glands enables heat transfer to the skin and results in sweat production. Further, sweating rates were found to be significantly high in tropically adapted breeds compared to other livestock breeds in the temperate zone (Sejian *et al.*, 2013). These are the various morphological features in farm animals which helps them to maintain homeostasis during extreme climatic stress conditions.

2.6.2 Behavioral Adaptive Responses

The major behavioral responses to livestock adaptation are feed intake, defecating and urinating frequency, lying time, standing time, shade seeking behavior and increased frequency of drinking (Aleena *et al.,* 2016). In addition, seeking shade was the primary behavioral response by the animals to cope with the effect of heat stress. During extreme summer season, the animals which are in the shaded condition showed lower core body temperature and lower respiration rates as compared to those animals which are directly exposed to heat stress (Sejian *et al.*, 2013). Similarly, it was also observed that darker colored animals mostly prefer shade rather than the lighter skin colored animals. Moreover, the animals adjust its standing posture during heat stress in order to control the airflow around the body and to maximize the surface area in order to control the heat dissipation (De dear *et al.*, 1997).

India is a tropical country having hot and humid climatic conditions which usually support the extensive system of rearing through grazing of animals (Nardone *et al.*, 2010). Further, it would affect the livestock production due to the severity of extreme environmental stressors. When the animals are exposed to hot humid environmental conditions, reduced feed intake was recorded. Particularly, in lactating cows, feed intake started to decline at a temperature of 30^0C in a temperate climatic conditions with rapid reduction in feed intake to the tune of 40% (Arias *et al.*, 2018). Hence, it could help the animal to regulate its metabolic heat production by balancing the negative energy mechanisms pertaining to its body weight and the body condition score (Roche *et al.*, 2009). In an effort to adapt to hot environmental condition the dairy animals shown a reduced rumination during heat stress condition (Arias *et al.*, 2018). In addition, urination and defecation are a common natural physiological process of the animal. Further, the frequency of urination and defecation differ with the factors affecting the livestock such as water intake, feed intake, type of feed, environmental temperature and disease conditions (Alam *et al.*, 2013). There are also reports which established the effect of combined stresses (Heat and nutritional stress), increased respiratory and cutaneous evaporative cooling mechanisms leading to severe dehydration and thus resulting in the reduction in their urinary and defecation frequency (Sejian *et al.*, 2013; Shilja *et al.*, 2016).

Likewise, the reduction in lying and standing time might result in the physiological changes and cause a negative impact on the animal health. The higher standing time during heat stress is an adaptive behavioral mechanism in animals to avoid the additional heat load from the ground, which also further favors the heat dissipation mechanism from the body (Wheeler *et al.*, 1992). Shilja *et al.* (2017) denoted that, the highest lying time was recorded in small ruminants with restricted feeding subjected to heat stress. Further, optimum level of nutrition are important for the farm animals to support the behavioral responses that could help the animals to cope with the extreme environmental conditions (Meunier *et al.*, 2001).

2.6.3 Physiological Adaptive Responses

The physiological responses to heat stress in diary animals involve the efficient integration of several organs to regulate the metabolic process for maintaining the homeostasis. The animals implement certain physiological adaptive responses to cope with the stress condition. Further, it could help the animal to regulate the metabolic heat production by controlling the thermoregulatory mechanisms (Aleena *et al.*, 2016). Also, increased respiration rate, sweating rate and rectal temperature are the typical examples of physiological adaptive responses in farm animals (Indu *et al.*, 2014). In addition, the body temperature is also considered as an important physiological thermoregulatory mechanisms which can reflect the adaptive potential of an animal (Srikandakumar *et al.*, 2003).

It is well established fact that the indigenous breeds are highly adapted to extreme climatic conditions (Sejian *et al.*, 2013; Archana *et al.*, 2016). Generally, it was reported that genetic potential of the indigenous breeds helps to regulate the various physiological responses as compared to other cross breeds under similar environmental conditions. Also, significantly increased respiration rate and pulse rate was reported in different goat breeds (Shilja *et al.*, 2017). However, the rapid increase in the respiration frequency in exotic breeds also indicated their greater vulnerability to extreme tropical environment. Further, the Sahiwal breed was established to exhibit higher heat tolerance and lower magnitude of thermal indicators than crossbreds in the arid regions (Abeygunawardena and Dematawewa, 2004). In addition, the increased rate of rectal temperatures in summer confirms the lower adaptive capacity of the temperate breeds (Renaudeau *et al.*, 2012). The increase in respiration rate (RR) in the afternoon is a general observation in livestock exposed to heat stress. The increase in heart rate (HR) in the afternoon was also found in sheep at a rate of 105.67 mov/min and 126.4 mov/min in the morning and 133.2 mov/min in the afternoon (McManus *et al.*, 2009). Also, the research findings have shown that the sheep start to present open mouth polypneia when RR is above 41°C (Jonasson *et al.*, 2006).

The higher RR is observed in cattle exposed to increased ambient temperature and relative humidity (Hammond *et al.*, 2015). The increased RR of animal during heat stress condition is an attempt to increase the respiratory evaporative cooling mechanisms by dissipating body heat (Sejian *et al.*, 2017). In a comparative study, the RR were 15.738±0.795, 18.158±0.795 and 29.818±0.795 in Sahiwal and 15.779±1.136, 22.979±1.136 and 47.299±1.136 in Karan Fries cattle during winter, spring and summer seasons respectively (Das *et al.,* 2016). This indicates that irrespective of breeds the animals depends extremely on the RR to maintain body temperature during exposure to different climatic extremities. Also, the positive correlation between RR and RT indicates that the respiratory mechanisms was very important for thermogenesis and maintain the homeostasis back to its normal condition (McManus *et al.*, 2009). Also, the skin temperatures in different area of the animal during morning in heat stress groups was generally significantly lower as compared to the respective control groups while reverse trend was observed during afternoon (Jonasson *et al.*, 2006). These highly significant changes in skin temperatures between the groups indicate its significance for assessing the adaptive nature of the different breeds (Aleena *et al.,* 2016). Similarly, the results of increased skin temperature due to heat stress was also reported in goats (Shilja *et al.*, 2016). This higher skin temperature could be directly attributed to the vasodilatation of skin capillary bed to stimulate the blood flow to the skin periphery for facilitating heat dissipation to the environment (Shilja *et al.*, 2016). In a study conducted on three indigenous breeds, Malabari breed showed significantly lower skin temperature than Salem Black and Osmanabadi goats during heat stress condition (Aleena *et al.,* 2016). The goats having light colored coat have greater advantage over dark coat colored pertaining to thermoregulatory mechanisms in tropical environment (Fadare *et al.*, 2012). In addition, a strong positive correlation for all the physiological traits with THI indicates the significance of assessing the heat tolerant capacity of indigenous breeds of goat. Among the various physiological variables, RR and RT are considered to be the ideal physiological markers for assessing the severity of heat stress in farm animals (Sejian *et al.*, 2013).

2.6.4 Blood biochemical adaptive response

Heat stress significantly alters the levels of Hb, PCV, plasma and glucose level in the blood. Also Toghyani *et al.* (2006) denoted that both Hb and PCV increased significantly in goats during exposure to severe thermal stress. The effect of heat stress increases the level of oxygen consumption of the animals by influencing the respiration rate (Collier *et al.*, 2008). Further, the increased level of oxygen consumption reduces the partial pressure of carbon dioxide in blood and ultimately results in respiratory alkalosis (Sejian *et al.*, 2013). Conversely, Sejian *et al.* (2011) reported that heat stress had no negative impact on hematological parameters in calves. Further a strong positive correlation between PCV and Hb concentration, indicates the significance of these parameters for heat tolerance in Brazilian sheep (McManus *et al.*, 2009). Banerjee *et al.* (2011) reported that, the lower hemoglobin concentration in calves may be due to haemodilution. High hemoglobin values are associated with the most adaptive capability of the animal in harsh climatic conditions and it has been suggested that this characteristic might be an index of superior heat tolerance. The higher blood glucose level in the calves might be due to an increased glucose oxidation (Collier *et al.*, 2008) and this finding was highly correlated with findings by Vijayakumar *et al.* (2011) who found a significantly higher blood glucose level in buffalo heifers during heat stress exposure. Further are also report\s which suggested the influence of heat stress on plasma total protein, total cholestrerol, urea, ALP and ACP (Sejian *et al.*, 2012; Sejian *et al.*, 2013).

2.6.5 Neuro-endocrine Adaptive Response

Neuro-endocrine regulation is one of the principal adaptive responses shown by the animal in harsh climatic conditions (Mahesh Gupta *et al.*, 2013). Hypothalamo-pituitary-adrenal (HPA) axis plays a significant role in the release of several neurotransmitters and hormones which regulates the thermo regulatory mechanisms in animals (Afsal *et al.*, 2018). The activation of the HPA axis was related to several factors such as heat stress, nutritional stress and disease occurrence. The corticotrophin releasing hormone (CRH), adreno corticotropic hormone (ACTH) and glucocorticoids are the primary products of HPA axis which ultimately controls the stress response pathways in animals (Sejian *et al.*, 2010). Adrenocorticotropic hormone is the important regulator of HPA axis which helps in the production and secretion of cortisol. Also several research findings shown that the hormones produced from the adrenal and thyroid glands are recognized to have significant role in thermoregulation and metabolic response in livestock animals (Aleena *et al., 2*016; Afsal *et al.*, 2018). Further, the activation of HPA axis might result in enhanced production of glucocorticoids such as cortisol, which is indicated as the major stress relieving hormone and also identified as a biomarker for assessing the severity of stress in farm animals (Shilja *et al.*, 2017). In addition, aldosterone is a steroid hormone released from the adrenal cortex and involves in the regulation of electrolyte mechanism in the animal body (Sharma *et al.*, 2013). Moreover, the animals during heat stress conditions undergo severe dehydration and which might result in the activation of renin-angiotensin-aldosterone pathway to restore the fluid and electrolyte balance (Akerman *et al.*, 2016). Similarly, pineal gland is a neuro endocrine transducer, which is responsible for the melatonin production and influences the seasonal changes pertaining to the reproductive capability in different animals (Reiter *et al.*, 1991). Similarly, thyroid gland produces two type of hormones triiodothyronine (T3) and thyroxine (T4) which aid the regulation of metabolic activity in the animals. Generally the level of thyroid hormones are found to be reduced during heat stress exposure to reduce the metabolic heat production (Pragna *et al.*, 2017). Generally, lower activity of the thyroid gland is helps to reduce the metabolic energy expenditure of animals to coping extreme environmental conditions and also similar results of inhibited thyroid hormone concentration level was also reported in various livestock species like cattle, sheep and goats were exposed to thermal stress (Das *et al.*, 2016; Shilja *et al.*, 2017).

The somatotropic axis mainly consists of growth hormone (GH), insulin-like growth factors (IGF-I and II), their associated target organs and receptors. Further, it plays a crucial role in the control of metabolism and regulation of various physiological processes in animals (Afsal *et al.*, 2018). Ghrelin is an important factor which helps in the control of GH secretion and feeding behavior. When the nutritional status of an animal is at stake, the level of ghrelin increases, and it has an orexigenic effect in stimulating the appetite center thereby increasing the GH concentration (Afsal *et al.*, 2018).

The IGF-1 hormone level is an indicator of nutritional status in many animal species and the level of IGF-1 is controlled by both GH and nutritional intake (Levine *et al.*, 2014). Likewise, the leptin concentration in blood responds to changes in nutritional status in livestock. Leptin has multiple role in regulating physiological and behavioral responses. Also, it plays a pivotal role in the control of body growth and adaptation. Leptin act as a unique nutritional signal to growth axis and high leptin levels were observed to inhibit the feed intake through binding to specific receptor in the hypothalamus (Dixit *et al.*, 2004). Finally result in the short-term feed restriction and reduction in the synthesis of leptin in adipose tissue. The reduced leptin concentration in feed-restricted animals could be activate the appetite center in the hypothalamus and stimulate feed intake and initiate adaptive mechanisms by synthesizing glucocorticoid production to favor hepatic gluconeogenesis and decrease thyroid activity to maintain the homeostasis in animals. Leptin is now recognized as an inhibitor of stress axis activity (Itai *et al.*, 2018). The changes occurred in the somatotropic axis, suggesting that plasma levels of IGF-1 and leptin were identified to be the indicators for assessing the severity of stress in farm animals. Likewise, the cortisol, T3, T4, IGF1, GH are identified as biological markers for assessing the severity of stress in farm animals. Moreover, it could be incorporated into future genetic breeding programme for producing thermo-tolerant livestock breeds.

2.6.6 Cellular Adaptive Response

Cellular response is one of the genetic adaptive mechanisms shown by the animal to overcome the severity of heat stress (Tzanavari and Karalis, 2015). The various cells and tissues respond to temperature above the thermo-neutral zone resulting in changes in the genetic characteristics to impart thermo-tolerance (Simons and Klopack, 2015). Also, the thermo tolerant genes like heat shock proteins (HSP) and slick hair gene expression indicate the severity of heat stress in farm animals (Srikanth *et al.*, 2017). The HSPs are activated by heat shock factors and their expression has increased further when cells are exposed to extreme hot and humid climatic conditions. The general response of cells to heat stress includes: inhibition of DNA synthesis, transcription, RNA processing, translation, progression of cell cycle, disruption of cytoskeletal elements, protein denaturation and changes in membrane permeability. It is a well-established fact that the changes in gene expression are an integral part of the cellular response to heat stress (Pockley and Henderson, 2018).

When the farm animals are exposed to heat stress, there are certain genes which preferentially expressed under the harsh environmental conditions like slick hair gene, ATP1B2 and HSPs (Archana *et al.*, 2017). During stressed conditions in the cell, HSPs interact with denatured proteins and inhibit the synthesis of cytotoxic protein aggregates, thereby maintaining the protein homeostasis of a cell. In addition, HSP70 concentration in blood is also identified as a reliable indicator of chronic stress in feedlot cattle (Gaughan *et al.*, 2013). Further, it is identified and considered to be a confirmatory molecular marker in assessing the stress adaptation in different farm animals (Sejian *et al.*, 2017). The HSPs are molecular chaperones which controls the cellular growth, maturation, and avoid degradation of proteins. Oxidative stress affects the antioxidant defense resulting in production of free radicals and reactive oxygen species (ROS), during heat stress (Archana *et al.*, 2017). Heat stress also was shown to raise both of Thiobarbituric acid-reactive species (TBARS) and malondialdehyde (MDA) levels in broilers, buffalos and dairy cows which are the major products of lipid peroxidation. Antioxidant enzymes activities, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were observed to be increased during heat stress exposure in livestock (Patsoukis *et al.*, 2005). Elevated concentration of SOD and GPX concentration were significantly higher in prepartum cows during summer season (Archana *et al.*, 2017). Antioxidant activity was analyzed during winter and summer season in growing calves, heifers and lactating Murrah buffaloes with significantly higher concentration of GPX reported during summer (Ganaie *et al.*, 2013).

The activities carried out by molecular chaperones include the cellular growth, maturation, and avoid degradation of proteins. The transcription of heat shock genes is mostly by heat shock factor 1 (HSF1). Also, inactive HSF1 is present in the cytosol, during heat stress it translocate into the nucleus and binds to promoters of heat shock elements (HSE) (Santoro, 2000). Further, it induces transcription leading to an increase in HSP expression. The activation of HSF controls the cell cycle, protein translation and glucose metabolism. It is now well accepted that HSPs helps in mediating cellular responses to heat stress. The cellular response is one of the primary pathway by which livestock tries to cope with the heat stress challenges (Afsal *et al.*, 2018). In addition, HSP70 is identified as the ideal molecular marker for assessing the severity of heat stress in farm animals (Archana *et al.,* 2016).

The advanced RNA sequencing technology (RNA-seq) is a recently developed method for identifying, profiling and quantifying RNA transcripts (Wang *et al.*,2009). Further, it is used to analyze the frequently changing cellular transcriptome and might help identifying different gene expression patterns involved in adaptive response of different farm animals. There are extensive research interests in the cellular mechanisms of animal to cope with heat stress and maintain homeostasis.

2.7 Importance of studying heat stress impact in indigenous livestock breed

Indigenous breeds are well adapted to different climatic conditions and diseases occurrences as comparing to cross breeds. There are considerable difference for heat tolerance between breeds and even between individuals within a breed (Mackinnon *et al.*, 1991). The native breeds are better heat tolerant in comparison to exotic breeds and its cross in terms of survival and performance because of the inability of the exotic genes to express/adapt under tropical conditions (Binsiya *et al.*, 2017). Sejian *et al.* (2013) suggested that, indigenous breeds reared in tropical and arid regions can sustain in the harsh environmental conditions. Hence, the farmers prefer indigenous livestock due to their extreme adaptive nature and resilience capacity (Allison *et al.*, 2004). Also, the indigenous goats reared under hot and humid conditions perform better than other pure and cross breed of goats (Silanikove *et al.*, 2000). In addition, the indigenous breeds alter their metabolic activity to minimize the body heat during heat stress (Wheelock *et al.*, 2014). Likewise, the reduced nutrient intake accounted for only 50% of heat stress-induced decreases in milk yield, and feed intake-independent shifts in post absorptive glucose and lipid homeostasis might contribute to the supplementary reduction in milk yield (Wheelock *et al.*, 2014). Indigenous cattle breeds are used for crossbreeding in order to improve the productivity and tolerance for subsistence in harsh climate (Scholtz and Theunissen, 2010). Similarly, African *Bos Taurus* indigenous to West and Central Africa, have a unique adaptive capability towards adverse climatic conditions (Nardone *et al.*, 2010). Pragna *et al.* (2017) indicated that Salem black breed which is native to Tamil Nadu had shown less impact when exposed to heat stress as compared to Osmanabadi breed indigenous breeds of Karnataka state in India. Similar study conducted in Aardi goats which is an indigenous breed of Saudi Arabia, proved that several physiological changes such as increase in skin temperature, increased heart rate and respiratory rate, decrease in T3 and T4 and increase in cortisol concentration further helps in eradicating the severity of heat stress (Samawi *et al.*, 2014). The *Bos indicus* are more heat tolerant characterized by shorter hair with greater diameter and lighter coat color in comparison to *B. Taurus*. Further, the HSP transcripts in Sahiwal cows showed minimum change in expression with change in season whereas in HF with higher abundance of major HSPs such as like HSP90, HSP70, HSP60 and HSP40 during summer season revealed their lower heat tolerance (Binsiya *et al.*, 2017). In many parts of world where, pure breeding with indigenous breeds are the only viable option for producing suitable breeds which can survive and produce optimally in adverse climatic and nutritional conditions (Scholtz and Theunissen, 2010).

2.8 Goat as ideal model from climate change perspective

Among the livestock species, goat was considered better adapted because of their ability to produce, survive and reproduce in harsh climatic conditions (Silanikove and Koluman, 2015). Furthermore, goats possess thermo-tolerance, drought tolerance, disease resistant and ability to survive in scarce pastures which make them adaptable to any harsh situations, compared to other livestock species such as cattle, sheep and buffalo (Banerjee *et al.*, 2014). Goats have a wide range of ecological adaptability due to their anatomical and physiological characteristics (Silanikove, 2000). Goats are also considered to be the most suitable species from the climate change perspectives as they are tipped to ensure the sustainable food security of the poor and marginal farmers (Debele *et al.*, 2013). Breed differences was also established in goats for adaptation to heat stress and these differences were established both in phenotypic and genotypic traits (Sejian *et al.*, 2016). Indigenous breeds have higher thermo-tolerant capacity as compared to the cross bred and pure bred animals (Archana *et al.*, 2017). In a recent study on three different indigenous goat breeds, Salem Black goat was established to be better adapted breed to heat stress as compared to both Malabari and Osmanabadi breeds of goat (Pragna *et al.*, 2017). Similarly, increased RR in both Sirohi and Jhakrana breeds of goat as compared to Barbari breed while the higher RT in Barbari goat breed compared to both Sirohi and Jhakrana breeds of goat during heat stress clearly indicates the breed differences in adaptation within a species (Kumar *et al.,* 2017). Climatic stress of tropical and temperate extremes has helped to develop the hardy breeds which are resistant to the extreme stressful environmental conditions (Sejian *et al.*, 2016) and Sirohi, Barbari and Salem black are few well-known goat breeds which can tolerate extreme climatic conditions (Banerjee *et al.*, 2014; Pragna *et al.*, 2017; Rout *et al.*, 2013). Research efforts are needed for identifying the better adaptive and productive breed by testing these goat breeds for their survival in different agro ecological zones and these efforts might help in producing better climate resilient breeds in the changing climate scenario.

2.9 Malabari Goat

Malabari breeds are well adapted to the hot and humid conditions of Kerala state. Also, the goats are mostly inhabit in Calicut, Kannur, Waynad and Malappuram districts of Kerala state of India. This breed is supposed to have originated centuries back by breedıng with native feral goats and Arab, Surti and Mesopotamian goats along with the native goats of Western cost. They survived in a climate of hot and humid where average monthly temperature ranges from 23.7 to 30.7°C. The humidity ranges from 75 to 80%. Additionally, as per the 17th livestock census conducted in 2003 the population of goats in Kerala state was 12.22 lakh. Approximately, 10% of the goat population of the state belongs to Malabari breeds of goat. Malabari goats are reared primarily for the meat production. The Malabari breed of goat is famous for its low fat content meat and milk production (Radhika *et al.*, 2018). The coat color of these goats varies from complete white, white and brown and black with brown variations. About 40% of the goats have long hair and consist of elongated beard (Prasad *et al.*, 2018). The horns are slightly twisted, directed upward and backward sometimes curved downward touching the skin. Further, the muzzle is pinkish red and eye lids are pinkish white. The nose is straight and the ears are directed outwards and downwards which can be extended up to the nose. Malabari goats shown early maturity and conceive at an age of 8 to10 months. When goats are kept together the heat symptoms are exhibited by most of the goats together in the flock. The male starts breeding at an age of 9 to 12 months. Also, the average age at first kidding has been reported as 365 days while inter kidding interval is 258 days. In addition, the Malabari breed possess a good prolificacy at a rate of 50% twinning, 25% triplets and 5% quadruplets. Further, higher birth percentage has been reported more than 60% at All India Coordinated Research Projects centers for Malabari goats. However, there is no specific breeding and kidding season for Malabari goats. The milk yield varies from 0.5 to 1.5 liters/day and most of the milk has been used for feeding the kids. The study was undertaken to characterize the breed for its genetic adaptive mechanism, particularly HSP70 in different vital organs of Malabari goats during heat stress conditions.

2.10 Factors influencing expression patterns of different thermos-tolerant genes with special reference to climate or heat stress in livestock

India is a tropical country which consists of various types of climatic system and topographical area. Geographic location and climate are established to be the most crucial factors particularly affecting the homeostasis of an animal (Sejian *et al.*,2013). Additionally, the farmers practice extensive system of rearing and hence, the animals are exposed to the extreme hot and humid environmental conditions. Furthermore, climate of the particular region indirectly influence the disease outbreaks and thereby affect the immune system of the animals making it more vulnerable to major environmental extremities (Aleena *et al.*, 2016). Among the various thermo-tolerant genes, HSP70 is the predominant gene studied in wide varieties of farm animals. There are different factors which influence the expression pattern of HSP70 gene expression. Genetic factor is one of the primary factor which influence the expression pattern of HSP70 gene. In a recent study by Archana *et al.* (2018) the breed variations was established for the expression pattern of HSP70 in skeletal muscles of Osmanabadi and Salem Black goat breed. The expression pattern of HSP70 in heat stress group was similar to the control group animals in contrast to the significantly higher expression pattern of HSP70 in heat stressed group of Osmanabadi breed. Further, Shilja *et al.* (2018) established the role of optimum nutrition on the expression pattern of HSP70 in Osmanabadi goats. It was established in this study that when nutrition was not

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compromised, the animals were able to mount appropriate heat stress response in terms of exhibiting significantly higher expression pattern of HSP70 gene in PBMC of Osmanabadi goats. Further, the expression pattern of HSP70 gene also depends on the target tissue for its expression (Shilja *et al.*, 2016; Niyas *et al.*, 2017; Archana *et al.*, 2018). Similarly, the expression pattern of HSP70 gene also was found to be influenced by both age and sex in farm animals (Archana *et al.*, 2017).

2.11 Expression pattern of different thermo-tolerant genes in goats and other livestock

Cellular exposure to heat stress leads to number of abnormalities in the functioning of cells which aid to modify the cell functions, govern the metabolic process, induces oxidative cell damage and activates both apoptosis and necrosis pathways, finally results in the survival of different types of cells. The response of ruminant livestock to heat stress has been well documented (Archana *et al.*, 2017; Banergee *et al.*, 2015). Oxidative stress observed during summer in livestock animals is attributed to HS. In addition, the expression pattern of different types of thermo-tolerant genes and higher heat shock proteins (HSP) concentration are specified to be the vital response through which the cell sustains the effect of HS. Further, it helps in identifying as a full proof biomarker for assessing the severity of stress in different farm animals (Sejian *et al.*, 2013).

The HSP is one of the cellular proteins found most abundantly in animals during heat stress condition. The higher expression pattern of HSPs provide protection against hyperthermia, circulatory shock and cerebral ischemia during heat stress which signifies the crucial role of HSPs in cytoprotection (Archana *et al.*, 2017). The HSPs have chaperonic activity ensuring the folding, unfolding and refolding to avoid the denaturation proteins due to extreme heat stress. In addition, hydrophobic protein sequences liberated by denaturation gets bounded with the HSPs which otherwise would interact with other neighbour proteins resulting in loss of protein function.

Oxidative stress affects the antioxidant defense resulting from overproduction of free radicals and reactive oxygen species (ROS). Likewise, the increase in ROS production, especially the superoxide anion is mainly observed during heat stress. Also, the severity of heat stress influences the Thiobarbituric acid-reactive species (TBARS) and Malondialdehyde (MDA) levels in broilers, buffalos and dairy cows which are the major products synthesized through lipid peroxidation (Archana *et al.*, 2016). Antioxidant enzymes activities, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were observed to increase in heat stress in livestock (Birben *et al.*, 2012). Elevated concentration of SOD and GPX concentration was significantly higher in prepartum cow during summer season (Awadeh *et al.*, 1998). Antioxidant activity studied during winter and summer season in growing calves, heifers and lactating Murrah buffaloes shown significantly higher concentration of GPX in all three groups during summer season.

The biological function of NOX proteins has been characterized in various farm animals. Reactive oxygen species (ROS) are important mediator of cell growth, adhesion, differentiation, migration and apoptosis. NADPH oxidases of the NOX family play diverse role in different biological processes, such as host defense, signal transduction and hormone synthesis (Leto and Geiszt, 2006). In addition, NOX family genes play diverse role in different pathway as signaling molecules in cellular response to growth factor, cytokines and hormones. Also, higher expression pattern of ENOX2 gene was established in animals during heat stress. In addition, the Jamunapari goats shown higher expression of ENOX 2 gene as compared to Sirohi breeds. This could be due to the better adaptive capability of Sirohi breeds to heat stress as compared to Jamunapari breeds. Also, the expression pattern of ENOX 2 gene was tissue and breed specific. NOX isoforms have been observed in different vascular tissues.

The tropical cattle breeds such as the Senepol, Carora, and Romosinuano have adapted to hot and humid climate through natural selection toward a short, fine sleek hair coat which helps the animal to maintain lower body temperatures under heat stress

(Olson *et al.*, 2003). Generally, slick-haired Criollo Limonero exhibit fewer hair follicles and shorter hair than Bos indicus cattle as well as increased sweat glands (Landaeta-Hernandez *et al.*, 2011). Moreover, there are potential SNPs and haplotype blocks as new diagnostic markers for identifying the state of inheritance of SLICK for guided breed management (Gautier *et al.*, 2012). Identifying SKP2 as a candidate gene lends new insight into the potential mechanisms for keratinocyte development or SPEF2 involvement in signal transmission and their potential influence on thermo-regulation in tropical cattle

Heat stress would cause alterations in the expression pattern of several genes while persistent heat stress potentiates this effect to survive the animals in harsh climatic conditions. Heat stress response is a complex phenomenon which involves alternation in gene expression profiling and immune response (Sharma *et al.*, 2013). Toll like receptors (TLRs) are mostly expressed on antigen presenting cells like monocytes, macrophages, dendritic cells and B lymphocytes (Vandana *et al.*, 2018). The TLRs recognize microbial markers like protein, carbohydrate, lipid, nucleic acids and which have a unique combination, further helps in the complex signaling mechanisms to activate a wide variety of transcription factors and inflammatory cytokines (Takeda and Akira, 2003). The TLR mRNAs expression patterns have been demonstrated in ruminant livestock (Sophia *et al.*, 2016; Vandana *et al.*, 2018). Heat stress also modulates the expression pattern of pro-inflammatory cytokines like IL 2 mRNA expression of lymphocytes in poultry species (Vandana *et al.*, 2018). It has been well established that, TLRs play a vital role in the activation of innate immune response during heat stress in mice and goat (Paul *et al.*, 2015). Among all TLRs, TLR 2 and TLR 4 recognize the damage-associated molecular patterns (DAMPs) to produce several pro-inflammatory cytokines to induce the host immune response during thermal stress (Kawai and Akira, 2010). Heat stress also modulates the expression pattern of pro-inflammatory cytokines such as IL-2 mRNA expression of lymphocytes inpoultry species (Han *et al.*, 2010).

2.12 Heat shock protein as ideal biological marker for quantifying heat stress response in goat

Heat shock proteins are important from cellular adaptation to heat stress perspectives in different farm animals (Archana *et al.*, 2016). The regulation and synthesis of HSP is influenced by both environmental and genetic factors (Liu *et al.*, 2011). Further it is very essential to, understand the role of HSPs in farm animals as a potential indicator for assessing the severity of heat stress (Mishra and Palai, 2004). The main advantage of HSP70s as a molecular biomarker is that it can be used for assessing the negative impacts of various stresses which compromises the growth and reproductive efficiency in different animals (Werner and Nagel, 1997). Further, sex and age of the livestock species can induce variations in expression levels of HSP70 (Rout *et al.*, 2014). In addition, the identified characteristics of HSP70 can be incorporated in the future genetic breeding programmes for producing genetically sound breeds. Also, understanding the underlying mechanisms of HSP70 associated with different immune regulatory process could help to develop appropriate disease controlling measures (Sorensen *et al.*, 2003). Furthermore, the genetic markers governing cellular adaptation are more applicable than the phenotypic traits to stress in the breeding programs (Archana *et al.*, 2016). The various research findings pertaining to HSP70 gene expression signifies the importance of this gene in livestock adaptation during different climatic conditions.

2.13 Expression pattern of HSP70 in different organs in goat or livestock

The HSP70 is one of the most abundant and highly characterized heat shock protein family which gets expressed in response to different stressors, and plays a crucial role in environmental stress tolerance and adaptation in farm animals (Banerjee *et al.*, 2014). The expression pattern of HSP70 was studied in various organs in livestock species. The HSP70 gene expression level was significantly higher in the testes of yak, followed by the brain, kidney, heart, lungs and liver. Also, the expression in spleen was found to be very less. In addition, the highest expression levels of the HSP70 gene and proteins were shown in the kidney, heart and cerebellum of yak (Lio *et al.*, 2017). The higher production of HSP70 has been shown to protect against cerebral vascular atherosclerosis, myocardial injury and other cellular damage due to various stresses (Deb *et al.*, 2015). There are literatures which indicate that, increased expression pattern of HSP70 was also observed in liver exposed to different stress conditions (Kiemer *et al.*, 2002).

In addition, HSPs are synthesized by cells in response to a variety of stimuli, including oxidative, metabolic and chemical stress (Somani *et al.*, 2017). It has been suggested that the expression of HSP70 was significantly higher in farm animals during summer season as compared to the winter in tropical regions, indicating its crucial role in animals for surviving in harsh climatic conditions (Dangi *et al.*, 2012). Likewise, HSP70 expression was higher in the liver and kidney for sustaining the regular body mechanisms. Similarly, the higher expression pattern of HSP70 was found in heart and kidney of goats during transportation stress (Zulkifli *et al.*, 2010). The up regulation of HSP70 expression at the cellular level is a contributing factor for better meat quality in goat as it provides protection to muscle glycogen content and thereby influences meat quality in ruminants (Rout *et al.*, 2016). However, further research efforts are needed in this field to establish the underlying biological mechanisms by which HSP70 influences the meat quality characteristics.

The expression pattern of HSP70 was also studied in Sirohi and Barbari breeds of goats and both the breeds has shown almost similar pattern for HSP70 gene expression (Banerjee *et al.*, 2014). In a comparative study, the Sirohi breed exhibited the higher mRNA level of HSP70 gene expression indicating the better adaptive capability as compared to the other breeds in spite of the four breeds being maintained under similar climatic conditions and feeding strategies. Likewise, HSP70 mRNA expression in the liver and kidney were significantly higher than the other tissues of goats (Nagayach *et al.*, 2017). The HSP70 mRNA expression pattern varied in different tissues of Barbari and Jamunapari goat breeds in summer and winter seasons indicating their season specific response to heat stress. This shows that the HSP70 mRNA expression is breed and tissue specific (Nagayach *et al.*, 2017). In another study, HSP70 protein concentrations in 22 tissue extracts of different organs were analyzed by ELISA and the concentration of HSP70 was found to be significantly higher in liver, kidney and heart followed by brain, spleen, lungs and testis. Similarly, kidney, liver and heart shown 1.5-2.0 fold higher HSP70 mRNA gene expression compared to the other organs. Conversely, Rout *et al.* (2016) reported that the breed and sex had no significant effect on HSP70 mRNA concentration in different organs during heat stress condition. However, Gerich *et al.* (2001) established that HSP70 gene can play a crucial role in controlling hepatic gluconeogenesis during heat stress exposure. Similarly Dangi *et al.* (2012) also established the expression pattern of HSP70 gene in tropical region goats (Dangi *et al.*, 2012). Further, there are reports which suggested that HSP70 could be used as an ideal biomarker for assessing the severity of heat stress in farm animals (Mishra *et al.*, 2014; Dangi *et al.*, 2012; Rout *et al.*, 2016).

In an another study, Shilja *et al.* (2017) established the HSP70 mRNA expression to be significantly higher in Osmanabadi goats exposed to different environmental stresses individually and in combination Similar result of nutritional stress induced the increase in HSP70 expression was also reported in laboratory animals (Aly *et al.*, 1994; Nadeau *et al.*, 2001). These results confirm that these changes were independent of hyperthermia indicating the role for HSP70 in any type of stress. In addition, the higher expression pattern of HSP70 was established during summer season (2.37 ± 0.12) compared to winter season (0.29 ± 0.04) in buffaloes (Aleena *et al.*, 2016).

2.14 Closing remarks

The above literature review clearly suggests that there are lot of research efforts are still needed to understand the basic molecular mechanisms which governs the adaptive capacity of farm animals. The ever changing climate puts the livestock production at stake and there is huge pressure on the researchers to develop research methodologies to impart greater resilience capacity to the farm animals in particular to improve livestock production. Developing suitable molecular biomarkers to quantify heat stress response and better resilience capacity may help to develop livestock breeds with extreme adaptive capacity to withstand the adverse impact of climate change. More research efforts are also needed further to establish the hidden intricacies associated with HSPs to provide a better understanding on the molecular mechanisms associated with livestock adaptation. As goat being projected as ideal climate change animals such research efforts in this species may help to sustain livestock production in testing environmental condition. This can help to fine tune the existing breeding policies with the use of more intense and appropriate molecular markers to provide greater resilience capacity to these animals which can help them to withstand the adversities associated with environmental stresses.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

The experiment was conducted in the experimental livestock unit of the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India located on latitude 77°36'25.3"E, longitude 12°57'04.3"N and altitude of 920 m above mean sea level. The mean annual maximum and minimum ambient temperature of this regime ranges between 15 to 36°C respectively. The mean annual relative humidity ranges from 20 to 85%. The average annual precipitation in this region varies between 200 to 970 mm with erratic distribution. The average annual minimum and maximum temperature ranges between 15-22°C and 27-34°C respectively. The study was conducted during the month of April to May. The maximum-minimum temperatures, relative humidity, dry and wet bulb temperature, pen surface temperature and temperature-humidity index (THI) during the study period (45 days) are listed in figure 1. THI was calculated by the formula described by McDowell (1972).

3.2 Animals

A total of 12 ten months to one year old Malabari female goats weighing between 12- 19 kg were used. Malabari goat breed is a meat purpose animal originated in the humid tropical region of southern India. The animals were brought from different locality and acclimatized to the current experimental location for a period of 45 days. These animals were maintained in well ventilated sheds following standard farm management procedures.

3.3 Experimental design

The study was conducted for a period of 45 days. Twelve animals were used in this study. The animals were randomly allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The animals were stall fed with a diet consisting of 60% roughage (Hybrid Napier) and 40% concentrate (Maize 36kg, wheat bran 37kg, soybean meal 25kg, mineral mixture 1.5kg, common salt 0.5 kg/ 100kg). The chemical compositions of diet offered to the experimental animals are described in table 1. The MC animals were maintained in the shed in thermo-neutral condition while MHS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h during the experimental period. The MC animals were fed and watered inside the shed while MHS animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily both inside and outside the shed throughout the study period. The animals were slaughtered at the end of the study and their adrenal, thyroid, liver, uterus and mesenteric lymph node samples were collected for gene expression study. The study was conducted after obtaining approval from the institute ethical committee for subjecting the goats to summer heat stress.

3.4 Weather parameters recording

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

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

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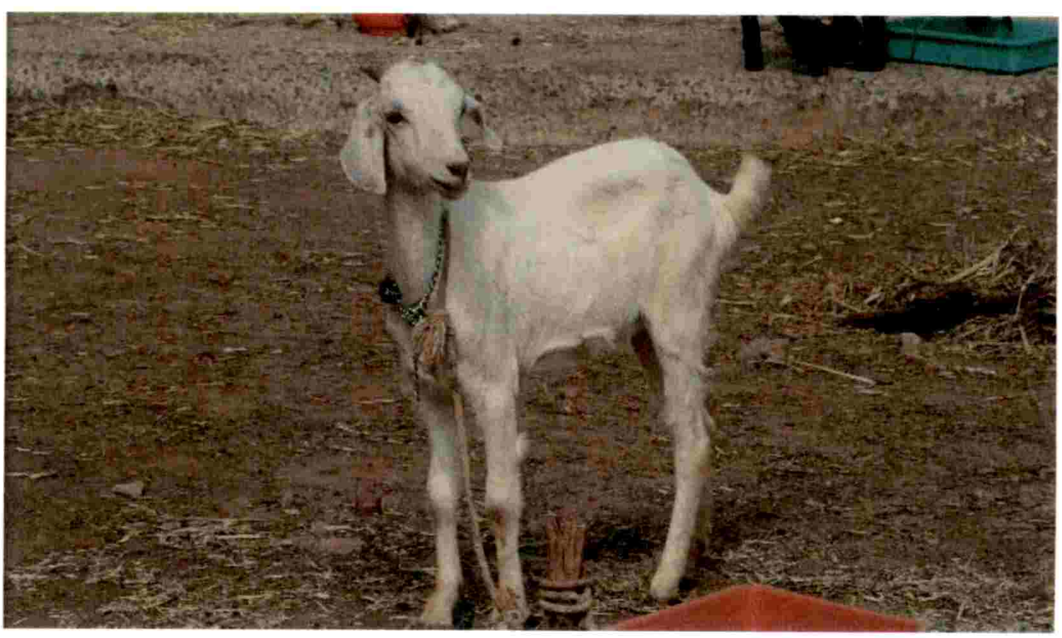
Plate 3.1: Pictorial representation of Malabari goat kept outside the shed

Plate 3.2: Pictorial representation of Malabari goat kept inside the shed



3.5 Expression Pattern of HSP70 mRNA in Adrenal, Thyroid, Liver, Uterus and Mesenteric Lymph Node

3.5.1 Principle

Samples are lysed and homogenized in lysis buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous 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 was spun through the column. Subsequently, impurities are effectively removed from them membrane by washing the column with wash buffers. Pure RNA was then eluted under low ionic strength conditions with nuclease-free waters

3.5.2 Sample collection and storage

The adrenal, thyroid, liver, uterus and mesenteric lymph node samples were collected from all the animals in each group immediately after slaughter. Further, the samples were cut into small pieces, washed in Phosphate Buffered Saline and immersed in RNA buffer (Zymo Research, USA) and snap chilled in LN2. Then samples were stored at - 80 °C till further use.

3.5.2 Sample preparation for RNA isolation

After thawing, the tissues were removed from the RNA shield (Zymo Research, USA) and immediately processed for RNA isolation. The total RNA was isolated from tissues using the Gene JET 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 with a Cole-Parmer LabGEN DTH homogenizer in lysis buffer and liquid nitrogen (–196 °C). After homogenization, 300μL of lysis buffer supplemented with β-mercaptoethanol (10μL/ml) in 2.0 ml DNase, RNase free micro centrifuge tube. To the lysate, 10μL of proteinase K in 590μL of Tris Ethylenediaminetetraacetic Acid buffer was added, then vortexed and incubated

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at 15-25 °C for 10 min. Then, the contents were centrifuged for 8 min at 12,000 g and the supernatant was transferred into a new RNase-free micro centrifuge tube. 450μL of ethanol was added and mixed well by pipette. Then 700μL of was transferred to a spin column with a 2 ml collection tube and centrifuged for 1 min at 12,000 g. The process was continued till all the lysate were allowed to move through the column. After discarding the flow through, 700μL of wash buffer 1 was added and centrifuged for 1 min at 12,000 g followed by two time washing with 600 and 250μL of wash buffer 2 followed by centrifugation at 12,000 g for 1 and 2 min, respectively. About 40μL of warm nuclease-free water was added to the membrane, and centrifuged at 10,000 rpm for 1 min to elute RNA. The purified RNA samples were stored at -80 °C until cDNA synthesis.

3.5.6 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 (20 U/μL, Invitrogen, USA) was added. After DNase treatment quality and quantity of the isolated RNA was analysed using Nano drop spectrophotometer. Integrity of the total RNA was checked using denaturing agarose gel (1%) electrophoresis and visualization under UV light. Two intact bands of 28 s and 18 s indicated good quality and intactness of RNA.

3.5.7 cDNA synthesis

The total RNA was reverse transcribed into cDNA using Maxima first strand cDNA synthesis kit for Real Time quantitative polymerase chain reaction (RTqPCR) (Thermo Scientific, Lithuania). The procedure was performed as per manufacturer's protocol with modifications are as follows: 4μL of 5x Reaction Mix, 2μL Maxima Enzyme Mix, 1μg of Template RNA for adrenal samples, while 1.5μg of Template RNA was used for liver sample and 20μL 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 in qPCR.

3.5.8 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/). The preferences were given to the primers binding to the exon-exon junction. The efficiency of the primers were checked using PCR EFFICIENY - an open source PCR efficiency prediction (Mallona *et al.*, 2011). The primers were titrated with different concentrations (10, 5, 2.5 and 1µM) for selecting optimum concentration to be used for qPCR experiments. Table 2 describes the primers used for gene expression studies. Primers used for amplifying the target regions of various genes are given in the Table 2 (published primers and newly designed primers).

3.5.9 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 20µL reaction was done in duplicates using 50 ng of template and 0.5µM primer concentrations. The real time qPCR reaction conditions were: enzyme activation at 95°C for 10 min and amplification cycle (40 cycles; initial denaturation at 95°C for 15 s, annealing at 60°C for 30sec and extension at 72°C for 30 s). The melt curve analysis was performed to check the non-specific amplification. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control, and the relative expression was analysed using the formula, 2ΔΔCT (Shilja *et al.* 2017).

3.5.10 Statistical analysis

The changes in relative expression of different genes in relation to the reference gene were analysed using SPSS (16.0) software using one-way analysis of variance (ANOVA). The significance level was set at $P<0.05$. The R^2 values were used to establish the correlation association between THI and various genetic traits with two levels of statistical significance set at $P<0.01$ and $P<0.05$

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Table 2: Primers used for Adrenal, Thyroid, Liver, Uterus and Mesenteric Lymph Node HSP70 mRNA gene expression. GAPDH used as reference gene to normalize the gene expression of target genes

Gene ID	Primers	Primer sequence (5″–3″)	Primer length (bp)	Product size (bp)	Accession No.
HSP70	F	TGGCTTTCACCGATACCGAG	20	167	NM001285703.1
	R	GTCGTTGATCACGCGGAAAG	20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG	20		

Note: bp – base pair; F – forward; R – reverse; GAPDH – Glyceraldehyde 3-phosph

RESULTS

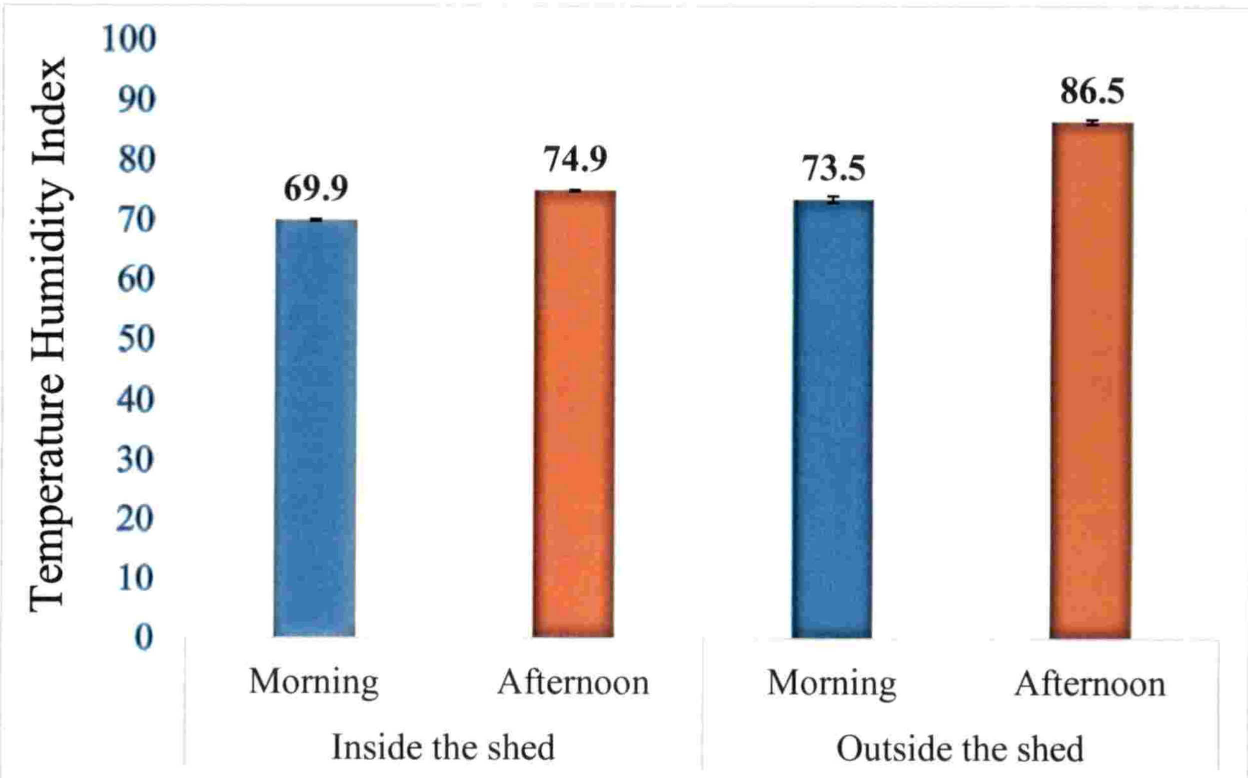
CHAPTER 4

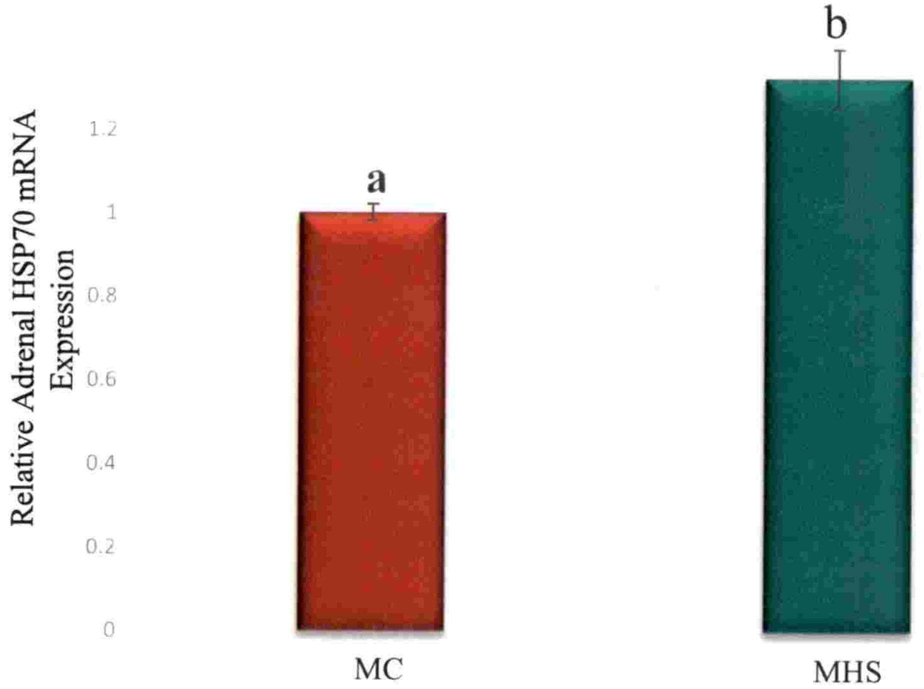
RESULTS

4.1 The THI Index

The THI values to depict the level of heat stress are depicted in Fig. 4.1. The THI values both inside and outside the shed in the morning are 69.9 and 73.5 respectively while in the afternoon the values were 74.9 and 86.5 respectively. The THI index inside shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant (P<0.01). The THI values description as per McDowell (1972) are: the values 72 and less are considered comfortable; THI values from 75-78 are considered stressful and THI above 78 considered extreme distress.

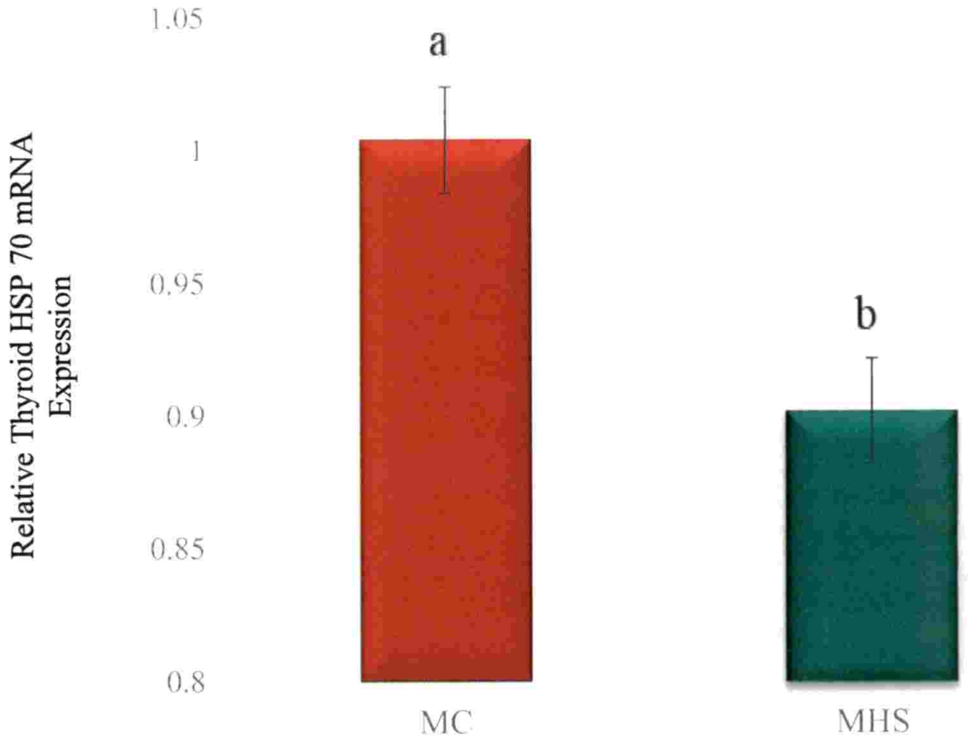
Fig.4.1: Description of average temperature- humidity index between both inside and outside the shed during the experimental period





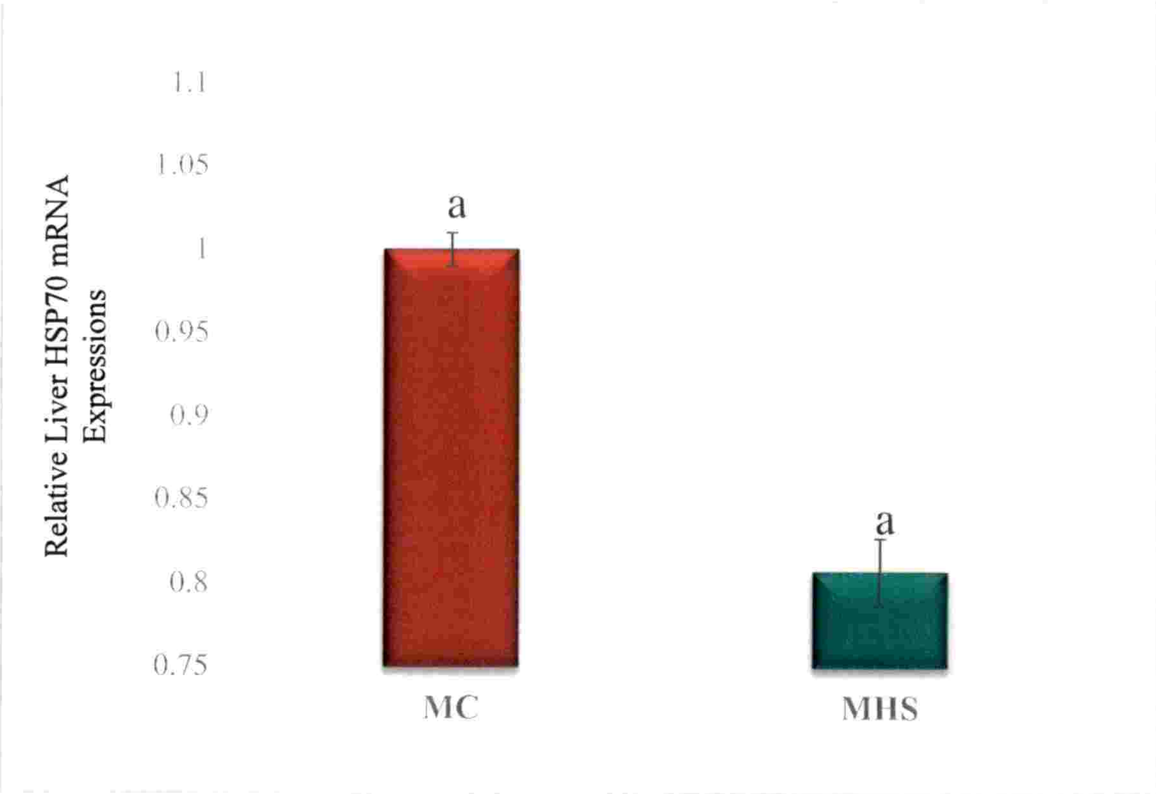
4.2 Relative adrenal HSP70 mRNA expression pattern between Controlled and Heat stressed group

Effect of heat stress on adrenal HSP70 mRNA expression in Malabari goats are depicted in Fig 4.2. The fold changes in expression pattern of adrenal HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of adrenal HSP70 in heat stress group was significantly up regulated in heat stress group as compared to the control group animals. Further a strong positive correlation ($P<0.05$) was obtained between THI and Adrenal (table 3).



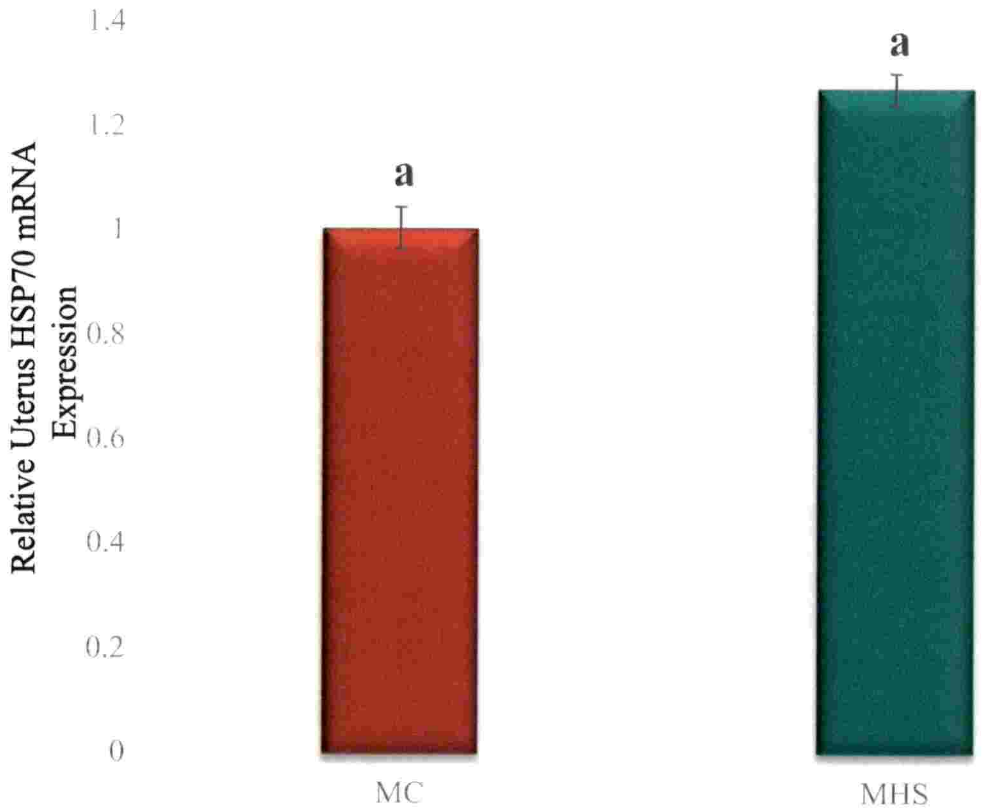
4.3 Relative thyroid HSP70 mRNA expression pattern between controlled and heat stressed group

Effect of heat stress on thyroid HSP70 mRNA expression in Malabari goats are depicted in Fig.4.3. The fold changes in expression pattern of thyroid HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of thyroid HSP70 in heat stress group was significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Further a strong negative correlation ($P<0.05$) was obtained between THI and Thyroid gland (table 3).



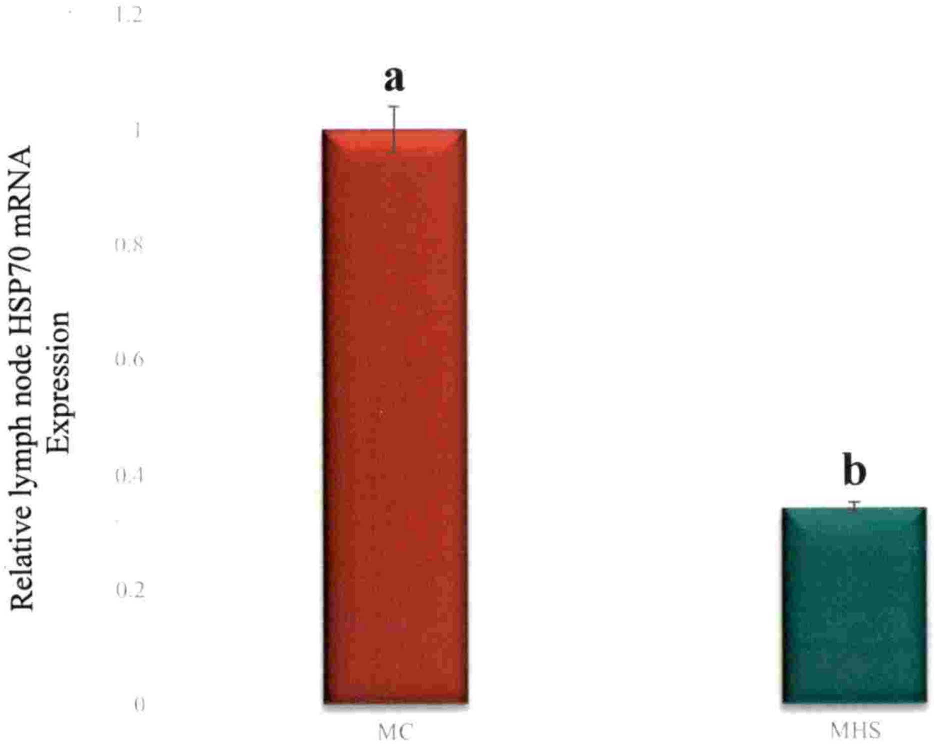
4.4 Relative hepatic HSP70 mRNA expression pattern between controlled and heat stressed group

Effect of heat stress on hepatic HSP70 mRNA expression in Malabari goats are depicted in Fig.4.4. The fold changes in expression pattern of hepatic HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Although, the expression pattern of hepatic HSP70 in heat stress group showed decreasing trend as compared to the control group, still the differences between the groups were not statistically significant. Further a strong positive correlation ($P<0.01$) was obtained between THI and Liver (table 3).



4.5 Relative Uterus HSP70 mRNA expression pattern between controlled and heat stressed group

Effect of heat stress on uterus HSP70 mRNA expression in Malabari goats are depicted in Fig 4.5. The fold changes in expression pattern of uterus HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of uterus HSP70 was non-significant between MC and MHS groups. Further a positive correlation was ($P<0.05$) obtained between THI and Uterus (table 3).



4.6. Relative mesenteric lymph node HSP70 mRNA expression pattern between controlled and heat stressed group

Effect of heat stress on Lymph node HSP70 mRNA expression in Malabari goats are depicted in Fig 4.6. The fold changes in expression pattern of lymph node HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of lymph node HSP70 in heat stress group was significantly (P<0.05) down regulated in heat stress group as compared to the control group animals. Further a strong negative correlation (P<0.01) was obtained between THI and mesenteric lymph node (table 3).

Table 3: Correlation association between THI and HSP70 mRNA gene expressions

	THI	Adrenal HSP70	Thyroid HSP70	Hepatic HSP70	Uterus HSP70	MLN HSP 70
THI	1					
Adrenal HSP70	0.800*	1				
Thyroid HSP70	-0.837*	-0.508	1			
Hepatic HSP70	0.982**	-0.673	0.868*	1		
Uterus HSP70	0.820*	0.939**	-0.714	-0.713	1	
MLN HSP70	-0.982**	-0.810	0.898*	0.955**	-0.884*	1

THI- Temperature humidity index; MLN- Mesenteric Lymph Node; HSP70- Heat shock protein 70; **Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

DISCUSSION

CHAPTER 5

DISCUSSION

Climate change is expected to have far reaching consequences in global food security. With the growing human population expected to be around 9.6 billion by 2050, the scientific community are under tremendous pressure to find solution to the problem. Livestock offers huge scope to ensure the food security in the future and small ruminants, in particular goats considered ideal climate resilient species which can contribute immensely in meeting the future food demands. Therefore, efforts are needed to identify local goat breeds which are efficient meat producers by countering the adversities associated with climate change. Efforts are needed to identify a climate resilient breed which apart from adapting to the environmental conditions must produce optimally to sustain production in the face of changing climate. Much of the research efforts needed preferably to explore the germplasms of local breeds which are efficient in adapting to the existing environmental conditions by acquiring their adaptive potential over generations. In this line, the current experiment is an important step towards identifying an indigenous goat breed which is well known for meat production in Southern India. Although considered well adapted to the existing hot humid tropical condition, still information are lacking at molecular level to establish its adaptive potential. The HSP70 is considered confirmatory biological marker for quantifying heat stress response which imparts the adaptive potential for an animal to survive the stress (Banerjee *et al.*, 2014; Mohanarao *et al.*, 2014; Shilja *et al.*, 2016). However, reports pertaining to establishing the HSP70 expression pattern which can give clue about the adaptive nature of indigenous animals are very meagre and these all the type of efforts needed to identify agro-ecological zone specific breed to sustain their production during adverse environmental condition. The results of this experiment are an important step towards establishing this hypothesis.

The THI index followed in the study clearly established the heat stress for the animals as any cumulative value above 75 as per McDowell (1972) model was considered extremely severe heat stress to animals and with the THI value of 86.5 recording during outside exposure in MHC group clearly indicated that these animals were subjected to extremely severe heat stress. This justifies the hypothesis of studying the HSP70 expression pattern in different vital organs in Malabari goats during heat stress exposure.

HSP70 was found to be the predominant HSPs getting expressed in adrenal gland under the influence of adreno-corticotropic hormone (ACTH) (Udelsman *et al.*, 1993). There are reports which suggest key role for HSP70 during heat stress in goat (Banergee *et al.*, 2014; Dangi *et al.*, 2014; Shilja *et al.*, 2016). HSP70 is one of the most abundant and best characterized heat shock protein family that consists of highly conserved stress proteins, expressed in response to stress, and plays crucial roles in environmental stress tolerance and adaptation in goat (Gupta *et al.*, 2013; Banerjee *et al.*, 2014; Mohanarao *et al.*, 2014).

The HSP70 gene expression pattern was found to be up regulated in the adrenal gland of MHS group as compared to the MC group. Similar to our finding Shilja *et al.*, (2017) also reported higher expression pattern of HSP70 in the adrenal tissue of heat stressed Osmanabadi goats. Adrenal gland is the primary stress relieving organ in animals and is the classical component of HPA pathway which ultimately culminates in release of glucocorticoids. Due to hyperactivity of adrenal gland to meet the increased glucocorticoid requirement, one would expect damage to the cellular component of adrenal gland triggering the release of more HSP70 for tissue damage control. This could be the reason for increased HSP70 expression pattern in adrenal gland in the current study. Enhanced HSP70 expression in the HS group may be a response to stressful environments and may improve cell survival by protecting proteins from degradation and facilitating their refolding (Dangi *et al.*, 2014). Further, Dangi*et al.* (2014) reported that HSP70 could play an important role during the initial phase of

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HS acclimation in goats. Further, a positive correlation of THI with adrenal HSP70 gene expression indicates the over functioning of adrenal gland and thereby reflects the higher HSP70 requirement for repairing of cellular damage.

In contrast to the adrenal HSP70, the expression pattern of the same in thyroid gland was found to be lower in MHS group as compared to MC group. This differential expression between the glands could be attributed to the functional differences of these glands. Adrenal glands are expected to go for hyperactivity mode to meet the increased demands of glucocorticoids while thyroid gland was expected to reduce its activity during heat stress exposure in an effort to reduce the metabolic heat production to cope with the hot external environment. Therefore, as a result of reduced activity in thyroid gland one would expect lower damage to its tissue at cellular level reflecting low HSP70 requirement for its repair. This could be the reason for the reduced HSP70 expression thyroid gland in the current study. No literatures are available to compare our results and expression of HSP70 in the thyroid tissue is the first report available in heat stressed livestock. This signifies the importance of the study by highlighting the expression pattern of HSP70 based on the functional differences of the tissues. A negative correlation established between THI and thyroid HSP70 gene expression clearly indicates the lower activity of thyroid gland in an effort to produce less metabolic heat.

There are reports signifying the importance of expressing HSP70 in liver due to its primary involvement in glucose metabolism and glucocorticoids favors hepatic gluconeogenesis to ensure regular glucose supply to vital organ functioning (Gerich *et al.*, 2001; Nagayach *et al.*, 2017). The hepatic mRNA expression pattern of HSP70 gene did not differ between MC and MHS groups. This was in contrast to the finding reported by Shilja *et al.*, (2017) in Osmanabadi goats. These authors established significantly higher HSP70 expression pattern in heat stressed Osmanabadi breed as compared to the control animals kept in comfort zone. This difference in these studies could be attributed the differences in their adaptive potential. This Malabari goat breed was established to be well known for their survival in hot and humid tropical environment. The better adaptive potential of Malabari breed could have evinced less physiological strain on the liver resulting in lower cellular damage reducing the demand for more HSP70 requirement. This could be the reason for the reduced hepatic HSP70 expression pattern in heat stressed Malabari goats. Similarly, Rout *et al.* (2016) also reported significantly higher level of HSP70 expression in liver of Barbari, Jakhrana, Jamunapari and Sirohi breeds of goats. Similar to our finding, these authors also correlated the expression pattern of hepatic HSP70 to adaptive potential of these breeds. Likewise, Banerjee *et al.* (2014) also conducted a similar study and reported significantly higher expression pattern recorded in Barbari breed and lower expression in Sirohi breed. This difference was attributed to their adaptive capacity as Sirohi was established to be better adapted breeds than Barbari breed. This indicates breed differences in the expression pattern of HSP70 in liver of different indigenous goats. Further, Nagayach *et al.* (2017) also conducted similar study in Barbari and Sirohi goats and observed that the hepatic HSP70 expression pattern was both breed and tissue specific. Further, a strong positive correlation of THI with hepatic HSP70 expression reflects the extreme adaptive nature of Malabari breed.

The uterine HSP70 mRNA expression pattern was comparable between MC and MHS groups in the current study. This could be attributed to the non-requirement of more HSP70 at uterine level because reproduction is not a vital function during adverse environmental condition. Growth and reproductive performances are generally compromised in livestock in an attempt to cope with heat stress challenges and this is brought about by regular energy and blood supply to vital organs which helps the animal to survive the stress (Sejian *et al.*, 2011). As a result of reduced functioning of reproductive organs, not much cellular damage is expected in these tissues reducing the demand for HSP70 to involve in cellular repair. This could be the reason for no difference in expression pattern of uterine HSP70 gene in the current study. There are no reports on heat stress induced changes in the expression pattern of HSP70 gene in uterine tissue of livestock to compare our results. It's a general observation that estradiol in its presence increases the HSP70 expression in uterus (Wu *et al.*, 1996). Further, estradiol was observed to be significantly lower in heat stress animals (Sejian *et al.*, 2011). This reduced estradiol concentration as a result of heat stress in the current study could be the reason for no increase in uterine HSP70 expression in goats.

Heat stress due to the increased production of glucocorticoids was found to suppress the immune response in goats (Paul *et al.*, 2015; Inbaraj *et al.*, 2016). Heat stress significantly down regulated the expression pattern of LN HSP70 gene in heat stressed Malabari goats. This is again an indication that the immune response is getting compromised during heat stress condition. Further, a strong negative correlation of THI with HSP70 expression clearly indicates the compromised immune response in heat stressed Malabari goats. Since functioning of LNs are not vital for animal survival one would expect reduced blood supply for functioning of lymph nodes. As a result of reduced functionality of these LNs the cellular damage would be less in these organs reducing the requirement for HSP70 to induce cellular repair. This could be the reason for reduced HSP70 expression in LNs of heat stressed Malabari goats.

SUMMARY AND CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

Understanding the regulation of heat stress at the molecular level andassessing the expression pattern of HSP70 gene in vital organs could pave way for establishing the underlying biological mechanisms by which the productive responses are compromised to support life sustaining activities in goats. Further, these attempts should target indigenous breeds as they play vital role in determining the livelihood securities of poor and marginal farmers in developing countries in particular. Malabari breed of goats are meat breed well known for their ability to survive and produce optimally in the hot humid tropics of Southern India and it plays a significant contribution to the economy of the local farmers. However, there are negligible efforts in establishing their adaptive potential and how they maintain production in their native track. Therefore, an attempt has been in this study to establish the expression patterns of HSP70 in some vital organs of indigenous Malabari goats in an effort to establish its role in maintaining the superiority of this breed to survive in harsh climatic condition

The study was conducted for a period of 45 days. Twelve animals were used in this study. The animals were randomly allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The MC animals were maintained in the shed in thermo-neutral condition while MHS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h during the experimental period. The MC animals were fed and watered inside the shed while MHS animals were fed and watered while they are exposed to summer heat stress in the outside environment. All cardinal weather parameters were recorded twice daily both inside and outside the shed throughout the study period. The animals were slaughtered at the end of the study and their adrenal, thyroid, liver, uterus and mesenteric lymph node samples were collected for gene expression study.

The THI values both inside and outside the shed in the morning are 69.9 and 73.5 respectively while in the afternoon the values were 74.9 and 86.5 respectively. The THI values for the entire study duration during morning were not stressful to the animals kept both inside and outside the shed. However, the obtained THI values ($P<0.01$) during afternoon indicated that the animals inside the shed were not stressed while the animals kept outside the shed were under extreme distress.

The fold changes in expression pattern of adrenal HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of adrenal HSP70 in heat stress group was significantly up regulated in heat stress group as compared to the control group animals. Adrenal gland is the primary stress relieving organ in animals and is the classical component of HPA pathway which ultimately culminates in release of glucocorticoids. Due to hyperactivity of adrenal gland to meet the increased glucocorticoid requirement, one would expect damage to the cellular component of adrenal gland triggering the release of more HSP70 for tissue damage control. This could be the reason for increased HSP70 expression pattern in adrenal gland in the current study. Further, a positive correlation of THI with adrenal HSP70 gene expression indicates the over functioning of adrenal gland and thereby reflects the higher HSP70 requirement for repairing of cellular damage.

The fold changes in expression pattern of thyroid HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. Further, the expression pattern of thyroid HSP70 in heat stress group was significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. In contrast to the adrenal HSP70, the expression pattern of the same in thyroid gland was found to be lower in MHS group as compared to MC group. This differential expression between the glands could be attributed to the functional differences of these glands. Therefore, as a result of reduced activity in thyroid gland one would expect lower damage to its tissue at cellular level reflecting low HSP70 requirement for its repair. This could be the reason for the reduced HSP70 expression thyroid gland in the current study.

A negative correlation established between THI and thyroid HSP70 gene expression clearly indicates the lower activity of thyroid gland in an effort to produce less metabolic heat.

Although, the expression pattern of hepatic HSP70 in heat stress group showed decreasing trend as compared to the control group, still the differences between the groups were not statistically significant. This Malabari goat breed was established to be well known for their survival in hot and humid tropical environment. The better adaptive potential of Malabari breed could have evinced less physiological strain on the liver resulting in lower cellular damage reducing the demand for more HSP70 requirement. This could be the reason for the reduced hepatic HSP70 expression pattern in heat stressed Malabari goats. Further, a strong positive correlation of THI with hepatic HSP70 expression reflects the extreme adaptive nature of Malabari breed.

The fold changes in expression pattern of uterus HSP70 gene expression in both control and heat stress groups are 1.0 and 0.3 respectively. The uterine HSP70 mRNA expression pattern was comparable between MC and MHS groups in the current study. This could be attributed to the non-requirement of more HSP70 at uterine level because reproduction is not a vital function during adverse environmental condition. As a result of reduced functioning of reproductive organs, not much cellular damage is expected in these tissues reducing the demand for HSP70 to involve in cellular repair. This could be the reason for no difference in expression pattern of uterine HSP70 gene in the current study.

Heat stress significantly down regulated the expression pattern of LN HSP70 gene in heat stressed Malabari goats. This is again an indication that the immune response is getting compromised during heat stress condition. Further, a strong negative correlation of THI with HSP70 expression clearly indicates the compromised immune response in heat stressed Malabari goats. Since functioning of LNs are not vital for animal survival one would expect reduced blood supply for functioning of lymph nodes. As a result of reduced functionality of these LNs the cellular damage would be less in these organs reducing the requirement for HSP70 to induce cellular repair. This could be the reason for reduced HSP70 expression in LNs of heat stressed Malabari goats.

Overall from the results obtained it was evident that the expression pattern of HSP70 gene was tissue and function specific in Malabari goats. The expression pattern of HSP70 gene was programmed in Malabari goats according to the requirement of the heat stressed animals. For example, in vital organ like adrenal which is very important for animal survival, the HSP70 expression pattern was higher while in productive organs which are not playing a role in animal survival such as lymph node the expression pattern was significantly lower. The non-significant influence of heatstress on the expression patterns of HSP70 in both liver and uterus reflects the extreme adaptive nature of Malabari breed to heat stress challenges. Further, a strong positive correlation between THI and HSP70 gene expression in adaptive organs such as adrenal and liver reflects the extreme adaptive nature of this breed to heat stress. Further, the lower expression pattern of thyroid HSP70 in heat stressed Malabari goats also reflects its superior adaptive capability to heat stress by reducing the metabolic heat production to cope with harsh outside environmental condition.

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ABSTRACT

HEAT SHOCK PROTEIN 70 EXPRESSION IN DIFFERENT VITAL ORGANS OF HEAT STRESSED MALABARI GOATS

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THESIS

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ABSTRACT

The study was conducted to establish the impact of heat stress on the expression pattern of heat shock protein 70 (HSP70) gene in different organs of Malabari goats. The study was conducted for a period of 45 days in twelve 10 months to one year old Malabari goats randomly allocated into two groups: MC (n=6; Malabari control) and MHS (n=6; Malabari Heat stress). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. The MC goats were placed in the shaded pens while MHS goats were exposed to heat stress in outside environment between 10.00 h to 16.00h.At the end of study period, all 12 animals were slaughtered and their adrenal, thyroid, liver, uterus and mesenteric lymph node (MLN) tissues were collected for HSP70 gene expression. The temperature-humidity index (THI) inside shed (74.9) proved that the animals were not stressed while in the outside environment (86.5) the animals were extremely distressed. Heat stress significantly increased the HSP70 expression pattern in adrenal while significantly lowered the HSP70 expression pattern in thyroid and MLN. However, heat stress did not influence the expression pattern of HSP70 gene in both liver and uterus. Further, a strong positive correlation was established between temperature-humidity-indexes (THI) and liver and uterus while negative correlation with thyroid and LN. Thus, it can be concluded that the expression pattern of HSP70 gene was tissue and function specific and it was programmed according to the requirement of the heat stressed animals of Malabari goats.

Keywords: Adaptation; Climate change; Heat stress; HSP70; Malabari Goat