EFFECT OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT GROWTH RELATED GENES IN MALABARI GOATS

by

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

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DECLARATION

I, hereby declare that this thesis entitled **"Effect of Heat Stress on the Expression Patterns of different Growth related Genes in 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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Angel P. Sunny

Dedicated to Sejian sir

&

My beloved family...

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SYMBOLS AND ABBREVIATIONS

UN	United Nations
GDP	Gross domestic product
IPCC	Intergovernmental panel on climate change
GH	Growth hormone
GHR	Growth hormone receptor
IGF-1	Insulin-like growth factor-1
LEP	leptin
LEPR	leptin receptor
THR-α	Thyroid hormone receptor-α
GHG	Greenhouse gas
oC	Degree Celsius
m	Meter
AT	Air temperature
RH	Relative humidity
SR	Solar radiation
TNZ	Thermo-neutral zone
DMI	Dry Matter Intake
ADG	Average Daily Gain
BCS	Body Condition Score
BMI	Body Mass Index
BW	Body Weight
SNF	Solids-not-fat
LH	Luteinizing hormone
FAO	Food and Agriculture Organisation
PCV	Packed cell volume
THI	Temperature humidity index
DBWG	Daily body weight gain
HG	heart girth
BL	Body length

WH	Withers height
N	North
E	East
kg	kilogram
mRNA	Messenger RNA
QTL	Quantitative trait loci
MAS	Marker assisted selection
HSP	Heat shock protein
ICAR	Indian Council of Agricultural Research
NIANP	National Institute of Animal Nutrition and Physiology
MC	Malabari control
MHS	Malabari heat stress
IACUC	Institutional Animal Care and Use Committee
CPCSEA	*Committee for the Purpose of Control and Supervision of Experiments on Animals*
LN_2	LiguidNitrogen
cDNA	Complementary DNA
RTqPCR	Real time quantitative polymerase chain reaction
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
NCBI	National Center for Biotechnology Information
ANOVA	Analysis of variance

INTRODUCTION

CHAPTER 1
INTRODUCTION

The human population is projected to increase from 7.2 to 9.6 billion by 2050 (United Nations, UN, 2013). Consequently, global demand for livestock products is expected to double by 2050. Livestock products are major agricultural commodity contributing immensely to global food security representing 33% of global protein consumption and 17% of global calorie consumption (Rosegrant *et al.,* 2009). Further, livestock sector contributes around 33 percent of global agricultural gross domestic product (GDP) and directly support the livelihoods of at least 600 million smallholder farmers (Thornton and Gerber, 2010). Although the livestock sector plays a substantial role in uplifting poor and marginal farmers, the susceptibility of livestock population to climate change threatens these advantages associated with livestock rearing (Henry *et al.,* 2012; Polley *et al.,* 2013).

Among the various climatic factors, increased temperature and relative humidity poses a formidable challenge to the development of livestock sector in the changing climatic scenario . According to the Intergovernmental panel on climate change (IPCC, 2014) the global average surface temperature is expected to rise by around 0.3 to 4.8 % by 2100. The potential negative impacts of heat stress which hampers livestock productivity includes, reduced growth, milk and meat production (Nardone *et al.,* 2010; Henry *et al.,* 2012; Sejian et al., 2018), reproduction (Nardone *et al.,* 2010), changes in quantity and quality of feed crop and forage (Chapman *et al.,* 2012; Polley *et al.,* 2013), water availability (Nardone *et al.,* 2010; Henry *et al.,* 2012), and disease occurrences (Nardone *et al.,* 2010; Gauly *et al.,* 2013). The threats of global warming together with increasing demand for food further accentuates the problems of heat stress in livestock (Renaudeau *et al.,* 2012).

Goat is considered a major source of income and it provides economic security to the poor and marginal farmers in the developing countries in particular.

Approximately 80% of the goats around the world are located in tropical areas of Asia and Africa (Silanikove *et al.,* 2010). Dairy cattle are more vulnerable to the severity of heat stress and hence goat production is gaining importance to ensure optimum economic return to the farmers due to their higher heat and drought tolerance, ability to survive in any pastures and high disease resistance capability (Escareno *et al.,* 2013; Silanikove and Koluman, 2015).

The somatotropic axis plays a pivotal role in regulating the pathway of energy metabolism, growth rate and body composition during postnatal growth in mammals which are controlled through specific genes (Farber *et al.,* 2006; Katoh *et al.,* 2007). The somatotropic axis comprises of growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor-1 (IGF-1), leptin (LEP), leptin receptor (LEPR) and thyroid hormone receptor-α (THR-α) genes and these genes operates in coordination to regulate the growth performance of livestock (Bagath *et al.,* 2016).

The impact of heat stress on growth performance of goats has been established fairly based on changes associated with phenotypic traits (Bagath *et al.,* 2016; Niyas *et al.,* 2017). However, not many reports are available on the genotypic traits which get altered on exposure to heat stress in livestock. A basic understanding of how the molecular mechanisms by which growth is getting compromised during heat stress condition are therefore required before one can consider and adopt possible improvements. As growth related modulations in goat is also mediated through the biological mechanisms on the functioning of the somatotrophic axis, it is very vital to understand the underlying molecular and endocrine mechanisms by which growth is regulated.

Therefore, research efforts are needed to identify different genotypic traits which govern the growth potential during heat stress. Since the meat industry predominantly relies on the growth potential of an animal, identifying such genetic markers are considered crucial to identify breeds with higher productive ability amidst the heat stress challenges. Such approach can make a substantial contribution

towards ensuring food security in the changing climate scenario. Hence, an attempt has been made in this study to elucidate the molecular mechanisms governing growth performance during heat stress in goats. The primary objective of the study was to establish the influence of heat stress on the expression patterns of different growth related genes in indigenous Malabari goats.

The objectives of the present study are:

1. To assess the expression patterns of different growth related genes in Malabari goats subjected to heat stress.
2. To evaluate the expression patterns of different metabolic activity controlling genes in heat stressed Malabari goats.
3. To establish the correlation between THI index and expression patterns of different growth related genes in heat stressed Malabari goats.

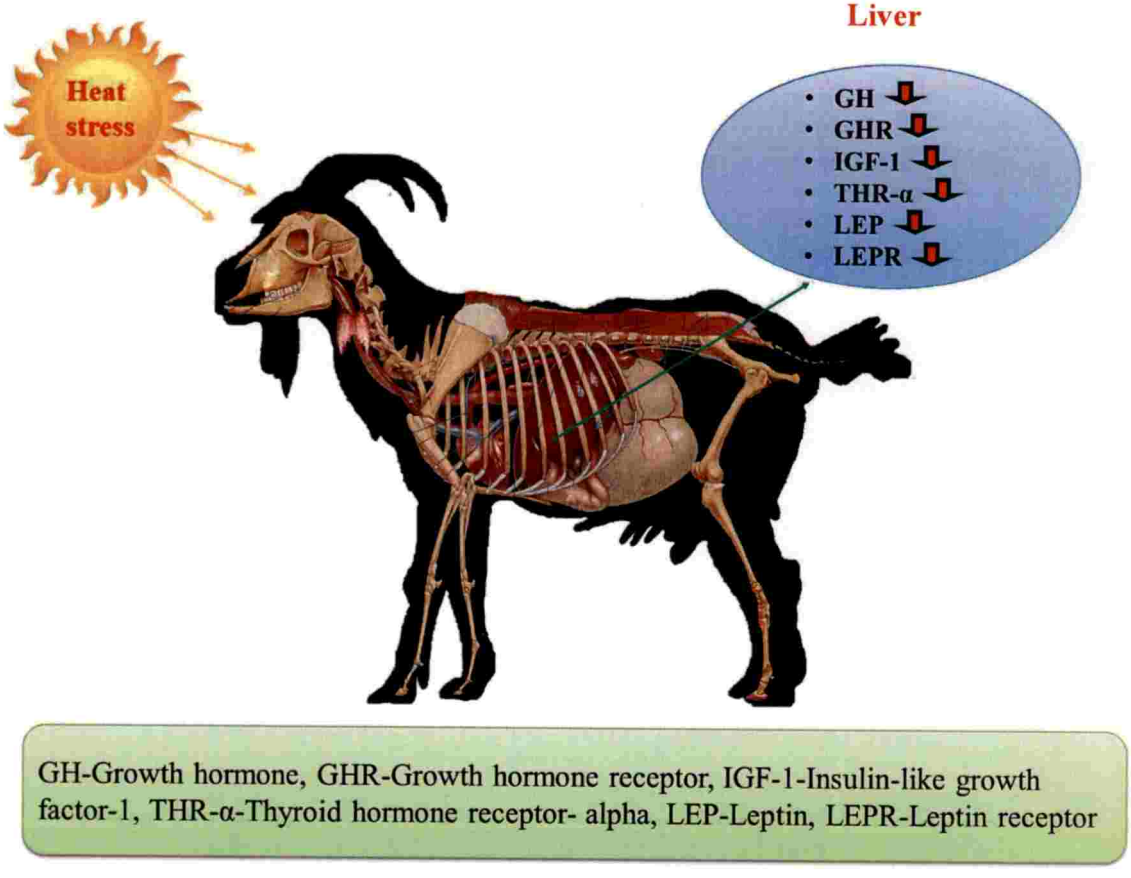

Fig. 1.1: Concept figure of the present study

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 General overview

While climate change is a global phenomenon, its negative impacts are more severely felt by poor people in developing countries who rely heavily on the natural resource base for their livelihoods. Climatic extremes and seasonal fluctuations in herbage quality and quantity are considered as imperative source of influence on the well-being of livestock in extensive production systems. This can result in impairing production and reproduction efficiency of grazing animals (Ali and Hayder, 2008). Therefore, grazing animals in extensive rearing can face nutritional imbalance during extreme summer months (Sejian *et al.*, 2015).

The role of livestock in rural communities is changing rapidly. Goats play a vital role in the livelihoods of small-scale farmers in developing countries (Kumar, 2007). Goats are found in many climatic regions of the world ranging from the arctic cold, temperate, deserts and mountains to subtropical and tropical dry and humid zones. The productivity of goats under the prevailing traditional production system is very low (Celi *et al.*, 2008). It is because they are maintained under the extensive system on natural vegetation on degraded common grazing lands and tree lopping. Even these degraded grazing resources are shrinking continuously as a result of climate change. Hence research efforts are desirable to identify the suitable goat breed that can withstand low pasture availability during summer season (Bagath *et al.*, 2016).

Energy homeostasis is controlled by a complex regulatory system of molecules that affect food intake that is critical for maintaining a stable body weight. The somatotrophic (growth hormone, GH; growth hormone receptor, GHR; insulin-

like growth factor, IGF-I) axis is considered to be one of the most important among them, because of their broad range of effects and central role in growth (Katoh *et al.*, 2007). A well-known cellular effect of GH is the enhanced biosynthesis of IGF-1 in the liver (Saleri *et al.*, 2005). Like GH, IGF-1 is also an important polypeptide growth factor and most of it is produced by the liver (Katoh *et al.*, 2004). In addition, IGF-1 also plays key roles in cellular transformation, organ regeneration, immune function, development of the musculoskeletal system and aging. Further, ghrelin is considered an important local hormone to control growth and has a potent or exigenic effect in both animals and humans and this effect is mediated through hypothalamic neuropeptide Y and Agouti-related peptide (Dimaraki and Jaffe, 2006).

The expression and secretion of the LEP are associated with body fat mass and are affected by alterations in feed intake (Zieba *et al.*, 2005). Food deprivation results in a decline in the circulating LEP, and if untreated, results in a cessation of reproduction in animals (Hyder *et al.*, 2013). The LEP stimulates the somatotrophic axis especially in nutritionally stressed animals (Zieba *et al.*, 2005). The LEP gene expression in relevant tissues is considered to be an indirect indicator of nutritional status of the animal (Hyder *et al.*, 2013). The animals have a characteristic physiological ability to survive the nutritional stress by altering their leptin levels which are required for maintaining the physiological normalcy (Zieba *et al.*, 2005).

Current information regarding how the growth related genes are affected during heat stress condition of indigenous breeds is limited, mostly extrapolated from developed countries. A basic understanding of how the molecular mechanisms by which growth is getting compromised during heat stress condition are therefore required before one can consider and adopt possible improvements. As growth related modulations in goat are also mediated through the biological mechanisms on the functioning of the somatotrophic axis, it's very important to understand the underlying molecular and endocrine mechanisms by which growth is regulated. This in turn, might pave way for identification of suitable biomarkers from the

somatotrophic axis for heat stress tolerance in goat. Hence an attempt has been made in this study to observe the influence of heat stress on the functions governed by growth related gene expression pattern in growing Malabari goats.

2.2 Devastating effects of climate change

The changes in climate have caused detrimental impacts on the ecosystems of all continents and across the oceans. Climate change refers to long-term statistical shifts of the weather, including changes and distribution of weather condition of a particular region. Human impacts on the climate system are clear, and the anthropogenic greenhouse gas (GHG) emissions have increased tremendously since the pre-industrial era.

The IPCC (Intergovernmental Panel on Climate Change, 2012) projected that the frequencies of extreme and anomalous weather events including heat waves, flood and drought are intensifying for the rest of this century, and these rapid changes in global climatic regimes are expected to have many negative effects over the impacts. The climate change has many consequences, including warming of both the atmosphere and ocean, melting of mountain glaciers, sea level rise and extreme weather events and these can result in food insecurity, water scarcity, damage to infrastructure, violent conflict, mass migration, injuries, mortality and more.

Pachauri *et al.* (2014), projected that by the end of the 21st century, the increase of average global temperature is in the range of 2.6°C to 4.8°C due to human-induced climate change. This projection was predicted to continue in the future. Further, the increase in global surface ocean acidification is likely to be 15 to 17%. Moreover, global mean sea level rise also will continue during the 21st century, and for the period 2081–2100 the rise will likely be in the ranges of 0.26 to 0.55 m.

In addition, climate projections predict more intensified droughts and longer dry period in many regions (Dai, 2013). Moreover, climate change can detrimentally

impact human health (Willox *et al.,* 2015), particularly from the infectious diseases perspectives (Bouzid *et al.,* 2014). Climate change is also considered one of the important drivers of biodiversity loss and Warren *et al.* (2013) predicted that about 0 to 54% of species could become extinct due to the devastating effects of climate change. There are many terrestrial, marine and freshwater species that have shifted their geographic ranges and altered their migration patterns, seasonal activities, interactions and abundances due to the ongoing climate change (IPCC, 2014).

Climate change governs the alterations in temperature, precipitation, atmospheric GHG concentration which impacts the agricultural productivity. There are several environmental stresses that arise due to the climate change which significantly influence the livestock productivity and reproductive efficiency, as well as health which eventually culminates in severe economic losses.

2.3 Climate change impact on animal agriculture

Among all the factors influencing livestock production, the climate is undoubtedly the most significant one which adversely influence their production. The predicted adverse effects of climate change on agriculture would also detrimentally impact the sustainability of the livestock production by changes in animal productivity including the growth, milk and wool productivity and reproduction capability, changes in production reliability, changes in feed resources, including their availability, quality, feed conversion efficiency and spatial as well as temporal distribution, changes in the quantity and quality of water resources and changes in disease occurrence (Godber and Wall, 2014; Ojima *et al.,* 2015).

Climate change can affect livestock production both directly and indirectly. The direct impacts include increased ambient temperature, photoperiod, and variability precipitation patterns. The indirect effects are increased scarcity of water resources, reduced feed resources, and increased emergence as well as the spread of new vector-borne diseases and parasites. Thompson (2010), established that the

increase in GHG in the atmosphere causing an increase in ambient temperature and that pose a clear and present danger to the distribution and abundance of livestock populations. The increasing temperature along with humidity results in heat stress, which has a wide range of negative effects such as reduced productivity and feed intake, reduced reproduction efficiency, increased susceptibility to pathogens, parasites as well as vectors and higher mortality (Niang *et al.,* 2014).Increasing fluctuation in rainfall patterns governs the water scarcity and also influences the forage quality; pasture yields and species composition of pastures. It also leads to an increased pests and diseases incidence, and variability in their distribution as well as transmission (Henry *et al.,* 2012). Moreover, extreme weather events such as heat waves, floods droughts and snow storm results in a drastic reduction in the livestock production.

The indirect impacts of climate change cause majority of the economic losses and, it is mainly due to the alterations in the nutritional environment. Climate change can potentially affect the reliability of forage production, quality and quantity of forage, water demand for forage crops, and large-scale rangeland vegetation patterns. Lamy *et al.* (2012), opined that climatological characteristics such as ambient temperature and rainfall patterns have a crucial impact on both the pasture and food resources availability cycle throughout the year and the disease and parasite outbreaks among animal populations. Moreover, higher temperatures induce lignin formation in plants consequently, the digestibility and degradation rates of fodder will be reduced, which ultimately culminate in lower feed conversion rates (Polley *et al.,* 2013; Das, 2016). Nardone *et al.* (2010), emphasized that the increase in temperature results in water consumption by two to three folds. Climate change also has a substantial impact on the availability, quantity, quality, river flow, snowmelt, groundwater and distribution of the rainfall. Furthermore, climate change poses a major threat to viability and sustainability of livestock production systems due to unexpected meteorological patterns, which significantly induce the outbreaks and

dissemination of new diseases (Baylis, 2006). However, animals can adapt to adverse climatic conditions, but the response mechanisms compromise their productive and reproductive functions including nutrient intake, weight gain, milk production and fertility (Halden and Schwab, 2008).

2.4 Heat stress as the major factor influencing livestock production

The major environmental factors influencing livestock production includes air temperature (AT), relative air humidity (RH), solar radiation (SR), atmospheric pressure as well as wind speed and all these factors determine the magnitude of heat stress to the animals. Heat stress is of major concern in the livestock sector because of its detrimental impacts on the productive and reproductive traits of the animals. Given the fact that global warming is happening at the rapid phase, animals getting exposed to severe heat stress in future are inevitable (Silanikove and Koluman, 2015).

High-productive animals reared under tropical region are negatively influenced by climatic factors and in particular high temperature and relative humidity were established to the detrimental factor which jeopardizes their production (Martello *et al.,* 2009).The magnitude of heat stress faced by the animals are determined by several factors including AT, RH, region, species, breeds, genetic potential, life stage, gender, and nutritional status. Heat stress also negatively affects the forage intake, feed conversion efficiency, milk production, reproductive efficiency and disease incidences.

Homoeothermic animals have a thermo-neutral zone (TNZ), which is a range of environmental temperatures that are beneficial to their normal physiological functions. When the temperature rises, the body temperature of the animal exceeds the range specified for their TNZ, and the total heat load exceeds the heat dissipation capacity of the animal resulting in imparting heat stress to the animals (Bernabucci *et al.,* 2010). Consequently, animals have developed adaptation mechanisms in order to withstand the negative impacts of heat stress, which may eventually lead to reduced

feed intake, increased water intake, reduced productive and reproductive performance and increased disease susceptibility (Nardone *et al.*, 2010).

2.4.1 Growth

Heat stress was shown to adversely impact growth performances in almost all livestock species across the globe. Heat stress declines the dry matter intake (DMI) of the animal resulting in negatively influencing their growth performance (Chase, 2006). Moreover, Sohail *et al.* (2010) established in a study that heat stress decreases the body weight (BW), growth rate and average daily gain (ADG) in livestock. Further, Padodara *et al.* (2013), also observed that increase in temperature adversely affects growth, puberty as well as the maturity of the animals. As per Mader (2007), a compensatory gain was reported in livestock after a mild period of heat stress. In addition to impacting the body weight, heat load was also found to deleteriously affecting the body condition scoring (BCS) and other allometric measurements (Nardone *et al.*, 2006).

2.4.2 Milk

Heat stress induces homeorhetic mechanisms that prioritize thermoregulation over other physiological processes consequently; cattle have reduced milk production and are more susceptible to metabolic disorders during the transition period (Baumgard and Rhoads, 2013). When the dairy animals are exposed to chronic heat stress, their feed intake reduced and this leads to negative energy balance, which is mainly responsible for the decline in milk production (Wheelock *et al.*, 2010). Das *et al.* (2016) emphasized in a study that the rise in AT has a direct negative impact on appetite centre of the hypothalamus of the dairy cattle. Further, Najar *et al.* (2010), established that the cumulative effects of heat stress on feed intake, metabolism and physiology of lactating cattle result in decreased milk synthesis. apart from influencing the milk yield, heat stress also was found to significantly influencing the milk composition, such as milk fat, solids-not-fat (SNF) as well as milk protein

percentage (Zheng *et al.,* 2009) and it also negatively affects the lactation length, calving interval and dry period of the animals (Singh *et al.,* 2013)

Furthermore, high productive dairy cattle such as Holstein or Holstein-Friesian cattle are highly susceptible to the heat load, due to their high metabolic rate, which results in increased metabolic heat production, making it difficult to maintain their thermal balance under heat stress exposure (Van Laer *et al.,* 2014). Tao and Dahl (2013) established that the negative impact of heat stress on lactation performance gets aggravated when heat stress exposure is confined to the dry period in dairy cow. This could be attributed to the carry-over effects of heat stress in the dry period to the subsequent lactation. Baumgard and Rhoads (2013), observed in a study that the decline in milk yields up to 50% in dairy animals was due to reduced feed intake, while, rest could be due to the metabolic adaptations to heat stress as these responses could alter the post-absorptive carbohydrate, protein, and lipid metabolism.

2.4.3 Reproduction

The majority of the alterations in the fertility are determined by environmental factors since the fertility traits in dairy animals have low heritability value (Thiruvenkadan *et al.,* 2010). Further, heat stress is one of the major environmental factors which has a deleterious impact on most aspects of reproductive function (Dash *et al.,* 2015), such as oestrous activity, conception rate, embryonic mortality, sperm motility and mortality (Hansen, 2009). Heat stress was found to negatively influencing both the conception and pregnancy rate of the Holstein cattle (El-Tarabany *et al.,* 2015), The increase in ambient temperature during summer reduces the length and intensity of estrus, increases incidence of the silent estrus and anestrous, decline the conception rate and reproductive activity in livestock (Kadokawa *et al.,* 2012).

There are several mechanisms which can prevent the growth of oocytes in heat stressed animals and the foremost is the reduction of preovulatory surge in estradiol and luteinizing hormone (LH) (Hansen, 2007). Singh *et al.* (2013), also emphasized that the oestradiol-17 beta concentration decreases during the summer season resulting in reduced intensity of estrus manifestation and finally, results in silent heat in buffaloes. Furthermore, heat stress significantly reduces estradiol synthesis, androstenedione production (Roth *et al.*, 2001), promotes apoptosis in granulosa cells and inhibits proliferation (Sirotkin, 2010). Khodaei-Motlagh *et al.* (2011) emphasized in a study that heat stress also reduces progesterone concentration, which is a major reason for abnormal oocyte maturation, implantation failure which eventually results in early embryonic death in livestock. The ovulatory failure, impaired oocyte quality, reduced progesterone production, embryonic development, and increased embryo mortality could be the possible reasons for the dramatic decline in fertility in heat stressed animals (Wolfenson *et al.*, 2000).

2.4.4 Health

Health is important to animal welfare since it indicates the animal's physiological functions (Fraser *et al.*, 1997), and climate change can cause substantial shifts in disease distribution pattern and disease outbreaks in previously unexposed animal populations that lead to the breakdown of endemic stability. Thornton *et al.* (2009), opined that variation in both the temperature and rainfall regimes influences the abundance as well as the distribution of disease-causing vectors. Further, heat load causes health issues, compromised animal welfare and in extreme condition may eventually result in mortality especially, in high producing animals (Silanikove and Koluman, 2015). Spooner *et al.* (2012), observed the significance of promoting health and strong biological functions as a component of animal welfare management practices, and its inextricable relationship with livestock production.

The direct and indirect effects of heat stress influences health of animals and affects their normal physiology, metabolism, immunity, and hormonal system.

Increase in ambient environmental temperature negatively affects the physiological mechanisms of rumen which increases the risk of metabolic disorders and health issues in animals. In addition, heat stress also affects health by reduced saliva production, declined DMI and altered digestion mechanisms (Soriani *et al.*, 2013; Nardone *et al.*, 2010). Furthermore, chronic heat stress causes prolonged inappetence which causes increased supply of total carbonic acid in the rumen and reduced ruminal pH which, finally resulting in subclinical and acute rumen acidosis (Kadzere *et al.*, 2002). Health issues such as liver lipidosis, impaired liver function (Basirico *et al.*, 2009) and subclinical as well as clinical ketosis (Lacetera *et al.*, 1996) are highly pronounced in animals of hot-humid regions. Further, increased AT results in increased incidence of lameness (Sanders *et al.*, 2009) and this could be attributed to the longer standing time (Privolo *et al.*, 2009). Lameness causes white line disease, thin soles, sole punctures, ulcers and increases early culling from the herd (Sanders *et al.*, 2009). Further, Dhakal *et al.* (2013) established in a study that the incidence of external parasites will be higher in warm temperate conditions. Jingar *et al.* (2014), observed increased incidence of mastitis in dairy cattle exposed to hot-humid conditions, which facilitates the survival and multiplication of pathogens carrier fly population.

2.5 Economic consequences of heat stress impact in livestock production

The livestock sector is one of the fastest emerging dominant agricultural subsectors in the world and it plays a major role in global economy (Thornton, 2010). Approximately 30% of the planet's ice-free terrestrial surface area is occupied by the livestock sector and it is serving as a major source of income for poor people and directly supports and sustains livelihood security of 766 million marginal farmers in the developing countries (Food and Agriculture Organization, FAO, 2015). Further, livestock sector is a major component of the agriculture it contributes around 36% of the global agricultural GDP, which is also projected to have an increase of 50-60% in the coming decades (Thornton, 2010). In India, livestock sector account for 40% of

the national GDP (FAO, 2009; Sejian *et al.,* 2016). Recent projections clearly showed that the global demand for livestock and its products may increase by around 70%, to meet the demand of rapidly growing human population which is projected to reach 9.6 billion by 2050 (FAO, 2015). Increasing urbanization, population, and purchasing power increase the requirements of livestock products and they satisfy the nutritional demand of the world through high quality meat and milk. According to FAO (2015), the livestock sector is a dominant source of world food economy and apart from their social, cultural and economic role; livestock food products also act as a source of complete protein and thus, help to maintain the balanced nutrition.

Increasing temperature may result in severe economic losses and may emerge as one of the major issues for farmers all over the world. Elevated temperature not only affects animal welfare but also impacts their economic returns (Kadzere *et al.,* 2002; St-Pierre *et al.,* 2003). Economic losses can result from reduced growth rate, meat, milk and egg production, semen quality, reproductive disorders and increasing mortality rates (Renaudeau *et al.,* 2012; Fouad *et al.,* 2016; Dittrich *et al.,* 2017). St-Pierre *et al.* (2003), estimated that heat stress causes total annual economic losses of $1.69 to $2.36 billion to global livestock industries of US. Further, Baumgard and Rhoads (2013) also projected that the economic impact of heat stress on global livestock production is approximately more than $1.2 billion. Key and Sneeringer (2014), estimated in their study that heat stress caused an annual cost between $1.69 and $2.36 billion in US livestock industries, and out of that around 40–60% costs arising from dairy sector alone. Heat stress also was found to cause economic losses to the tune of 2.4 billion dollars in US livestock sector, including dairy cattle, beef cattle, swine and poultry (Ghassemi Nejad *et al.* 2014, 2015). About 25% of reduction in animal productivity is expected due to prolonged heat stress exposures and this projected reduction in production as a result of reduced growth, meat and milk production and reproduction may cause severe economic burden for the poor and marginal farmers (Rojas-Downing *et al.,* 2017).

Elevated temperature may cause a significant economic burden to dairy industry, and the loss was estimated to be $900 million per year (Baumgard and Rhoads, 2013). St-Pierre *et al.* (2003), also assessed the impact of heat stress on the economy of US dairy industries and projected the loss of 897 to 1,500 million dollars for the entire US dairy sector. Further, Collier *et al.* (2007) also observed that the heat wave caused more than 1 billion dollars of economic burden to the Californian dairy farmers. Recent economic impact study of heat stress projected a loss of about $39,000 in an average dairy, which all together contribute 1.2 billion dollars annual production loss in the United States alone (Key and Sneeringer, 2014). A recent study projected the economic loss of $49.1 and $125.8 million/year by 2050 and 2100, respectively in US dairy industry and they attributed this loss primarily to the reduced DMI in these animals (Hristov *et al.*, 2018).

2.6 Heat stress impact on growth performance in livestock

Growth is defined as an irreversible positive change in the measured dimensions of the body, which is controlled by environmental and genetic factors (Brody, 1945). Bourdon (2000), opined that since last two to three decades, significance of growth performance in livestock has gained wider attention from the researchers all around the world. Growth is considered as one of the important selection criteria for the meat breed animals, especially the pre-weaning growth. Mpofu *et al.* (2017), established in their study that the decline in growth performance due to the altered environmental parameters is one of the major limiting factors that hampers the meat production industry in the tropical region.

Out of all the environmental factors, heat stress is the most crucial factor that reduces the growth performance of the livestock. In the tropical and sub-tropical regions of the world, animals which are raised under the extensive system of rearing are more prone to heat stress associated growth retardation. The hot season has been reported to cause heat stress in livestock and it negatively influences all aspects of

animal production in hot arid and tropical climates (El-Tarabany *et al.,* 2015; Habibu *et al.,* 2017). In order to adapt to the hot environmental conditions animals compromise their growth, productive and reproductive performance (Darcan and Silanikove, 2018). There are several studies reported that during the heat stress exposure, different growth variables are altered in the livestock (Popoola *et al.,* 2014; Habibu *et al.,* 2016; Niyas *et al.,* 2015; Pragna *et al.,* 2018). Growth variables such as growth rate, ADG, solids daily gain, live BW and dry BW, are impaired in livestock during higher temperature conditions. Several researchers have also established the negative correlation between growth and heat stress (Silanikove, 2000; Marai *et al.,* 2007; Renaudeau *et al.,* 2012). Baumgard and Rhoads (2013), observed that heat stress decreases growth, alters carcass quality, and reduces efficiency, thereby diminishing efforts by animal agriculture to produce high quality protein. Different growth variables such as ADG, BW, body mass index (BMI), BCS, allometric measurements and feed conversion efficiency are found to be decreased during heat stress exposures (Sejian *et al.,* 2010a; Niyas *et al.,* 2015; Pragna *et al.,* 2018).

Marai and Haeeb (2010), established that increased tissue catabolism and reduced anabolic activity are the main causes for the reduced growth performance in livestock. The increased tissue catabolism primarily occurs in adipose tissues and lean body mass due to heat stress induced increase in catecholamines and glucocorticoids (Habeeb *et al.,* 1992; Kandemir *et al.,* 2013). The decreased anabolism is fundamentally caused by a declined voluntary feed intake resulting in low metabolizable energy available for both maintenance and body weight gain. This leads to the decline in production per unit of feed consumed during heat stress exposure (Morrison and Lofgreen, 1979).

In order to maintain the homeostasis, animals respond to adverse environmental conditions in various ways, and one of the primary mechanism by which the animals responds to long term exposure to heat stress is by reducing the

DMI (Abdel-Samee and Diel, 1998, Beatty *et al.,* 2006). Thermal exposure of sheep results in biological functional changes, which include declined feed intake and utilization (Marai *et al.,* 2007). Elevated temperature reduces body size, carcass weight and fat thickness in livestock (Schütz *et al.,* 2014; Archana *et al.,* 2018). Niyas *et al.* (2015), reported in their study that body condition score (BCS) significantly reduced in Osmanabadi goats during the thermal exposure. Nardone *et al.* (2010), also observed reduced feed intake, growth and carcass weight in cattle during exposure to heat stress. Kamal *et al.* (2018), emphasized that the thermal exposure causes a reduction in feed intake, growth and BW, increase in water intake and even death in extreme conditions. High AT and SR causes declined DMI and ADG, reduced carcass weight fat thickness and increased disease incidence in steers (Mitloehner *et al.,* 2001). Mahjoubi *et al.* (2014) also emphasized that heat stress is partially responsible for reduced growth in lambs. Heat stress showed a negative correlation with the relative growth rate (r=0.708) in crossbred cattle calves and this could be attributed to the fact that animals used most of the available energy to maintain their homeothermy instead of growth (Aziz *et al.,* 2016). Heat stress also has a deleterious effect on the uterine environment and it significantly reduced the placentome size and total embryo cell number, which eventually resulted in a smaller size of neonatal animals.

2.6.1 Body weight

Out of all the growth parameters, BW is the first and foremost trait which gets hampered by the heat stress exposure in livestock. There are several studies reported about the drastic reduction in daily weight gain, growth rate and live BW of livestock during the heat stress exposure (Nardone *et al.,* 2010; Popoola *et al.,* 2014; Hooda and Upadhyay, 2014). When animals exposed to elevated temperatures, growth variables such as growth rate, BW, body solids daily gain and total body solids were impaired (Ismail *et al.,* 1995; Marai *et al.,* 1997a). There are several studies which established that elevated temperature could decline feed intake, BW and nutrient

digestibility, increase metabolic requirement and mortality rate in livestock (Nesamvuni, *et al.,* 2012; Chand *et al.,* 2014; Hu *et al.,* 2016).

Okoruwa (2014), established in his study that the BW of West African dwarf goats significantly decreased, and this reduction was attributed to the declined DMI during the heat stress condition. A reduction in the BW and packed cell volume (PCV) also was observed in Damascus and Balady goats during the thermal exposure for a period of 12 hours (Helal *et al.,* 2010). Saab *et al.* (2011) also established in his study that the live BW of Awassi rams reduced during the elevated temperature conditions. A recent study conducted by Hooda and Upadhyay (2014) reported significantly lower BW in heat stressed Alpine x Beetle kids in comparison with those reared under controlled conditions, and they attributed this to the reduction in feed intake and poor quality forage in the summer season. Gad (2013) also emphasized that heat stress significantly reduced the live BW, daily BW gain and total BW gain by 4.50, 24.76 and 24.88%, respectively. Habeeb *et al.* (2014) observed in his study that the summer season heat stress significantly reduced the live total BW gain in bovine calves by 30 kg at the rate of 333.9 g during 3 months of time period in comparison with the winter season, and the percentage of reduction is more than 45%. A similar observation was reported by Ocak *et al.* (2009), who established that loss in BW during heat stress exposure could be attributed to the reduction in their energy level and that limited available energy could be expended for heat dissipation by respiratory evaporation and it reduces the amount of water available for storage. Recent research reported that short and long term heat stress exposure during late gestation period results in lower birth weight in calves and this difference in weight was found to continue until the puberty (Monteiro *et al.,* 2016). The direct impact of fetal hyperthermia, shorter gestation length and fetal growth retardation by maternal heat stress induce placental function impairment and that eventually results in lower birth weight (Tao *et al.,* 2012). Heifers born to heat stressed cows had reduced starter intake and that causes lower ADG and BW when

compared with those from cooled cows and this can be attributed to the carryover effects of maternal heat stress on calf postnatal growth, nutrient absorption and utilization (Monteiro *et al.*, 2016). In an another study, Monteiro *et al.* (2013) also emphasized in their study that heifers born to heat stressed cows have a greater probability of leaving the herd before puberty due to growth retardation, malformation or sickness in comparison with those from cooled cows. Heat stress negatively impacts the BW in West African dwarf goats and the BW was found to be significantly reduced at higher temperature humidity index (THI) (27.50) conditions as compared to lower THI (23.50) conditions, which are 6.07 ± 0.12kg and 7.16 ± 0.17kg respectively (Popoola *et al.*, 2014).

2.6.2 Average daily gain

Habeeb *et al.* (1992) opined that heat stress is considered one of the major environmental factors detrimentally influencing ADG. During the periods of high AT (above 30°C) the ADG is reported to be severely affected (Mitloehner *et al.*, 2001). Gesualdi Junior *et al.* (2014) reported that the heat stress causes a reduction in ADG in livestock. A significant reduction in ADG reported in Sufflok lambs when exposed to heat stress than cold stress (Sun and Christopherson, 2001). A recent study by Johnson *et al.* (2015) established that ADG was reduced significantly in pigs during heat stress exposure.

Thermal exposure of 36.0 and 32.0°C caused a significant reduction in average daily body weight gain (DBWG) of buffalo calves by 22.6 and 16.5%, respectively (Habeeb *et al.*, 2012). A 36% of the reduction in ADG was observed in Afshari lambs exposed to heat stress conditions (Mahjoubi *et al.*, 2014). Habeeb *et al.* (2009) established in his study that the exposure to hot climatic conditions induced a significant decline in average DBWG of bovine claves by 25.5%. Habeeb *et al.*, (2011) also reported that the heat stress exposure during summer season induced a highly significant reduction in average DBWG. Habeeb *et al.* (2012) also reported

that heat stress causes a significant decline in DBWG of buffalo calves by 18.1, 17.41 and 8.65 % during 1st, 2nd and 3rd months of elevated temperature exposure, respectively. Bernabucci *et al.* (1999) also reported that heat stress significantly reduces DBWG in cattle. Similar results were also obtained by Atta *et al.* (2014), who reported that the DBWG values were significantly lower in summer than in winter during the first, second and third months and the reduced values are 55.2, 60.2 and 57.4% respectively. Habeeb *et al.* (2014) also observed in his study that averages of live DBWG of purebred and crossbred bovine calves were 283±9.3 and 478±38 g during the summer season and were 600±32 and 843±7.1 g during the winter season, respectively. The average DBWG was found to be reduced significantly during summer than winter in two breeds by 52.8 and 43.3.4%, respectively. The ADG of the Western African dwarf goats significantly increased (4.50±0.03g) at low THI condition and it can be attributed to the higher feed intake during favorable environment conditions (Popoola *et al.*, 2014).

2.6.3 Body mass index

Estrada Cortes *et al.* (2009), opined that BMI is considered as a good indicator of body energy reserve in animals. It has been reported that the degree of body fatness (BMI) influences the thermoregulatory ability of the animal during the heat stress conditions (Hayward and Keatinge, 1981; Mozaffarieh *et al.*, 2010). Further, Habibu *et al.* (2016) also reported a significant reduction of BMI in heat stressed Sahel and Red Sokoto goats.

2.6.4 Feed conversion efficiency

Feed conversion efficiency is another important factor which determines the economic value of meat. Padua *et al.* (1997), observed that the Suffolk lambs exposed to higher temperature showed a significant reduction in the feed conversion efficiency. A significant reduction in feed conversion efficiency also reported in Black Bengal goats during the thermal exposure (Alam *et al.*, 2013). Moslemipur

and Golzar-Adabi, (2017) also reported that the fattening Dalagh lambs exposed to heat stress showed a significant reduction in feed conversion efficiency. Padua *et al.* (1997), established that the daily feed intake and feed conversion are significantly reduced in Suffolk lambs under thermal exposure of 30.5 ◦C in a climatic chamber, when compared to a group under thermo-neutral conditions (19.3 ◦C), during spring. Hooda *et al.* (2014) also investigated that the environmental temperature has a negative impact on feed conversion ratio and ADG in livestock.

2.6.5 Allometric measurements

Allometric measurements of the body indicate the growth potential of the animals. Heat stress causes a decline in allometric parameters including heart girth (HG), body length (BL) and withers height (WH). A significant reduction in chest depth reported in Farafra sheep exposed to heat stress and this is due to the adaptive capability of the animal to dissipate excess body heat by increasing their body surface area (Ali and Hayder, 2008). Lacetera *et al.* (1994), reported a significant reduction in WH, oblique trunk length, hip width (−35, −26, −29%, respectively) and BCS (0.0 vs. +0.4 points) in Holstein Friesian calves exposed to heat stress in comparison with control group kept under thermo-neutral conditions. Renaudeau *et al.* (2004) observed that under hot environments (above 25 °C) sows reduces feed intake by 5–6 times compared with that at 18–25 °C and this leads to reduced growth and that results in eventually leaner carcasses at slaughter. Nevertheless, Hashem *et al.* (2013), reported a non- significant change in BL and HG in Black Bengal goats exposed to different temperatures such as zero, four and eight hours of heat exposure. Similar results of non-significant change in HG and BL in indigenous sheep of Bangladesh was also reported by Rana *et al.* (2014a).

2.7 Importance of studying the heat stress impact on indigenous livestock breeds

Darcan and Silanikove (2018) opined that indigenous breeds can adapt and produce optimally during adverse environmental condition in addition to producing

less methane as compared to exotic and cross bred animals. These classic advantages of indigenous animals make them the ideal animal model from climate change perspectives. Therefore, selection of indigenous breeds is a promising strategy to alleviate the negative impact of climate change in the animal agriculture. However, FAO (2015) projected that there is a clear decline in the indigenous livestock breed populations around the world. It has been reported that over 80% of the indigenous livestock breeds in Switzerland have become extinct (ProSpecieRara, 2016).

The vulnerability of the animals to a particular adverse environmental condition determined by their genetic potential and variations in the adaptive capability (Silanikove and Koluman, 2015; da Silva *et al.,* 2017). There are several studies which established that the indigenous livestock breeds perform and survive better than exotic breeds as well as their crosses which can be attributed to the inability of the exotic genes to adapt with tropical environmental conditions (Collier *et al.,* 2008; Baumgard and Rhoads, 2013; Kumar *et al.,* 2014). High producing, exotic animals may perform poorly due to the negative interaction between their genetic merit and adverse environmental conditions (Mpofu *et al.,* 2017). However, indigenous animal possess low average productivity with supreme adaptive capacity to extreme environmental conditions. The native breed possess increased tolerance to the heat stress exposure by deviating less energy for the adaptation mechanisms in comparison with exotic breeds (Baumgard and Rhoads, 2013).

Habibu *et al.* (2016) observed that indigenous breeds are well adapted to their agro-climatic conditions and possess superior ability to survive in adverse environmental conditions than the high yielding exotic breeds. Indigenous breeds reared in tropical and arid regions were reported to be the best tolerant to elevated ambient temperatures, than those living under temperate environments (Marai *et al.,* 2007). Native breeds which are evolved in the tropical and subtropical regions, are hardy and thrive well on poor forage and stressful conditions (Omondi, 2007). Sejian *et al.* (2010) also reported that the indigenous tropical sheep breeds are highly adapted to arid and semi-arid regions because of their efficient thermoregulatory

mechanisms. Further, Helal *et al.* (2010) also observed that indigenous breeds exhibit superior adaptive capabilities in their respective agro-ecological zones because of their higher genetic merit.

Although the production potential of native livestock breeds are less than the exotic and crossbred, they possess a stable production during heat stress conditions, where high yielding exotic and crossbred animals succumb. It has been reported by Silanikove (2000) that the indigenous goat possesses the ability to use the plants rich in lignin and consume less feed as well as water. Indigenous goats are likely to be more resistant to diseases, disease vectors and external parasites, which will change under extreme climate conditions such as heat stress and drought. It has also been reported by Alamer (2003) that the native breeds are more adapted to environmental fluctuations and disease outbreaks than other exotic and crossbred breeds.

A recent study by Nyamushamba *et al.* (2017) emphasized that indigenous cattle breeds are thermo-tolerant due to their particular thermoregulation characteristics such as high heat dissipation rate and low metabolic heat production. Additionally, Kim *et al.* (2017) reported that indigenous zebu cattle possess various adaptive traits such as coat colour, horn development, feeding behaviour, tick resistance and heat tolerance, which enable them to thrive in harsh conditions.

It has been observed that tropical breeds can survive even at a higher temperature (38°C), whereas crossbreds are able to perform better at a lower temperature (5-25°C) (Valente *et al.*, 2015). A recent study conducted by Gill *et al.* (2017) in indigenous and crossbred cattle established that *in vitro* cellular heat exposure caused a decline in cell count and viability in crossbreds, which reflects their higher susceptibility to elevated AT conditions. Maibam *et al.* (2017) established a significant reduction of HSP70 expression in indigenous Zebu cattle in comparison with Karan fries during the hot season. It has also been reported that crossbred cattle (Karan Fries) are highly susceptible to oxidative stress in comparison with native breeds (Tharparkar and Sahiwal) during summer (Sheikh *et al.*, 2017). Apoptosis enzymes are significantly higher in native breeds during summer and it

ensures the rapid removal of damaged cells, which indicates the superior skin defensive mechanism in indigenous breeds (Maibam *et al.*, 2017)

2.8 Significance of goat from climate change perspective

Goats are important small ruminant resources in the tropics and they act as an important source of income and nutrition for the rural poor and therefore they are generally recognized as the poor man's cow. Goats are multi-functional animals and they contribute immensely in securing the livelihoods of poor and marginal farmers particularly in the rural areas (Hirpa and Abebe, 2008; Mlambo and Mapiye, 2015). Aziz (2010) established that goats are the most ancient farm animals to be domesticated and they are believed to be associated with the man over 10,000 years. Goats can be reared for different purposes such as income generation, household consumption, religious purpose and security against crop failure, which contribute significantly to the economy of the people. FAO (2015) projected that there is a significant increase in goat population throughout the world, especially in the poor countries. Among all the livestock species population, goat holds the third position, about 861.9 million heads (Aziz, 2010; FAO, 2015).

Darcan and Silanikove (2018) established that goats are highly thermo-tolerant compared to all other livestock species, that enable them to cope with extreme harsh climatic conditions. Goats have differential adaptive mechanisms to survive in adverse environmental conditions. Goats are compacted and have larger surface area per unit weight, and that enable them for faster heat dissipation. Darcan and Silanikove (2018), reported that goats have better feed conversion efficiency than other ruminants and they can convert low quality feed resources into high quality protein. Goats are widely distributed throughout the world and they are highly suitable for the hot and arid regions of the world (Darcan and Silanikove, 2018). Small body size, low feed requirements and capability to conserve water help them to survive well during the scarcity periods (Hamzaoui *et al.*, 2012). Goat possesses

unique characteristics to survive in any agro-ecological zone because of their grazing behaviour, high feed conversion ratio, drought tolerance and extreme disease resistance (Debele *et al.*, 2013; Shilja *et al.*, 2016). These exemplary capabilities exclusively confirm, goats potential to be considered as the ideal climate animal to alleviate the impacts of climate change on animal agriculture. Goat rearing reduces the risk of economic loss because it involves low initial investment but gives high turnover due to its reduced body size, high prolific rate, less housing requirements and less management care (Omoike, 2006; Aphunu and Okojie, 2011).

2.9 Malabari goat

Goat production in Kerala is centered mainly on its native breed, Malabari which is also known as Tellicherry goats. The breed owes its name from the place of its origin. The Malabari breeds of goats are well adapted to the hot and humid tropical climate of Kerala, where average monthly temperature ranges from 23.7 to 30.7°C and the humidity ranges from 75 to 80%. The breeding tract of these goats lies at the longitude ranging from 11.15' to 11.52' N and latitude 75.25' to 75.49 E, widely distributed the coastal and midland areas in Northern Kerala, comprising of Calicut, Kannur, Wayanad and Malappuram districts of Kerala (Verma *et al.*, 2009; Bindu and Raghavan, 2010). Kaura (1952), observed that Malabari goat is supposed to have originated centuries back by mixing of native goats with Arab, Surti and Mesopotamian goats along with the native goats of Western Coast. Malabari goats are dual-purpose, small to medium sized (Jimcy *et al.* 2011), animals with medium-sized ears and small slightly twisted horns directed outward and downward (Alex and Raghavan, 2012; Alex *et al.*, 2013). The coat colour of Malabari goats widely varies from white, admixtures of white and brown, black and brown and black (Alex *et al.*, 2013). Around 40% of the goats have long hair and about 20% of animals have beard. They have good quality skin and their muzzle is pinkish red. The Malabari breed of goat is famous for its low fat meat and mainly reared as a meat breed. Alex and Raghavan (2012) established that Malabari goat is well known for its higher

growth rate, milk yield, high prolificacy and adaptability. Average BW of adult male and female goats is 38.96 and 31.12 kg respectively, and the milk yield ranges from 1-2 kg per day. Age at first kidding for Malabari goats was significantly lower. The Malabari breed of goat is having high prolificacy with 50% twinning, 25% triplets and 5% quadruplets, which reflects the high production potential of this breed (Thiruvenkadan *et al.*, 2008).

2.10 Factors affecting the expression pattern of different growth related genes with special reference to climate change or heat stress in livestock.

The expression patterns of different growth related gene expressions in livestock can be influenced by both the external and internal factors. The external factors comprise of climate, nutritional status and disease occurrences and the internal factors include species, breed, age, gender and genetic modifications.

Climate is one of the major factors which influence the growth and production performance of the animal. Climatic factors include AT, RH, air velocity and intensity of SR and all these variables determine the degree of heat stress in animals. There are several studies which reported that heat stress significantly influences the growth related gene expressions in livestock. Rhoads *et al.* (2010) established that heat stress significantly decreases expression levels of hepatic GHR and IGF-I mRNA in lactating Holstein cows. It has been reported by Weitzel *et al.* (2017), a lower level of THR gene expression pattern in dairy cows during heat stress conditions. A low LEP mRNA expression was also reported in heat stressed animals (Bartha *et al.*, 2005; Lacetera *et al.*, 2009).

Thorn *et al.* (2006) reported that nutrition level acts as an environmental cue that influences the somatic growth in livestock. The dietary and nutritional supplement can also influence the different growth related gene expression. Jaquiery *et al.* (2012) established lower levels of hepatic IGF-1 gene expression due to the nutritional deprivation in animals. It has also been reported by Bagath *et al.* (2016), that the GH expression in the pituitary and GHR gene expression in the liver lowered

during nutritional stress conditions in goats. Rhoads *et al.* (2010) also observed a significant decrease in hepatic GHR abundance and IGF-I mRNA abundance in Holstein cows due to reduced feed intake (Rhoads *et al.*, 2010).

Moreover, the genetic make-up of an animal also substantially determines the production potential of the animal. The difference in genotype affects the expression patterns of GH and IGF-1 in cattle (Pereira *et al.*, 2005). There are several studies which established the significant associations between growth traits and polymorphisms in the LEP gene in animals (Wang *et al.*, 2015; Martinez *et al.*, 2016). It has also been reported by (Tian *et al.*, 2013), the LEP gene mutations influence the carcass and meat quality traits in cattle.

Apart from genetic factors, breed differences also have a major role in expressing the desired growth related genes. Choudhary *et al.* (2007) established that breed differences significantly influence IGF expression patterns in cattle. The expression patterns of GH, GHR showed significant differences between European and Brangus steers (Baeza *et al.*, 2011). Further, Bagath *et al.* (2016) established the age influence on growth related gene expression pattern and established that the kids in the study were able to cope with heat stress to maintain production and they attributed this to the active growth phase of these kid during the period of study.

2.11 Expression patterns of different growth related genes in goats and other livestock

Martinez *et al.* (2016), established that growth is a complex process that involves the regulated coordination of a wide diversity of candidate genes. There are number of studies which identified several candidate genes associated with phenotypic variations in physiological growth pathways that are responsible for the differences in productive performance in livestock (Houba and Pas, 2004; Pereira *et al.*, 2005; Martinez *et al.*, 2016). Heat stress found to be one of the major exogenous factors detrimentally affecting different growth traits, by influencing the somatotrophic axis (Katoh *et al.*, 2007; Bagath *et al.*, 2016).

Heat stress influence on the GH mRNA expressions is primarily regulated through altered feed intake which was inversely proportional to the nutritional status of the animals (Rhoads *et al.,* 2010; Bagath *et al.,* 2016). Rhoads *et al.* (2010) also reported that heat stress is a crucial factor which found to be affecting the total GHR expression. It has been reported by Okuyama *et al.* (2017), the variation in hepatic GHR expression arises to compensate the release and action of GH during heat stress conditions. Gasparino *et al.* (2013) also established lower GHR mRNA expression patterns in well fed Japanese quails during exposure to elevated environmental temperature. Lucy *et al.,* (1998), observed that hepatic IGF-I gene expression was established to be the primary determinant underlying GHR abundance in dairy cattle. Several studies identified that the hepatic IGF-1 expression determines the fidelity of the hepatic GH-IGF axis (Radcliff *et al.,* 2003; Rhoads *et al.,* 2004 & 2010). Rhoads *et al.* (2010) also opined that an increase in stress hormone level due to the heat stress was found to be one of the reasons for the association between the primary growth axis genes.

The reduction in IGF-1 gene expression was established to be one of the primary reasons for the reduction in growth rate in animals during thermal exposure (Thorn *et al.,* 2006). Likewise, Rhoads *et al.* (2010), also observed a reduction in the hepatic IGF-1 gene expression pattern in heat stressed dairy cows (Rhoads *et al.,* 2010). It has been reported that hepatic IGF-I mRNA abundance was lower in animals which was attributed to the declined feed intake during the heat stress conditions (Collier *et al.,* 2008). Collier *et al.* (2012), established that the THR was found to be one of the major thermo-tolerant genes in livestock. A lower level of THR mRNA expression pattern was reported in dairy cows exposed to elevated temperatures (Weitzel *et al.,* 2017).

Agarwal *et al.* (2009) reported that the LEP gene can act as a nutritional cue to indicates the growth and energy metabolism of the animals during stressed conditions. There are several studies reported low LEP gene expression in heat

stressed animals which are attributed to the homeostatic mechanisms through which animals attenuate heat-tolerance by reducing the metabolic heat production by regulating the energy metabolism (Bartha *et al.*, 2005; Lacetera *et al.*, 2009). Bagath *et al.* (2016) also reported lowered LEP expression in heat stressed goats and linked this to the adaptive mechanisms of the stressed animals to consume more feed.

Nevertheless, there are several contrasting reports available for the increased LEP gene and LEPR gene expression in bovine species during heat stress conditions which was attributed to the severity of heat stress and the nutritional status of these animals (Kamigaki *et al.*, 2006; Lafontan and Viguerie, 2006; Bernabucci *et al.*, 2009). Moreover, it has also been reported that the influence of heat stress on LEP gene expression could be independent of the level of feed intake in the stressed laboratory animals (del Mar Romero *et al.*, 2009, Lee *et al.*, 2010). Several studies established that the molecular control of GH on the LEP gene expression provides lipostatic signal to regulate feed intake, body weight, reproduction, energy, expenditure and functions of the immune system in bovines (Garcia *et al.*, 2002; Nkrumah *et al.*, 2004). Further, a significant association between LEP gene and growth traits were established in heat stressed goats (Wang *et al.*, 2015; Martinez *et al.*, 2016). Tian *et al.* (2013) also reported that the LEP gene expression pattern was found to be associated with meat quality traits in cattle.

2.12 Biological markers for quantifying heat stress impact on growth performance in goats

Biomarkers are the biological substances which indicate the biological states, state of a protein or a change in gene expression. Conventionally biochemical markers have been used for the identification of superior animals with high genetic potential in livestock. Genetic markers are of prior importance in the recent revolutionary developments in the field of molecular genetics because it can be used for assessing the stress adaptation mechanism in animals. Biomarkers can be used as

a reference point in the breeding program to identify and to cross-breed for the improvement of genetic merit in livestock.

Molecular markers associated with desired traits can be identified by applying molecular techniques and these advanced techniques can identify genetic variation at specific loci and evaluate the relationship between production traits and genetic variation at quantitative trait loci (QTL) (Jiang *et al.,* 2002; Arora and Bhatia, 2006; Missohou *et al.,* 2006). Marker assisted selection (MAS) program could accelerate the rate of change in economically important traits. Two approaches of MAS are generally applied to identify molecular markers for economic traits. Primarily, genome scans facilitating anonymous DNA markers to identify QTL. Further, a candidate gene approach has been employed in order to find genes with an impact on desired traits (Rothschild *et al.,* 1998). The biological marker has potential to substantially enhance the accuracy of selection and increasing selection differences in breeding programs. Therefore, an overall improvement in livestock species can be achieved by the use of biological markers to a great extent.

There are several studies which identified different biological markers for growth traits in goats. Agarwal *et al.* (2009) established that mRNA expression levels of leptin could act as biomarkers for growth and feed conversion efficiency in goats, which has potential welfare applications. Further, Bagath *et al.* (2016) identified GH gene and GHR to be the indicators of nutritional status during heat stress exposure in Osmanabadi goats. A recent study conducted in two different indigenous breed goats by Archana *et al.* (2018) reported that heat shock protein 70 (HSP) genes and myostatin gene could serve as biological markers for meat production potential. Further, the GH genes associated with caprine growth traits also can be used as molecular markers in MAS in Boer goat bucks (Hua *et al.,* 2009). It has been reported that GH gene has been widely used as a biomarker in several goat breeds (Chitra and Aravindhakshan, 2004; Hua *et al.,* 2009; Wickramaratne *et*

al., 2010; Marini *et al.*, 2015 and, Singh *et al.*, 2015). A recent study also established that GH gene serves as a molecular marker and influence the weight traits and it could be used to select the goat weight in MAS program (Susilorini *et al.*, 2017). Zhang *et al.* (2012) observed that myostatin can act as a molecular marker for growth traits in Boer goat which could be useful for MAS program. Further, Zhang *et al.* (2018) reported that IGF-I gene could be used as a potential molecular marker for growth traits in Nanjiang Huang goats.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Animal

A total of 12 one-year old female Malabari goats were used for the study. Malabari is the indigenous breed of Kerala and it is a dual-purpose goat, well known for their adaptive capability in hot and humid conditions of Kerala. Plate 3.1 is the pictorial representation of the experimental goat.

3.2 Experimental design

The study was conducted in the experimental livestock unit of the ICAR-National Institute of Animal Nutrition and Physiology (ICAR-NIANP), Bengaluru, India. Twelve healthy female Malabari goats were used in the study and the animals were allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The duration of the study was for a period of 45 days. The animals were provided with the diet comprising of 60% roughage (Hybrid Napier) and 40% concentrate. The concentrate mixture comprises of Maize, wheat bran, soybean meal, mineral mixture, common salt to the tune of 36 Kg, 37, Kg, 25 Kg, 1.5 Kg and 0.5 Kg respectively for a 100 Kg concentrate mixture. During the experimental period, the MC animals were kept in the shed in comfortable condition and the MHS animals were kept outside the shed exposed to summer heat stress between 10:00 h to 16:00 h. The THI was calculated by the formula as described by McDowell (1972). The MC animals were fed and watered inside the shed while MHS animals were provided with feed and water when they were kept exposed to heat stress in outside environment. The cardinal weather variables were recorded both inside and outside the shed twice daily.

Plate 3.1: Pictorial representation of the experimental goat

At the end of the study the animals were slaughtered and their uterine samples were collected in aseptic condition for subjecting them to gene expression. The ownership of the experimental animals was with the Director of ICAR-NIANP. These animals were maintained in the experimental livestock unit of the institute and they were used in the study after taking permission from the institute director. All handling and management procedures performed in the study were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC, ICAR-NIANP). In addition, the study was conducted after obtaining approval from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India for subjecting the animal to heat stress (NIANP/IAEC/2/2017).

3.3 Expression of growth related genes in liver

The animals were slaughtered at the end of the study and representative liverine samples were collected from each animal. Immediately after collection the tissue samples were cut into small pieces and washed in phosphate buffered saline. The samples were then kept immersed in RNA shield (Zymo Research, USA) and snap chilled in liquid nitrogen (LN_2) and kept stored at –80 °C till further use.

The tissues samples were thawed after removing them from RNA shield (Zymo Research, USA) and processed for RNA isolation. The total RNA was isolated from tissues using the GeneJET RNA Purification Kit (Thermo Scientific, Lithuania) and the procedure was carried out as per manufacturer's protocol. Total RNA was treated with DNase (TURBO DNA-free, Ambion, USA) to eliminate the genomic DNA contamination. The purified total RNA samples were stored at –80 °C until cDNA synthesis. The Maximafirst strand cDNA synthesis kit (Thermo Scientific, Lithuania) was used to reverse transcribe the total TNA into cDNA. The cDNA was subjected to real time quantitative polymerase chain reaction (RTqPCR).

Table 3.1. Primers used for GH, GHR, IGF-1, THR- α, LEP and LEPR gene expression. GAPDH used as reference gene to normalize the gene expression of target genes.

Gene ID	Primers	Primer sequence (5"- 3")	PrimerLength (bp)	Product Size (bp)	Ta (° C)	Accession No	Reference
GH	F	GCCCAGCAGAAATC AGACTTG	21	133	56	NM_001285586.1	Bagath *et al.*, 2016
	R	CATAGACACGGTCC GAGGTG	20				
GHR	F	CTGTTTCAGGATTGT CTGCCG	21	159	60	NM_001285648.1	Bagath *et al.*, 2016
	R	AAGCTGGTGTGGCT TCACTC	20				
IGF-1	F	CTTGAAGCAGGTGA AGATGCC	21	132	60	NM_001285697.1	Bagath *et al.*, 2016
	R	AGAGCATCCACCAA CTCAGC	20				
THR-α	F	ATGTTCTCCGAGCT GCCTTG	20	105	60	KF_589923.1	Newly Synthesized
	R	TGTCGCTCTCGGGG TCATA	19				
LEP	F	GGCTTTGGCCCTATC TCTCC	20	120	60	XM_005679433.1	Bagath *et al.*, 2016
	R	CGGACTGCGTGTGT GAGATG	20				
LEPR	F	GCTCTGCTTTTGACG ACTCC	20	196	60	AY_846770.1	Newly Synthesized
	R	ATAAGCCCTTGCTC CTCCTC	20				
GAPDH	F	GGTGATGCTGGTGC TGAGTA	20	265	60	AF030943	Shaji *et al.*, 2017
	R	TCATAAGTCCCTCC ACGATG	20				

Note: Ra-Annealing Temperature; bp – base pair; GH-Growth hormone; F – forward; R – reverse; GHR-Growth hormone receptor; IGF-1- Insulin like growth factor-1; THR-α-Thyroid hormone receptor-α; LEP- Leptin; LEPR- Leptin receptor; GAPDH – Glyceraldehyde 3-phosphate dehydrogenase.

Specific primers were synthesized for the target genes using NCBI primer design software (Primer3, http://bioinfo.ut.ee/primer3/) and Primer3 and BLAST websites (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) were used to check the specificity of the primers. Different primers used for amplifying the target regions of various genes in the study are described in table 3.1.

The relative quantitative expression patterns of target genes were studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA) using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene as internal control. The relative expression patterns of target genes in comparison to the housekeeping gene were analyzed as per the formula 2ΔΔCT (Shaji *et al.*, 2017).

3.4 Histopathological observation

All the animals were slaughtered at the end of the study period and their liverine tissues were collected for histopathological sectioning. Care was taken to collect representative tissue sample from the same site of liver in all experimental animals. Immediately after collection the tissue samples were kept in 10% formalin and processed for obtaining histopathological sections. The tissue sections were stained using Haematoxylin and Eosin (H and E) stain as per the method described by Luna (1968). The results were interpreted by comparing between MC and MHS sections and the representative lesions were photographed. Based on the degree of histological changes the scores were given. The scoring pattern for the histological section was based on the method of Gibson-Corley *et al.* (2013). Three different scoring patterns were followed on a scale of 0-3 point as 0-normal; 1- mild; 2-moderate and 3-severe changes.

3.5 Statistical analysis

The quantitative relative expression pattern between target genes and housekeeping gene were analyzed by one-way analysis of variance (ANOVA) using SPSS version 18.0 software. The level of statistical significance was set at $P<0.05$.

Pearson's correlation coefficient test was used to assess the correlation coefficient between the THI and all genotypic traits using R^2 values by setting two levels of statistical significance at $P<0.01$ and $P<0.05$. Again one-way ANOVA was used to assess the degree of changes associated with histological sections between MC and MHS groups.

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 was 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 to 78 are considered stressful and THI above 78 considered extreme distress.

4.2 Real time amplification plot of growth related genes

Amplification plot showed distinct variation of log (ΔRn) for different genes against PCR cycle number is depicted in Fig4.2.1. There was no amplification in non-template control (NTC). Multicomponent plot also showed the difference between the amplified and the non-amplified genes based on the graph pattern using the SYBR green dye is depicted in Fig 4.2.2. Different genes (GH, GHR, IGF-1, THR-α, LEP, LEPR) showed different Tm in the melt curve graph is depicted in Fig 4.2.3. The melt curve showed that the PCR reaction is free from primer-dimer artifacts based on the clear distinct curve which was absent in NTC.

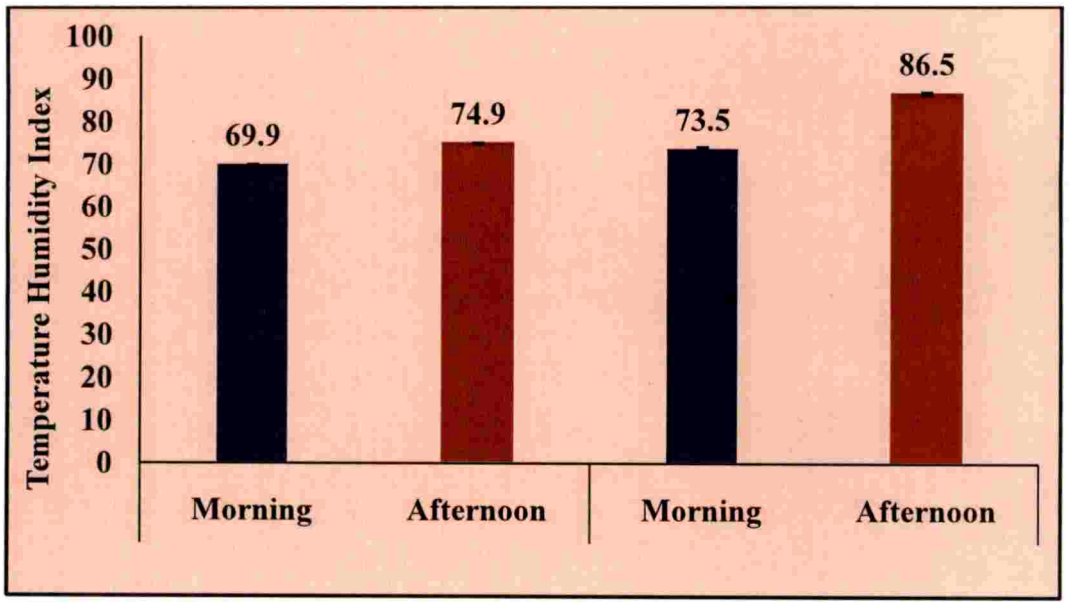

Fig. 4.1. Average temperature humidity index (THI) for the study period both inside and outside the shed

The THI values were calculated as per method described by McDowell (1972). Accordingly, the formula used was THI = 0.72 (T_{db} + T_{wb}) + 40.6 where, T_{db} = Dry bulb temperature in °C; T_{wb} = Wet bulb temperature in °C. The THI values 72 and less are considered comfortable; THI values between 75-78 are considered stressful and THI above 78 considered extreme distress

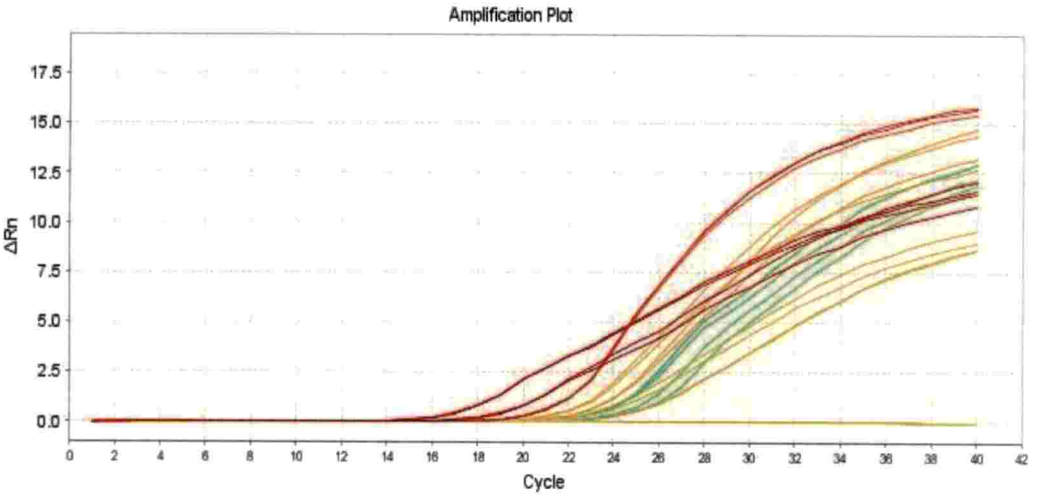

Fig. 4.2a. Description of amplification plot showing distinct variation of log (ΔRn) for different genes against PCR cycle number.

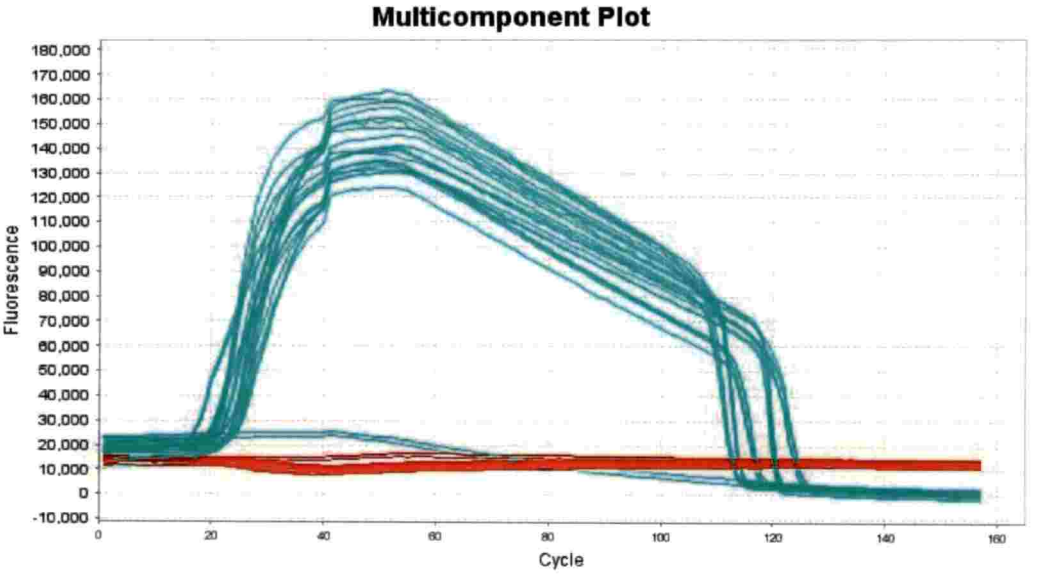

Fig. 4.2b. Description of Multicomponent plot also showing the difference between the amplified and the non-amplified genes based on the graph pattern using SYBR green

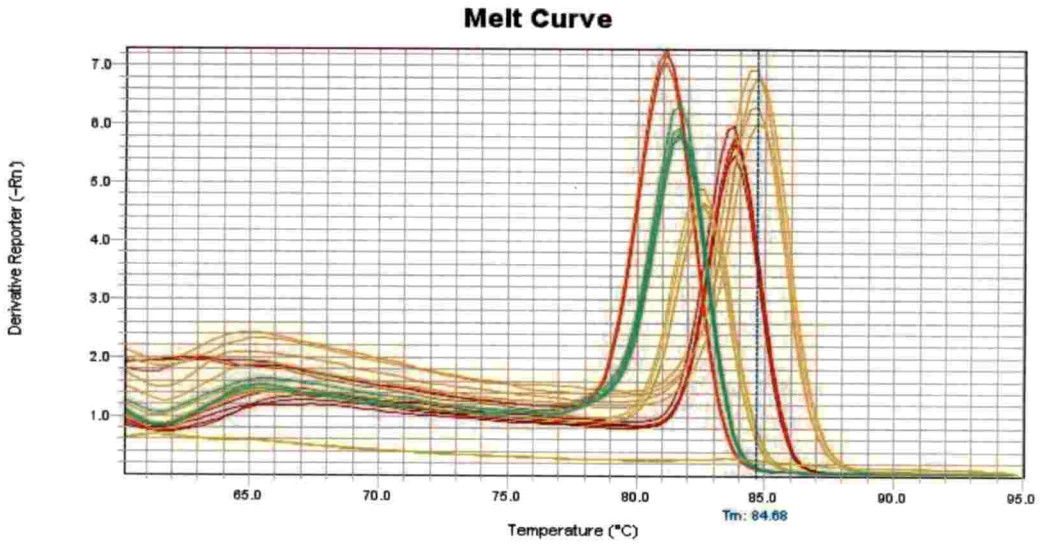

Fig. 4.2c. Description of different growth related genes showing different Tm in Melt curve.

4.3 Relative hepatic GH mRNA expression pattern

The relative hepatic GH mRNA expression patterns between control and heat stress groups of Malabari goats are illustrated in Fig. 4.3. The fold changes in expression patterns of hepatic GHR gene of control and heat stress groups are 1.0 and 0.26, respectively. The hepatic GH expression pattern was significantly ($P<0.05$) lower in heat stress group as compared to the control group animals. In addition, a strong negative correlation ($P<0.05$) was established between THI and GH gene expression pattern (Table 4.1).

4.4 Relative hepatic GHR mRNA expression pattern

The relative hepatic GHR mRNA expression patterns between control and heat stress groups of Malabari goats are illustrated in Fig. 4.4 The fold changes in expression patterns of hepatic GHR gene of control and heat stress groups are 1.0 and 0.27, respectively. The quantitative expression pattern of hepatic GHR was significantly ($P<0.05$) lower in heat stress group as compared to the control group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and GHR gene expression pattern (Table 4.1).

4.5 Relative hepatic IGF-1 mRNA expression pattern

The relative hepatic IGF-1 mRNA expression patterns between control and heat stress groups of Malabari goats are depicted in Fig. 4.5. The fold changes in the expression pattern of the hepatic IGF-1 gene in control and heat stress groups are 1.0 and 0.46, respectively The hepatic IGF-1 gene expression pattern was found to be 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 established between THI and IGF-1 gene expression pattern (Table 4.1).

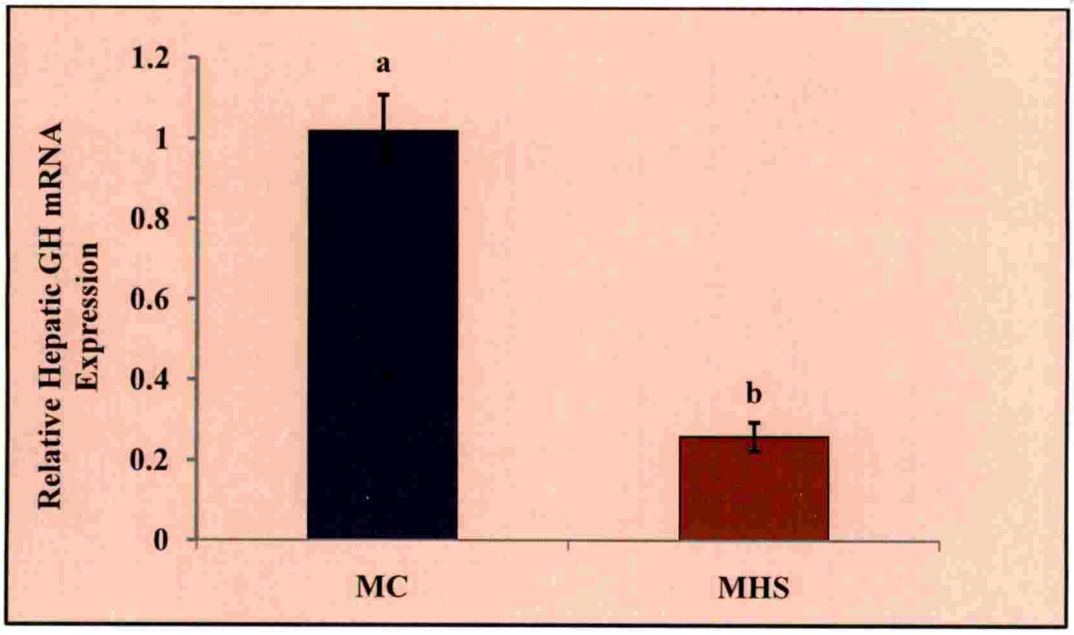

Fig. 4.3. Relative hepatic GH mRNA expression patterns in control and heat stressed Malabari goats

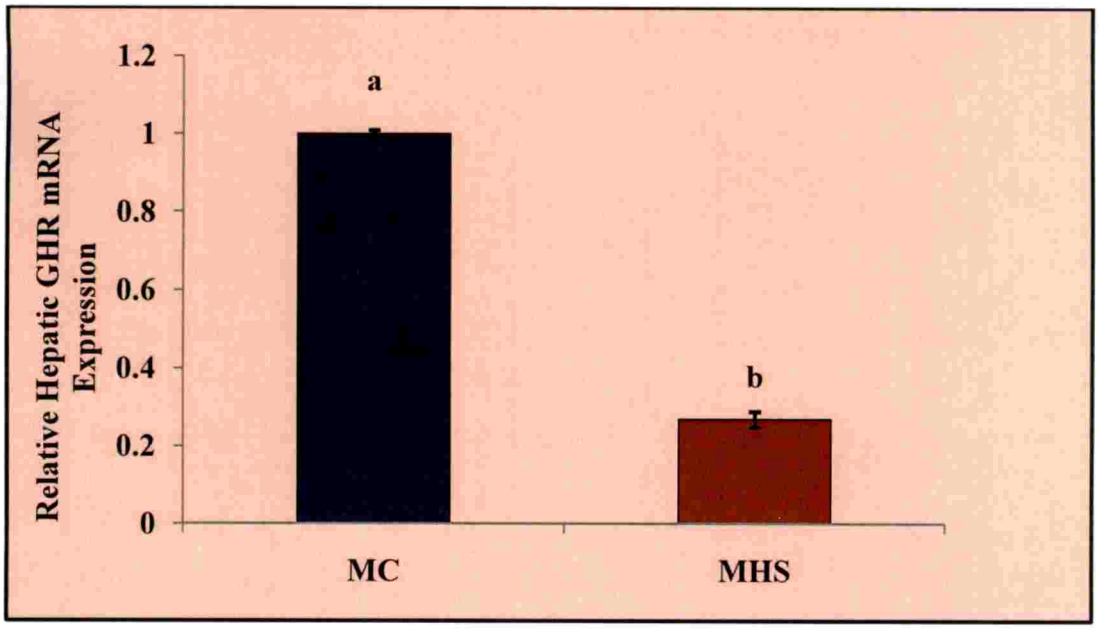

Fig. 4.4. Relative hepatic GHR mRNA expression patterns in control and heat stressed Malabari goats

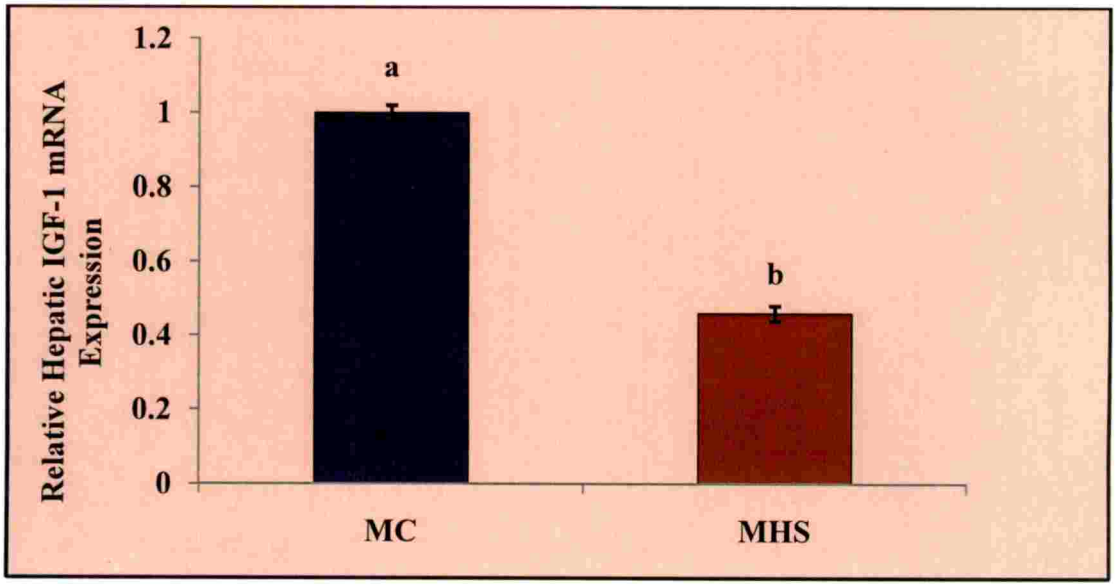

Fig. 4.5. Relative hepatic IGF-1 mRNA expression patterns in control and heat stressed Malabari goats

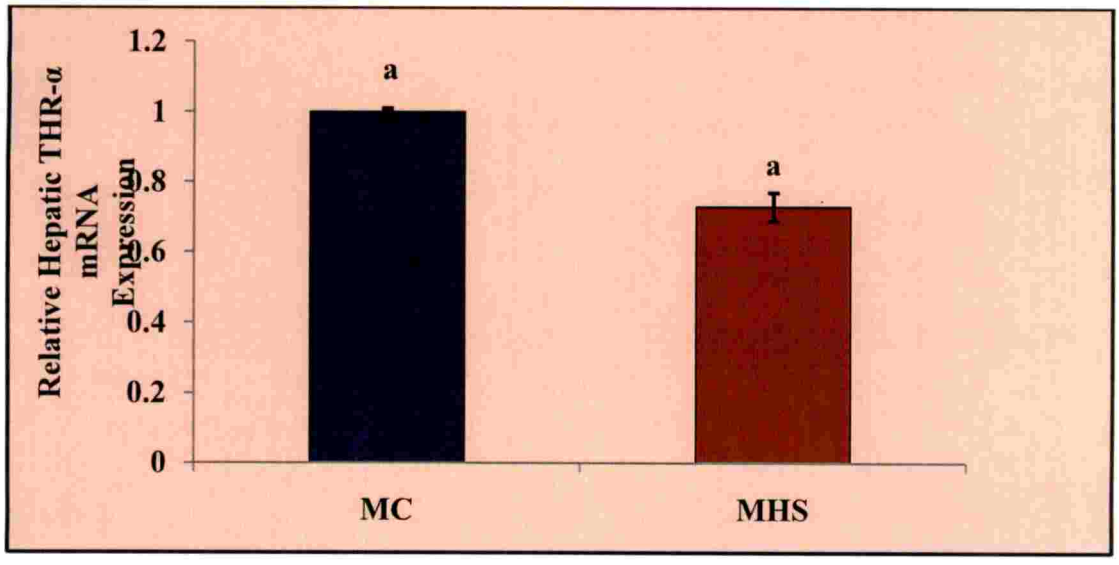

Fig. 4.6. Relative hepatic THR-α mRNA expression patterns in control and heat stressed Malabari goats

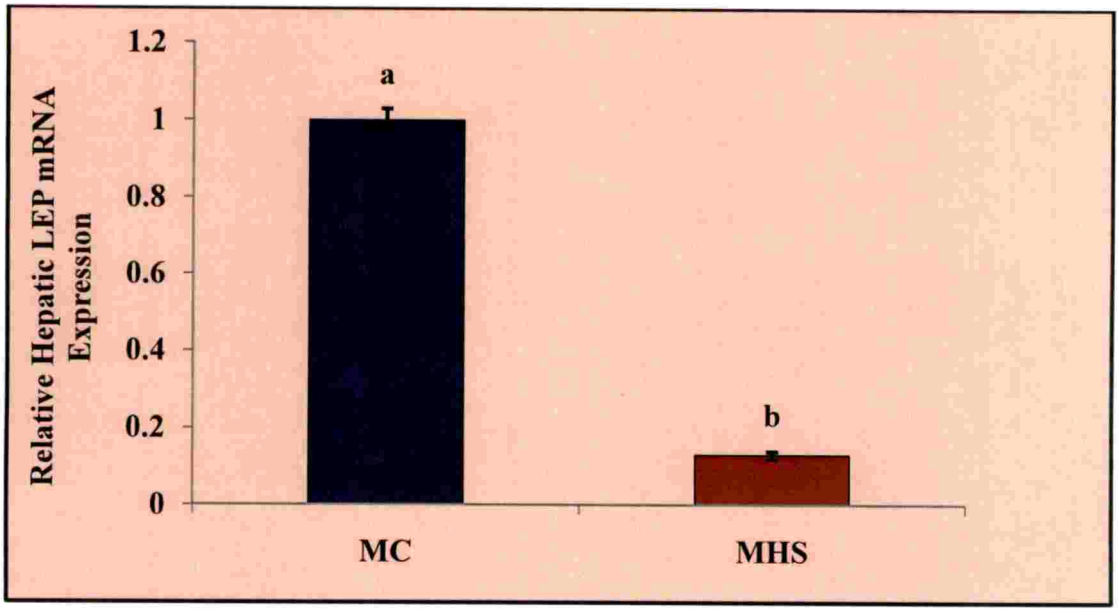

Fig. 4.7. Relative hepatic leptin mRNA expression patterns in control and heat stressed Malabari goats

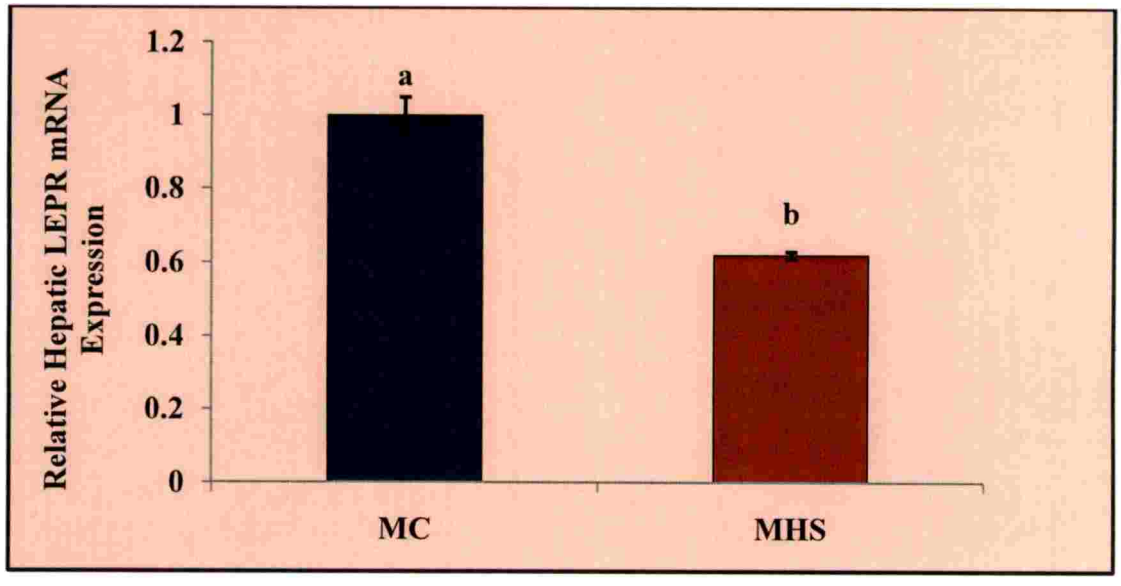

Fig. 4.8. Relative hepatic LEPR mRNA expression pattern in control and heat stressed Malabari goats

4.6 Relative hepatic THR-α mRNA expression pattern

The relative hepatic THR-α mRNA expression patterns between control and heat stress groups of Malabari goats are described in Fig. 4.6. The fold changes in the expression patterns of relative hepatic THR-α in both control and heat stress groups are 1.0 and 0.73, respectively. The expression pattern of the hepatic THR-α was found to be non-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 established between THI and THR-α gene expression pattern (Table 4.1).

4.7 Relative hepatic leptin mRNA expression pattern

The relative hepatic LEP mRNA expression patterns between control and heat stress groups of Malabari goats are depicted in Fig. 4.7. The fold changes in the expression patterns between both the control and heat stress groups are 1.0 and 0.13, respectively. Further, the hepatic LEP gene expression pattern was found to be 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 established between THI and LEP gene expression pattern (Table 4.1).

4.8 Relative hepatic LEPR mRNA expression pattern

The relative hepatic LEPR mRNA expression patterns between control and heat stress groups of Malabari goats are elucidated in Fig. 4.8. The hepatic LEPR mRNA expression pattern also showed similar trends like that of THR-α gene expression. The fold changes in the expression patterns between the control and heat stress groups are 1.0 and 0.62, respectively. Similar to the THR-α gene expression, the LEPR gene also was found to be significantly down regulated in heat stress group as compared to the control group animals. Further, a strong negative correlation

(P<0.05) was established between THI and LEPR gene expression pattern (Table 4.1).

4.9 Histological section of liver

Histopathological section showing changes in liver between the MC and MHS group Malabari goats are depicted in Fig. 4.9. The hepatocytes of liver in MHS group showed more fatty changes (P<0.05) compared to MC group. Further, the hepatic degenerative changes also were more pronounced in MHS group (P<0.05) compared to MC group.

Table 4.1: Correlation association between THI and different growth related genes

	THI	**GH**	**GHR**	**IGF-1**	**THR-α**	**LEP**	**LEPR**
THI	1						
GH	-0.908*	1					
GHR	-0.999**	0.917**	1				
IGF-1	-0.998**	0.895*	0.998**	1			
THR-α	-0.900*	0.883*	0.914*	0.919**	1		
LEP	-0.996**	0.867*	0.994**	0.998**	0.895*	1	
LEPR	-0.885*	0.611	0.874*	0.898*	0.744	0.923**	1

THI- Temperature humidity index; GH- Growth hormone;GHR- Growth hormone receptor; IGF-1- Insulin like growth factor-1; THR- Thyroid hormone receptor-α; LEP- Leptin; LEPR- Leptin receptor; **Indicates statistical significance at P<0.01; *Indicates statistical significance at P<0.05

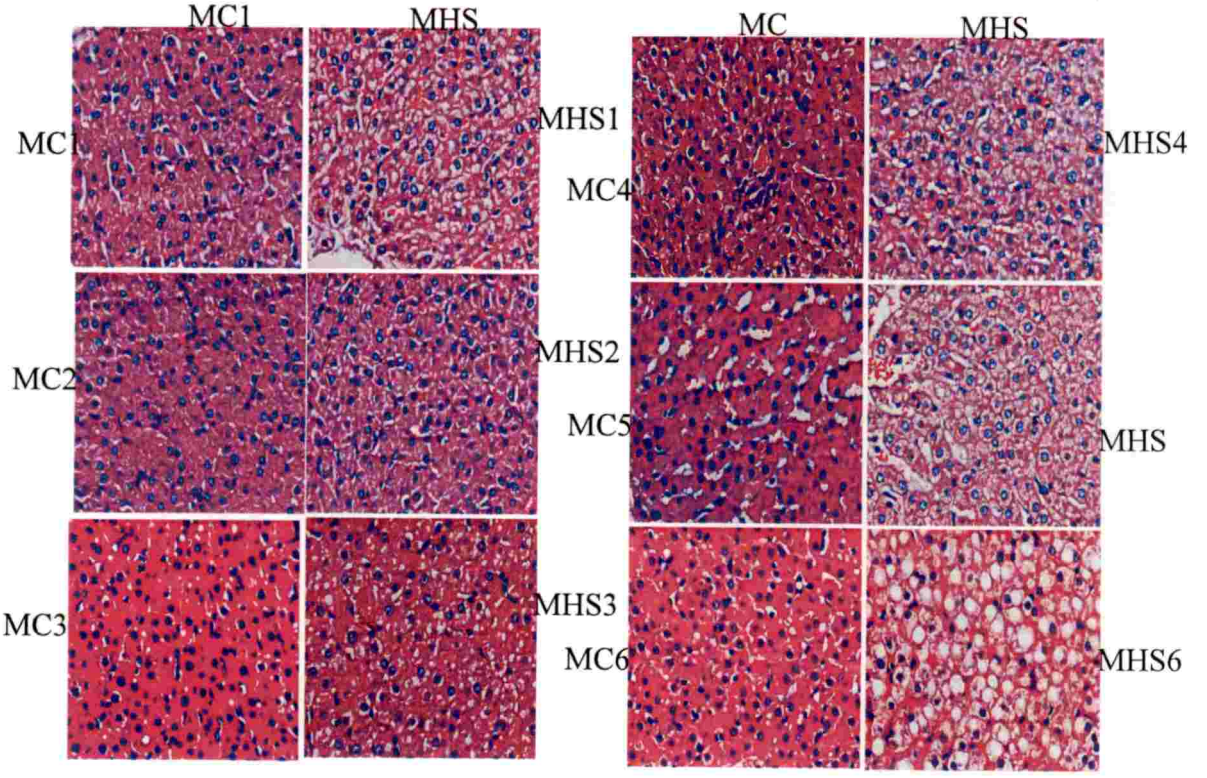

Fig. 11. Histopathological section showing changes in liver between the control and heat stress group of Malabari goats.

The hepatocytes of liver in stress group showed more fatty changes compared to normal group. The hepatic degenerative changes were more pronounced in stress group compared to normal group. MC-Malabari control; MHS-Malabari heat stress; MC-1-6: Six animals in MC group; MHS-1-6: Six animals in MHS group

DISCUSSION

CHAPTER 5

DISCUSSION

Growth traits in livestock animals are quantitative variables, often controlled by a large number of genes (Martinez *et al.,* 2016). Several studies have identified candidate genes associated with phenotypic variations in growth traits that might be responsible for the differences in productive performance observed among animals maintained under the same environmental conditions (Houba and te Pas 2004; Pereira *et al.,* 2005; Martinez *et al.,* 2016). However, not much of research reports are available pertaining to establishing expression profiles of growth related traits during heat stress in livestock. Summer heat stress is a major factor which negatively influences the growth performance, possibly by affecting the components of the somatotrophic axis (Katoh *et al.,* 2007; Bagath *et al.,* 2016). The current study offers the first thorough insight into the expression patterns of different growth related gene expression during heat stress exposure in goats. In the changing climate scenario, efforts are needed to concentrate on identifying livestock which can withstand the adversities associated with climate change and produce optimally in varied environmental condition. Malabari goats in Southern India are well known for its adaptation in hot and humid tropical environment and are primarily reared for meat purpose. Therefore, growth traits are of prime importance in these animals, which are mainly raised for meat production. Genes encoding GH, GHR, IGF-1, LEP, LEPR and THR have been associated with physiological growth pathways in cattle and other species. In this line, the findings from this study provide some crucial initial information on how different growth related genes are expressed when goats are subjected to heat stress. This information might be of high value in assessing the growth performance of goats and may provide useful information pertaining to nutrient supplementation to the heat stressed goats. These candidate genes which

form the molecular basis for controlling muscle growth in Malabari goats may underpin genomic breeding for improved growth rates.

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 growth performance in Malabari goats during heat stress exposure.

On a comparative basis, lower GH mRNA expression pattern was established in heat stressed Malabari goats as compared to the control group goats. It is generally believed that GH level is more sensitive to nutritional stress as compared to heat stress in livestock (Sejian *et al.,* 2014; Bagath *et al.,* 2016). Further, the effects of heat stress on the GH level are primarily mediated by altering the feed intake in heat stressed animals and GH mRNA expressions are inversely proportional to the nutritional status of the animals (Rhoads *et al.,* 2010; Bagath *et al.,* 2016). Since the feed intake was not measured in this study, it could be speculated that the heat stress was not severe enough to induce changes in the GH level. Although the THI shows that the MHS groups were subjected to extreme heat stress, still Malabari goats are well known for their survival in the hot humid tropical environment wherein the magnitude of heat stress could be further severe. Further, the corresponding lower hepatic GHR expression in the heat stressed animals supports this notion. Therefore, the reduced GH gene and GHR gene expression in heat stress groups could be attributed to the extreme adaptive nature of this indigenous Malabari goat.

In the current study, the hepatic GHR expression showed down regulation in heat stressed goats as compared to the control group. This was in contrast to the previous finding in the same laboratory on Osmanabadi goats (Bagath *et al.,* 2016). The differences in the result between the studies could be attributed to the nutritional

stress in the previous study than the heat stress in the current study. This signifies the importance of optimum nutrition to maintain growth performance in goats. The significantly lower GHR in the current study in the Malabari goats could be attributed its heat tolerance capability and this could be due to their indigenous nature. This shows that when nutrition was not compromised the animals were able to counter heat stress without compromising production. Similarly, Rhoads *et al.* (2010) observed heat stress to be one of the major exogenous factors affecting the GHR abundance and established that when the dairy animals were well fed it down regulated the total GHR expression during heat stress condition. The altered hepatic GHR expression during heat stress indicates that changes were occurring to compensate for the release and action of GH (Okuyama *et al.,* 2017). Similarly, changes in tissue GH responsiveness do not always correlate with changes in GHR abundance, indicating that abundance or activity of intracellular signaling proteins is also regulated. In dairy cattle, the liver-specific GHR1A transcript is regulated by physiological state and appears to be the primary determinant underlying GHR abundance (Lucy *et al.,* 1998). Likewise, in well fed Japanese quails also the same result of higher GHR mRNA expression patterns were obtained during exposure to hot environmental temperature (Gasparino *et al.,* 2013). Further, it has been established in dairy cattle that hepatic IGF-I gene expression appears to be the primary determinant underlying GHR abundance (Lucy *et al.,* 1998). The similar trend in hepatic IGF-1 expression in the current study proves this association between these genes to determine the fidelity of the hepatic GH-IGF axis (Radcliff *et al.,* 2003; Rhoads *et al.,* 2004, 2010). Further, heat stress induced increase in stress hormone also was established to be one of the reasons for this association between these primary growth axis genes (Rhoads *et al.,* 2010). This warrants further experiments in this line to determine the long-term effect of stress hormones on hepatic GHR gene expression. In addition, a strong negative correlation between THI and both GH and GHR gene expression clearly indicated the sensitivity of these variables to heat stress in Malabari goats.

Lower expression pattern of IGF-1 mRNA was recorded in heat stress group as compared to control group. Growth is a complex process that involves the regulated coordination of a wide diversity of neuroendocrine pathways. The IGF-1 is an important component of the somatotrophic axis that plays a key role in postnatal growth and metabolism in mammals (Sejian *et al.,* 2014). Given its growth-stimulating and anabolic effects (Thorn *et al.,* 2006), the reduction in the level of IGF-1 and IGF-1 gene expression are likely to be the primary reason for the reduction in growth rate in heat stressed animals. Similar heat stress induced reduction in the expression pattern of the hepatic IGF-1 gene was established in dairy cows (Rhoads *et al.,* 2010). Collier *et al.* (2008) established hepatic IGF-I mRNA abundance was lower in heat stressed animals and they linked this to the reduced feed intake in heat stressed animals. Further, IGF-1 being considered a biological marker for growth governance and a strong negative correlation of THI with this variable in the study clearly indicated the compromised growth performance in heat stressed Malabari goats.

The thyroid hormone system and thyroid hormone mediated signaling pathway plays a pivotal role in the control of substrate utilization and thus body temperature of heat-stressed animals (Weitzel *et al.,* 2017). The THR was established to be an important thermo-tolerant gene in livestock (Collier *et al.,* 2012). The THR-α mRNA expression patterns are comparable between the MC and MHS groups. This again indicates that the growth performances are not much compromised due to heat stress in this breed. The other possible reason for this could be that the magnitude of heat stress may not have been severe enough to induce changes in the THR-α expression pattern. However, Weitzel *et al.* (2017) reported a lower level of THR mRNA expression pattern in heat stressed dairy cows. In general, adaption mechanisms in heat stressed animals are oriented towards lowering the thyroid activity in an effort to reduce the metabolic heat production to cope with harsh external environment. However, the comparable THR expression between MC

and MHS groups clearly indicates the extreme adaptive nature of this breed. Although the expression pattern of THR-α was not significant between MC and MHS, a negative correlation between THI and THR-α indicates the severity of heat stress on this variable to negatively influence the growth in Malabari goats.

The LEP gene secreted from adipocytes are involved in several physiological functions, including regulation of energy expenditure, body temperature regulation and whole body metabolic balance (Supakorn, 2009). The lower hepatic LEP gene and its corresponding LEPR expression in MHS group as compared to MC indicates depleted energy resources in these animals as thermo-regulatory mechanisms are the biologically costly process and require constant energy supply for maintaining vital body functions. Likewise, heat stress associated low LEP gene expression was also established by other researchers and they attributed this to the may represent one of the homeostatic mechanisms through which heat-stressed animals attenuate thermo-tolerance by controlling the metabolic heat production by altering the energy metabolism (Bartha *et al.,* 2005 Lacetera *et al.,* 2009). Similar observation of lower LEP expression was also established in goats by Bagath *et al.* (2016) indicating it as an adaptive mechanisms of the stressed goats to consume more feed. The LEP gene therefore, can act as a nutritional cue to reflect the growth and energy metabolism of the stressed animals (Agarwal *et al.,* 2009). However, contrasting reports of increased LEP gene and its receptor expression in heat stressed bovine species were also observed by other researchers (Kamigaki *et al.,* 2006; Lafontan and Viguerie, 2006; Bernabucci *et al.,* 2009) and they attributed this to the nutritional status of these animals and the severity of heat stress. Further, it was also observed in laboratory animals that the effect of heat stress on LEP gene expression could be independent of the level of feed intake in the stressed animals (del Mar Romero *et al.,* 2009, Lee *et al.,* 2010). Therefore, the lower LEP gene and its receptors expression in heat stress group of Malabari goats from this study provide the initial evidence of the molecular mechanisms through which heat stress induces the changes in the LEP and its

receptor gene expression pattern to regulate energy and lipid metabolism in goats to support vital thermo-regulatory mechanisms. There are reports suggesting the molecular control of GH on the LEP gene expression in bovines which in turn provides lipostatic signal to regulate body weight, feed intake, expenditure energy, reproduction, and functions of the immune system (Garcia *et al.,* 2002; Nkrumah *et al.,* 2004). Further, a significant association between LEP gene and growth traits have also been established in goats (Wang *et al.,* 2015; Martinez *et al.,* 2016) and the expression pattern of this gene was also found to be associated with meat quality traits in cattle (Tian *et al.,* 2013). These authors attributed the energy metabolic regulation role of LEP during heat stress through its action on the hypothalamic-pituitary-adrenal axis, with subsequent effects on the growth process of different species (Delavaud *et al.,* 2002). Further, a negative correlation of THI with LEP and LEPR gene indicates the negative energy balance in the heat stressed Malabari goats.

The highest degree of degenerative changes was also recorded in the liver of MHS group as compared to MC group. This indicates the hepatic damage and burden on the MHS group goat liver to cope with heat stress. Similarly, Shilja *et al.* (2017) also reported more degenerative changes in the livers of heat stressed Osmanabadi goats. This indicates the sensitivity of hepatocytes to heat stress challenges and this effect was more pronounced in goats irrespective of breeds.

SUMMARY AND CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

The impact of heat stress on growth performance of goats has been established fairly based on changes associated with phenotypic traits. However, not many reports are available on the genotypic traits which get altered on exposure to heat stress in livestock. A basic understanding of how the molecular mechanisms by which growth is getting compromised during heat stress condition are therefore required before one can consider and adopt possible improvements. As growth related modulations in goat is also mediated through the biological mechanisms on the functioning of the somatotrophic axis, it is very vital to understand the underlying molecular and endocrine mechanisms by which growth is regulated. Therefore, an attempt has been made in this study to elucidate the molecular mechanisms governing growth performance during heat stress in goats. The primary objective of the study was to establish the influence of heat stress on the expression patterns of different growth related genes in indigenous Malabari goats.

Twelve animals were randomly allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The study was conducted for a period of 45 days. 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. The animals were slaughtered at the end of the study and their liver tissues were collected for gene expression study.

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 hepatic GH expression pattern was significantly ($P<0.05$) lower in heat stress group as compared to the control group animals. Although the THI shows that the MHS groups were subjected to extreme heat stress, still Malabari goats are well known for their survival in the hot humid tropical environment wherein the magnitude of heat stress could be further severe. Therefore, the reduced GH gene expression in heat stress groups could be attributed to the extreme adaptive nature of this indigenous Malabari goat. In addition, a strong negative correlation ($P<0.05$) was established between THI and GH gene expression pattern.

The quantitative expression pattern of hepatic GHR was significantly ($P<0.05$) lower in heat stress group as compared to the control group animals. The significantly lower GHR in the current study in the Malabari goats could be attributed its heat tolerance capability and this could be due to their indigenous nature. This shows that when nutrition was not compromised the animals were able to counter heat stress without compromising production. The altered hepatic GHR expression during heat stress indicates that changes were occurring to compensate for the release and action of GH. Similarly, changes in tissue GH responsiveness do not always correlate with changes in GHR abundance, indicating that abundance or activity of intracellular signaling proteins is also regulated. Further, a strong negative correlation ($P<0.01$) was established between THI and GHR gene expression pattern.

The hepatic IGF-1 gene expression pattern was found to be significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Given its growth-stimulating and anabolic effects, the reduction in the IGF-1 gene expression is likely to be the primary reason for the reduction in growth rate in heat stressed Malabari goats. Further, IGF-1 being considered a biological marker for growth governance and a strong negative correlation of THI with this variable in the

study clearly indicated the compromised growth performance in heat stressed Malabari goats.

The THR-α mRNA expression patterns are comparable between the MC and MHS groups. This again indicates that the growth performances are not much compromised due to heat stress in this breed. The other possible reason for this could be that the magnitude of heat stress may not have been severe enough to induce changes in the THR-α expression pattern. In general, adaption mechanisms in heat stressed animals are oriented towards lowering the thyroid activity in an effort to reduce the metabolic heat production to cope with harsh external environment. However, the comparable THR expression between MC and MHS groups clearly indicates the extreme adaptive nature of this breed. Although the expression pattern of THR-α was not significant between MC and MHS, a negative correlation between THI and THR-α indicates the severity of heat stress on this variable to negatively influence the growth in Malabari goats.

The hepatic LEP gene expression pattern was found to be significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Similar to the LEP gene expression, the LEPR gene also was found to be significantly down regulated in heat stress group as compared to the control group animals. The lower hepatic LEP gene and its corresponding LEPR expression in MHS group as compared to MC indicates depleted energy resources in these animals as thermo-regulatory mechanisms are the biologically costly process and require constant energy supply for maintaining vital body functions. The LEP gene therefore, can act as a nutritional cue to reflect the growth and energy metabolism of the stressed Malabari goats. Therefore, the lower LEP gene and its receptors expression in heat stress group of Malabari goats from this study provide the initial evidence of the molecular mechanisms through which heat stress induces the changes in the LEP and its receptor gene expression pattern to regulate energy and lipid metabolism in goats to support vital thermo-regulatory mechanisms. Further, a

negative correlation of THI with LEP and LEPR gene indicates the negative energy balance in the heat stressed Malabari goats.

The hepatocytes of liver in MHS group showed more fatty changes ($P<0.05$) compared to MC group. This indicates the hepatic damage and burden on the MHS group goat liver to cope with heat stress. Further, the hepatic degenerative changes also were more pronounced in MHS group ($P<0.05$) compared to MC group. This indicates the sensitivity of hepatocytes to heat stress challenges and this effect was more pronounced in goats irrespective of breeds.

The findings from this study provide some crucial initial information on how different growth related genes are expressed when Malabari goats are subjected to heat stress. This information might be of high value in assessing the growth performance of goats and may provide useful information pertaining to nutrient supplementation to the heat stressed goats. Further, a strong negative correlation was established between THI and all the growth related gene expression in the study reflecting the molecular mechanism for compromised growth performance during heat stress in goats. In addition, the study also established that the expression pattern of GH, GHR, IGF-1, LEP and LEPR genes could serve as reliable indicators for reflecting growth performance in indigenous Malabari goats.

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ABSTRACT

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EFFECT OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT GROWTH RELATED GENES IN MALABARI GOATS

by

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ABSTRACT OF THE THESIS

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ABSTRACT

The impact of heat stress on growth performance of goats has been established fairly based on changes associated with phenotypic traits. However, not many reports are available on the genotypic traits which get altered on exposure to heat stress in livestock. Therefore, the study is an attempt to elucidate the molecular mechanisms governing growth performance during heat stress in goats. The primary objective of the study was to establish the influence of heat stress on the expression patterns of different growth related genes in Malabari goats. The study was conducted for a period of 45 days in twelve 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. All animals had access to *adlibitum* feed and water and they were fed and watered individually. 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 16.00 h. At the end of study period, all 12 animals were slaughtered and their liver tissues were collected for gene expression and histopathological studies. The temperature-humidity-index (THI) inside the shed (74.9) proved that the animals were not stressed while in the outside environment (86.5) the animals were extremely distressed. The hepatic growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor-1 (IGF-1), leptin (LEP) and leptin receptor (LEPR) gene expression patterns were significantly ($P<0.05$) lower in heat stress group as compared to the control group animals. In addition, negative correlation ($P<0.05$) was also established between THI and all the growth related gene expression in the study. The hepatic histopathological section showed more fatty and degenerative changes ($P<0.05$) in hepatocytes in MHS group as compared to MC group. The study offers the first thorough insight into the expression patterns of different growth related genes during heat stress exposure in goats. Further, the study established GH, GHR, IGF-1, LEP, LEPR genes to be the ideal markers to reflect growth potential in Malabari goats. The findings from this study provide some crucial initial information on how different growth related

genes are expressed when Malabari goats are subjected to heat stress. This information might be of high value in assessing the growth performance of goats and may provide useful information pertaining to nutrient supplementation to the heat stressed goats.

Keywords: Climate change; goat; growth hormone; heat stress; IGF-1; Leptin