INFLUENCE OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT CYTOKINE GENES IN MALABARI GOATS

by

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(2013-20-113)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

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DECLARATION

I, hereby declare that this thesis entitled **"Influence of Heat Stress on the Expression Patterns of different Cytokine 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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Rasha Mol V.P

Dedicated to

Sejian Sir

And

My Beloved

Family

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SYMBOLS AND ABBREVATION

Abbreviation	Expansion
α	Alpha
β	Beta
γ	Gamma
$	Dollar
%	Percentage
°C	Degree Celsius
ΔRn	Distinct Variation of Log
ADG	Average Daily Gain
ANOVA	One-Way Analysis Of Variance
AR5	Fifth Assessment Report
ATP	Adenosine Triphosphate
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
C	Control
cDNA	Complementary DNA
CH_4	Methane
CMI	Cell Mediated Immunity
CO_2	Carbon Dioxide
DNA	Deoxyribonucleic Acid

F	Forward
FAO	Food and Agriculture Organisation
Fig	Figure
FMD	Foot and Mouth Disease
FSH	Follicle Stimulating Hormone
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDP	Gross Domestic Product
GHG	Greenhouse Gas
GnRH	Gonadotropin Releasing Hormone
H and E	Haematoxylin and Eosin
h	Hour
HPA	Hypothalamo-Pituitary-Adrenal
HS	Heat Stress
HSP	Heat Shock Proteins
HIS	Humane Society International
IACUC	Institutional Animal Care and Use Committee
ICAR	Indian Council of Agricultural Research
ICAR-NIANP	Indian council of agriculture research-National Institute of Animal Nutrition and Physiology

IFAD	International Fund for Agricultural Development
IFN	Interferon
IL	Interleukin
ILRI	International Livestock Research Institute
IPCC	Intergovernmental Panel on Climate Change
kcal	kilocalorie
kg	Kilogram
LH	Leutinizing Hormone
LN_2	Liquid Nitrogen
m	Meter
MAS	Marked Assisted Selection
MLN	Mesenteric Lymph Node
mm	Millimetre
mRNA	Messenger Ribonucleic Acid
N_2O	Nitrous oxide
NASPA-CCN	National Adaptation Strategy and Plan of Action on Climate Change for Nigeria
NCBI	National Center for Biotechnology information
NK	Natural Killer cells
NTC	Non-Template Control

OIE	Office International Epizootics
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
QTL	Quantitative Trait Loci
R	Reverse
RNA	Ribonucleic Acid
RTqPCR	Real Time Quantitative Polymerase Chain Reaction
RVFV	Rift Valley Fever Virus
SPSS	Statistical Package for the Social Sciences
Ta	Annealing temperature
TBE	Tick Born Encephalitis
T_{db}	Dry Bulb Temperature
Th	T helper
THI	Temperature Humidity Index
TNF	Tumor Necrosis Factor
T_{wb}	Wet Bulb Temperature
UNFCC	United Nations Framework Convention on Climate Change
UNPD	United Nations Development Programme
US	United State

USA	United States of America
USGCRP	United States Global Change Research Program
VBD	Vector-borne disease

INTRODUCTION

CHAPTER 1

INTRODUCTION

Climate change is the most challenging issue human kind had ever experienced which pose risks to both the global ecological balance as well as economic security. Global climate change is mainly instigated by greenhouse gas (GHG) emissions that result in warming of the atmosphere (IPCC, 2013). According to fifth assessment report (AR5) of Intergovernmental Panel on Climate Change (IPCC), the global surface temperature is expected to rise by 0.3-4.8 °C by the end of 2100 (IPCC, 2013). The rising temperature along with the increased intensity of weather and climatic extremities ultimately affects all the ecosystems on earth and particularly the negative impacts on the agricultural and its allied sector yields threatens the global food security.

Livestock is an important agricultural components widely believed to play a significant role to global food security. According to FAO (2015), the global demand for livestock products are expected to double by 2050 due to rising human population and their improved standard of living. Among the various factors, climate change is considered the most intriguing factor which negatively affects the livestock production (Nardone *et al.,* 2010). Further within the climatic variables, heat stress was established to be the most crucial factor affecting the livestock production (Sejian *et al.,* 2016).

Among the livestock species, small ruminants like goat are mainly reared by poor and marginal farmers and they play a substantial role in securing their livelihoods (Mengesha and Tsega, 2012). Goat has exemplary capability to survive in any agro-ecological zone because of their extreme disease resistance, dexterous grazing behaviour, high feed conversion efficiency and drought tolerance (Shilja *et al.,* 2016). In addition, rearing of goat involves low investment with maximal output mainly because of its small size, prolific breeding, less housing requirements, less feed requirements, less management

care as compared to large ruminants (Aziz, 2010). The native goat breeds in particular are hardy with high thermo-tolerance and disease resistance that help them to cope with harsh environmental conditions.

Heat stress was found to alter the immune functions of livestock and increase the susceptibility of the animals to infectious diseases. The stress signals act on the hypothalamo-pituitary-adrenal (HPA) axis to alter the immune responses in livestock. The chronic heat stress indirectly causes immune suppression in livestock and makes them vulnerable to diseases (Sophia *et al.,* 2016). The end product of HPA axis, the glucocorticoid acts on the immune cell receptors to modulate immune response in livestock (Webster and Glaser, 2008). Further, the stress hormone also modulates the cytokine release and this may act as indicators for the levels of compromised immune responses.

According to Shini *et al.* (2010a), the pro-inflammatory cytokines in poultry namely, interleukin-1β (IL-1β) and IL-18 are increased during acute stress and decreased in chronic stress condition. Further, the glucocorticoids also enhance IL-10, which is normally found at the end of immune response (Marchant *et al.,* 1994). The tumor necrosis factor-alpha (TNF-α) is required to initiate innate immune response through inhibition of p38 MAPK pathway which helps in maintaining animals stability. Further, heat stress induced glucocorticoids also acts to inhibit the TNF-α in mice (Abraham *et al.,* 2006).

Moreover, the pro-inflammatory cytokine interferon-β (IFN-β) which is essential for innate immune response is down regulated during the heat stress condition (Jin *et al.,* 2011). Also, the IFN-γ which is the key component of humoral immune response was found to be decreased during heat stress condition (Ju *et al.,* 2014).

In the changing climatic scenario, heat stress is the predominant stress affecting livestock production through emergence of different vector borne diseases (VBDs). Heat stress weakens the animal's immune system and makes them more prone to diseases. Even though, the compromised immune system

during heat stress in animal has been observed by various researchers, the impact of heat stress in particular on immune system related gene expression has not been elucitated. Further, the heat stress mediated immune suppression at molecular level has not been dealt in detail in goats. Therefore, research efforts pertaining to quantifying the impact of heat stress on immune system related gene expression are crucial for development of superior thermo-tolerant breeds without compromising their health. Fig.1.1 is the hypothetical figure describes the expression patterns of different cytokine genes during heat stress exposure in goats. Not much research efforts are available on heat stress effect on the cytokine gene expression patterns in goats. Hence, an effort has been made in this study to determine the impact of heat stress on different cytokine gene expression pattern in heat stressed Malabari goat breed. This study would establish the hidden intricacies of heat stress influencing different cytokine gene expression pattern in goat breed. In addition, this study helps to identify suitable immunological markers which may reflect the compromised animal health during heat stress condition. Promotion of such breed might prove to be a worthful attempt to ensure the livelihood securities of poor and marginal farmers. Therefore, the study was conducted with the primary objective of establishing the differences in relative expression patterns of different lymph node associated cytokine genes between the normal and heat stressed Malabari goats.

The objectives of the present study are:

1. To assess the expression patterns of different interleukins gene in Malabari goats subjected to heat stress.
2. To evaluate the expression patterns of different cytokine genes in heat stressed Malabari goats.
3. To establish the correlation between temperature humidity index (THI) and expression patterns of different cytokines in heat stressed Malabari goats.

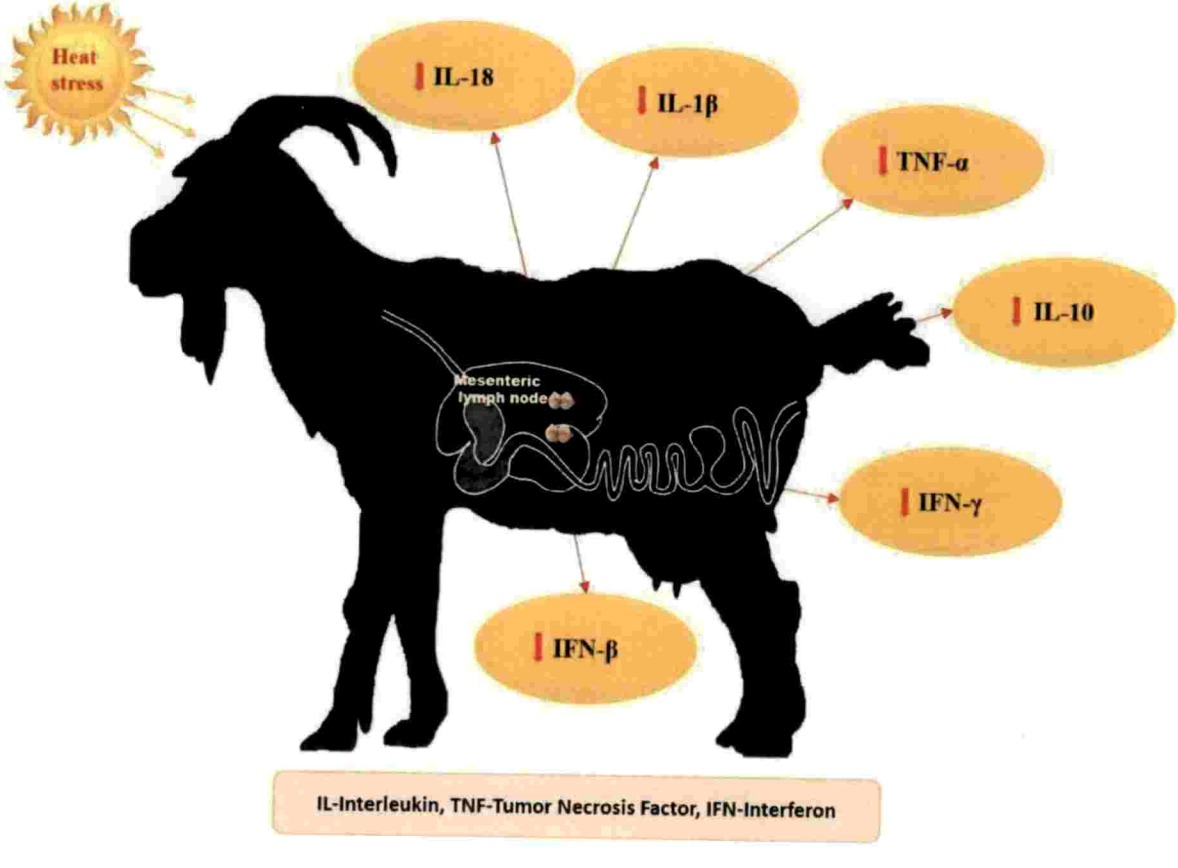


Fig 1.1 Hypothetical figure describing the expression patterns of different cytokine genes during heat stress exposure in goat

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Livestock sector is the source of livelihood for about 1.3 billion world population contributing about 53% of world agricultural gross domestic product (gross domestic product) (FAO, 2009). Among livestock, small ruminants are mainly reared by small, marginal and landless farmers of developing countries. The native goat species are hardy animals with disease resistance mechanisms that allow them to survive in the harsh climatic conditions in tropical and sub-tropical regions.

Animals reared in the tropical environments are frequently subjected to heat stress due to high temperature combined with high relative humidity (Sejian *et al.,* 2011). These stressors impair production, reproduction (Martin *et al.,* 2004) and also compromise the immune system, thus increasing the animals susceptibly to diseases (Deng *et al.,* 2012).

Abiotic stressors such as heat and nutritional stress have a major impact on livestock productivity (Sejian *et al.,* 2011). In the changing climate scenario, other factors like solar radiation, photoperiod, and humidity also synergize with the above stressors. Temperature influences the animals' productive and reproductive traits in a major way. In particular, heat stress is one of the crucial factors affecting livestock productivity (Rivington *et al.,* 2009). Furthermore, the concept of global warming is alarming the planet earth day by day. According to the latest report released by IPCC (2013), there is an average of about 1.53°F (0.85°C) rise in global surface temperature from 1880-2012. Heat stress affects animal productive performances like milk yield, meat quality and reproductive performances like age at maturity, ovulation failure, embryo mortality etc (Shinde and Sejian, 2013). It also weakens the animal's immune system and makes them more prone to diseases. Although this has been observed by various researchers,

the impact of heat stress on immune gene expression and process of heat stress mediated immune suppression at molecular level has not been dealt in detail in livestock. In order to withstand the existing and emerging pathogen challenges, the animal's immune system should be in a right tone. Further, to mitigate immune suppression by means of various nutritional and hormonal interventions, an in depth understanding of the immunological pathways affected by heat stress and the mechanism by which it is affected is mandatory.

The pro-inflammatory cytokines namely IL-6, IFN-ß which contributes to innate immune response were down regulated by heat stress (Jin *et al.,* 2011). The cytokine genes for both T helper1 (Th1) (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cell responses were down regulated during heat stress (Liu *et al.,* 2012). Heat stress experiments in Bama miniature pigs revealed that IL-12, the key cytokine gene to initiate cellular immune response was up regulated but IL-2 and IFN-γ which are also involved in cell mediated immunity (CMI) process were down regulated, revealing differential expression of immune cytokine genes (Ju *et al.,* 2014). Heat stress reduces the blood flow to intestine, reduces the integrity of intestinal epithelium, causes villi desquamation, reduce the villi height and crypt depth (Yu *et al.,* 2013). Furthermore, the innate immune components like mucosal barrier, toll like receptors, secretory Ig A, intestinal intraepithelial lymphocytes production (Deng *et al.,* 2012), expression of cytokines responsible for humoral and cell mediated immune response were down regulated in intestine by heat stress. Reduction of intestinal immune function enabled bacterial translocation to mesenteric lymph node (Liu *et al.,* 2012). Research efforts are needed to quantify immune responses to different environmental stresses, particularly in light of the changing climate where there are sudden outbreaks of different diseases in livestock.

2.1 Climate change and its consequences

Climate change refers to statistical changes in weather over time and it can include long-term changes in rainfall, wind, temperature, or other patterns (Treut and Somerville, 2007). The changes in climate remain to be the most unprecedented challenge faced by human in the 21st century (Ayinde *et al.,* 2011). The climate change has become more threatening not only for the sustainable development of the socio-economic and the agricultural activity but also to the totality of human existence (Adejuwon, 2004).

Climate change mainly attributed to the changes in the emission of GHGs which includes carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) through different activities (Steinfeld *et al.,* 2006). Generally, these GHGs are not harmful rather their presence in the atmosphere helps to sustain life on the planet by keeping the earth warm (Forster *et al.,* 2007; EPA, 2008). Since the pre industrial era, the anthropogenic GHG emissions have increased which is mainly driven by economic and population growth. Between 1970 and 2004, GHG concentrations rose about 70% (Rogner *et al.,* 2007). This culminated in up surging of GHGs concentration in the atmosphere to the level never before in last 800000 years (IPCC, 2014). The burning of fossil fuel and other GHG emitting activities are also contributes towards this increase in the level of GHGs in the atmosphere resulting in greenhouse effect. The increased rate of greenhouse effect arising from the anthropogenic activities results in global warning, which is believed to be the root cause for the current climate change. Continuous emission of GHGs may cause further warming and long lasting changes in all components of climate system, which increases the frequency of climatic events and weather extremes and irreversible impacts for people and ecosystem. Rapidly increasing human population is also tipped to be the major contributing factor for the present adversities of climate change.

According to UNFCC (2007), the GHG emission could rise by 25 -90 % at the end of 2030 in comparison with 2000 which may result in warming of the earth surface by 3°C during this year. The scientific studies of tree rings, coral reefs, and ice cores shows that average global temperature has up surged substantially as the industrial revolution began in the mid- 1700s (HSI, 2011). The IPCC predicted that, even 1 - 2.5°C rise in temperature may cause serious effect in agriculture and allied sector including reduced crop yield in tropical areas leads to risk of hunger, rapid spread of newly emerging diseases such as malaria as well as increasing the risk of extinction of plants and animals around 20-30% (UNFCC, 2007). Since 1950, there have been increasing occurrences of extreme weather events such as more heavy precipitation, frequent occurrence of heat and cold waves and expansion of drought affected areas resulting in the emergence of new diseases ultimately increasing the number of deaths (Schneider *et al.,* 2007; IPCC, 2014). Further, hurricane intensity has increased since 1970 onwards (Trenberth *et al.,* 2007). Since the beginning of industrial era, the CO_2 uptake of the ocean increased tremendously which culminated in ocean acidification, the pH of ocean surface water has decreased by 0.1 and 26% increase in acidity. Further, 75 m of the ocean surface is warmed by 0.11°C (0.09 to 0.13) per decade over the period 1971 to 2010 globally (IPCC, 2014). The glaciers started retreating, sea level is raising, sea ice is melting, tundra is thawing, and increasing the number of extinction is the other impact of climate change (IPCC, 2007). The raising of sea level and reduction of sea-ice leads to loss of habitat for the polar bears which result in the risk of extinction of species (Fischlin *et al.,* 2007).

In several regions extreme weather events and rainfall are becoming more common while others are experiencing more extreme heat waves and droughts. Agriculture and allied sector strongly rely on climatic variables especially the temperature and precipitation levels and therefore changes from the normal level cause may eventually cause huge loss to these sectors. These include impacts on agriculture and forestry, effects on water supplies, damages from sea-level rise to coastal areas and expenditures to protect them, increased mortality risks, effects

on fisheries and wetlands, and effects of changes in conventional air and water pollutants. These impacts of climate change are likely to intensify further in the coming decades. Limiting the effect of climate change risk would require substantial and sustained reductions in GHG emissions together with adaptation strategies.

2.2 Climate change impacts on animal agriculture

The agricultural ecosystems are highly susceptible to the effects of climate change (NASPA-CCN, 2011). Livestock is a contributor to the climate change and simultaneously livestock production system is also sensitive to the same phenomenon (Reddy, 2014). Climate change affects the livestock both directly and indirectly. Climatic variables such as temperature, humidity, precipitation, wind speed and other climatic factors are directly affecting the productive (growth, milk, and wool) and reproductive performance of animal (Rojas-Downing *et al.,* 2017). Indirectly climate change influences the production by altering the quantity and quality of feed and through emergence of sudden disease outbreaks (Niggol and Mendelsohn, 2008). It also alters the immune function of the animal and makes them susceptible to infectious diseases. The exposure to the stress either enhances or suppresses the immune function in the animal (Sophia *et al.,* 2016).

2.2.1 Impact on fodder availability

Climate change negatively affects the distribution as well as the availability of fodder. The climate change affects the quality and quantity of the crops and this particular impact was determined by the location, production system and the species (IFAD, 2010). The feed quality and quantity is mainly affected by increase in atmospheric CO_2 and temperature as well as dry condition due to variations in concentrations of water soluble carbohydrates and nitrogen (Chapman *et al.,* 2012). The concentration of CO_2 may improve the forage quality by enhancing the crude protein content and digestibility of C3 plants than C4 plants (Thornton *et al.,* 2009). The up surge of temperature may increase the

lignin and cell wall components in plants which directly affect the digestibility and degradation rate leading to decreasing nutrient availability for the animal (Polley *et al.,* 2013; Sanz-Saez *et al.,* 2012). The increased temperature of 2° C may negatively affect the fodder growth and livestock production in arid and semiarid region but it may positively impact in the temperate region (Thornton, 2010).

The other important factor affecting the quality and quantity of forage is the length of the growing season which determines the duration and periods of availability (Rojas-Downing *et al.,* 2017). The climatic extremities may affect the root growth, leaf growth rate, even lead to drastic reduction in the yield of crops (Thornton *et al.,* 2015). The continuous exposure to warmer temperature and elevated CO_2 as well as the non-availability of water due to the changes in the precipitation pattern drastically decline the crop and forage growth which negatively affect the livestock sector (Giridhar and Samireddypalle, 2015).

2.2.2 Impact on Growth

Growth is the increase in the live body mass which is controlled both genetically as well as environmentally (Marai *et al.,* 2007). Climate change is an important limiting factor that reduces the growth performance of the animal which is threatening the meat industry in the tropical region (Mpofu *et al.,* 2017). Environmental stresses reduce the feed intake as well as increase the tissue catabolism are the major reason for the growth retardation in livestock during stresses (Popoola *et al.,* 2014). The increase in tissue catabolism could be attributed to the increase in catecholamines and glucocorticoids after exposure to heat stress in livestock.

Among the growth parameters, body weight is the first and foremost variable affected by environmental stresses in livestock (Pragna *et al.,* 2018). Environmental stressors reduce body weight, average daily gain (ADG) and body condition of livestock. Popoola *et al.* (2014) reported reduced ADG in West African dwarf goats and they attributed this to the decreased feed intake of the

animal during heat stress exposure. In a recent study comparing the growth potential of three different indigenous goat breeds during heat stress exposure significantly reduced all growth variables expect body mass index (BMI) (Pragna *et al.,* 2018). In another study Habibu *et al.* (2016) reported a significant reduction in BMI in Sahell and Red Sokoto goats during heat stress condition. There are also reports suggesting the significant influence of heat stress on the body condition scoring of the animals (Sejian *et al.,* 2010; Pragna *et al.,* 2018).

2.2.3 Impact on Milk Production

One of the principal negative influences of heat stress in livestock is its effect on milk production. Climate change especially increased temperature adversely affects the milk production as well as its composition which results in economic losses to the farmers (Rojas-Downing *et al.,* 2017). Milk production is the most sensitive productive process to heat stress as it decreases the yields upto 35-40% are a common occurrence during environmental induced hyperthermia in dairy animals (Wheelock *et al.,* 2010). The THI is negatively correlated with milk yield, as the THI increases from 68 to 78 there is decrease of dry matter intake as well as milk synthesis by 9.6% and 21% respectively (Spiers *et al.,* 2004; Bouraoui *et al.,* 2002).

The up surge in temperature leads to increase in body temperature of the animal resulting in decreased milk yield (Masama, 2016). Increasing frequency of stressful days may negatively impact the both the yield and production of milk in cattle and buffaloes (Chauhan and Ghosh, 2014). Heat stress causes decline in feed intake but increases the demand for energy and protein requirements for maintenance. In addition, the energy requirements for maintenance of dairy animal also increase by 30 % during stressful condition. Therefore, the intake of the animal may not be sufficient to cover the daily requirement for the production of milk.

During stressful days, the dairy animals reduce their feed intake which results in inducing negative energy balance is considered responsible for declining the milk production (Wheelock *et al.,* 2010). Decline in the milk yield are pronounced and milk quality variables such as reduced fat content, lower-chain fatty acids, solid-non-fat, and lactose contents are affected. Generally, the higher production animals are more sensitive to increased ambient temperature (Masama, 2016). According to Rhoads *et al.* (2009) the decrease in dry matter intake by 0.85 kg per cow for every 1°C increase above thermo-neutral zone and similarly the decrease in milk yield were recorded to be 36%. Upadhyay *et al.* (2007) reported that, the milk yield drastically declined at mid and early stage of lactation and the milk yield varied from 10-30 % in first lactation and 5-20 % in subsequent lactation periods in Murrah buffaloes. Further, thermal stress is also responsible for marked decrease in carbohydrate, lipid, and protein metabolism which could be attributed to the reduced feed intake in heat stressed animals (Baumgard and Rhoads, 2013).

2.2.4 Impact on Meat Production

The animal meat and carcass quality characteristics are governed by several intrinsic and extrinsic factors. The extrinsic factors affecting the meat quality include stresses such as environmental factors (Guerrero *et al.,* 2013). During the stressful condition, the animal exhibit several adaptive mechanisms which compromise the productive functions of animal (Sejian *et al.,* 2016). The quality of beef was reported to be reduced when the beef cattle was exposed to summer season induced heat stress with the ambient temperature of 34.3±1.67°C and 48.8+7.57% relative humidity (Balamurugan *et al.,* 2018).

Increased temperature decline the muscle pH which results in altering all physico-chemical attributes such as cooking loss, water holding capacity, meat colour and shear force (Gregory, 2010). Further, heat also influences the meat quality at the cellular level including changes in expression of different heat shock proteins (HSP) (Chauhan *et al.,* 2014). Archana *et al.* (2018) reported that a wide

variety of carcass and meat quality characteristics are affected by heat stress exposure in three different indigenous goat breeds.

The HPA axis is stimulated and leads to increased secretion of catecholamines and cortisol which depletes the energy reserves and induces severe dehydration and catabolism of protein all of which culminates in deteriorating the meat quality (Nikbin *et al.,* 2016).

2.2.5 Impact on Reproduction

Climate change negatively affects the reproductive efficiency of both male and female livestock (Dash *et al.,* 2015; Rojas-Downing *et al.,* 2017). Heat stress can reduce dry matter intake to indirectly inhibit gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH) and leutinizing hormone (LH) secretion from the hypothalamo-pituitary system. Increased temperature compromises the reproductive function including poor expression and intensity of oestrus due to reduced estradiol secretion from the dominant follicle developed in a low LH environment (Balamurugan *et al.,* 2017).

The conception rate of dairy animals was reported to be declined with increasing ambient temperature (Schuller *et al.,* 2014). The oocyte growth and quality by altering progesterone secretion, impairment of embryo development and pregnancy rate of cows and pigs are affected by increased temperature (Ronchi *et al.,* 2001; Barati *et al.,* 2008; Nardone *et al.,* 2010). Further, the compromised oocyte growth alter the secretion of LH, FSH and ovarian dynamics during the oestrus cycle which impair the embryo development and increase embryonic mortality in livestock (Nardone *et al.,* 2010). In addition, the pregnancy during heat stress slows the growth of the fetus and increases fetal loss. Heat stress can also directly compromise the uterine environment to cause embryo loss and infertility in livestock.

The male reproductive performance is also affected equally for the heat stress. Heat stress also was associated with lower sperm concentration and quality in livestock (Karaca *et al.,* 2002; Kunavongkrita *et al.,* 2005). The testes must be

2-6 °C cooler than core body temperature for the production of fertile sperm. Therefore, increased testicular temperature during heat stress exposure may hamper the fertile sperm production by altering the seminal and biochemical parameters leading to infertility in livestock (Kebede, 2016). Cardozo *et al.* (2006) reported seasonal influence in the testicular volume, hormonal profiles, semen quality and sexual behaviour that affect the reproductive performance of male. Further, Bhakat *et al.* (2014) also reported reduced male fertility which lowered the conception as well as fertility rates per insemination of male.

2.2.6 Impact on livestock water availability

Global agriculture utilizes 70% of fresh water resources, making the sector as the world's largest water consumer (Thornton *et al.,* 2009). Nevertheless, world's demand for water is moving towards increased competition due to water scarcity and depletion, where 64 % of the global population will undergo water-stressful conditions by 2025 (Rosegrant *et al.,* 2002). Water is an important input for agriculture, livestock, human life, industries and all other living beings. As temperatures rise, animal requires more water to maintain good health status and sustain in the changing climatic scenario. As a result of its spatial and temporal variation, its availability, affordability and accessibility poses a great challenge to users and water planners. These drawbacks are further intensified by the devastating effects of climate change (Upadhyaya, 2016).

2.3 Heat stress as a major factor influencing livestock production

Numerous factors affect livestock productivity such as genetics, environment, nutrition, health status, age, sex etc. The productive capacity of the livestock species varies with age, sex as well as the level of stress they are exposed to. Most of the hybrid breeds are more productive than indigenous breeds because of their superior genetic potential. Currently, the rates of genetic changes are improved in crossbred verities because of the technological advancement to improve the productive traits of animals such as milk yield (Simm *et al.,* 2004). Further, livestock disease occurrence is another important factor

which hampers animal production causing severe economic problem to the farmers.

The outbreak of new diseases reduces the productive and reproductive performance of the animal. The sudden outbreak of diseases may cause direct losses such as reduced fertility, stunting, changes in herd structure and death while indirectly cause additional costs for drugs and vaccines, labour costs and profit losses due to denied access to better markets and use of suboptimal production technology (Rushton, 2009).

Climate change is the major predisposing factor hindering economic viability of livestock production (Henry *et al.,* 2012). As the climate continue to warm, the frequency, duration, and severity of extremities such as heat waves, hotter day and nights etc may increase (IPCC, 2013). Among the climatic variables, ambient temperature seems to have a critical role in negatively influencing the livestock production (Reynolds *et al.,* 2010). The increase in temperature alter the species distribution, time of reproduction, migration events, population size and increase in the frequency of pest and disease outbreak (Henry *et al.,* 2012). Therefore, heat stress is considered as the single most crucial factor affecting the livestock sector.

High temperature coupled with relative humidity hampers the cellular function and impairs various functions of animals (Amundson *et al.,* 2006). Further, heat stress also reduces the feed intake which alters the metabolic and digestive functions of the animal thereby impairing their productive functions (Mader *et al.,* 2003). The up surged temperature strongly affects the productive and reproductive performance of the animal and also impair the normal behaviour, immune system and the physiological functions of the animal (Nienaber and Hahn, 2007; Rashamol *et al.,* 2018).

2.4 Economic Consequences of Heat Stress Impact on Livestock Production

The human population is expected to up surge in the coming decades within the range of 7.96 - 10.46 billion and an average of 9.15 billion at the end of 2050 (UNPD, 2008). The rapid increase in the population growth results in food insecurity especially in developing countries. The other important factor which increases the food demand is urbanisation. Livestock is an important sector which helps to satisfy the food demand of growing population because agriculture sector contributes about 53% of the GDP (World Bank, 2009). Further, livestock is the fast growing agriculture sector in developing countries like India (Rosegrant *et al.,* 2009). However, agriculture sectors are more sensitive and vulnerable to heat stress which results in severe economic loss to agriculture oriented countries (USGCRP, 2009; IPCC, 2014). The estimated annual economic loss in US alone due to heat stress ranges from $1.7 to $ 2.4 billion (St-Pierre *et al.,* 2003).

The demand of the livestock product is doubling in sub-Saharan Africa and South Asia, that is, 200 kcal per person per day in 2000 to around 400 kcal per person per day in 2050 (Van Vuuren *et al.,* 2009). Further, the total demand for meat production is tripled from 45 to 134 million tons in the year of 2020 (World Bank, 2009). Livestock products are the most efficient provider of nutrients and in the same way livestock is the most vulnerable sector to climate change (Thornton and Gerber, 2010). Among the climatic variables, heat stress continues to be a major economic problem for the livestock industry.

The effect of heat stress is evident in tropical region where most of developing countries are located than sub tropical region (Muller *et al.,* 2010). As a result, these regions faces extended period of heat stress, and making significant economic loss, food insecurity, climatic extremes, and death (Johnson *et al.,* 2015). Heat stress reduces feed intake, body weight gain, and reproductive efficiency, in livestock sector (Baumgard and Rhoads, 2013). Therefore, it may cause financial burden to livestock keepers by decreasing the milk and meat production, reproductive efficiency and outbreak of diseases.

The annual losses were primarily determined by estimating the loss of the productive function such as meat production, Milk synthesis and reproduction. According to Collier *et al.* (2012), the prolonged expose of dairy animal to heat stress result in economic loss due to declined milk yield up to $3375 per year. A study by St-Pierre *et al.* (2003) evaluated the annual economic impact of heat stress on dairy cows and found that without heat abatement strategies in place, the nation's dairy industry incurred an economic loss of $1507 million. Qi *et al.* (2014) estimated that, a 1.0 °C increase in ambient temperature during summer season leads to 4.5 % reduction in production output of livestock species.

The economic loss associated with dairy production system was 25 times greater than the losses incurred in beef production system (Scharf *et al.,* 2014). The average annual losses in milk production across the state were $ 89.01 while the beef production was only $ 3.05 during heat stress exposure (Scharf *et al.,* 2014). Reproductive processes in both the sexes are very sensitive to environment changes. Heat stress causes infertility in farm animals and this represents a major source of economic issue to the farmers.

2.5 Animal Adaptation to Heat Stress

Climate change results in several environmental issues which negatively affects the productive performance of livestock. In livestock sector, stress is considered as a reflex reaction which happen when the animal undergone severe climatic and environmental changes and this results in unfavourable conditions and even may result in mortality of the animal. This discomforts faced by the animal due to climate change is restored by generating certain mechanisms. In order to maintain the thermal comfort zone for animal during stress condition, they initiate compensatory and adaptive mechanisms such as behavioural and physiological changes to re-establish homeothermy and homeostasis which promote welfare and favour survival in a specific environmental condition (Indu *et al.,* 2015).

Animal exhibits several adaptive mechanisms in order to withstand the climatic stresses in the changing climatic scenario (Alameen *et al.,* 2012). Animals expose to particular stress may cope up either through behavioural, physiological, molecular or the combinations of different adaptive mechanisms. Adaptation is broader terms which describe the ability of the animal to cope to the stresses by modifying their physiological, behavioural and genetic characteristics that make the animal more suitable for a specific environment. Adaptive mechanisms exhibited by the animal depend on number of factors such as exposure to stresses, intensity, duration, frequency, physiological and health status of the animal and environmental restrains (Etim *et al.,* 2013).

Animals deviates the energy for the adaptive mechanisms to cope with the adverse climatic condition resulting in compromised productive functions such as milk production, meat production, and reproduction. The animal's adaptive capabilities are determined by changes in the morphological, behavioural, physiological, neuro-endocrine, blood biochemical and cellular and molecular responses which help them to survive in a specific environment (Das *et al.,* 2016). Adaptation refers not only the tolerances capacity of the animal but also their ability to maintain production in harsh environmental condition (Hoffmann and Sgro, 2011).

Morphological adaptation include changes in the morphological characters of the animal such as includes coat, fur depth, hair type, hair density, fat storage in hump or tail especially under desert conditions, skin colour and body size (Khalifa, 2003). The four dimension of adaptive capacity of livestock are: the ability to make informed assessment of imminent threats, the ability to make to an informed choice, from a range of options, about the best response measure, being capable of deploying the preferred option and being free to implement this option (Rashamol and Sejian, 2018).

Behavioural responses are one of the primary mechanism by which the animals survives the stressful environment. The impact of heat stress leads to behavioural changes such as reduction of feed intake, increased lying time, increasing the drinking frequency, reduced standing time and exhibit shade seeking behaviour etc (Athira *et al.,* 2017). The changes in the behavioural response indicate the severity of the stress the animal is exposed to. Further, physiological adaptive mechanism is also considered as one of the primary adaptive responses that the animal exhibit to cope to the environmental stresses (Rashamol *et al.,* 2018). Physiological adaptive mechanisms include increasing the respiration rate, rectal temperature, pulse rate, heart rate, skin temperature, sweating rate (Rashamol *et al.,* 2018).

Endocrine Response is the major regulators for animal adaptation by activating adrenal and thyroid gland. The stress induces changes in the pituitary hormone secretion which result in altered metabolism, immune competence and behavioural changes in the animal (Binsiya *et al.,* 2017). Further, cellular adaptation is an acute systemic response to heat stress which plays a significant role in imparting thermo-tolerance capacity to the animal during stress condition (Archana *et al.,* 2017).

Resilient capacity of animal is the ability of an animal to regain the normal biological function after the exposure to harsh environment. Resilience capacity helps the animal to bounce back to the original condition and perform better in the changing climatic scenario. The traditional farming practices and biodiversity help the animal to be more resilient and cope to the climatic changes. Among the agriculture sector, livestock possess more resilient capacity than any other sectors because of the potential to strengthen the resilience to the changing climate. The major inherent traits involved in the adaptive and resilient mechanisms are long legs, large surface area, short hair coat, lower metabolic rate, higher sweating rate, body conformation, higher capacity for maintenance of heat balance, and higher feed efficiency, higher tolerance to dehydration and adipose tissue depots and capacity to alter the hormone and biochemical profiles to adapt to a particular

environment (Rashamol and Sejian, 2018). Further to claim the animals are adapted, only if the animal's phonotypic and genotypic characters are in normal condition such as normal physiological responses, proper feed intake, and good health status coupled with standard production in climate changing condition.

Global warming, climate change and extreme weather events have an adverse effect on biodiversity, distribution of animals and micro flora, all of which may increase the likelihood of emergence of zoonotic agents and infectious disease outbreaks (Sachan and Singh, 2010). Climate change is expected to cause an increase in weather-related disasters and extreme weather events, such as droughts, heat waves, storms, desertification, and increases in insect infestations. Long-term changes in climate will jeopardize the future of all animals.

Climate change is responsible for the emergence and proliferation of the many disease such as malaria and zoonotic parasitic diseases including Leishmaniasis, cryptosporidiosis, giardiasis, trypanosomiasis, schistosomiasis, filariasis, onchocerciasis, and loiasis (Patz *et al.,* 2000). The most sensitive diseases are those that are indirectly transmitted, that is, those requiring either a vehicle for transfer from host to host (eg, water- and food-borne disease) or an intermediate host or vector as part of its life cycle.

Global climate change predictions suggest that far-ranging effects might occur in the population dynamics and distributions of livestock parasites, provoking fears of widespread increases in disease incidence and production loss (Morgan and Wall, 2009). The majority of infectious diseases are those that require either a vehicle or vector for host to host transmittance during their life cycle (eg, water- and food-borne disease).

Livestock are sensitive to temperature variations and it is the major of cause of economic loss due to climate change (Reilly *et al.,* 2003). Increased temperature is the major cause of reduction in livestock productivity. An estimation of economic losses per year in USA was $2.4 billion with minimal heat stress abatement. Climatic restrictions on vectors, environmental habitats and

disease causing agents are important for keeping many animals diseases in check (Stem *et al.,* 1989). Alterations of temperature and precipitation regimes may result in a spread of disease and parasites into new regions or produce an increase in the incidence of disease, which, in turn, would reduce animal productivity and possibly increase animal mortality (Baker *et al.,* 1998). Baylis and Githeko (2006) described the potential of how climate change could affect parasites and pathogens, disease hosts, and disease vectors for domestic livestock. The potential clearly exists for increased rate of development of pathogens and parasites due to spring arriving earlier and warmer winters that allow greater proliferation and survivability of these organisms.

Warming and changes in rainfall distribution may lead to changes in spatial or temporal distributions of those diseases sensitive to moisture such as anthrax, blackleg, haemorrhagic septicaemia, and VBDs.

2.6 Climate change and disease occurrence in livestock

Climate change, environmental changes, changes in human demographics and behaviours, and the rise of global trade and travel are most-often-cited drivers for the emergence of infectious diseases in human and animal populations (Wolfe *et al.,* 2005; Chomel *et al.*, 2007; Woolhouse and Gaunt, 2007; Jones *et al.*, 2008; Reperant, 2010). Warmer global temperatures may allow an expansion of the geographic range within which both the mosquito and parasite could survive with sufficient abundance for sustained transmission. Model predictions indicate that a 3 °C global temperature rise by 2100 could increase the number of annual malaria cases by 50-80 million (Martens *et al.,* 1995).

Climate change is widening viral disease among farm animals, expanding the spread of some microbes that are also a known risk to humans. According to OIE, several countries are indicating that climate change has been responsible for atleast one emerging or re-emerging disease occurring on their territory. Little data exist to evaluate the effects of climate change on infectious diseases of animals. However, some data exist from other parts of the world (Khasnis and

Nettleman, 2005; Epstein, 2001; Kutz *et al.,* 2005), and a recent report from the World Organization for Animal Health (OIE) addresses the impact of climate change on the epidemiology and control of animal diseases (OIE, 2008).

Sensitivity of the vector to climate change could change the range, season and incidence of many zoonotic diseases (CDC, 2008). Some few examples are:

1. Increased night temperature could increase the flight activity of the vector for example malaria (Purse *et al.,* 2005)
2. Night temperature could increase the replication and transmission cycle of viral pathogen (Baylis and Githeko, 2006)
3. Cycle of massive humidity followed by drought provide breeding site of vector and pathogen to facilitating them for disease outbreaks (Baylis and Githeko, 2006)
4. Alteration in the precipitation range could help in the migration of arthropod vector to different landslides to convert the disease outbreaks from endemic to epidemic (Trape *et al.,* 1996).

2.7 Heat stress impact on immune response in livestock

Variations in temperature and rainfall are the two most significant climatic variables affecting livestock disease outbreaks. Temperature increases and changes in rainfall patterns have an impact on the persistence and patterns of occurrence of bacteria, viruses, parasites and fungi and the patterns of their corresponding food borne diseases (Tirado *et al.,* 2010). Such changes also have an impact on microbial ecology and growth, plant and animal physiology and host susceptibility which may result in the emergence, redistribution and changes in the incidence and intensity of plant and animal diseases and pest infestations, all of which could impact foodborne diseases and zoonoses (FAO, 2008).

Warmer temperatures will benefit free living bacteria and parasites whose survival and development is limited by temperature. Warmer temperatures could promote survivability, shorter development rates and transmission. Insects such as mosquitoes and ticks that transmit disease agents may also benefit from climate

change as well as the diseases they spread (Bradley *et al.,* 2005; Randolph *et al.,* 2008; Sumilo *et al.,* 2009). Wall and Ellse (2011) say there is a widespread assumption that a generally warmer environment will result in higher parasite abundance and increased disease incidence.

Change on the spread and emergence of animal diseases as global temperatures increase, the effects will be quite complex and vary from region to region. Though the extent of these effects is uncertain, it is known that those communities and regions with the least resources, such as rural agricultural areas, will be the most vulnerable to climate change. Warmer and wetter weather (particularly warmer winters) will increase the risk and occurrence of animal diseases, as certain species who serve as disease vectors, such as biting flies and ticks, are more likely to survive year-round. Certain existing parasitic diseases may also become more prevalent, or their geographical range may spread, if rainfall increases. This may contribute to an increase in disease spread, including Zoonotic diseases.

2.7.1 Climate change and types of disease outbreaks

Climate change is considered a major threat to human health and wellbeing, with increasing evidence of it affecting infectious diseases (Dufour *et al.,* 2006; Gale *et al.,* 2009). Increased temperature have supported the transmission of vector from warmer area to the cooler areas for example malaria and livestock tick born diseases i.e, babeiosis, theileriosis, anaplasmosis, bluetongue disease in Europe. Heavy rainfall also supports the transmission of vector to the wetter areas for example Rift valley fever. There are many disease emerging rapidly from the last decade those are continue to spread over large landslides for example ovine chlamydiosis, caprine arthritis, equine infectious anemia, Eqine Influenza, Marek's disease, Bovine viral diarrhea etc. Outbreaks of diseases such as Foot and Mouth Disease (FMD) or Avian Influenza affect very large numbers of animals and contribute to further degradation of the environment and surrounding communities' health and livelihood.

The three most mentioned diseases were bluetongue, spread among sheep by biting midges; Rift Valley fever, a livestock disease that can also be picked up by people handling infected meat; and West Nile virus, which is transmitted by mosquito from infected birds to both animals and humans. African livestock productivity has been severely affected by vector-borne livestock diseases, such as nagana (Trypanosomiasis), known as sleeping sickness. Nagana is transmitted to people and animals by the tsetse fly. Approximately 30% of Africa's 160 million cattle population and comparable numbers of small ruminants are at risk (ILRI, 2005). Cutaneous myiasis (blowfly strike) is common disease of livestock and would be expected to be highly sensitive to even small changes in climate (Wall and Ellse, 2011). Rift Valley Fever: Rift Valley fever virus (RVFV) is an emerging zoonotic disease of significant public health, food security, and overall economic importance, particularly in Africa and the Middle East. In infected livestock such as cattle, sheep, goats and camels, abortions and high death rates are common.

2.7.2 Vector borne diseases

Increasing temperatures have supported the expansion of vector populations into cooler areas, either into higher altitude systems (for example, malaria and livestock tick-borne diseases i.e. babeiosis, theileriosis anaplasmosis) or into more temperate zones (for example, the current outbreak of bluetongue disease in northern Europe). Changes in rainfall pattern can also influence an expansion of vectors during wetter years. This may lead to large outbreaks of disease, such as those seen in East Africa due to RVFV, which is transmitted by a wide variety of biting insects. The potential complexity of climate change influences with other factors associated with vector populations is well illustrated by the distribution of tsetse flies in sub-Saharan Africa. Tsetse flies transmit African trypanosomes widely in livestock (ruminants, equids, and pigs). Tsetse flies are very sensitive to environmental change, either due to climate or direct human impacts on habitat.

Climate change will impact not only on the distribution and abundance of arthropod vectors but also on the interaction between the virus and its vector. Climate change induces changes in the environment that promote the spread of contagious diseases through increased contact between animals, or increased survival or availability of the agent or its intermediate host. The distribution and prevalence of VBDs may be the most significant effect of climate change. In the context of VBDs, the predicted temperature increases and changes in other climatic variables are likely to have profound effects. Like the disease organisms themselves, the vectors are cold-blooded and so peculiarly liable to be influenced by climatic variables, especially temperature and humidity. The transmission of VBD depends upon the three stages that is (i) Infectious agent (ii) the Vector (ii) the host. The OIE categorize 66 diseases that affect the ovine, equine, bovine, swine, caprine in which 22 are consider as a VBDs.

The VBDs are widespread in Europe and are the best studied diseases associated with climate change. In both human and animal health, it is in the area of VBDs, that link between climate change and disease has become most apparent. In a number of cases, arthropod vectors such as ticks, midges and mosquitoes that can transmit various viral and protozoal diseases to animals and humans are expanding their ranges into new geographic areas. Furthermore, in temperate regions, warmer winters are allowing these vectors to survive year round thus increasing the likelihood that cycles of infection will be continuous rather than be interrupted by winter freeze. Twenty two of 66 listed diseases in the OIE animal health terrestrial code affecting bovine, swine, caprine, ovine and equine alone or more than one species are VBDs

2.7.3 Tick borne diseases

Tick born Encephalitis (TBE) is caused by an arbovirus of the family Flaviviridae and is transmitted by ticks, Ixodes ricinus that act as both vector and reservoir. Similar to VBD this disease is also accelerated by the climatic changes mainly with increased day and night temperature. A common example of TBE is

FMD. There are seven distinct types of FMD virus that are distributed in various landslides of the world.

2.7.4 Parasitic Diseases

Ecological disturbances exert an influence on the emergence and proliferation of several parasitic diseases of humans and animals including, leishmaniasis, cryptosporidiosis, giardiasis, trypanosomiasis, schistosomiasis, lariasis, onchocerciasis, filariasis, and fascioliosis. Each environmental change, whether occurring as a natural phenomenon or through human intervention, changes the ecological balance and context within which disease hosts or vectors and parasites breed, develop, and transmit disease. Climate changes could also influence disease distribution indirectly through changes in the distribution of livestock. Areas becoming more arid would only be suitable for camels and small ruminants. If these species are forced to aggregate around water points, the incidence of parasitic diseases could increase. The most important adaptive trait of tropical livestock is disease resistance.

Two of the most important disease resistance traits have been identified for trypanosome tolerance in African ruminants and helminth resistance, particularly in certain breeds of sheep across tropical and temperate regions. Particularly for trypanosome tolerant breeds, climate change may decrease the importance of this trait in sub-humid zones of West Africa. One potential danger is that if climatic changes lead to selection against trypanosome tolerance in the short to medium term, these adaptive traits that have developed over millennia will be lost if future conditions lead to greater disease risk in the longer-term. The combined effects of environmentally detrimental changes in local land use and alterations in global climate disrupt the natural ecosystem and can increase the risk of transmission of parasitic diseases to the human population.

Climatic variables are able to affect the prevalence, intensity and geographical distribution of zoonotic helminthes, directly influencing free-living larval stages and indirectly influencing mainly invertebrate, but also vertebrate

hosts. The impact of climate change appears to be more pronounced in trematodes, and is mainly shown by increased cercarial production and emergence associated with global warming. Fascioliasis, Schistosomiasis and cercarial dermatitis caused by avian schistosomes have been most important (Singh, 2010). Alveolar echinococcosis is currently the only cestode disease that climate change has been found to influence. Nematodiases, including heterakiasis, different trichostrongyliases and protostrongyliases, anchylostomiases and dirofilariases are the helminth disease most intensively analysed with regard to climate change.

2.8 Effect of heat stress on immune responses

Several scientists dealt in detail about the impact of heat stress on the various behavioural and physiological parameters in livestock species (Lu, 1989; Kadzere *et al.,* 2002; Marai *et al.,* 2006; Yahav, 2009). They reported different results in which the heat stress is a factor as it either suppress or enhance the immune system. Both the adaptive and innate immune responses are affected in heat stressed animals. Any stressful condition stimulates the endocrine system to increase the catecholamines and glucocorticoids secretions which modulate the cytokine release which regulate the different immune responses.

2.8.1 Heat stress and innate immunity

Literatures pertaining to heat stress influence on livestock immune responses are very negligible. Heat stress negatively affects the relative weights of lymphoid organs such as spleen, thymus and cloacal bursa (Aengwanich, 2008). The mechanical barriers which act as the first defence lines in innate immune response are mucosa and skin. Quinteiro-Filho *et al.* (2010) reported that, heat stress exposure caused mild acute lymphocytic enteritis in poultry.

An important component of innate immune system called natural killer cells (NK) cells present in the systemic circulation as well as in the lymphoid organs such as lymph node, spleen and bone marrow destruct the tumour cells and infectious agents like bacteria, fungi and viruses. A study by Won and Lin, (1995) reported, chronic heat stress reduce splenic NK cell cytotoxic functions in

mice because of the glucocorticoid influence in the immune cell (Won and Lin, 1995). Jin *et al.* (2011) studied pro-inflammatory cytokines such as IL-6 and IFN-ß that contribute to innate immune response were down regulated by heat stress exposure in mice.

Heat stress increases the secretion of glucocorticoids which acts as inhibitor of the pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 initiating the innate immune responses by the inhibition of p38 MAPK pathway which maintains the stability of the animal (Abraham *et al.,* 2006). Further, glucocorticoid also enhances anti-inflammatory cytokines like IL-10 normally found out at the end of the immune response (Marchant *et al.,* 1994). Catecholamines acts through adrenergic receptors to elevate cAMP levels which reduce the cytotoxic functions (Whalen and Bankhurst, 1990).

2.8.2 Heat stress and adaptive immunity

Both the humoral and cell mediated immune response represent the adaptive immune response in animals (Sophia *et al.,* 2016).

The Th1 cells are responsible for cell mediated immune response and Th2 involved in humoral immune response. The level of cytokine gene expression alters both the immune function from Th1 and Th2 and vice versa. The Th1 cells secrete IFN- γ, IL-2, TNF-β which contributes to cellular immunity and Th2 secretes IL-4, IL-10 and IL-13 which controls humoral immunity. The IL-12 and IFN-γ together converts uncommitted Th0 cells to Th1 cell whereas the cytokines IL-4 and IL-10 induce Th2 cell production (Elenkov and Chrousos, 1990). The release of major cytokines involved in Th1 based cell mediated immunity such as IL-12 and IFN-γ are inhibited by the action of glucocorticoids (Elenkov *et al.,* 1996). According to Wu *et al.* (1998), the expression pattern of IL-12 receptors in NK cells and Th1 cells are down regulated by the action of glucocorticoids thus altering the immune system by shifting immune function from Th1 to Th2 cells.

Dendritic cells are potent APCs and link the innate and adaptive immune response as they are involved in phagocytosis and antigen presentation. Dendritic

cells also increase the expression of MHC Class II and co-stimulatory molecules such as CD80, CD83 and CD86 on their cell surface. Glucocorticoids also act as an inhibitor of the expression of these molecules on DCs which prevent its maturation (Girndt *et al.,* 1998). Also adrenaline and nor-adrenaline promotes the release of catecholamines during exposure to heat stress which prevents the production of IL-12, development of Th1 cells and Th2 differentiation (Elenkov *et al.,* 1996).

2.9 Importance of studying the heat stress impact on indigenous livestock breed

Indigenous animals are well known for their survival in the specific agro-ecological zone of their origin. They are extremely adapted breed with the ability to withstand harsh environmental condition in contrast to cross bred and exotic breed. Indigenous animals are well known for their adaptive capacity when exposed to harsh environmental conditions as compared to the cross bred and pure bred animals. The low investment to rear indigenous animals is considered a main advantage over exotic /pure breeds as they require huge initial investments. They exhibit superior adaptive nature in terms of their thermo-tolerance, drought resistance and disease resistances ability. In addition, they also have the ability to survive on limited pasture available during summer season.

In contrast, the increased temperature makes the exotic or cross bred animals more susceptible to diseases which decrease their immunity. Production performance is the one aspect where the hybrids score over than native breeds. But during exposure to adverse environmental condition which is not congenial to milk production the exotic animals reduce the yield more than the indigenous breeds. Heat stress related problems such as fertility and production performance are exacerbated in exotic animals. The heat stress also reduces the feed intake of the animal which culminates in reduced milk and meat yield up to 25% and this reduction was more pronounced in exotic animals.

A study on the impact of heat stress on the physiological, haematological and behavioural characteristics on native Tharparkar and hybrid Karan Fries reported that the native breed exhibit better adaptation capacity than the hybrid (Pandey *et al.,* 2017). Further, Sansthan and Rollefson (2005) reported that, there are several indigenous livestock species which perform well in the unfavourable condition because of their genetic superiority which helps them to survive in a specific environment.

Vechur is an indigenous breed of cattle in Kerala which exhibit superior adaptive capacity and high disease resistance ability with low feed intake. The small fat globules in the milk of this breed make their milk more easily digestible. Variations in physiological adaptability were also established between indigenous, cross bred and pure bred animals and the results from these studies indicated that, the indigenous livestock breeds were tipped to be exhibiting less physiological variability as compared to their counterparts during the exposure to climate change. Therefore, in the changing climate scenario it is evident that, the conservation of indigenous breed is very essential because of their hardy nature and they are better suited to withstand the adverse environmental conditions.

As per FAO (2015) guidelines, efforts are needed to study the impact of heat stress on the indigenous animals because it may yield important biological markers which could be used in breeding programmes to conserve these germplasms. Studying their genetic traits in different season helps to identify the biological markers which improve their adaptability and productive capacity and it can be used for the breeding program in marked assisted selection (MAS) to develop new breed with superior thermo-tolerance ability.

In addition, efforts are also needed to shift these breeds to different locality with an intention of assessing their ability to survive in different harsh environmental condition which might pave way for identifying region specific breeds. Further, it also helps to judge the survivability of different indigenous breeds and among them the best breed which can survive and produce optimally

cutting across the agro-ecological zone can be selected as an ideal animal model which can be disseminated to the farmers ensuring their livelihood in the climate changing scenario. Based on these advantageous over exotic animals, it is very essential to study in a systematic way the impacts of heat stress on indigenous breeds both in their native track as well as shifting them to different agro-ecological zones to assess their survivability in varied climatic regions to select an appropriate region specific breed to help the local farmers.

2.10 Significance of goat from climate change perspectives

Small ruminants are the backbone of the rural economy which constitutes 80% of the goat's population in developing countries (Shilja *et al.,* 2016). Asia holds the largest goat population (545 million) followed by Africa (245 million), which together account for about 93% of the global goat population (Devendra and Solaiman, 2010). Currently, the economic importance of the goats had tremendous progress because of their adaptive capacity in extreme climatic condition. Goat meat contains nutritious richness and the high value protein content make their meat indispensible role in forming a healthy and well balanced diet, and is considered optimal for human growth and development (Adeyemi *et al.,* 2015).

Over 10,000 years, goats are associated with human being in a symbiotic relation and it is the earliest farm animal to be domesticated (Aziz, 2010). Goats are important for the prevailing climate change perspective when comparing with other livestock species. They have the ability to survive in harsh environment condition because of the small body size and water conserving capability (Hamzaoui *et al.,* 2012). Goats are mainly reared for the production of meat, milk and hide. Rural poor farmers and women mainly depend on goats because of the huge initial investment and the difficulty associated with rearing large ruminants than the small ruminants. The rearing of goat needs low investment initially and provide maximum output mainly because of the small size of the animal, prolific breeding, less housing requirement, less feed and less management requirement.

Therefore, goats are considered to contribute immensely for the livelihood securities of poor and marginal farmers. Hence, goat is considered as poor man's cow in the climate change scenario.

In developing countries like India, small ruminants play a huge role for securing the economic status (Agarwal *et al.,* 2014). Goats have several advantages for maintain their production in extreme climate conditions. Especially, they effectively convert feed sources into desired output as compared to other animals. The other important trait of goat breed is the low methane emission than the other ruminant species.

Goat have exemplary capability to survive in any agro-ecological zone because of their skilful grazing behaviour, extreme disease resistance, drought tolerance, and high feed conversion efficiency (Shilja *et al.,* 2016; Debele *et al.,* 2013). The physiological factors influences the goats ability to maintain productivity in difficult condition such as low metabolic heat production, tolerance to water deprivation, anatomic and morphologic structure which enable efficient utilization of low quality feeds, skin and hair type, sweat gland capacity, reproductive capacity and, resistance to diseases and parasites and these advantages makes the goat sought after species for climate change adaptation. Further, their ability to survive in scarce pastures may be useful in tropical countries like India. The local goat breeds possess high genetic potential with resistant capacity to drought and diseases under changing climatic condition (Silanikove, 2000; Silanikove and Koluman, 2015).

2.11 Malabari goat

Malabari is an important indigenous goat breed originated in the tropical India. This goat breed is a native breed of Kerala mainly inhabits in Calicut, Kannur, Wayanad and Malappuram districts. Malabari breed is medium to small sized animals with varied coat colour ranging from white to admixtures and black. This breed is well adapted to the hot and humid conditions of Kerala state. This breed has good heritability for reproductive trait, which is the most important

production aspect of small ruminants (Thiruvenkadan *et al.,* 2008). This goat breeds exhibit excellent reproductive capabilities with the possibility of twinning and triplets (Alex and Raghavan, 2012).

The average body weight of Malabari buck weighs between 45 to 50 kg and doe weighs between 35 to 40 kg. The breed is reared mainly for production of meat and well known for their good quality skin and palatable meat (Verma *et al.,* 2009). The Malabari breed of goat is famous for high prolificacy and low fat meat (Bablu, 2002, ICAR, 2008). The milk yield of the Malabari breed ranges from 1-2 kg/day. Therefore, this breed is considered a dual purpose breed with good source of meat and milk (Jindal, 1984).

2.12 Heat stress impact on the cytokine production

Heat stress has detrimental effect on the immune system of livestock by altering the immune functions and making the animal more prone to diseases (Lacetera *et al.,* 2005; Lacetera *et al.,* 2006; do Amaral *et al.,* 2009). There are different types of cytokines present in the body with different functions for controlling the immune function of the animal. The IL-1β is generally considered as pro-inflammatory cytokines which stimulates HPA axis oriented stress response (Arimura *et al.,* 1994). The effect of heat stress on IL-10 could attribute shifting of humoral immunity to cell mediated immunity (Ohtsu *et al.,* 2015).

The IL-18 has a specific role in modulating the immune response in animals which was considered a very unique and distinct feature comparing to other cytokines (Alboni *et al.,* 2010). Any alteration in IL-18 level may directly reflect the immune status of the animals because of its uniqueness in its function. The IFN cytokines act as the first line of defence against the virus, bacteria, and parasites to modulate the immune system (Sophia *et al.,* 2016). The IFN-β plays a crucial role in maintaining the innate immune response. Heat stress was also established to be one of the primary factors which negatively influence the expression pattern of IFN-γ indicating the production of the same is compromised during heat stress condition (Hu *et al.,* 2007).

Cytokines are essential proteins of immunity which recognized as endogenous signalling molecules that mediate the cellular defence against inflammatory response by increased temperature (Hietbrink *et al.,* 2006). Cytokines are produced by activating macrophages in response to bacterial products which include IL-1 and TNF-α which activate lymphocytes and increase antibody production in animal (Strong, 2014).

The TNF-α activates vascular endothelium and increases vascular permeability, which results in increased effector cells immigrating to the site of infection. The IL-1, IL-6 and TNF-α are important cytokines which induce acute response helping the host to defend itself from the pathogens by activating the phagocytic cells (Möller and Villiger, 2006). The increases in the cytokine concentration reflect the adaptive response before calving because inflammatory cytokines are related to the stress-induced acute-phase response in cattle (Lomborg *et al.,* 2008).

The innate immunity can be evaluated through the ability of neutrophils to phagocytize and destroy pathogens (do Amaral *et al.,* 2011). External stress signals, which act as indicators of stress or injury in other cells, employ mediators such as catecholamines, endotoxin, alarmins, ATP and pro-inflammatory cytokines, such as TNF-α and IL-1β (Welc and Clanton, 2013).

The production of IL-1β protein was suppressed in rat by the rapid and prolonged exposure of the animal to heat stress (Nguyen *et al.,* 1998). Increase in the serum concentrations of TNF-α and IL-10 was identified in lactating cows during exposure to heat stress (Zhang *et al.,* 2014). Similarly, Sahin *et al.* (2010) also reported the increased concentration of serum TNF-α in heat stressed quails. Another study in hen reported increased level of TNF-α and IL-1 concentration in serum throughout the experimental period (Deng *et al.,* 2012). Further, study in experimental animal by Lin *et al.* (1994) and Leon, (2006) showed marked increase in IL-6, IL-1 and TNF-α concentration during harsh environmental conditions.

The elevated concentration of cytokine such as TNF-α were observed in various pathological condition includes endotoxemia and infection and several reports have shown correlation between concentrations of TNF and its soluble receptor (Van Zee *et al.,* 1992; Spinas *et al.,* 1992). The production of glucocorticoids by the HPA axis inhibits IL-1 release at both the expression and the post-translational level (Knudsen *et al.,* 1987; Gewert *et al.,* 1999).

Heat stress induced by the release of inflammatory cytokines, especially IL-1, IL-6 and TNF-α from the macrophages or blood monocytes at the site of inflammatory lesions or infections. The liver is the main site of synthesis of most acute phase proteins. Cytokines therefore act as mediators between the local site of injury and the hepatocytes to produce and release the acute phase proteins (Jain *et al.,* 2011). In contrast, Strong (2014) reported there is no significant difference in the plasma concentration level of IL-1β, IL-6, and TNF-α among the group of hen at 27 week of age. Further, Retzlaff *et al.* (1994) demonstrated the relationship between glucocorticoids and cytokine under in-vitro condition.

A study in Sahiwal and Karan Fries cows reported that the IL-10 concentration was higher on the day of calving (Sheikh *et al.,* 2016). Heat stress experiments in mouse shown IL-1β concentration was elevated at initiation of heat stress (Hashim, 2010). The IFN-β plays critical role in the development and maintenance of innate immune response (Thornley *et al.,* 2007; Vanden Bush *et al.,* 2009; Popko *et al.,* 2010). The increased IL-1β production was identified in both the hypothalamus and plasma in heat stress group as compared to those of control rabbits. Further, the higher level of glucocorticoids suppresses the synthesis and release of cytokine production of IL-6 and TNF-α (Richards *et al.,* 2001). A study by Benschop *et al.* (1994) reported that activation of the sympathetic nervous system suppresses the dendritic cells and monocytes function by inhibiting the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α.

An experiment in laying hens subjected to 12 day of heat stress up to 34°C reported increase in IL-1 and TNF-α concentration in serum (Deng *et al.,* 2012). In addition, the heat stress activates the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which ultimately results in hemorrhage and necrosis of different organs in rodents (Bouchama and Knochel, 2002). The concentration of IL-1β, IL-6 and IL-18 increased in heat stressed hen during the study period (Shini *et al.,* 2010a). In an in vitro study by Ju *et al.* (2014) found decreasing level of IFN-γ in controlled peripheral blood mononuclear cell (PBMC) over the period of the experiment. Further, the level of IFN-γ in PBMC was initially decreased followed by significantly increased levels during prolonged heat stress exposure (Ju *et al.,* 2014).

2.13 Expression pattern of different cytokine

Heat stress signaling pathway leads to expression of a peculiar cytokine pattern consisting of increased IL-6 and IL-10 expression and decreased TNF-α, IL-1β expression. In contrast, the endotoxemic pathway increases the expression of IL-1β, IL-6, TNF-α genes (Lang *et al.,* 2003). The pro-inflammatory cytokines called IL-6, IFN-ß contributes to innate immune response were down regulated by heat stress (Jin *et al.,* 2011). During heat stress, both Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cell responses were down regulated (Liu *et al.,* 2012). An experiment in Bama miniature pigs shown that, IL-12 was up regulated but IL-2 and IFN-γ were down regulated due to heat stress exposure revealing differential expression of immune cytokine genes (Ju *et al.,* 2014). Heat stress exposure for 15 days significantly reduced the expression level of IFN-γ in the spleen of broiler chicken (Ohtsu *et al.,* 2015).

A study in mice determined the effect of chronic heat stress on the innate immune response associated cytokines such as IL-6, IL-10, and IFN-β in the spleen after exposure to 38 ± 1◦C or 24 ± 1◦C (Jin *et al.,* 2011). This study established that the IL-6 and IFN-β mRNA levels were down regulated significantly compared to controlled group but the level of IL-10 was significantly

up regulated. Further, in the same study the mRNA expression level of TNF-α doesn't show any difference between the groups (Jin *et al.,* 2011). Shini *et al.* (2010b) demonstrated the exposure of heat stress in hen and reported significantly increased the mRNA expression pattern of the pro-inflammatory cytokines such as IL-1β, IL-6, IL-18.

The chronic heat stress retarded the maturation of dendritic cells and reduces the mRNA levels of IL-6 and IFN-β significantly in mice (Jin *et al.,* 2011). Further, it was established that the expression pattern of the IL-10 was higher in heat stress and varied between the breeds and the level of exposure (Sheikh *et al.,* 2016). Experiment has shown that at elevated temperatures, there was increased expression pattern of pro-inflammatory cytokines such as IL-1, IL-6, IL-2, TNF-α, granulocyte and macrophage colony-stimulating factor and IFN which supports a role for the cytokines in the pathophysiological response to heatstroke (Dinarello *et al.,* 1986; Velasco *et al.,* 1991).

The expression pattern of pro-inflammatory cytokines of IL-1β, IL-6, and IL-18 in the spleen and blood lymphocytes increased in chickens subjected to induced stress (Shini and Kaiser, 2009). Tao *et al.* (2013) reported the increased TNF-α expression pattern in heat stressed cow during transitional period. Further, Kang *et al.* (2011) suggested that the up regulation of TNF-α in tissue could be an indicator of heat stress in chicken.

Helwig and Leon, (2011) studied IL-1 expression in spleen and liver from heat stressed mice and reported higher level of IL-1β expression in both spleen and liver. This indicates heat stress influences IL-1 expression irrespective of whether the target organ being primary or secondary lymphoid organs. Further in the same study, the IL-18 expression also was found to be increased during heat stress exposure. Similarly, Sutinen *et al.* (2014) and Dinarello, (2006) reported, higher IL-18 expression during stressful condition. In contrast, reduced IL-1β gene expression was reported in heat stressed chicks by Meng *et al.* (2013). Further, Caroprese *et al.* (2017) correlated the hyperthermia induced increased

level of HSP70 expression to the increased level of regulatory cytokine IL-10 expression. The IL-10 is generally considered an anti-inflammatory cytokine and its levels were established in ruminant species mainly during pregnancy stress especially between the prepartum and postpartum periods (Wiegers *et al.,* 2005; van Engelen *et al.,* 2009; Brodzki *et al.,* 2015).

Heat stress induced significant reduction in expression pattern of IFN-β was also reported in mice by Jin *et al.* (2011). The heat stress associated glucocorticoid can inhibit the expression of IFN-γ which is considered one of the major cytokines involved in Th1 based cell mediated immunity. Liu *et al.* (2012) also established the down regulation of the IFN-γ cytokine during stressful condition in rat. Srinivasan *et al.* (2016) reported the heat stress induced increase in production of glucocorticoids brings this down regulation of IFN-γ gene expression. Thompson *et al.* (2014) reported significantly higher IL-10 expression and no change in the expression levels of IL-1β and TNF-α in heat exposed dairy cows. Further, Chauhan *et al.* (2014) also studied the expression pattern of different cytokines in sheep and established significantly higher TNF-α expression in the skeletal muscles of heat stressed sheep. In an another study, heat stress was found to down regulate the expression pattern of IFN-γ and no change was observed in the IL-10 and IL-18 expression in the spleen of broiler chicken (Ohtsu *et al.,* 2015). Also it was established that the expression level of IL-18 could be altered due to increased glucocorticoid secretion (Shini *et al.,* 2010a; Ohtsu *et al.,* 2015).

2.14 Biological marker for quantifying heat stress impact on immune response in goat

Variations in the expression patterns of gene could be used as a strategy to identify biological markers to reflect the condition which can be used as an aid in the selection of parental stock to propagate the supreme germplasm (Supakorn, 2009). Studying the genetics of an individual at DNA level and application of molecular genetics may provide opportunities for genetic improvement. Genetic

improvement of breeding depends on the selective breeding of the superior phenotypes. The accuracy of the breeding program and selection of the breed for desired characteristics can be improved by applying molecular markers. Molecular markers can help to evaluate the efficiency of the livestock performance for the purpose of genetic improvement as well as for genetic diversity of the animal (Dekkers, 2004; Groeneveld, 2010).

The immunological markers identified should be verified by the independent population before applied for an MAS program (Jamli, 2017). The identification of immunological marker and selective breeding to incorporate those markers in goat may increase their thermo-tolerance and disease resistance capabilities in harsh climatic condition (Vaiman *et al.,* 1996; Malveiro *et al.,* 2001; Fang *et al.,* 2008). Identification of genes that influence the biological response to disease make better understanding of the underlying physiological process by which the susceptibility of infection and the associated immune response could contribute to the development of genetic tools to fight against diseases (Bressani, 2014).

Application of molecular genetic techniques already resulted in discovery of several genes that have major effect on some interesting quantitative traits and genetic markers which are linked to quantitative trait loci (QTL) that helps the animal to survive and produce optimally in the climate changing scenario (Tambasco *et al.,* 2003; Womack, 2005). Currently, the advancement in genomic tools results in discovering individual gene or a group of genes to improve the genetic make-up of the animal. Cytokine are the cell-signaling proteins that play an important role in the immune system participating in intracellular communication (Bressani, 2014).

Several studies in different species identified IL-18 as an important immunological marker for assessing heat stress influence on the animal (Dinarello, 2006; Alboni *et al.,* 2010; Shini *et al.,* 2010b; Ohtsu *et al.,* 2015; Sutinen *et al.*, 2014). The increased expression pattern of TNF-α helped the

animals to survive in stressful condition by resisting to diseases. Therefore, TNF-α considered as a potential marker to identify the effect of heat stress on livestock (Tao *et al.,* 2013; Chauhan *et al.,* 2014). Further, IFN-β and IFN-γ expression pattern are also altered due to prolonged exposure to heat stress condition (Jin *et al.,* 2011; Liu *et al.,* 2012; Ju *et al.,* 2014; Ohtsu *et al.,* 2015; Srinivasan *et al.,* 2016). Consequently, IFN-β and IFN-γ are also being considered an important immunological marker reflecting the compromised immune status during exposure to heat stress.

The above literature review clearly indicates the dearth of information pertaining to climate change or for that matter heat stress associated influence on immune responses in domestic livestock. There is still a huge gap that needs to be bridged to elucidate the entire mechanisms by which heat stress impacts the immune response in livestock. Therefore, it will be a worthful attempt to investigate the impact of heat stress on immune response in ruminant species as these animals being the important productive animals to ensure food security in near future. Goat being projected as the ideal climate change model animal, any research efforts involving attempt to establish the heat stress impact on immune responses will be of immense value as this is the area of research wherein still many hidden intricacies are yet to be identified. Assessing the different cytokine expression pattern in particular may give a clue about the initial information of how the animals cope to the heat stress challenges by maintaining or altering their immune response mechanisms. Generation of these baseline information would be very vital as the scientific community battles in its effort to identify a breed with relatively higher thermo-tolerant and disease tolerant ability.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

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

3.2 Animals and Housing

A total of 12 ten months to one year old Malabari female goats weighing between 12-19 kg were used. Malabari goat breed is a meat purpose animal originated in the humid tropical region of Kerala in Southern India. Both Plate 3.1and Plate 3.2 represents the female Malabari goat breed inside and outside the shed in the experimental condition.

The animals were brought from different locality and acclimatized to the current experimental location for a period of 45 days. These animals were maintained in well ventilated sheds following standard farm management procedures.

Plate 3.1: Pictorial representation of Malabari goat outside the shed expose to heat stress

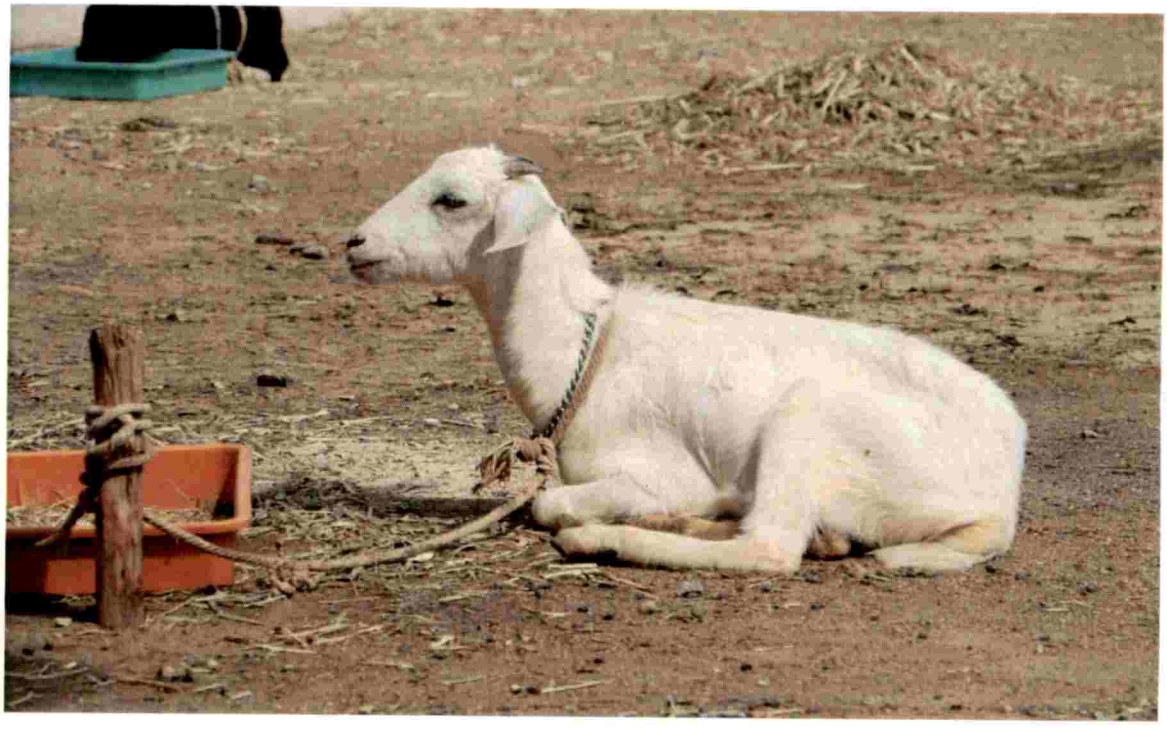


Plate 3.2: Picture representing the female Malabari goats inside the shed with comfort condition

3.3. 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, C (n=6; Malabari control), and HS (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 C animals were kept in the shed in comfortable condition and the HS 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 C animals were fed and watered inside the shed while HS 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. At the end of the study the animals were slaughtered and their MLN samples were collected in aseptic condition for subjecting them to gene expression. The study was conducted after obtaining approval from the Institutional Animal Care and Use Committee (IACUC) for subjecting the animals to heat stress (NIANP/IAEC/2/2017).

3.4 Expression of different cytokines in mesenteric lymph nodes

The animals were slaughtered at the end of the study and representative MLN 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 LN_2 and kept stored at –80 °C till further use.

The MLN 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 Maxima first strand cDNA synthesis kit (Thermo Scientific, Lithuania) was used to reverse transcribe the total DNA into cDNA. The cDNA was subjected to real time quantitative polymerase chain reaction (RTqPCR).

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 $2^{-\Delta\Delta CT}$ (Shilja *et al.,* 2016). Plate 3.2 depicts the pictorial representation of gene expression study of all target genes.

3.5 Histopathological observation

All the animals were slaughtered at the end of the study period and their MLN tissues were collected for histopathological sectioning. Care was taken to collect representative tissue sample from the same site of MLN 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 C and HS 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 C and HS groups.

Table 3.1: PCR primer pairs and reaction conditions used in the gene expression study

Gene ID	Primer Type	Primer sequence (5"- 3")	Ta (°C)	Reference
IL-1β	F	CCTTGGGTATCAGGGACAA	6	(Baron *et al.*,
	R	GGGTATGGCTTTCTTTAGG	0	2014)
IL-10	F	ACTGTTCAGATAATGCACCCCAG	6	(Bin-Tarif *et al.*,
	R	TTCTTACACTGCACAGAGATGGTTAC	0	2014)
IL-18	F	CCAGATGGTTCTCCTGCTGTGT	6	(Li *et al.*, 2008)
	R	GACCAATACGGCATCTTCCTTC	0	
TNF-α	F	CTCCGGCCTAACTCTCTCCT	6	(Baron *et al.*,
	R	AGGCCCACCCTTAGCTACAT	0	2014)
IFN-β	F	GGAATACCTGGACTATGCTGA	6	(Bin-Tarif *et al.*,
	R	CCTCACTTCCCTACATCCCT	0	2014)
IFN-γ	F	GGTGATGCTGGTGCTGAGTA	6	(Bin-Tarif *et al.*,
	R	TCATAAGTCCCTCCACGATG	0	2014)
GAPDH	F	GGTGATGCTGGTGCTGAGTA	6	(Smeed *et al.*,
	R	TCATAAGTCCCTCCACGATG	0	2007)

Ta: Annealing temperature; IL: Interleukin; TNF: Tumor Necrosis Factor; IFN: Interferon; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase used as reference gene to normalize the gene expression of target genes

Plate 3.2: Pictorial representation of gene expression study of all target genes.

RESULTS

CHAPTER 4

RESULTS

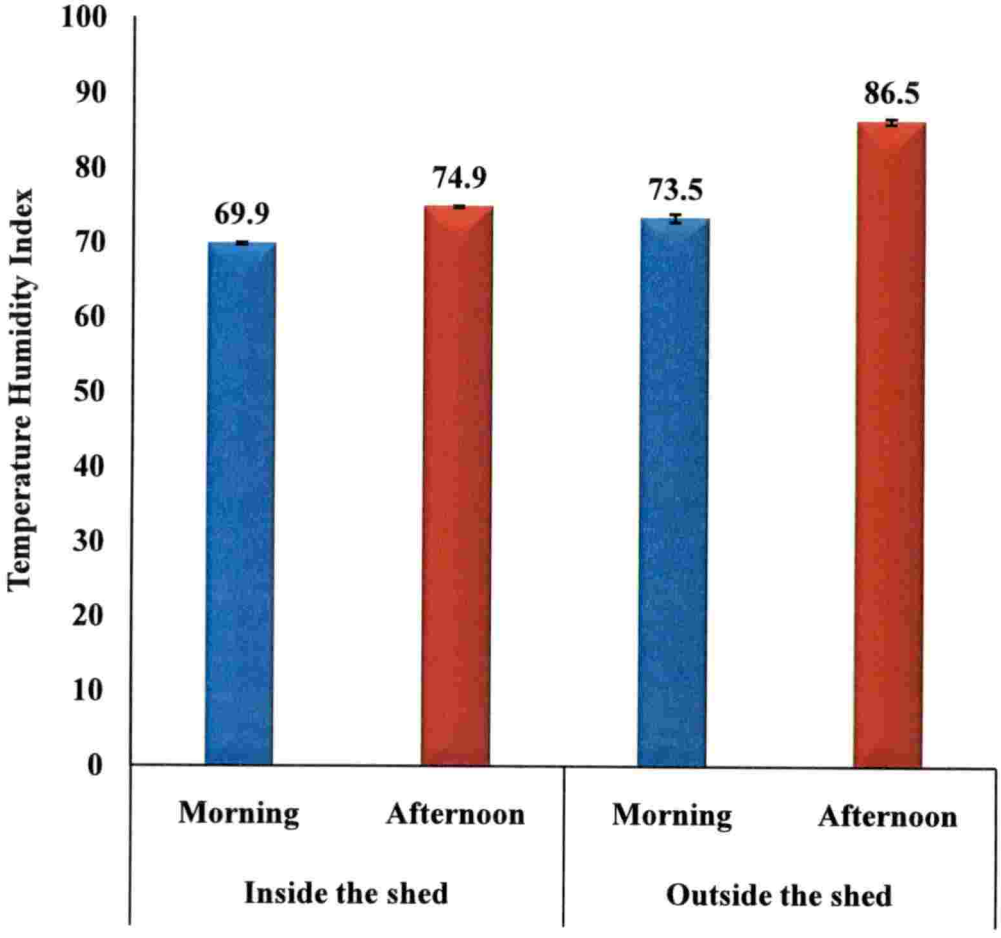
4.1 Temperature-humidity index

The THI values calculated during the study period to describe the level of heat stress were depicted in Fig.4.1. The THI values descriptions 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. 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 the shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P<0.01$).

4.2 Real time amplification plot of target genes

Amplification plot showed distinct variation of log (ΔRn) for different genes against PCR cycle number depicted in fig.4.2.(a). 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 depicted in fig 4.2.(b). Different genes (IL-1β, IL-10, IL-18, TNF-α, IFN-β, IFN-γ) showed different Tm in the melt curve graph depicted in fig 4.2.(c). 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: Mean temperature humidity index (THI) for the entire duration of the study both inside and outside environment



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

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

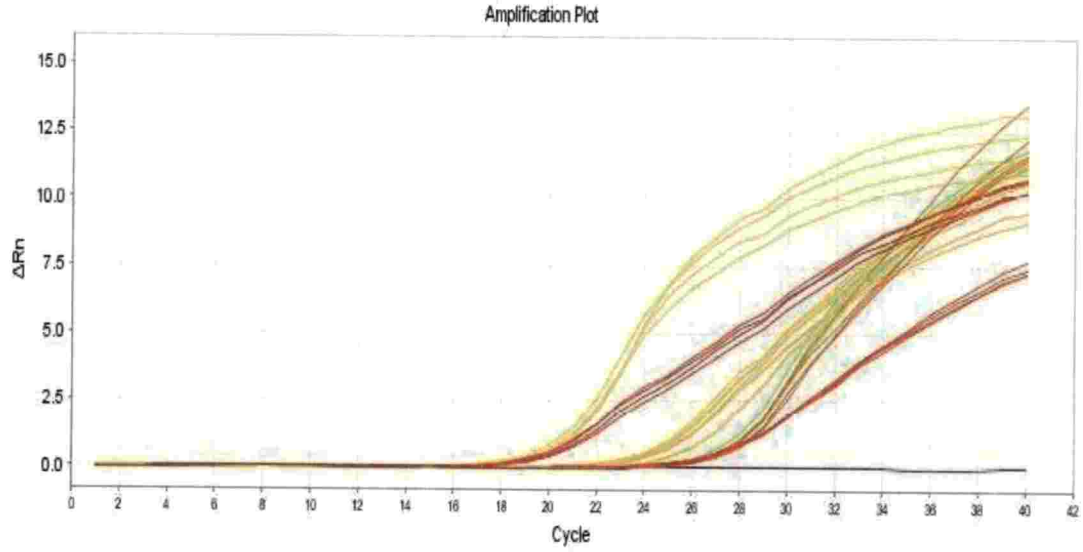


Fig. 4.2.(b) Description of Multicomponent plot also showing the difference between the amplified and the non-amplified genes

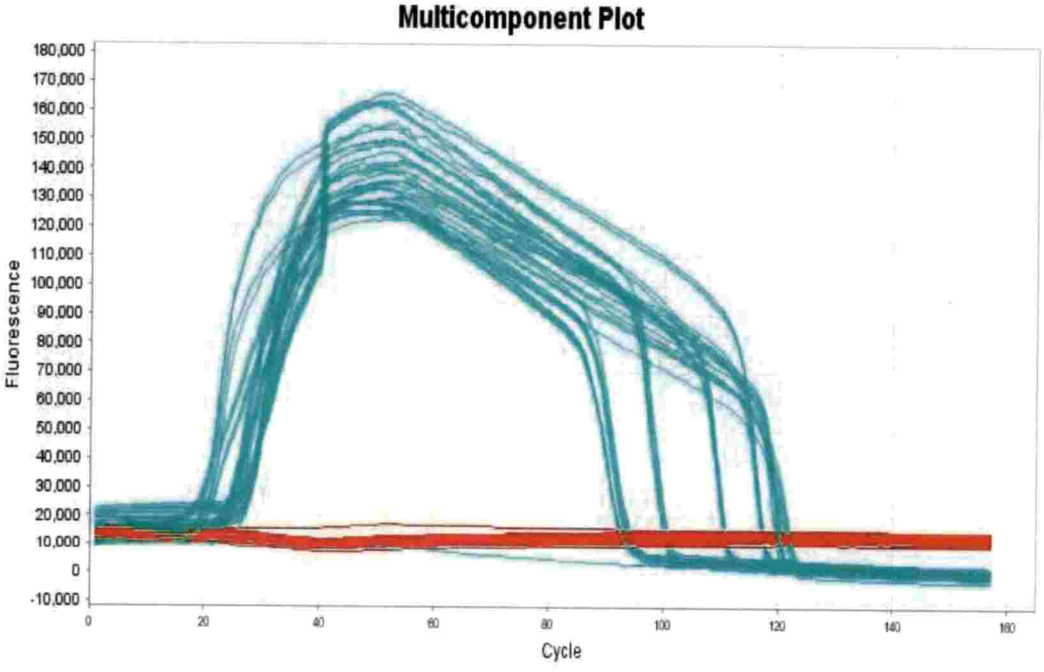
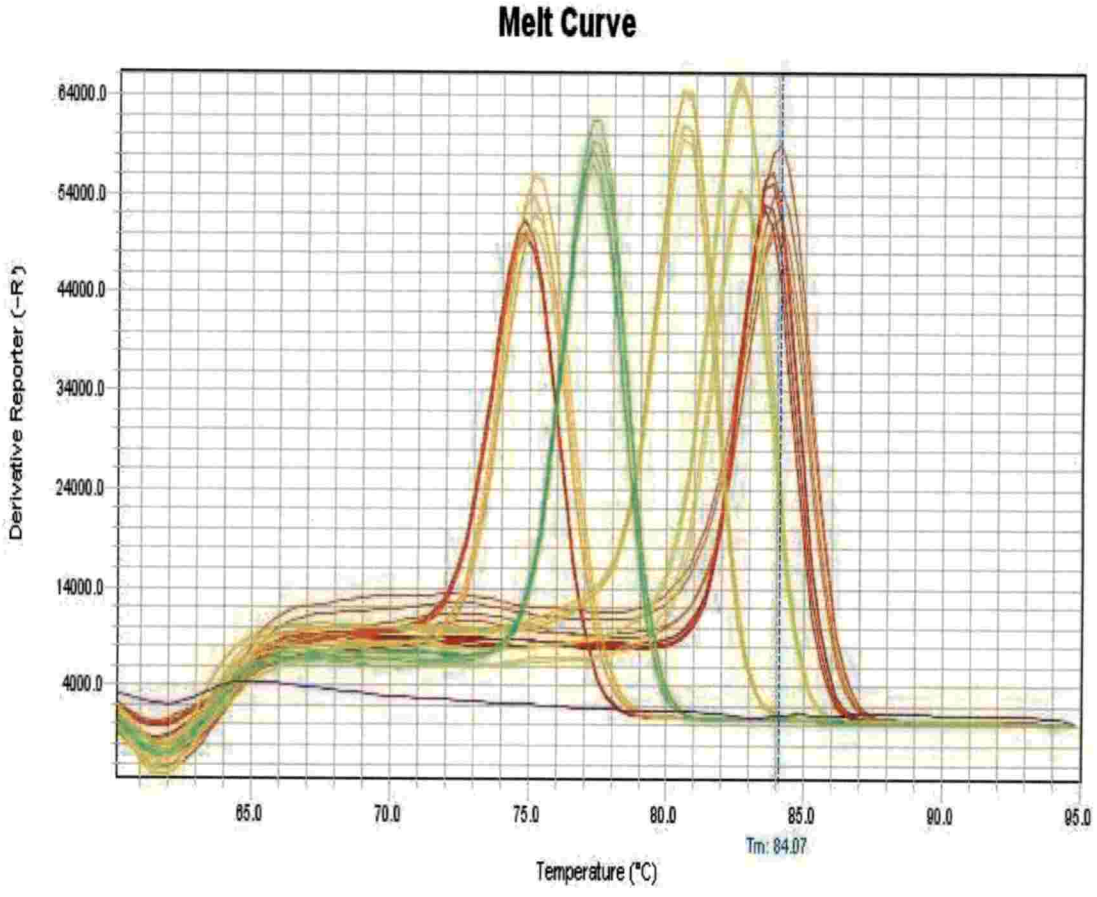


Fig. 4.2.(c) Description of different cytokine gene showing different Tm in the melt curve graph



4.3 Relative expression pattern of IL-1β mRNA

The effect of heat stress on the IL-1β gene expression patterns between C and HS groups of Malabari goats are described in Fig. 4.3. The fold changes in expression patterns of IL-1β in both C and HS groups are 1.0 and 0.82, respectively. Although the expression pattern of IL-1β gene in HS group showed trends of down regulation as compared to the C group, still the differences between the groups were not statistically significant. Further, a negative correlation ($P<0.05$) was established between THI and IL-1β gene expression pattern (Table.4.1).

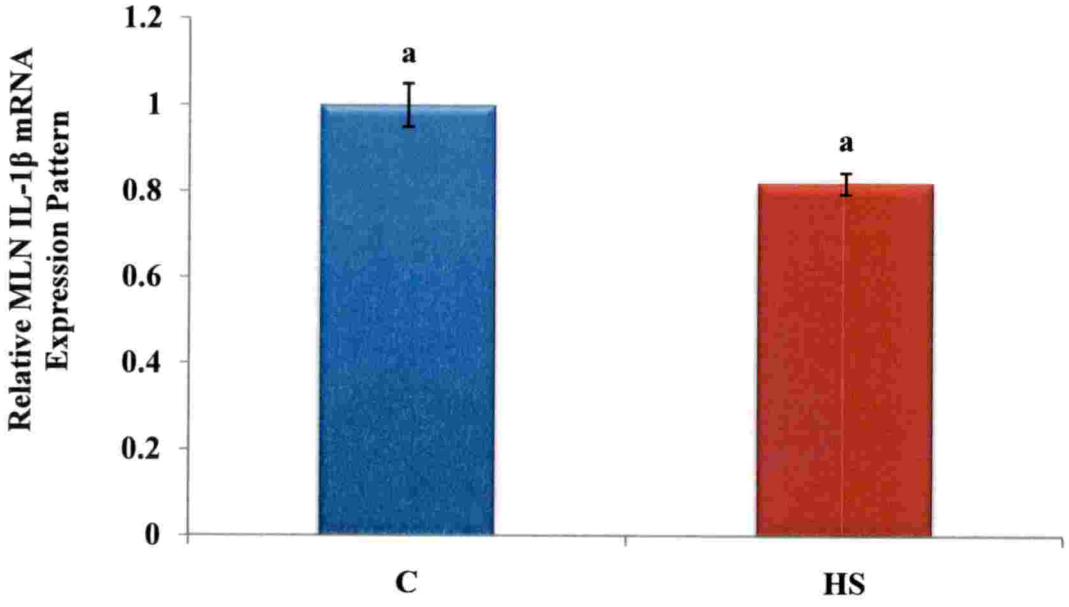
4.4 Relative expression pattern of IL-10 mRNA

The effect of heat stress on the IL-10 gene expression patterns between C and HS groups of Malabari goats are described in Fig.4.4. The fold changes in expression patterns of IL-10 in both C and HS groups are 1.0 and 0.97, respectively. Although the expression pattern of IL-10 gene in HS group showed trends of down regulation as compared to the C group, still the differences between the groups were not statistically significant. Further, no significant correlation was established between THI and IL-10 gene expression pattern (Table.4.1).

4.5 Relative expression pattern of IL-18 mRNA

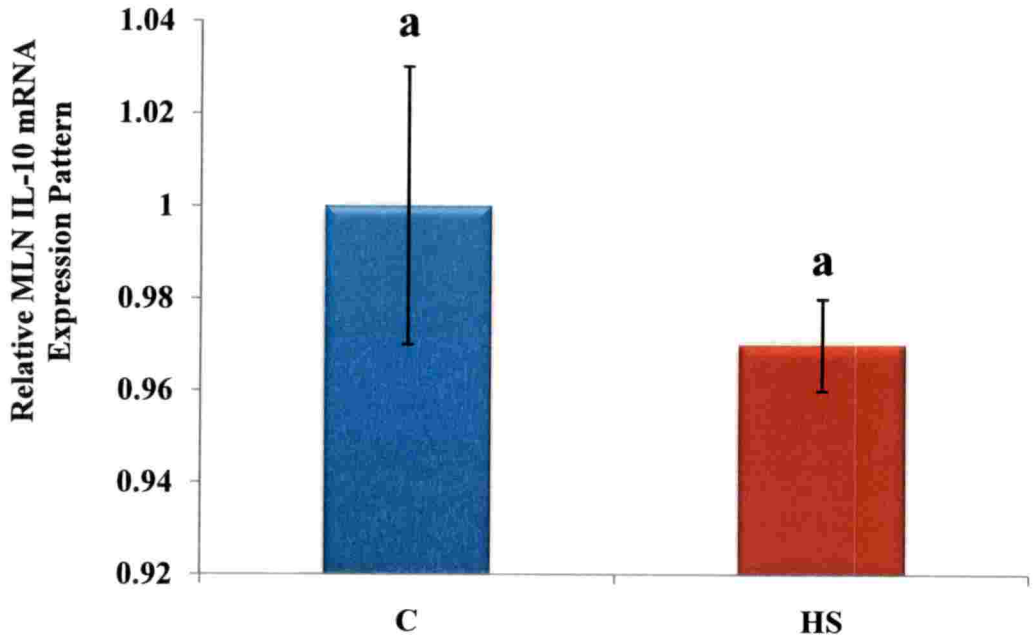
The effect of heat stress on the IL-18 gene expression patterns between C and HS groups of Malabari goats are described in Fig.4.5. The fold changes in expression patterns of IL-18 in both C and HS groups are 1.0 and 0.69, respectively. The expression pattern of IL-18 gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and IL-18 gene expression pattern (Table.4.1).

Fig. 4.3: Relative MLN IL-1β mRNA expression patterns between C and HS group of Malabari goats



The values bearing similar superscripts do not differ significantly with each other.

Fig.4.4: Relative MLN IL-10 mRNA expression patterns between C and HS group of Malabari goats



The values bearing similar superscripts do not differ significantly with each other.

4.6 Relative expression pattern of TNF-α mRNA

The effect of heat stress on the TNF-α gene expression patterns between C and HS groups of Malabari goats are described in Fig.4.6. The fold changes in expression patterns of TNF-α in both C and HS groups are 1.0 and 0.29, respectively. The expression pattern of TNF-α gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and TNF-α gene expression pattern (Table.4.1).

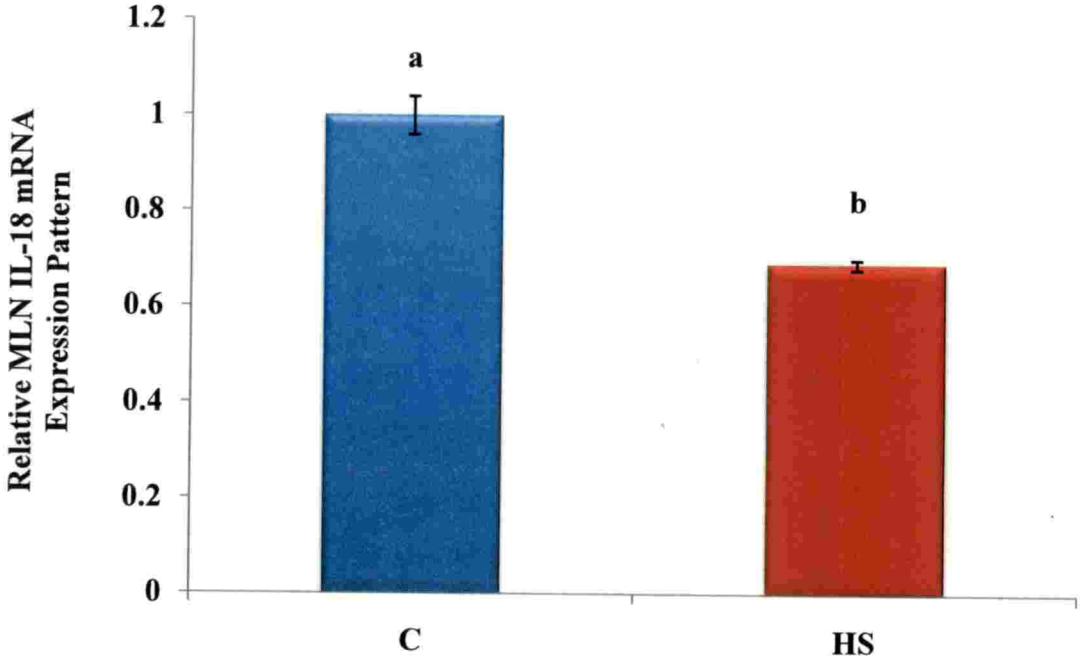
4.7 Relative expression pattern of IFN-β mRNA

The effect of heat stress on the IFN-β gene expression patterns between C and HS groups of Malabari goats are described in Fig.4.7. The fold changes in expression patterns of IFN-β in both C and HS groups are 1.0 and 0.62, respectively. The expression pattern of IFN-β gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and IFN- β gene expression pattern (Table.4.1).

4.8 Relative expression pattern of IFN- γ mRNA

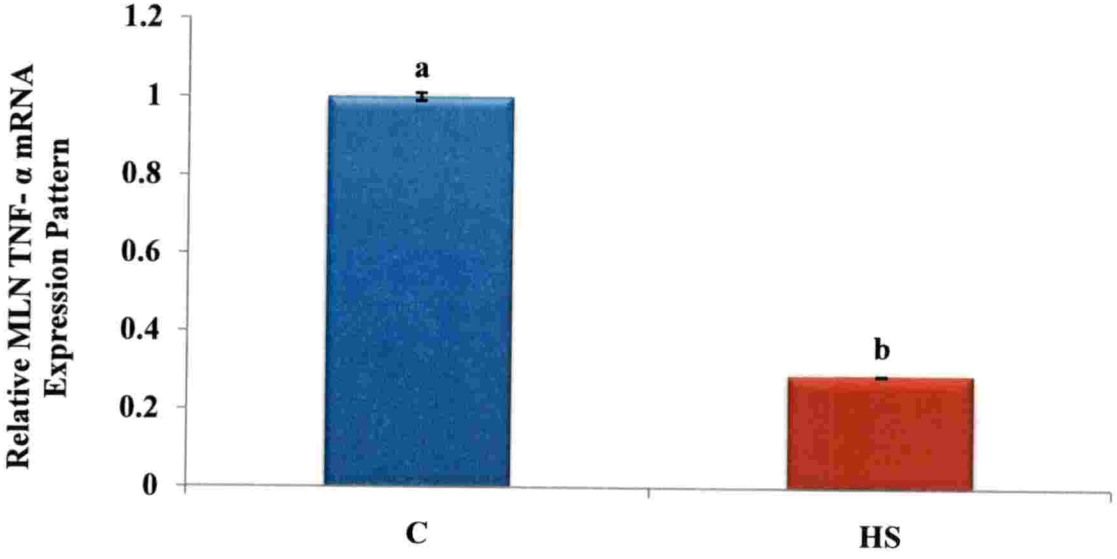
The effect of heat stress on the IFN- γ gene expression patterns between C and HS groups of Malabari goats are described in Fig.4.8. The fold changes in expression patterns of IFN- γ in both C and HS groups are 1.0 and 0.66, respectively. The expression pattern of IFN- γ gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and IFN- γ gene expression pattern (Table.4.1).

Fig.4.5: Relative MLN IL-18 mRNA expression patterns between C and HS group of Malabari goats



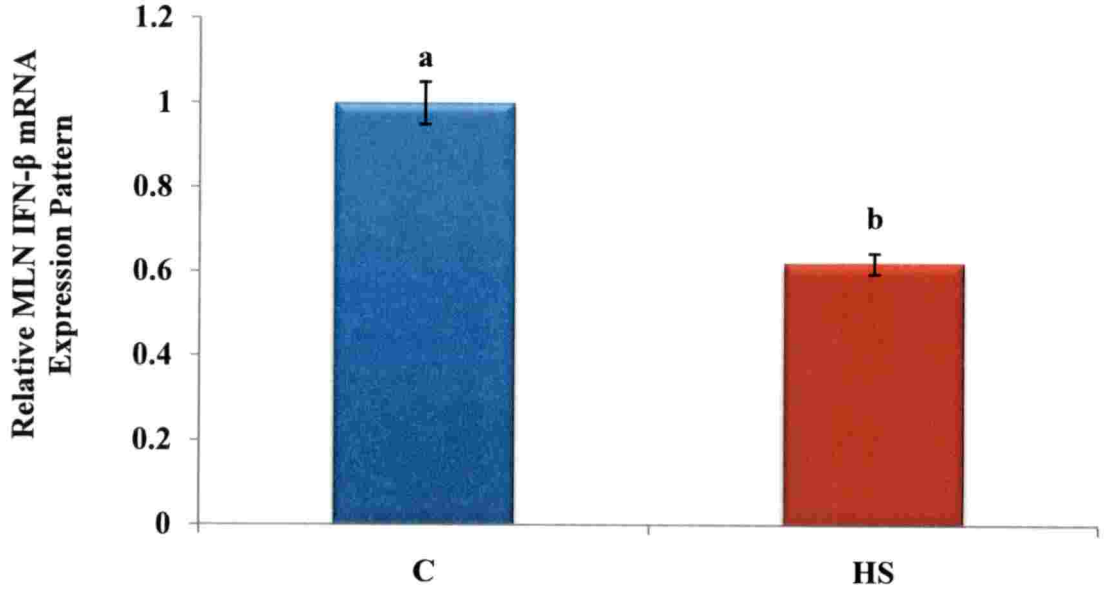
The values bearing different superscripts differ significantly at P<0.05.

Fig.4.6: Relative MLN TNF-α mRNA expression patterns between C and HS group of Malabari goats



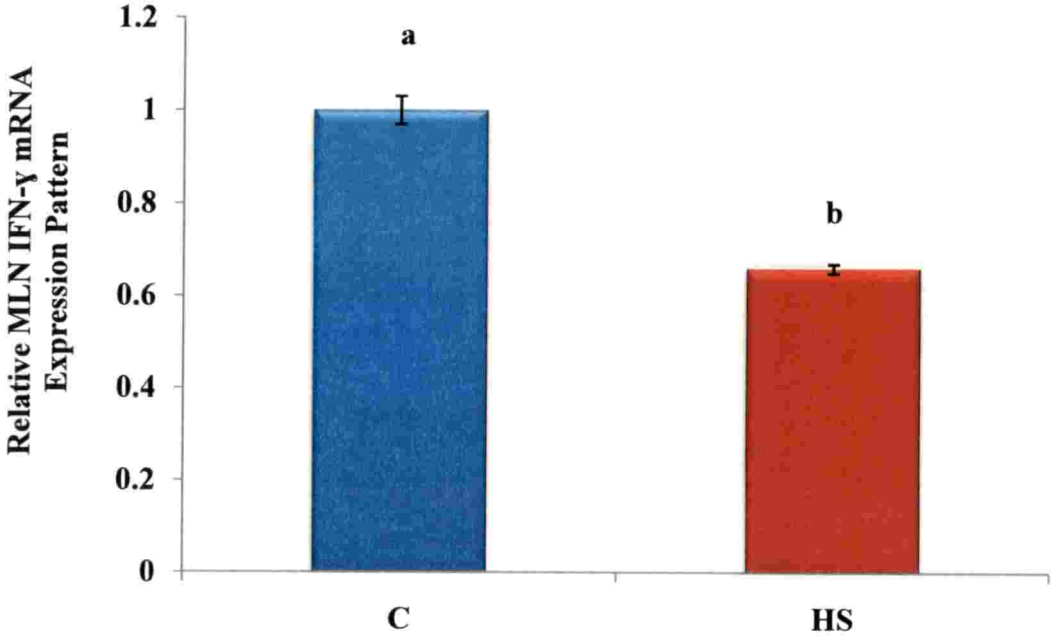
The values bearing different superscripts differ significantly at P<0.05.

Fig.4.7: Relative MLN IFN-β mRNA expression patterns between C and HS group of Malabari goats



The values bearing different superscripts differ significantly at P<0.05.

Fig.4.8: Relative MLN IFN-γ mRNA expression pattern between C and HS group of Malabari goats

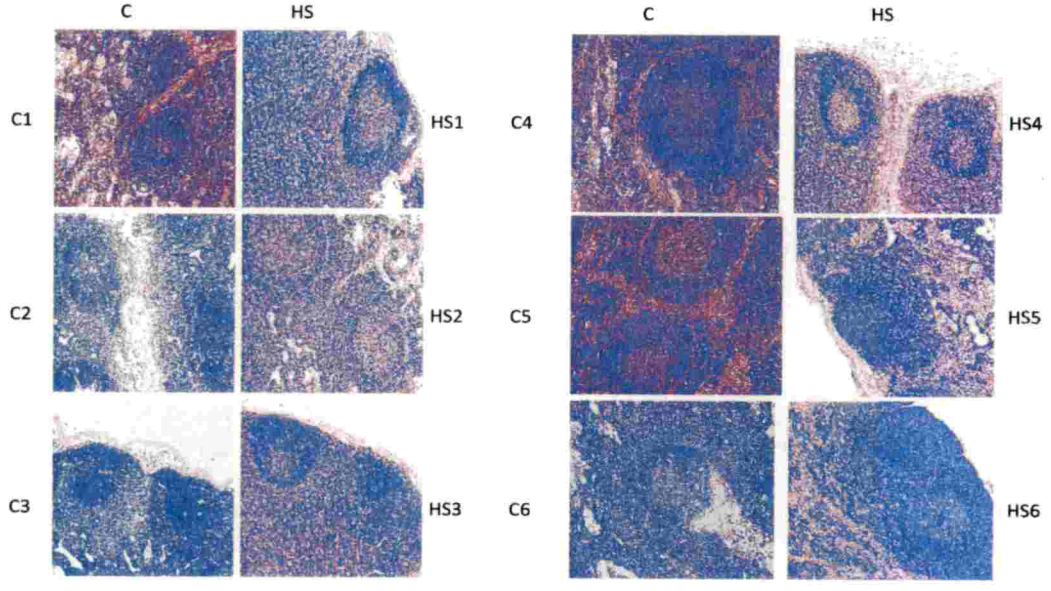


The values bearing different superscripts differ significantly at P<0.05.

4.9 Histopathological changes

Histopathological section showing changes in MLN between the C and HS groups Malabari goats are depicted in Fig.4.9. The MLNs showed paucity of lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centre of HS group ($P<0.05$) compared to C group.

Fig 4.9: Histopathological section of MLN in C and HS groups of animals



Histopathological observations between C and HS groups: The MLN pieces were collected from C and HS group goats immediately after sacrifice in buffered 10% formalin and processed to obtain H&E stained sections. The MLN showed paucity of lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centres of HS group compared to C group. C-Control; HS-Heat Stress; C-1-6: Six animals in C group; HS-1-6: Six animals in HS group; MLN-Mesenteric Lymph Node

Table.4.1. Correlation association between THI and different cytokine genes expression

	THI	IL-1β	IL-10	IL-18	TNF-α	IFN-β	IFN-γ
THI	1						
IL-1β	-0.833*	1					
IL-10	-0.273	0.723	1				
IL-18	-0.992**	0.772	0.155	1			
TNF-α	-0.999**	0.850*	0.305	0.987**	1		
IFN-β	-0.997**	0.866*	0.314	0.986**	0.998**	1	
IFN-γ	-0.985**	0.757	0.121	0.999**	0.979**	0.980**	1

THI- Temperature humidity index; IL- Interleukin; TNF- Tumor Necrosis Factor; IFN- Interferon.

**Indicates statistical significance at P<0.01;

* Indicates statistical significance at P<0.05.

DISCUSSION

CHAPTER 5

DISCUSSION

Heat stress is responsible for alterations of the animals' homeostasis and activates physiological compensatory mechanisms driven by the activation of central nervous system to cope with changed environmental condition and to maintain vital functions (Sejian *et al.,* 2017). It also weakens the animal's immune system and makes them more prone to diseases. Although this has been observed by various researchers, the impact of heat stress on immune gene expression and process of heat stress mediated immune suppression at molecular level has not been dealt in detail in livestock (Sophia *et al.,* 2016). Cytokines modulate the immune system to fight against the invading pathogens during various conditions. Further, cytokines play a central role in the cross-talk between altered homeostasis and reassembling and balancing of the immune responses passing through signals of oxidative imbalance (Caroprese *et al.*, 2017). Various stressors including heat stress induce the endocrine system to increase catecholamines and glucocorticoids (Indu *et al.,* 2015). These hormones modulate the cytokine release and thereby regulate immune responses (Webster *et al.*, 2002). Further, previous findings on the subject suggest the pivotal role of diet in the regulation of immune responses during heat stress response, probably by altering the expression of different genes involved (Chauhan *et al.,* 2014). Thus, the heat stress-immune system interactions need to be studied thoroughly in order to introduce various management and nutritional strategies to alleviate the ill-effects of heat stress in farm animals.

The current study offers the first thorough insight into the expression patterns of different immune system related gene expressions during heat stress exposure in goats. Livestock production in the changing climate scenario needs to target to identify appropriate breed which can withstand the negative impacts of climatic stresses. Breeds with genetic superiority to control effectively their

immune system to cope with climate associated disease occurrences is considered crucial as very few studies investigated the relation between increased heat load and immunological responses in livestock. Malabari goats in Southern India are well known for its adaptation in hot and humid tropical environment and for their disease resistance. In this line, the findings from this study provide some crucial initial information on how different immune related genes are expressed when goats are subjected to heat stress. This information might be of high value in assessing the immune status of goats and may provide useful interpretation pertaining to identification of immunological biomarkers in heat stressed goats. These candidate genes which form the molecular basis for controlling the immune system in Malabari goats during heat stress may be used as immunological markers for developing appropriate breeding program to establish a goat breed with superior immune competency.

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 HS group clearly indicated that these animals were subjected to extremely severe heat stress. This justifies the hypothesis of studying the cytokine expression in Malabari goats during heat stress exposure.

The results showed that the MLN IL-1β mRNA expression was comparable between C and HS groups. Although non-significant, reduction trend was obtained for the level of IL-1β mRNA expression in HS group. Similarly, Meng *et al.* (2013) reported reduced IL-1β gene expression in heat stressed chicks. The IL-1β is generally considered pro-inflammatory cytokines which stimulates HPA axis oriented stress response (Arimura *et al.,* 1994). However, no change in its level of expression in the current study indicates that heat stress was not able to induce changes in its expression level of IL-1β. Thompson *et al.* (2014) reported no change in the expression levels of IL-1β level in dairy cows subjected for cooling during dry period which coincided with summer months.

The no change in IL-1β gene expression level could be attributed to the cooling effect in heat stress cows. However, in our study without intervention point for heat stress no change in the expression levels of IL-1β between C and HS groups could be attributed to the extreme adaptive nature of Malabari goat breed to heat stress challenges in maintaining the pro-inflammatory cytokine IL-1β level. Further, it may also be speculated that the decreasing trend obtained for IL-1β expression may be significant if more number of animals were used in the study. However, Jin *et al.* (2014) established increase in expression pattern of IL-1β gene and they attributed this to vitamin supplementation in dairy cow. In our study also the animals had access to *ad libitum* feed and the feed offered were total maintenance ration including mineral mixture. Further, Chauhan *et al.* (2014) also indicated that the cytokine expression increases in heat stressed sheep provided with feed supplements. The provision of balanced diet to all animals in the current study could be another reason for nullifying the heat stress effect on IL-1β level leading to a comparable level in its expression between C and HS groups. Further, a negative correlation between THI and IL-1β indicates the sensitivity of this particular cytokine to heat stress in Malabari goats.

A similar trend to that of IL-1β was obtained for IL-10 expression pattern between C and HS groups. A similar result of heat stress induced no change in expression pattern of IL-10 in heat stressed broiler chicken (Ohtsu *et al.,* 2015). The reason for non-significant effect of heat stress on IL-10 expression level could be attributed to shifting of humoral immunity to cell mediated immunity during heat stress (Ohtsu *et al.,* 2015). Moreover, heat stress was reported to reduce the production of antibodies (Khajavi *et al.,* 2003), suggesting that the humoral immune system is deactivated by heat stress. Therefore, the immune system and/or Th1/Th2 polarization might be disturbed under heat stress in these animals. A similar observation of shifting of humoral immune response to cellular was also established in heat stressed pigs (Ju *et al.,* 2014). However, Thompson *et al.* (2014) reported significantly higher IL-10 expression dairy cows during heat stress exposure. Further, Caroprese *et al.* (2017) correlated the

hyperthermia induced increased level of HSP70 expression to the increased level of regulatory cytokine IL-10 expression. The differences between their studies and ours could be attributed to the indigenous nature of Malabari goat breed to maintain the level of IL-10 during heat stress. The IL-10 is generally considered an anti-inflammatory cytokine and its levels were established in ruminant species mainly during pregnancy stress especially between the prepartum and postpartum periods (Sheikh *et al.,* 2016; vanEngelen *et al.,* 2009; Brodzki *et al.,* 2015). Further, zinc supplementation in the diet was found to reduce the level of IL-10 in dairy cows (Sheikh *et al.,* 2016). Similarly, in our study also the dietary composition consists of zinc supplementation and this could have reduced the effect of heat stress on the IL-10 expression pattern to obtain a comparable level of its expression between the C and HS groups. Further, a non-significant correlation between THI and IL-10 cytokine gene expression denotes the adaptive potential of Malabari goats without compromising the production of IL-10 during heat stress exposure.

The reduced IL-18 expression level in HS group as compared to C group indicates the sensitivity of this gene for heat stress. However, there are reports suggesting higher IL-18 expression during stressful condition (Sutinen *et al.,* 2014; Dinarello *et al.,* 1986). Further, Ohtsu *et al.* (2015) did not observed any change in the expression level of IL-18 in heat stressed broiler chicken. Also, it was established that the expression level of IL-18 could be altered due to increased glucocorticoid secretion (Shini *et al.,* 2010b; Ohtsu *et al.,* 2015). It was also observed that IL-18 has a specific role in modulating the immune response in animals which was considered very unique and distinct from other cytokines (Alboni *et al.,* 2013). Because of its uniqueness in its function any alteration in its level may directly reflect the immune status of the animals. The significantly lower IL-18 expression level might reflect the compromised immune response during heat stress in Malabari goats. Therefore, the significantly lower IL-18 gene in goats subjected to heat stress indicates that it could be a potential biomarker in assessing the impact of heat stress on the immune response in goats.

In addition, a strong negative correlation between THI and IL-18 clearly proves the compromised immune function in heat stressed Malabari goats.

The TNF-α mRNA expression level was lower in HS group as compared to the C group goats. However, Chauhan *et al.* (2014) demonstrated significantly higher TNF-α expression in heat stressed sheep. The differences in TNF-α between these studies could be attributed to the differences in the tissues of their expression as Chauhan *et al.* (2014) expressed the TNF-α in the skeletal muscle while in our study it was done in primary lymphoid organ the MLN. Similarly, Tao *et al.* (2013) also reported significantly higher PBMC TNF-α in heat stressed dairy cows and again the difference with our study could be attributed to the difference in tissue samples used for expression between the studies. Our finding has greater significance because of the central role of MLN in controlling innate immune response. Therefore, TNF-α could be another potential biomarker for assessing the immune status during heat stress in indigenous goats. In addition, a strong negative correlation between THI and TNF-α clearly proves the compromised innate immune response in heat stressed Malabari goats.

The IFN act as the first line of defence against the Virus, bacteria, and parasites and modulate the immune system (Sophia *et al.,* 2016). The IFN-β mRNA expression in HS group was lower than the C group. Similar heat stress induced significant reduction in expression pattern of IFN-β was also reported in mice by Jin *et al.* (2011). The IFN-β plays crucial role in maintaining the innate immune response. Therefore, it could be inferred that the reduction in IFN-β in Malabari goats after exposure to heat stress might indicate the compromised innate immune response in these goats. This again indicates the sensitivity of IFN-β to heat stress challenges in goats and reduction in its level might indicate the compromised immune response in these animals. Therefore, this gene too might serve as yet another biological marker reflecting compromised immune response in goats. This indicates the immune response is at stake even in extremely adapted indigenous Malabari goats.

The hepatic IFN-γ gene expression also showed similar trend as that of IFN-β with significant reduction in expression in HS group as compared to its C group. Heat stress was also established to be one of the prime factors negatively influencing the expression pattern of IFN-γ (Hu *et al.,* 2007). Further, heat stress associated glucocorticoids can inhibit the expression of IFN-γ which is considered one of the major cytokines involved in Th1 based cell mediated immunity. In addition, Liu *et al.* (2012) also established the down regulation of the IFN-γ cytokine. Heat stress experiments in Bama miniature pigs also revealed differential expression of immune cytokine genes with significant down regulation of IFN-γ gene (Ju *et al.,* 2014). Similar heat stress induced down regulation of IFN-γ was also reported in the spleen of broiler chicken by Ohtsu *et al.* (2015). This shows the trend for IFN-γ expression during heat stress exposure in primary lymphoid organs followed the same pathway of down regulation cutting across species. It was postulated that the heat stress induced increase in production of glucocorticoids brings this down regulation of IFN-γ gene expression (Srinivasan *et al.,* 2016). From the above discussions it is very clear that heat stress induced lower IFN-γ gene expression may reflect the suppressed innate immune response in goats and therefore, IFN-γ gene may also be considered an important immunological biological marker for heat stress. In addition, a strong negative correlation of THI with both IFN-β and IFN-γ also proves the compromised innate immune response in heat stressed Malabari goats.

Lymph nodes functions as filters of tissues and tissue fluids and are sites of origin and production of lymphocytes for normal physiological functions (Elmore, 2007). The decreased lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centres of HS group suggests decreased mucosal immunity in the intestine which may lead to predisposing the stress group to enteric infections. Similarly, it has also been observed that heat stress reduces the lymphocyte distribution in lymph nodes and this was attributed to the increased glucocorticoid production during the stressful condition (Viswanathan and Dhabhar, 2005; Al-Rahman and Baqey, 2016).

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Heat stress weakens the animal's immune system and makes them more prone to diseases. Even though, the compromised immune system during heat stress in animals has been observed by various researchers, the impact of heat stress in particular on immune system related gene expression has not been elucidated in farm animals. Therefore, research efforts pertaining to quantifying the impact of heat stress on immune system related gene expression are crucial for development of superior thermo-tolerant breeds with potent immune competency. Hence, the study was conducted with the primary objective of establishing the impact of heat stress on the MLN associated different cytokine gene expression patterns and the related histopathological changes in Malabari goats.

The study was conducted for a period of 45 days. Twelve animals were used in this study. The animals were randomly allocated into two groups of six animals each, C (n=6; control), and HS (n=6; heat stress). The C animals were maintained in the shed in comfort condition while HS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h during the experimental period. The C animals were fed and watered inside the shed and HS animals were fed and watered outside while they are exposed to summer heat stress in the outside environment. The animals were slaughtered at the end of the study and their MLN were collected for gene expression study.

The current study offers the first thorough insight into the expression patterns of different immune system related gene expressions during heat stress exposure in goats. Malabari goats in Southern India are well known for its adaptation in hot and humid tropical environment and for their disease resistance. In this line, the findings from this study provide some crucial initial information on how different immune related genes are expressed when goats are subjected to

heat stress. The THI index inside the shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P<0.01$).

Although the expression pattern of IL-1β gene in HS group showed trends of down regulation as compared to the C group, still the differences between the groups were not statistically significant. Further, a negative correlation ($P<0.05$) was established between THI and IL-1β gene expression pattern. The IL-1β is generally considered pro-inflammatory cytokines which stimulates HPA axis oriented stress response. The non-significant change in the expression levels of IL-1β between C and HS groups could be attributed to the extreme adaptive nature of Malabari goat breed to heat stress challenges in maintaining the pro-inflammatory cytokine IL-1β level.

A similar trend to that of IL-1β was obtained for IL-10 expression pattern between C and HS groups. Further, no significant correlation was established between THI and IL-10 gene expression pattern. The reason for non-significant effect of heat stress on IL-10 expression level could be attributed to shifting of humoral immunity to cell mediated immunity during heat stress. The non-influence of heat stress on IL-10 expression may also be due to the indigenous nature of Malabari goat breed to maintain the level of IL-10 during heat stress.

The expression pattern of IL-18 gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. The significantly lower IL-18 expression level might reflect the compromised immune response during heat stress in Malabari goats. Therefore, the significantly lower IL-18 gene in goats subjected to heat stress indicates that it could be a potential biomarker in assessing the impact of heat stress on the immune response in goats. In addition, a strong negative correlation between THI and IL-18 clearly proves the compromised immune function in heat stressed Malabari goats.

The expression pattern of TNF-α gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. This finding has greater significance because of the central role of MLN in controlling innate immune response. Therefore, TNF-α could be another potential biomarker for assessing the immune status during heat stress in indigenous goats. In addition, a strong negative correlation between THI and TNF-α clearly proves the compromised innate immune response in heat stressed Malabari goats.

The expression pattern of IFN-β gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, a strong negative correlation ($P<0.01$) was established between THI and IFN-β gene expression pattern. The IFN-β plays crucial role in maintaining the innate immune response. Therefore, it could be inferred that the reduction in IFN-β in Malabari goats after exposure to heat stress might indicate the compromised innate immune response in these goats. This again indicates the sensitivity of IFN-β to heat stress challenges in goats and reduction in its level might indicate the compromised immune response in these animals. Therefore, this gene too might serve as yet another biological marker reflecting compromised immune response in goats. This indicates the immune response is at stake even in extremely adapted indigenous Malabari goats.

The expression pattern of IFN- γ gene in HS group was significantly ($P<0.05$) down regulated as compared to the C group animals. Further, heat stress associated glucocorticoids can inhibit the expression of IFN-γ which is considered one of the major cytokines involved in Th1 based cell mediated immunity. Heat stress induced lower IFN-γ gene expression may reflect the suppressed innate immune response in goats and therefore, IFN-γ gene may also be considered an important immunological biological marker for heat stress. In addition, a strong negative correlation of THI with both IFN-β and IFN-γ also proves the compromised innate immune response in heat stressed Malabari goats.

The MLNs showed paucity of lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centre of HS group ($P<0.05$) compared to C group. The decreased lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centres of HS group suggests decreased mucosal immunity in the intestine which may lead to predisposing the stress group to enteric infections.

The study is the first of its kind in establishing the impact of heat stress on different cytokine gene expression pattern in Malabari goats. The significantly lower levels of IL-18, TNF-α, IFN-β and IFN-γ in the HS group as compared to C group indicates the compromised immune response in goats. Further, a strong negative correlation of IL-18, TNF-α, IFN-β and IFN-γ with THI supports the argument of compromised immune response in heat stressed Malabari goats. In addition, the study identified these IL-18, TNF-α, IFN-β and IFN-γ to be reliable immunological marker for quantifying heat stress impact on immune response in goat. The information generated in the study might be of high value in assessing the immune status of goats and may provide useful interpretation pertaining to identification of immunological biomarkers in heat stressed goats. These candidate genes which form the molecular basis for controlling the immune system in Malabari goats during heat stress may be used as immunological markers for developing appropriate breeding program to establish a goat breed with superior immune competency.

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ABSTRACT

INFLUENCE OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT CYTOKINE GENES IN MALABARI GOATS

by

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ABSTRACT OF THE THESIS

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ABSTRACT

Heat stress acts as detrimental factor for immune functions in farm animals. The impact of heat stress in particular on immune system related gene expression has not been elucidated in farm animals. Therefore, research efforts pertaining to quantifying the impact of heat stress on immune system related gene expression are crucial for development of superior thermo-tolerant breeds with potent immune competency. Hence, a study was conducted with the primary objective to determine the impact of heat stress on different mesenteric lymph node (MLN) associated cytokine gene expression patterns and histopathological changes in heat stressed goat. To satisfy this objective, a study was conducted in twelve 10 months to one year old Malabari breed goats for 45 days duration. The goats were randomly allocated into two groups: C (n=6; Control) and HS (n=6; Heat stress). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. All animals had access to *ad-libitum* feed and water and they were fed and watered individually. The C goats were placed in the shaded pens while HS goats were exposed to heat stress in outside environment between 10.00 h to 16.00 h. The animals were slaughtered at the end of the study and their representative MLN samples were collected for assessing the different cytokine gene expression and histopathological changes. The expression patterns of interleukin-1β (IL-1β) and IL-10 were comparable between C and HS groups. However, the expression patterns of IL-18, tumor necrosis factor-α (TNF-α), interferon-β (IFN-β) and IFN-γ were significantly ($P<0.05$) down regulated in HS group as compared to the C group animals. In addition, a strong negative correlation ($P<0.01$) was also established for THI with IL-18, TNF-α, IFN-β and IFN-γ gene expression in the study. The histopathological changes of MLNs showed paucity of lymphocyte distribution in the follicular areas as wells as decreased density of lymphocytes in the germinal centres of HS group ($P<0.05$) compared to C group. The findings from this study clearly indicated the compromised immune functions during heat stress exposure in Malabari goats.

This is evident from the down regulation of most of the cytokine gene expressions as well as through the histopathological changes observed in MLN. The study also identified IL-18, TNF-α, IFN-β and IFN-γ genes to be the ideal immunological markers for quantifying the heat stress mediated immune response in goats. These candidate genes which form the molecular basis for controlling the immune system in Malabari goats during heat stress may be used as immunological markers for developing appropriate breeding program to establish a goat breed with superior immune competency.

Keywords: Climate change; Cytokines; Heat Stress; Goat; Immunity; Interleukins