IMPACT OF HEAT STRESS ON DIFFERENT TOLL LIKE RECEPTORS GENE EXPRESSION IN MALABARI GOATS

by

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(2013-20-119)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

BSc-MSc (Integrated) CLIMATE CHANGE ADAPTATION

FACULTY OF AGRICULTURE
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2018

DECLARATION

I, hereby declare that this thesis entitled **"Impact of Heat Stress on different Toll like Receptors Gene Expression 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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EXTERNAL EXAMINER

Acknowledgements

At the verge of my thesis completion I would like to express my sincere gratitude towards several people who have contributed to the final result and made this thesis writing fascinating and extremely rewarding.

I would like to start with the person who made the biggest difference in my life, my mentor Dr. V. Sejian I was privileged to have him as my research supervisor. Only his exemplary guidance, unceasing encouragement and his indefatigable support made me possible to complete my thesis. His tireless enthusiasm and inspiring guidance during my research work will always remain a source of inspiration to me. He has been a living role model to me, taking up new challenges every day, tackling them with all his grit and determination and always thriving to come out victorious. I also would not forget the care he gave like a family member. He is a great icon of integrity and hard work, as well as a great philanthropist. No words to thank you sir, I owe a lot to you.

I express my heart-felt gratitude to director of ICAR-NIANP, Dr. Raghavendra Bhatta for providing an opportunity to conduct my research work in a venerated institute like NIANP and making the facilities available for carrying out research. His constant encouragement during the odd discussions has always provided me an inner strength to accomplish something in science.

I extend my sincere word of thanks to Dr. M Bagath, Dr. G. Krishnan and Dr. C. Devaraj for their insightful comments and encouragement throughout my study period and also express my deep sense of gratitude and respectful regards for the untiring help during my gene expression study and critiques and appreciation without their precious support it would not be possible to conduct this research.

I am highly thankful to the head Dr. I.J. Reddy and Dr. J.P. Ravindra for their indispensable support throughout the completion of my project.

I owe my special thanks to Dr. Giridhar, for providing feed for the experiment and for all the constant support throughout my study period

I extend my sincere word of thanks to AAO and AFO who have gone out of way to help me with several official matters.

I am obliged to Dr. Manjunatha Reddy for the tremendous help offered during the study period.

I express my humble gratitude towards NIANP, ACCER, KAU and CAADECS for extending all the facilities for my work.

I sincerely express my gratitude from the core of my heart to my seniors Pragna, Aleena, Archana and Niyas Ikaa who lead me to choose this pathway and for all their support and motivation during the initial days of my project work and have lived by example to make me understand that hard work is the key to

success. Also my special thanks to Dr. Savitha for being the most loving senior throughout this one year.

I am immensely indebted to my colleagues Amitha, Afsal, Angel and Rasha who had been there for moral support and motivation, which drives me to give my best in last 5 years. I owe a lot to them for being with me in thicks and thins of life, I find myself lucky to have friends like them and will ever cherish all the moments with them.

I would not forget to mention Munisamy who always lend the helping hand in all the critical situations during my research period.

I extend my sincere thanks to my friends Yallappa, pushpa Rani, Thirumalasamy, Remya, Neil wincer, Mridula and Naveen for extending their support, sympathetic attitude and unfailing help during the course of entire study. And would not forget to mention Anthoni Akka for the care and affection throughout this one year

I would like to thank all the members and staffs of the Experimental Livestock Unit for their immense help and cooperation throughout the experiment. I also take this opportunity to thank all the administrative staffs who have helped during the critical periods

I owe my most sincere gratitude to Dr. (Prof) P.O. Nameer for his constant encouragement and valuable suggestions throughout my study period. I owe my special thanks to all the former special officers who have helped me immensely.

I take this opportunity to express my deep sense of gratitude and respectful regards to Hon'ble Vice chancellor, Registrar and Director of KAU for providing all the facilities and encouragement.

My acknowledgement will never be complete without the special mention of my teacher Mrs. Krishnapriya who was there with me in my ups and downs and the motherly care during all the four years of my academic career.

I also take this opportunity to thank all the teaching staffs who have been there for the development of ACCER and my academic career. I express my sincere gratitude to Saju sir, Sajitha miss and Unni chettan for all the help and support during my academic career.

I am obliged to Dr. Beena (Assistant Prof) KVASU for the encouragement, support and valuable advice, you gave throughout our association.

I am sincerely indebted to Dr. Girish Varma, Dr. Rao for the support and positive appreciation throughout the course of the investigation. My earnest thanks to towards Hon'ble Vice chancellor, Registrar and Dean of KVASU and all the teachers for the encouragement and support.

It's my fortune to gratefully acknowledge the support of my batch KAURAVAS for the support and generous care throughout the academic tenure. They were always beside me during the happy and hard moments to push me and motivate me. I also express my special thanks to my entire three senior batches for the care and affection and for setting the best example for the juniors and also would not forget my juniors for the fruitful help.

I will never forget the smiling faces of my dear friends Aswathy, Megha, Iram, Deeju and Hanna for all the good moments and I happily cherish all the memories that we shared together, the boasting moments, small pranks, thank you all for the great influence you made in my life.

Finally, I acknowledge the people who mean a lot to me, my parents, and my brother for showing faith in me and giving me liberty to choose what I desired. I salute them for the selfless love, care, pain and sacrifice they did to shape my life. They were willing to support any decision I made. I would never be able to pay back the love and affection showered upon by my family.

Ultimately, I pay my obeisance to GOD, the almighty to have bestowed upon me good health, courage, inspiration, zeal and the light for a successful life.

I owe this thesis to my Mentor and my Family

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SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of Variance
APCs	Antigen Presenting Cells
ADG	Average Daily Gain
BIRC3	Baculoviral IAP Repeat Containing 3
C	Control
Ca	Calcium
CAE	Caprine Arthritis
CASP8	Caspase 8, Apoptosis-Related Cysteine Peptidase
CD80	Cluster of Differentiation 80
cDNA	Complementary DNA
CO_2	Carbon dioxide
CpG ODN	CpG oligodeoxynucleotides
DAMPs	Damage associated molecular patterns
DC	Dentritic cell
DNase	Deoxyrobonuclease
EIA	Equine influenza, Marek's disease (MD)
FMD	Foot and Mouth Disease
FSH	Follicle-stimulating hormone
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GHG	Greenhouse Gas
HSFs	Heat shock factors
Hb	Hemoglobin
HSP	Heat Shock Proteins
HPA	Hypothalamic–pituitary–adrenal axis

IFNAR2	Interferon-alpha/beta receptor
IgA	Imunoglobulin A
IgG	Immunoglobulin G
IL15	Interleukin-15
IL2/6	Interleukin-2/6
IL8	Interleukin-8
IPCC	Intergovernmental Panel on Climate change
k Da	Kilo Dalton
LN	Lymph Node
LN2	Liquid nitrogen
MC	Malabari Control
Mg	Magnesium
MHS	Malabari Heat stress
MLN	Mesenteric Lymph node
mRNA	Messenger Ribonucleic Acid
MyD88	Myeloid Differentiation Primary Response protein 88
NEFA	Non-Estrified Fatty Acid
NFIL3	Nuclear factor, interleukin 3
Ng	Nano gram
^{o}C	Degree Celsius
P	Phosphorous
PCV	Packed Cell Volume
PAMPs	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PRRs	Pattern Recognition Receptors
PR	Pulse Rate

qPCR	Quantitative real-time PCR
ROS	Reactive Oxygen Species
RT	Rectal Temperature
RBCs	Red Blood Cells
RT	Respiration Rate
RNase	Ribonuclease
RTqPCR	Real Time quantitative polymerase chain reaction
THI	Temperature Humidity Index
T4	Thyroxin
TIR	toll/IL-1 receptor
TLR	Toll –Like Receptors
T3	Tri iodothyronine
USA	United States of America
UV	Ultra Violet
WHC	Water Holding Capacity
WBCs	White Blood Cells
µL	Microlitre

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INTRODUCTION

CHAPTER 1

INTRODUCTION

Climate change is among the most critical challenges of our time. It jeopardizes the rural poor in developing countries, who rely heavily on the natural resource for their survival (FAO, 2017). The enduring effect of climate change influences the survival of the species, ecosystem and the sustainability of the livestock sector (Chaidanya *et al.*, 2015). Generally, climate change is associated with rising temperature. Both climate change and global warming cause pernicious effect to the entire livestock population (Sejian *et al.*, 2016). Climate change causes both direct and indirect effect on livestock. Heat stress is one among the most significant direct effect of climate change and seems to have adverse effect on production and reproduction and health status in farm animals (Sophia *et al.*, 2017). In addition, heat stress reduces the feed intake, increased water consumption which leads reduced weight gain, poor breeding efficiency, low milk production and susceptible to diseases (Sejian *et al.*, 2018). Further, the production is compromised as a part of energy divergence for adapting the animal to the prevailing climatic condition thus leading to huge economic loss (Shaji *et al.*, 2015).

Goats are versatile animals that can produce meat, milk, hide, fibre and manure. The economic significance of goat production has been increased during the last decades all over the world. Rearing of goat is much easier as compared to other livestock as they are well adapted to hot-humid to cold-arid climatic conditions and is tolerant to heat stress. They emit less methane than other domestic ruminants. Moreover, the goats can thrive well, by consuming shrubs, herbs and other poor-quality roughages, due to their increased digestibility of crude fibre (Shaji *et al.*, 2015; Darcan and Silanikove, 2017).

Malabari goats are one of the recognised breeds in India and is widely distributed among Malabar region of Kerala and also reared in different parts of Tamil Nadu. These breeds have a unique genotype exhibiting very high

multiple birth percentage and have high milk yielding capacity (Sundaram *et al.*, 2012).

Economic importance of goat production has been increased during the last decades all over the world, predominantly in countries that are routinely exposed to harsh environment. Goats reared in the tropical humid climate are subjected to multiple stressors due to high temperature combined with high relative humidity inadequate feed and fodder with low-quality nutrients and unavailability of drinking water (Sejian, 2013; Topp and Doyle, 1996). These stressors impair production performance, reproduction and also compromise the immune system, thus making them susceptible to wide range of diseases (Deng *et al.*, 2012; Meng *et al.*, 2013). Further, the impact of heat stress on the foreseen effects of immune system is very high, but minor information is only available regarding the livestock response to heat stress at a cellular level.

The changing climate makes the animals vulnerable to diseases, hence it is crucial to have an innate defence mechanism (Paul *et al.*, 2015). Toll-like receptors (TLRs) play a crucial role in pathogen recognition through the broadly specific innate immune system (Brubaker *et al.*, 2015). The immune system responds to stress by enhancement or suppression of immune functions (Dhabhar, 2009). The TLRs are a family of at least 10 proteins that functions as an initial response to stress, significant in research efforts to combat infectious and inflammatory disease. They enable the differentiation between self and non-self-components through Pattern Recognition Receptors (PRRs). The TLRs are one among them and widely been studied to identify specific signature molecules in microbes. These receptors in important organs and its expression pattern in response to particular stimuli is one of the factors determining the disease resistance capability of an animal (Tirumurugaan *et al.*, 2010).

According to Paul *et al.* (2015), TLR plays a pivotal role in goats in activating their innate response during heat stress. The TLRs are usually expressed in antigen cells like monocytes, macrophages, dendritic cells and B

lymphocytes. Among them TLR2 and TLR4 identify the damage associated molecular patterns (DAMPs) to induce immune response during heat stress by producing pro-inflammatory cytokines (Bharati *et al.*, 2017). Tirumurugaan *et al.* (2010) studied that TLR5 mRNA expression had been shown to play an important role in innate response to flagellated bacteria.

Further, research efforts are needed to quantify immune responses to environmental stresses in different indigenous goats will be of practical relevance. Hence the present study was designed to establish the impact of heat stress on different TLR genes expression in lymph node of Malabari goats. Such an attempt may yield fruitful results and can establish the impact of climate change on livestock immune response. The study was conducted with the primary objective of establishing the expression pattern of different cell surface and intra cellular TLRs in Malabari goats subjected to heat stress and also to establish the correlation between THI index and expression patterns of both cell surface and intracellular TLRs in Malabari goats subjected to heat stress.

The objectives of the present study were:

Objectives

a) To assess the expression pattern of different cell surface TLRs in Malabari goats subjected to heat stress.

b) To assess the expression pattern of different intra cellular TLRs in Malabari goats subjected to heat stress.

c) To establish the correlation between THI index and expression patterns of both cell surface and intracellular TLRs in Malabari goats subjected to heat stress.

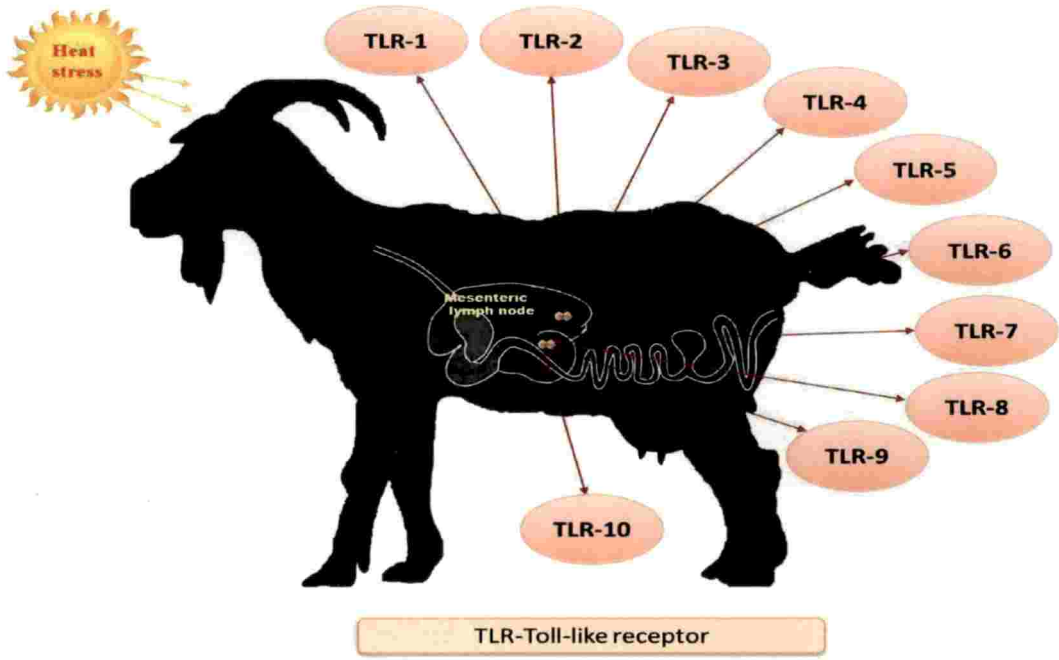

Fig1.1. Hypothetical figure describing the expression pattern of different Toll like receptors in Malabari goat

REVIEW OF LITERATURE

CHAPTER 2

Review of Literature

Climate change acts as a major threat to climate sensitive sectors such as agriculture and animal husbandry. With the changing climatic scenario, the frequency and duration of exposure of livestock to abiotic and biotic stressors increases. Abiotic stressors such as heat and nutritional stress have a major impact on livestock productivity (Sejian *et al.* 2011). In particular, heat stress is one of the crucial factors affecting livestock productivity (Rivington *et al.*, 2009). Heat stress affects animal productive performances like milk yield, meat quality and reproductive performances like age at maturity, ovulation failure, embryo mortality etc (Sandercock *et al.*, 2001). 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 (Tirumurugaan *et al.* 2010; Sophia *et al.*, 2016a).

Stressful stimuli are known to change the immune function. The immune system responds to stress by enhancement or suppression of immune functions (Dhabhar, 2009). Stress affects both innate and adaptive immune response in animals. The immune system does not respond directly to stress but act via neuroendocrine system. The stress related hormones act on the immune cell receptors to modulate the immune response. Innate immune response is one of the primary immune response which helps to tackle the pathogens that enter the host animals (Vahanan *et al.*, 2008).The PRR play a crucial role in this process and they help to identify the conserved molecular signatures called PAMPs (Pathogen Associated Molecular Patterns) present in the pathogens and help to kill the pathogen through phagocytosis (Basset *et al.*, 2003). Toll like receptors (TLRs) are the most studied among the PAMPs (Raja *et al.*, 2011).

The TLRs are a family of at least 10 proteins that function as central mediators of the innate immune response to diverse pathogens as well as to

endogenous molecules released by injured or dying cells (Tirumurugaan *et al.* 2010; Paul *et al.*, 2015).

Animals reared in tropical environments are generally subjected heat stress (Sejian *et al.*, 2010). Heat stress greatly affects animal production, reproduction and immune status particularly in the extensive system of rearing. Most studies which have investigated the effects of environmental stress on livestock have generally studied the impact of heat stress only on production and reproduction activities of the livestock (Sophia *et al.*, 2016b). However, reports on impact of heat stress on immune response are very scanty. Hence considerable research efforts are needed to establish the impact of heat stress on the immune response in livestock. Only such attempt may yield fruitful results if one tries to establish the impact of climate change on livestock immune response. Since goat has been projected as animals to deal with in the climate change perspectives, research efforts establishing impact of heat stress on immune response in different indigenous goats will be of practical relevance (Sophia *et al.*, 2016b). Hence the present study was designed to establish the impact of heat stress on different TLR genes expression in lymph node of Malabari goats.

2.1. Climate change and its adverse impacts

Global climate change is overwhelming during the past few decades that compromise the biological balance of the earth. The primary cause for this is due to the greenhouse gas (GHG) emissions that lead to warming of atmosphere (IPCC, 2013). Greenhouse gases are an integral part of the atmosphere that protects the earth from the direct solar radiation. However, during the past few decades there is a substantial increase in the level of GHG that has been released into the atmosphere both through natural and anthropogenic source (Naqvi and Sejian, 2011). Increase in the amount of GHGs pertains to warming of the earth by elevating the temperature. According to the IPCC fifth assessment report, by the year 2100 the mean global temperature would rise in the range of 0.3° C and

4.8°C (IPCC, 2013). Thus, the warming of the climate system jeopardizes the ecosystem on the earth.

The climate system has undergone unprecedented changes over decades which include extreme events such as increased concentration of GHGs, drought, flood, ocean acidification, sea level rise, melting of glaciers, changes in the rainfall pattern, infestation of the epidemic diseases, and threat to food security (Naqvi and Sejian, 2011; IPCC, 2014). Further, the number of cold days and nights has decreased and the number of warm days and nights has increased tremendously over the years (Donat and Alexander, 2012; Hansen *et al.*, 2012). Moreover, incidences of floods are evident in high latitudes while the droughts have been observed in the tropics and sub tropics (Seneviratne, 2012; IPCC, 2014). In addition, warming of ocean occurs as a result of climate change resulting in increased water level. Consequently, the rise in sea level, melting of glaciers and ice sheets takes place (IPCC, 2014). The IPCC (2014), has also concluded that there is a substantial increase in the rainfall pattern over the mid latitude regions that can lead to flooding. In addition to all the above consequences, climate change also increases the vulnerability of human population in coastal areas to new emerging diseases.

2.2. Impact of climate change on livestock production

Livestock is considered as one of the important agricultural sectors in developing countries (Balamurugan *et al.*, 2018). Livestock products play a major role in the agricultural commodity of the global food security (Rosegrant *et al.*, 2009). Approximately 1 billion people depend on the livestock sector and around 1.1 billion people are employed in this sector (Hurst *et al.*, 2005). It also provides essential nutrients through milk, meat and egg. This has tremendously increased the demand of livestock products for the past few decades (Chauhan and Ghosh, 2014). In the coming years the milk production is expected to shoot from 664 million tonnes to 1077 million by the end of 2050 and meat production will double from 258 to 455 million tonnes (Alexandratos and Bruinsma, 2012, Owens

et al., 2000). Therefore, livestock sector has become one of the key components of agriculture sector.

The enduring effect of climate change jeopardizes the livestock sustainability globally. This may be due to increase in CO_2 (Carbon dioxide) concentration, rise in air temperature, precipitation variation (Polley *et al.*, 2013). Consequently, the animals posses several adaptation mechanisms like panting, increase in water intake, reduced feed intake and alterations in the endocrine status, which ultimately hampers the production potential of the animal (Gaughan and Cawsell-Smith, 2015; Rath *et al.*, 2015). The seasonal changes in the rainfall pattern and high temperature pertains to large fluctuations in the availability and quality of pasture which in turn reduces the feed intake and suppress the production and animal health (Getu, 2015).Climate change has both direct (growth rate, milk production, reproduction, meat production, immunity status) and indirect (quantity and quality of livestock feed stuffs such as pasture and forage, dearth of available water) impact on livestock (Rust, 2013; Fereja, 2016).

2.2.1. Direct impacts

2.2.1.1. *Milk Production*

Climate change adversely affects milk yield that pertains to huge economic loss to farmers (Balamurugan *et al.*, 2018). Increase in temperature is the most significant factor that affects milk production in dairy cows (Chauhan and Ghosh, 2014). Impact of climate change is profoundly seen in high producing cows rather than the low producing cows due to high metabolic heat production (Rojas-Downing *et al.*, 2017). Nardone *et al.* (2010) observed inclination in temperature also decreased the milk yield in ewe, goat, and buffalo milk production (Nardone *et al.*, 2010). Thermal stressed animals show a low level of hepatic glucose synthesis and NEFA (Non Estrified Fatty Acids) in the blood that reduces the glucose supply to mammary glands which acts the main cause for lower milk yield (Balamurugan *et al.*, 2018). Moreover, reports suggest that high producing exotic breeds get more affected by the increasing temperature as

compared to the local breeds (Lacetera *et al.* (1996). Heat stress impairs both the milk yield and the contents of the milk components (Nardone *et al.*, 2010). Reports also emphasized that heat stress gradually reduced the quality of milk (Bernabucci *et al.*, 2013).

2.2.1.2. *Meat Production*

Heat stress negatively affects the livestock meat production (Nardone *et al.*, 2000). Gregory (2010) observed that heat stress reduces the muscle pH which in turn affects all the physico-chemical attributes such as cooking loss, water holding capacity (WHC), meat colour and shear force. Further, Chauhan *et al.* (2014) reported that extreme heat stress also influence the cellular level including alterations in the expression pattern of genes, such as heat shock proteins (HSPs), that play a role in the growth performance of livestock. However, Hashem *et al.*, 2013; Rana *et al.*, 2014 reported that heat stress did not influence body and carcass weight in goats and sheep. But in a recent elaborate study by Archana *et al.* (2018) established breed differences for the impact of heat stress in goats. These authors established the impact of heat stress on carcass characteristics, primal cuts, organoleptic attributes, proximate composition and different gene expression patterns in skeletal muscle. Based on the results it was concluded that Salem Black breed possessed superior adaptive capacity over Osmanabadi breed in terms of maintaining meat production potential (Archana *et al.*, 2018). Further the study also established HSP70 and myostatin genes could serve as ideal biomarkers for reflecting the heat stress influence on meat quality attributes in goats.

2.2.1.3. *Reproduction*

Under adverse conditions reproduction is one of the first physiological parameters to be affected. High temperature impairs the production potential of the animals which in turn results in the reduction of the reproductive capacity of both male and female animals (Al-Dawood, 2017). Elevated temperature alters the reproductive rhythm through hypothalamo- hypophyseal–ovarian axis in livestock

species (Kebede, 2016). As per Naqvi *et al.* (2012) conception rates of cattle may drop from 20-27% under thermal stress. Heat stressed animal poses poor oestrus expression due to the reduction in the secretion of oestradiol. Thermal stress compromises oocyte growth by varying the progesterone secretion, luteinizing hormone (LH) secretion, follicle-stimulating hormone (FSH) and ovarian dynamics during the oestrus cycle. Heat stress compromises the ovarian functions and embryonic development (Naqvi *et al.*, 2012). During gestation period heat stress induces slow growth of the foetus and enhances the chance of foetal loss. Reproductive process in male animal is sensitive and heat stress reduces the quality of sperm production as well as decrease the sperm quality. In males, thermal stress hampers the spermatogenesis by hindering the proliferation of spermatocytes (Sejian *et al.*, 2016). Thermal stress increases the testicular temperature that induces low sperm output, decreased sperm motility and morphological abnormalities of the spermatozoa (Niyas *et al.*, 2015).

2.2.1.4. *Livestock Diseases*

Animal health status can be affected by the climate both directly and indirectly, (Nardone *et al.*, 2010). Elevated temperature and changes in the rainfall pattern leads to outbreak of diseases and vector borne pests such as flies, ticks and mosquitoes (Thornton *et al.*, 2009). In addition, transmission of diseases reaches to the peak during warmer conditions (Thornton *et al.*, 2009). Diseases such as babesiosis, theileriosis, anaplasmosis, Rift Valley fever and bluetongue disease has been widely identified. Further, they also get prone to diseases such as ovine chlamydiosis, caprine arthritis (CAE), equine infectious anemia (EIA), equine influenza, Marek's disease (MD), and bovine viral diarrhea. Moreover, under hot and humid conditions there is a sudden increase in the internal parasite population. There are also widespread diseases that spreads rapidly such as avian influenza or foot and mouth diseases that contributes to the degradation of the surrounding environment (Rojas-Downing *et al.*, 2017). Emergence of new diseases is one of the major concerns of the farmers in the changing climatic condition. They consider that diseases to which they are not used are

stirring in the area and claiming lives of many livestock. Furthermore, this could increase the vulnerability of livestock to diseases (Yilma *et al.*, 2009). Rise in ambient temperature induces lameness in animals (Cook *et al.*, 2007). These climatic and seasonal fluctuations are also correlated to mastitis in dairy animals (Sinha *et al.*, 2017).

2.2.1.5. *Livestock adaptation*

During high temperature animals posses certain adaptive mechanisms that reduce the effects of climate change. However, these mechanisms tend to deviate their energy thus compromising their production potential. Adaptability can be of different types such as anatomical, morphological, physiological, feeding behaviour, metabolism, and performance (Silanikove and Koluman, 2015). The adaptability in small ruminants is determined by vital parameters like RT, heart rate, and respiratory rate (Niyas *et al.*, 2015). Heat stress affects the hypothalamic–pituitary–adrenal axis (HPA). According to Moberg (2000), increase in temperature invokes changes in the pituitary hormones such as growth hormone, prolactin, thyroid hormones, mineralocorticoids, glucocorticoids, catecholamines and antidiuretic hormones. Cellular adaptation is an integral mechanism and HSPs are activated when the animal is exposed to heat stress. The HSPs protect the cells from degrading under high ambient temperatures (Rowlinson, 2010). Reports suggest that goats in tropical regions showed higher expression of HSP70 mRNA (Guptal *et al.*, 2013; Shilja *et al.*, 2016).

2.2.2. Indirect Impacts

The indirect effects of climate change that influences the livestock production is the alteration in the feed and water availability (Chapman *et al.*, 2012). In extensive system of rearing under hot climate, where there is a prolonged thermal stress the pasture availability are limited in quantity and quality and the existence of animal diversity would be under stake. Perhaps stresses reduce the forage yield, alter its nutritional content and alter the species composition of the pasture. Increase in temperature and CO_2 adversely affects the

quantity and quality of feed which mainly depends on the location and species (Chapman *et al.*, 2012). Rise in temperature results in the herbage growth changes with a higher change in the C3 plants and less on the grain yields (Thornton *et al.*, 2009, 2015; Chapman *et al.*, 2012). Elevated temperature and dry conditions due to water soluble carbohydrates and nitrogen influences the quality of crops and forages. This rise in temperature increases the concentration of lignin and cell wall components in plants that decrease the nutrient availability pertaining to low digestibility (Polley *et al.*, 2013; Sanz-Saez *et al.*, 2012). However, abundance of CO_2 in the atmosphere improves the quality of C3 plants as compared to C4 plants (Polley *et al.*, 2013). In addition, extreme weather events may affect the structure of roots, change the leaf growth rate and decrease the overall yield. An increase in 2^{o} C impairs the pasture growth in the arid and semi arid regions whereas it has a positive influence in the humid and temperate regions (Rojas-Downing *et al.*, 2017).

Water availability is another major issue that influences the livestock sector. Animals accounts of about 8% of global human water use, when the temperature increases the water consumption rate of animals also increases thus creating a limited access to water (Nardone *et al.*, 2010). To tackle this issue the animals should be brought up in a livestock system that consumes less water. Alteration in the rainfall pattern and temperature affects the availability of feed quantity and quality, grazing ranges, weed, pest and disease. As the sea level rise due to climate change the saline water intrudes the freshwater, this adds to salination, biological and chemical contaminants to the water bodies. Saline water can affect the metabolism, and digestibility of the animals (Nardone *et al.*, 2010). Excess intake of nitrite can hamper both cardiovascular and respiratory systems correspondingly excess intake of heavy metals can impair the excretory, skeletal and nervous system of animals. Mere researches are only available on the implications of availability of water, thus appropriate mitigations strategies could help to maintain a sustainable livestock system.

2.3. Heat stress as the major factor influencing livestock production

Stress is an impulse action of animals under harsh environment that threatens their survival. All animals have their own thermo-neutral zone under which the normal body temperature is maintained and minimum energy expenditure. Therefore, when the temperature exceeds the upper critical temperature the animal is said to be under heat stress (Rojas downing *et al.*, 2017). For the past few decades heat stress is the major concern that hampers the production potential especially the high producing animals (Belhadj *et al.*, 2016). Heat stress mainly affects the livestock producers by jeopardizing the milk production, growth, reproduction, meat production and animal health (Sejian *et al.*, 2016). Correspondingly, heat stress increases the body temperature, respiration rate (RR), pulse rate (PR) and rectal temperature (RT) which inturn reduces the feed intake and increase the water intake (Das *et al.*, 2016). Effects of heat stress are generally aggravated when the high environmental temperature was coupled with high relative humidity (Marai *et al.*, 2007a; Belhadj *et al.*, 2016). Thus, a standard point for heat stress was established through temperature humidity index (THI) and if the value exceeds 72 the animals starts showing signs of heat stress and this threshold was determined by its level of production (Valtorta, 2002).

Heat load increases the rate of disease outbreak and mortality rate, especially when combined with humidity. Elevated temperature alters the physiological mechanisms of rumen which adversely influence the ruminants as it invokes metabolic disorders and further health problems by reducing saliva production that causes changes in the digestion patterns thus decreasing the feed intake (Das *et al.*, 2016). Further, during heat stress respiratory rate and sweating rate rises as a result the amount of CO_2 expired increases leading to a low concentration of blood bicarbonate concentration leading to a condition known as respiratory alkalosis (Das *et al.*, 2016). Perhaps oxidative status helps the animal to sensitize heat stress for metabolic diseases that gradually decrease the production and reproduction ability of the animal. This happens when heat stress

interrupts the concentration of pro-oxidants and antioxidants which results in the overproduction of free radicals and reactive oxygen species (ROS) which further decrease the antioxidant defence (Ganaie *et al.*, 2013). The primary indicator of immune response like white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), glucose and protein concentration in blood get distorted during heat stress (Das *et al.*, 2016). Sejian *et al.* (2016) emphasized the significant variation of Hb, PCV plasma glucose total protein and albumin in Malpura ewes when exposed to thermal stress.

It is pertinent that heat stress hampers all the production parameters of the animal especially it affects high producing animals. Rise in the temperature adversely affects the milk production and its composition (Wheelock *et al.*, 2010). Heat stress influences the rostral cooling centre of the hypothalamus to stimulate medial satiety centre. In response the animal reduce the feed intake that is directly responsible for the decline in the milk yield (Das *et al.*, 2016). Further, Bouraoui *et al.* (2002) established it's not only the yield but also the milk quality variables are affected by heat stress. These authors reported decreased milk fat, solid non fat and milk protein percentage on exposure to heat stress in dairy cows. Hamzaoui *et al.* (2012) also emphasized heat stress induced reduction in protein (6-13%) and lactose (1-5%) in dairy goats. Despite milk production, summer stress also causes infertility in animals. Thermal stress declines the length and intensity of oestrus besides increasing the incidence of anestrous and silent heat. Moreover, animals exposed to heat stress lose their competence for fertilization and affects the development of blastocyst stage (Gendelman and Roth, 2012). Recently, Lacerda and Loureiro (2015), depicted decrease in the fertility rate due to reduction in the quality of oocytes and embryos through the direct and indirect effects of heat stress. Correspondingly, conception rates dropped from 40% to 60% during winter to 10-20% or lower during summer, depending on the severity of the thermal stress (Das *et al.*, 2016). Further, in male heat stress reduces the concentration of testosterone, sperm mortality, sperm production, and abnormal spermatozoa thus reducing the quality of sperm (Hansen, 2009). Bhakat *et al.* (2014) observed that heat stress adversely influences the bio-physical

characteristics of semen in Karan Fries bull. Consequently, Sejian *et al.* (2016) observed that heat stress induces slow growth rate in animals including loss of body condition scoring. Thermal stress induced increase in concentration of corticotrophin releasing hormone stimulates the release of somatostatin which results in the reduction in both growth hormone and thyroxin level (Al-Dawood, 2017). Belhadj *et al.* (2016), reported that animals exposed to heat stress reduced the body weight and average daily gain (ADG) and growth rate in lambs. Balamurugan *et al.* (2018) indicated reduction in the dry matter intake, ADG, carcass weight, fat thickness and finally reduces the meat quality under summer stress. Heat stress compromises the meat quality, carcass characteristics and organoleptic quality of sheep and goats (Rana *et al.*, 2014). These authors observed that heat stress aggravates adrenaline which stimulates peripheral vasodilatation and muscle glycogenolysis, prolonged exposure leads to higher pH and darker meat. Similarly, thermal stress and anaerobic metabolism develops hyperthermia that results in an early and stronger rigormortis. Furthermore, when temperature rises animals tend to dehydrate which leads to dearth of water in the animal body leading to poor meat quality by making it darker in colour through shrinkage of the myofibrils and dryness of muscles leading to less cooking loss (Al-Dawood, 2017).

2.4. Heat stress associated economic loss in livestock farms

Heat stress jeopardizes the production potential as the temperature as the animals when exposed to environmental temperature above the thermal comfort zone may result in deviating their energy for adaptive processes. Therefore the heat stressed animals tend to compromise their production such as milk, meat and reproduction. The loss in feed intake was tipped to be the primary factor during heat stress which reduces production in Animals. Apart from the production loss, heat stress also acts as predisposing factor for few diseases and increases the risks of lameness which again reduce their production (St-Pierre *et al.*, 2003). These productive losses associated with heat stress may result in severe economic loss to the farmers of both developed and developing countries especially in the tropical

regions. For example, the US livestock industries were struck by economic crises due to the heat stress impact on livestock. St-Pierre *et al.* (2003) estimated about a decade ago the annual economic loss to livestock industry as a result of heat stress to be $897 and 369 million for dairy and beef industries, respectively.

Dairy cows are highly susceptible to temperature variations, particularly to sudden increases in temperature or long exposure to extreme heat. This increase in temperature raises the metabolic rate and exacerbates the survivability of dairy cows. This vulnerability can cause a reduction in the milk production from 1.5 to 2 L of milk per cow per day as well as increases the morbidity and mortality (Bishop *et al.*, 2015). De Vries (2004) emphasized that there was a reduction in the milk production of utmost 15% and 53% of reduction in the conception rate in Florida. Further, the data from Israel depicted a 7% decrease in the dairy industry and 51% in the reproduction (Flamenbaum and Galon, 2010). In addition, heat wave resulted in a drastic loss in the production of $253–$337 with respect to the average milk prices of $0.75 /L with 75 milch cows (Bishop *et al.*, 2015). Heat stress also established to be severely affecting the meat production and especially the meat industry is bit vulnerable given the fact of huge demand for meat to feed the growing human population. Exposure to extreme temperature is associated with the undesirable meat characteristics (Scharf *et al.*, 2014). In comparison to the economic loss in dairy sector, national annual losses associated with beef industry were only $3.05 (Scharf *et al.*, 2014). These researchers also opined that by ameliorating the heat stress condition in livestock farms associated with beef production, it is possible to reduce the loss to the tune of $125 per cow per year (De Vries, 2014).

2.5. Animal adaptation to heat stress

When the animal is exposed to high ambient temperatures which is above their thermal comfort zone the animal tend to pose several thermoregulatory mechanisms that help them to survive in the harsh climatic conditions. Adaptation is the ability of the animal to both survive and produce optimally

during prolonged exposure to stressful condition (Athira *et al.*, 2017). There are different types of adaptive mechanisms such as morphological, behavioural, physiological, biochemical adaptation, neuro-endocrine and cellular and molecular responses (Athira *et al.*, 2017; Sanin *et al.*, 2016).

Physical attributes favours an animal to adapt to their existing temperature, known as morphological adaptation. Morphological adaptation includes: coat colour, fur depth, hair density; hair type and subcutaneous fat on the hump or tail portion, skin colour, body size (Niyas *et al.*, 2015). Animals having light coloured coat such as white, light red absorb less heat as compared to dark colour and woolly coats. Similarly, smooth, thin, and short hair coat enhances more heat dissipation (Fanta, 2017). Sweat glands are considered to be the potential source for heat dissipation were Johnson and Hales (1983) opined that increase in blood flow to sweat gland enables heat transfer that promotes sweat production. Reports also suggest that, coat depth is associated with the heat loss, where increase in 3 to 10 mm of coat depth reduced the sensible heat loss in cattle by 17% at 20°C (Niyas *et al.*, 2015).

Animals opt for behavioural adaptations in order to reduce the need for evaporative cooling (Asres and Amha, 2014). Reduction in the feed intake is a behavioural change adapted by the animal, since feeding leads to heat increment and act as a source for heat production in ruminants. Athira *et al.* (2017) indicated that when the environmental temperature rose between 25-26°Cthe feed intake was found to be reduced in the lactating cows. Panda *et al.* (2016) reported in their study that goat exposed to heat stress significantly reduces their defecation and urination frequency. Similarly, Alam *et al.* (2011) indicated that there was a drastic decline in the urination and defecating frequency in black Bengal goats when exposed to heat stress. The heat stressed animals also increases their standing time as a way to increase the evaporative heat loss from the body surface (Kamal *et al.*, 2016). However, this condition may be reversed on provision of sprinklers and fans in the shed to alleviate heat stress as reported in buffalo heifers by Vijayakumar *et al.* (2011) establishing a significantly increased lying time.

Further, animal seeks shade during heat stress, to reduce the intensity of solar radiation (Fanta, 2017; Asres and Amha, 2014; Athira *et al.*, 2017). Furthermore, drinking frequency of the heat stressed animals also rises with increase in each degree celsius. Devendra *et al.* (1979) emphasized that, during severe heat stress water consumption of livestock raises by 20-30%. Thermal stress increased water consumption by 0.81 L per animal per day (Ali *et al.*, 1994; Mitloehner et al., 2001). Livestock mostly prefers moderate temperature water for drinking during stressful conditions.

Animals also exhibit certain physiological mechanisms when exposed to extreme heat stress such as increased heart rate, RR and RT. The increased heart rate in heat stressed animals allows the blood to flow to the peripheral vessels to dissipate body heat. Increased ambient temperature elevates the respiratory rate which promotes the evaporative cooling mechanisms like sweating and panting resulting in increased drinking frequency in order to maintain the water balance (Sanin *et al.*, 2016).

Animal alters blood biochemical profile so as to withstand heat stress and the extreme heat stress conditions. Sejian *et al.* (2014) opined that thermal stress significantly varied the level of Hb, PCV, plasma glucose, total protein and albumin and established them to be associated with thermo-tolerance in sheep. Similarly, Mazzullo *et al.* (2014) also added that, the value of the RBC and Hb count decreased drastically in cow during the extreme heat stress period. Decrease in the level of PCV during summer season indicates haemodilution effect or more water transport in the circulatory system for evaporative cooling mechanism (Gupta *et al.*, 2013). In addition, heat stress results in oxidative stress that increases the production of free radicals and reactive oxygen species. Helal *et al.* (2010), also reported that the chronic heat stress increased the concentration of plasma total protein, albumin, and globulin in goats as a result of vasoconstriction and reduced plasma volume during heat stress. Increase in the environmental temperature was established to be directly proportional to the plasma cortisol (Silanikove and Koluman, 2015). The Heat stressed animals also tend to reduce

the metabolic heat production by reducing the concentrations of thyroid hormones such as tri iodothyronine (T3) and thyroxin (T4) (Magdub *et al.*, 1982).

Gene expression is an integral part of cellular response to heat stress; HSPs play a key role in cell survival under thermal stress. Therefore, HSP is considered to be the ideal and confirmatory biological stress marker for heat stress in livestock. They protect the cells from degrading when exposed to heat stress. The heat shock factors (HSFs) acts as triggering mechanism to initiate the HSP synthesis and HSP gene expression pattern in livestock (Mishra *et al.*, 2010). Dangi *et al.* (2012) also observed that when goats are exposed to heat stress they showed higher HSP70 mRNA expression pattern which helps them to cope with the harsh environment. However, this depends on various factors including temperature, wind velocity, body condition, health status, and genetics of the animal (Sanin *et al.*, 2016). Therefore, research efforts are needed to elucidate the hidden intricacies of regulation of adaptation processes at cellular and molecular levels in various livestock species.

2.6. Climate change and disease occurrences in livestock

Environmental factors such as ambient temperature, relative humidity and rainfall have been implicated to alter the immune status of the animal (Sevi and Caroprese, 2012). Acclimatization of animal to the various environmental challenges results in reduction of feed intake and alteration of normal physiological functions linked with impaired health status(Nardone *et al.*, 2010).Climate change affects both directly and indirectly the immune system of animals (Nardone *et al.*, 2010). Alterations in the temperature and rainfall may lead to emergence and proliferation of diseases in the livestock sector as several diseases vectors like biting flies and ticks, are more likely to survive year-round (Sejian *et al.*, 2016). This change in the environmental parameters overwhelms diseases such as malaria and zoonotic parasitic diseases including leishmaniasis, cryptosporidiosis, giardiasis, trypanosomiasis, schistosomiasis, filariasis, onchocerciasis, and loiasis (Patz *et al.*, 2000). Das *et al.* (2016) reported that, when temperature rises animals tend to spend more time standing, hence causing

lameness. Further, the animals will suffer from thin soles, white line disease, ulcers, and sole punctures which finally leads to death. In addition, incidence of external parasites was observed during summer season. Further high temperature coupled with humidity showed a prevalence of mastitis in dairy animals. Jingar *et al.* (2014) indicated that cows subjected to heat stress and high relative humidity (RH) showed mastitis whereas, Murrah buffaloes were not much affected. Similarly, Sevi and Caroprese (2012) also observed a negative impact of heat stress and humidity on the udder health of sheep.

Extreme weather events may cause emergence of both zoonotic as well as infectious diseases, which easily spread to the human population (Sachan and Singh, 2010). Disease vectors also transport to new area for instance malaria, livestock tick borne diseases, rift valley fever and bluetongue disease in Europe. Diseases such as ovine chlamydiosis, CAE, EIA, equine influenza, MD, and bovine viral diarrhea may occur during the changes in the rainfall pattern (Sejian *et al.*, 2016). Moreover, diseases such as foot and mouth disease or avian influenza affects widely the animal population and also affects the environment. There are different types of diseases that affect the animal population such as vector borne diseases, tick borne diseases, and parasitic diseases. Environmental challenges promote the spread of contagious diseases through mingling with the diseased animal or its intermediate host. Vector borne diseases are mainly transmitted through infectious agent, vectors and host (Baumgard *et al.*, 2012). Tick borne Encephalitis is caused by of the family Flaviviridae and is transmitted by ticks, Ixodes ricinus that act as both vector and reservoir. Parasitic disease is also influenced by the changing climatic scenario. Environmental changes affect free-living larval stages, other invertebrates, and vertebrate hosts. Nematodiases, including heterakiasis, different trichostrongyliases and protostrongyliases, anchylostomiases, and dirofilariases are some of the parasitic diseases which has correlation with environmental variables (Baumgard *et al.*, 2012).

Animals subjected to heat stress alter the glucose and lipid metabolism, liver function and oxidative status; as a result, animals are prone to metabolic

diseases and infectious diseases. Nardone *et al.* (2010), evidenced that animal exposed to heat stress showed changes in the liver functions. Thus, the cholesterol, albumin secretion reduced along with the changes in enzyme activities (Bernabucci *et al.*, 2006). In addition, Martin *et al.*, 1975 observed that thermal stress impaired the colostral immunoglobulin in the lactating cow which resulted in huge mortality of new born. Bewket *et al.* (2015) reported, increased incidence of diseases during when animals are under poor nutrition condition due to inadequate feed supply and increased heat stress. Similar study reported by Zelalem *et al.* (2009), foot and mouth disease, black leg were identified as the major diseases in cattle, whereas, Coenurus cerebralis and general septicemia hampers the sheep and goat population in Borane area. Severe heat stress hampered the cell mediated immunity in high yielding dairy cows. Increase in temperature and humidity favours the growth of mycotoxin producing fungi that causes acute disease episodes when consumed in large quantities. This toxin affects the function of liver, kidney, gastric mucosa, brain and reproductive tract (Nardone *et al.*, 2010). Livestock diseases causes both direct losses through death heat stress, stunting, reduced fertility, and changes in herd structure and indirect losses through additional costs for drugs and vaccines, added labour costs and profit losses due to denied access to better markets and use of suboptimal production technology (Chauhan *et al.*, 2014).

2.7. Heat stress impact on immune response in livestock

Stress enhances or suppresses the immune status of the animal (Dhabhar, 2009). Heat stress plays a major role in altering the immune functions of the animal. The changes in the environmental condition promote the growth and interaction between host and pathogen that breaks the immune barrier of the host and induces disease conditions (Sophia *et al.*, 2016b).

WBCs, RBCs, Hb, PCV, glucose and proteins are considered to be the primary indicators of immunity that gets affected during heat stress. Das *et al.* (2016) reported that thermal stress increases the WBC count and decreases the RBC count due to thyromolymphatic involution. Rejeb *et al.* (2016) reported that

number of leukocyte decreased drastically during heat stress. Heat stress also reduces neutrophiles and eosinophiles while increasing the lymphocytes and monocytes in cows (Broucek *et al.*, 1985).

Heat stress affects the immunity of foetus, with significant changes after the birth. Animals born to the Heat stressed dams are lighter in weight than the normal ones, due to early birth (Tao and Dahl, 2013). Heat stress reduces the immunoglobulin concentration in the uterus of pregnant cows which hamper the growth, health and survival of the foetus (Tao and Dahl, 2013). First 18 hours after birth is the most crucial period for the foetus as it is during this period they are susceptible to diseases and determines the immunity of the animal. At this point the passive transfer of immunoglobulin from dams to offspring occurs and the intestinal epithelium becomes permeable to colostral proteins. When animals are exposed to heat stress during late postpartum, reduces the concentrations of IgG (Immunoglobulin G), IgA (Immunoglobulin A), milk proteins and fatty acids in colostrums (Nardone *et al.*, 1997). Further this could also reduce the intestinal absorption of the offspring that leads to mortality (Sophia *et al.*, 2016b). In addition, heat stress significantly affects the udder health of the animals, as heat stress impedes the movement of leukocytes to mammary gland which lead to increase in pathogens in milk (Zhang *et al.*, 2014). However, Dahl and collier (2017) indicated that heat stress had no effect on the milk production of ewes. do Amaral *et al.* (2011) indicated that heat stress hampers the cows during the dry period

Further, climate change increases the disease occurrences which are categorised into different types such as vector borne diseases, parasitic diseases, virus diseases and fungal diseases. Rise in temperature increases the population of pathogens hence increasing the risk of infection (Sophia *et al.*, 2017). Elevated temperature provides the ideal environment for vectors like ticks, flies, midges temperature in favour of reproduction and increased frequency. Hot and humid conditions favour the growth of tick population like *Boophilus microplus, Haemophysalis bispinosa and Hyalomma anatolicum* (Basu and Bandhopadyay,

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2004). Heat stress reduces the incubation period of the parasites such as *Culicoides* (Wittman & Baylis, 2000). Elevated temperature coupled with relative humidity raises the chances of mastitis in cows which causes severe economic loss to dairy industry (Singh *et al.*, 1996).

When temperature and humidity rises animals experience change in the endocrine system in order to promote for the mechanisms that attributes to heat loss. Thus, the level of cortisol and prolactin hormones rises with the increase in temperature. Alterations in these hormones influences the gene expression associated with heat stress (Collier *et al.*, 2008). The HSPs are produced by the cells as a response to heat stress and they are categorised according to their molecular weights approximately 90, 70 and 27 k Da and are referred to as HSP90, HSP70 and HSP27. HSPs protect the cells from degrading during the stressful conditions. In addition, they help in protein synthesis and support the innate immune mechanisms in animals (Sevi and Caroprese, 2012).

Heat stress also activates TLR signaling pathway and as a result up regulates genes such as CD80, CASP8, RIPK1, IFNAR2, and BIRC3 (Eicher *et al.*, 2004; Zhouetal., 2005). Apart from these genes, few additional interleukin genes such as NFIL3, IL8, and IL15 are also over expressed during heat stress. Further, Ju *et al.* (2014) reported that activation of TLR and dysregulation of cytokine in heat stressed animal has been reported to suppress the immune system and increased vulnerability to various diseases in pigs. Therefore, environmental stresses are considered one of the primary factors which makes the animals susceptible to pathogens.

2.8. Importance of studying heat stress impact in indigenous livestock breed

Indigenous breeds are well adapted to the environmental stresses and diseases occurrences as comparing to exotic breeds. Indigenous breeds posses certain adaptive mechanisms to sustain in their existing environment. Marai *et al* (2007b) suggested that, indigenous breeds reared in tropical and arid regions can sustain in extreme climates. Therefore, farmers prefer indigenous livestock due to their adaptive nature and resilience capacity (Assan, 2014). Bernabucci *et al.*

(2010) reported that indigenous goats reared under hot and humid conditions perform better than other domesticated goats. Indigenous cattle breeds are used for crossbreeding in order to improve the productivity and tolerance for subsistence in harsh climate (Scholtz and Theunissen, 2010). Kadim *et al.* (2007) observed that indigenous sheep in tropical and sub-tropical regions reproduce throughout the year. Landim cattle native to Mozambique showed extreme tolerance to high ambient temperature, longer dry period along with resistance to Foot and mouth diseases (FMD) (Mwai, *et al*, 2015). Wheelock *et al* (2010) indicated that indigenous breeds alter their metabolic activity to minimize the body heat during heat stress. African *Bos taurus* indigenous to West and Central Africa, have a distinctive evolutionary adaptation capability towards adverse climatic conditions (Mwai *et al.*, 2015). Orma Boran, an indigenous East African zebu breed is adapted to drought and thermal stress and also resistant for tick infestation (Mwai *et al.*, 2015). Kugonza *et al.* (2011) suggested that most African local breeds may not be much productive but are highly tolerant to adverse climatic conditions. Pragna *et al.* (2018) indicated that Salem black breed indigenous to Tamil Nadu had shown less impact when exposed to heat stress as compared to Osmanabadi breed native to Karnataka state in India. This study also established that indigenous breed alters their metabolic rhythmicity in order to adapt to the thermal stress. Similar study conducted in Aardi goats which is an indigenous breed of Saudi Arabia, proved that several physiological changes such as increase in skin temperature, increased heart rate and respiratory rate, decrease in T3 and T4 and increase in cortisol concentration could decrease the affect of heat stress (Al-Samawi *et al.*, 2014). From this study it was also summarized that this breed is highly productive when heat stress is controlled (Al-Samawi *et al.*, 2014). Rashid *et al.* (2013) emphasized that, indigenous goats are well adapted to acute heat stress whereas; chronic heat stress had adverse effects on the physiological functions. Indigenous Barki goats and sheep from Egypt also acclimatized well to the local hot and dry climatic conditions (Kim *et al.*, 2016). In a recent study, Archana *et al.* (2018) reported that heat stress did not influence the meat quality and quantity of Salem black breed; in contrast heat stress

negatively affected the meat quality of Osmanabadi breed. This study attributed the superiority of Salem black breed to maintain meat production characteristics to the lower HSP70 expression pattern during heat stress. However, Rana *et al.* (2014a) established negative impact of heat stress on both meat quality and carcass characteristics in indigenous sheep. Further, in an another study these authors also established the adverse impact of heat stress on the adaptive capability as reflected by changes in the blood biochemical variables in indigenous goat of Bangladesh (Rana *et al.*, 2014b).

2.9. Significance of goat from climate change perspectives

Goat is considered to be an ideal climate change animal and also known as poor man's cow (Bhattarai, 2012). They highly contribute to the agriculture sector and play a key role in the livelihood of poor and marginal farmers (Sundaram *et al.*, 2012). Goat is a versatile animal; it can produce meat, milk fibre, hide and manure. Less investment and management on goat production system can give a huge output. Goats are considered to be hardy breed as it can thrive in drought and heat stress condition (Shilja *et al.*, 2015). Despite its high tolerance goats can also survive by feeding on shrubs, herbs and low quality pastures due to its high crude fibre digestibility (Shilja *et al.*, 2016). As a result goats can be used to improving the quality of rangelands and also helps in controlling weeds (Norman, 1991). Darcan and Silanikove (2018) reported that goats can survive in adverse conditions by producing low metabolic heat production, tolerance to water stress, by altering their anatomical and morphological structure, resistance to disease occurrence and feeding on shrubs and other poor quality feed. They start their reproduction phase from the age of one year. Moreover, goats emit less methane as compared to other ruminants, making them the ideal climate change animal. Weaning weight, growth rate and ADG are some of the economical significant traits in goats (Sundaram *et al.*, 2012).

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Indigenous goats are highly resistant to environmental stresses. Their physiological factor allows them to maintain their reproductive capacity under stressful conditions. Indigenous breeds are well adapted to sudden fluctuation in the environment and disease outburst as compared to the exotic and cross breeds (Pragna *et al.*, 2018). To minimize their internal heat load goats tend to alter their metabolic activities (Wheelock *et al.*, 2010). Oppon-Anane *et al.* (2008) established that, the indigenous West African dwarf goat shows early maturation and they are prolific and non seasonal breeders with high resistivity. Similarly, Selolo *et al.* (2015) also suggested that the indigenous goat in South Africa showed valuable morphological traits that assisted them to survive in different agro ecological zones which can be used for breeding programmes. Further, Baruwa (2013) indicated that goat meat is mostly consumed around the world not only due to its taste but also it does not have any religious taboos as compared to other red meat. In addition, goat milk is also considered to have high medicinal value. Bhattarai (2012) reported that goat milk has higher amount of Ca, Mg and P as compared to cow and human milk. Goat meat is well known for its flavour, tenderness, aroma and juiciness and also it is rich sources of protein, vitamins, minerals and essential fatty acids (Webb *et al.*, 2005).

2.10. Malabari goats

Malabari goats are also known as Tellicherry goats and they are one among the recognised breeds of goats in India. The breed owes its name to area where they reside from, Malabar region of Kerala. Malabari goats are widely distributed in Malabar region of kerala and also reared in Calicut, Kannur, Waynad and Malapuram districts of Kerala state and different places of Tamil Nadu (Verma *et al.*, 2009; Thiruvenkadan *et al.*, 2008; Sundaram *et al.*, 2012). This breed is well adapted to hot and humid conditions of Kerala and to facilitate their survival in this harsh environment they possess small to medium body size. They are commonly seen with a wide range of coat colour ranging from complete white, admixtures of white and brown, black and brown to complete black (Verma *et al.*, 2009). Around 40% of the Malabari goats have long hair and about 20% of

both male and female have beard. Their horns are twisted either upwards or downwards touching the skin. Their features include pinkish red muzzle, pinkish white eyelid, straight nose, long ears reaching up to nose with folded tips, thin and small tail, small udder with medium teats. Adult female weight is about 20-25 Kg and adult male weight is about 30-35 Kg (Verma *et al.*, 2009).

Malabari goats are dual purpose goats and its meat is famous for its low-fat content (Verma *et al.*, 2009). This breed also was considered as milch breed of goats in Kerala with an average milk yield of 43.78 kg for an average lactation length of 143.5 days (Sundaram *et al.*, 2012; Thiruvenkadan *et al.*, 2008). Their breeding tract lies between longitude ranging from 11.15' to 11.52' N and latitude 75.25' to 75.49 E. This breed has a unique genotype exhibiting higher multiple birth percentages, with high prolificacy. Their age of first kidding is 8 month and inter- kidding extents upto 14 month and has 65% of kidding rate that produce twins or triplets. They have single and multiple birth percentages pooled to 50.5 and 49.5 % respectively (Thiruvenkadan *et al.*, 2008).A recent study conducted by Vandana *et al.* (2018) established that Malabari goat has a superior thermo tolerant capacity by maintaining their immune status during heat stress.

2.11. TLR significance in goat/ livestock

TLRs play a significant role in pathogen recognition through the innate immune mechanism. The TLRs are a family of 10 proteins which plays significant role in controlling immune response during stressful conditionin livestock (Sophia *et al.*, 2016a). Cell surface TLRs also activates phagocytosis, inflammation and apoptosis in response to pathogen detection which initiates the immunity through induction of pro-inflammatory mediators (Paul *et al.*, 2015). Chao (2009) established that, TLR4 enhance T cell response to burn the injuries, embed inflammation, sterile injury and alloimmune responses in tissue transplantation. Cole *et al.* (2011) observed that TLR3 was responsible for protecting the integrity of the blood vessel wall. Further, Heijden *et al.* (1998) established that intra cellular TLR plays a crucial role in auto-immune disorders. Initiation of TLR exacerbates and ameliorates airway reactivity and inflammation

as in case of asthma in animal models. In addition, Nadeem *et al.* (2016) emphasised that TLR7 augment antioxidant network in the lungs which protects against ROS-mediated airway reactivity and inflammation. Yu *et al.* (2010) reported that TLR repairs tissue injuries and enhances regeneration mainly in liver and intestinal epithelium. Further, TLR2 is responsible for wound healing and regulation of homeostasis (Rakoff-Nahoum and Medzhitov, 2009).

Shchebliakov *et al.* (2010) emphasized that TLRs suppresses tumor growth and enhances the subsistence of malignant cells and induces resistance to chemotherapy. Paulos *et al.* (2007) also reported that TLRs plays a crucial role in cancer immunotherapy. In addition, TLR acts as a natural adjuvant to vaccines that contain live or dead viruses or bacteria (Van Duin *et al.*, 2006). Sophia *et al.* (2016a) has emphasized that the transmembrane TLR proteins can identify the pathogen that enters the host and binds to the microbial molecules. The MyD88 (Myeloid Differentiation Primary Response protein 88) is a protein coding gene that is linked with the TIR (toll/IL-1 receptor) domain of TLRs; MyD88 is an adapter molecule which plays a significant role in TLR signal transduction (Medzhitov and Janeway, 2002). The TLRs enhances bridging of innate immunity and adaptive immunity by identifying the invader pathogens and stimulates the antigen presenting cells (APCs) that hampers the phagocytosis pertaining to destruction of foreign particle. The presence of all the TLRs on the immature dentritic cell (DC) helps in enhancing the resistance to pathogens (Duin *et al.*, 2006). However, environmental stresses like heat stress changes the adaptive immunity from cell mediated to humoral immunity, where the immunity gradually ceases (Sophia *et al.*, 2016b).

2.12. Factors influencing expression patterns of different TLRs with special reference to climate / heat stress

The response of animals to stress depends on the various factors such as genetic makeup, environmental stress, nutrition, species, age, sex, and breed (Sophia *et al.*, 2017).

Genetics of an animal have huge influence in the expression pattern of different TLRs. Marple *et al.* (1972) reported that animal responds to each stress based on their genetic makeup. Genetics plays a crucial role during stress as the genetically sound animals can thrive for subsistence using important genetic markers which imparts them thermo-tolerance and ability to maintain immune status (Singh *et al.*, 2014). The TLR1 has over expressed in spleen of heat stressed Osmanabadi goat breed (Sophia *et al.*, 2016b). Paul *et al.* (2015) reported that heat stress over expressed TLR1 in Black Bengal goats. A recent study by Vandana *et al.* (2018) established that TLR has up-regulated in mesenteric lymph node when exposed to heat stress in Malabari goat. Further, Tirumurugan *et al.* (2010) also established thermal stress induced up-regulation of TLR5 in lung, skin, uterus and jejunum in Tharparker cattle.

Species difference also was established for the impact of heart stress on the immune response in livestock. Tharparker cattle showed higher expression pattern for TLR4 when subjected to heat stress (Bharati *et al.*, 2017). Ju *et al.* (2014) reported that heat stress up-regulated TLR4 in PBMC of Bama miniature pigs. Similarly, higher expression trend for TLR3was observed in Black Bengal goats when exposed to heat stress (Paul *et al.*, 2015). Vandana *et al.* (2018) established that heat stress has down-regulated the expression pattern of TLR1 in Malabari goat breed. In addition, Sophia *et al.* (2017) indicated that heat stress did not influence the TLR4 gene expression in hepatic tissue of Osmanabadi goats.

In a study conducted in *adlibitum* fed Osmanaabadi goat breed, Sophia *et al.* (2017) observed over expression of hepatic TLR3 gene during heat stress indicating that nutrition enhances the immunity of the animal and the animals do not compromise their immune status. This shows that supplementing with additional nutrition during heat stress periods may be highly beneficial for maintaining the immune status in the goat (Sophia *et al.*, 2017). In addition to breed difference, the expression pattern of different TLRs also differs according to the target tissues of its expression. Tirumurugan *et al.* (2010) indicated that heat

stress up-regulated TLR5 in lung, skin, uterus and jejunum in Tharparker. Further, Vandana *et al.* (2018) established up regulation of TLR4 in lymph node of heat stressed Malabari goat. Sophia *et al.* (2016b & 2017) also established differences in expression patterns of different TLRs between spleen and liver tissues in Osmanabadi goats.

Paul *et al.* (2015) reported that age influenced the expression pattern of TLR in Black Bengal goats. During heat stress TLR1 has over expressed at age 2-5 years as compared to 0-2 and above 5 years. In addition, Renshaw *et al.* (2002) established age had influenced significantly in a decreasing trend of TLRs in splenic and peritoneal cells. Sex also was found to influence TLR expression pattern in animals. Rettew *et al.* (2008) observed that estrogen enhanced the expression patterns of different TLRs. For example, Yao *et al.* (2007) established increased expression pattern of TLR2 both during estrus and diestrus period in murine.

2.13. Expression patterns of different TLRs in goats/other livestock

The TLR1 was up regulated in uterus, skin, lymph node, PBMC and lungs of Kanni breed goats when exposed to heat stress (Tirumurugan *et al.*, 2010). Similarly, Sophia *et al.* (2016b) also reported that TLR1 showed over expression in spleen of heat stressed goat. The TLR1 showed significantly higher expression in Osmanabadi under heat stress as compared to nutritional stress and combined stress group indicating that when nutrition was not compromised the heat stressed animals were able to mount immune response appropriately (Sophia *et al.*, 2017). Further, Paul *et al.* (2015) indicated that TLR1 was over expressed in Black Bengal goats during exposure to heat stress. However, Vandana *et al.* (2018) established that heat stress suppressed TLR1 expression in Malabari goats showing that heat stress compromised the immunity of Malabari breed which was in contrast to the above findings. Bama miniature pigs showed a higher expression of heat stress PBMC when subjected to heat stress (Ju *et al.*, 2014). In contrast, Sophia *et al.* (2017) reported that Osmanabadi breed had no effect on

TLR2 expression pattern when exposed to heat stress which shows the highly adaptive nature of this breed. Paul *et al.* (2015) established that TLR2 was up-regulated in heat stressed Black Bengal goat. Similarly, TLR2 was over expressed in Tharparkar cattle when subjected to chronic heat stress (Bharati *et al.*, 2017). Tirumurugan *et al.* (2010) indicated that TLR2 was down-regulated during heat stress in uterus, lung and skin. A recent study by Vandana *et al.* (2018) emphasized that TLR2 could be recognised as a reliable marker to assess the immune status of the Malabari goats during heat stress as TLR2 has over expressed during heat stress. Paul *et al.* (2015) identified that, TLR3 was up-regulated in PBMC during heat stress in goats and this may help to fight against viral infection. Similar trend was observed in spleen of Osmanabadi goats exposed to heat stress indicatingTLR3 could serve as ideal marker for assessing the severity of heat stress (Sophia *et al.*, 2016b). In another study, Sophia *et al* (2016b) observed that TLR3 has shown higher expression in liver of *ad libitum* fed Osmanabadi goats during thermal stress again indicating that this breed could sustain their immunity when nutrition is not compromised. Further, TLR3 was down-regulated in uterus and jejunum of goats under heat stress (Tirumurugan *et al.*, 2010). In addition, TLR3 showed non-significant effect in lymph node of Malabari breed when exposed to heat stress (Vandana *et al.*, 2018). In another study Ju *et al.* (2014) indicated that TLR4 was up-regulated in PBMC of Bama miniature pigs when subjected to heat stress. Similarly, Paul *et al.* (2015) established higher expression of TLR4 during summer season in Black Bengal goats. Hence, this study proved that TLR4 had enhanced the cytokines that helped in host subsistence. Bharati *et al.* (2017) also observed over expression of TLR4 in Tharaparker cattle during chronic and acute heat stress, further proved that his breed was thermo tolerant to heat stress. However, Sophia *et al.* (2017) established that heat stress did not influence the TLR4 gene expression in hepatic tissue of Osmanabadi goats. In contrast, Slawinska *et al.* (2016) established that decreasing trend in TLR4 expression during heat stress. Similarly, Sophia *et al.* (2016b) and Tirumurugan *et al.* (2010) reported lower expression of TLR4 gene in the spleen of Osmanabadi goat and uterus and jejunum of Tharparker cattle

during heat stress exposure. Vandana *et al.* (2018) also established a higher expression of TLR4 in lymph node of heat stressed Malabari breed indicating the immune-suppressive effects of heat stress in this breed. Sophia *et al.* (2016b) observed over expression of TLR5 in spleen of Osmanabadi breed during summer season. In addition, Sophia *et al.* (2017) also proved that TLR5 had no effect on liver indicating tissue specific expression pattern for TLR5. Similarly, Paul *et al.* (2015) also established no effect of heat stress on TLR5 expression in Black Bengal goats. However, Tirumurugan *et al.* (2010) observed up-regulation of TLR5 in various tissues such as lung, skin, uterus and jejunum in Tharparker cattle indicating species differences in the expression pattern of TLR5. Contrastingly, Vandana *et al.* (2018) established lower expression of TLR5 in Mesenteric lymph node of Malabari goats during heat stress.

Sophia *et al.* (2016b) reported over expression of TLR6 in the spleen of Osmanabadi goat during heat stress. Similarly, Srikanth *et al.* (2017); Paul *et al.* (2015) and Sophia *et al.* (2017) also established over expression of TLR6 in Holstein calves; Black Bengal and Osmanabadi goats respectively during heat stress. Paul *et al.* (2015); Sophia *et al.* (2016a, 2017) established up regulation of TLR7 and TLR8 during heat stress in Black Bengal and Osmanabadi goats respectively. Further in several studies it was established that heat stress did not influence the expression pattern of TLR10 (Paul *et al.* 2015; Sophia *et al.* 2016a, 2017; Vandana *et al.*, 2018).

2.14. Type of TLRs to be considered biological markers for quantifying heat stress impact on immune response

Due to the environmental challenges highly adaptive animal with high production potential is the need of hour. Advance development in the field of molecular biology makes the impossible possible to identify a breed with higher thermo-tolerance using these advanced tools. Molecular markers refer to DNA fragment specific to certain location in the genome and are called as genetic markers. Biological markers are of various types including morphological,

phenotypic, biochemical and molecular markers. During climate change scenario, animals compromise their immune status and become susceptible to various diseases. As a result scientific community are looking to establish various molecular markers to assess the immune status of animals during stressful condition in an effort to improve their productivity.

In a recent study, Vandana *et al.* (2018) has emphasized that TLR2 could be considered as an immunological marker which can be used to assess the immune status of the animal. Similarly, TLR3 also could be served as an ideal marker to quantify heat stress, further it can be used for marker assisted selection program (Sophia *et al.*, 2016b; Srikanth *et al.*, 2017). Genes such as TLR8 and TLR10 may also act as biological markers to assist breeding programs to develop a goat breed which can with heat stress and heat stress and maintain immunity (Sophia *et al.*, 2017). In addition, IL2/6 could also serve an immunological marker to quantify heat stress and can be used in selective breeding programs for inducing thermo- tolerance (Bharati *et al.*, 2017). The above discussed various TLRs could serve as ideal biological markers to assess the immune status during heat stress challenges in livestock. Once validated in different species these markers can used in selective breeding to evolve a breed which can maintain the immune status during climate change associated heat stress.

2.15. Closing Remark

The above discussed literature review clearly describes the very little information available on heat stress associated immune response in domestic livestock. This reflects the huge knowledge gap in this particular research area and warrants more research hypothesis that needs to be set and proved. This may provide valuable clue for future animal breeding program to evolve a breed with supreme thermo-tolerance and disease resistance capabilities. Toll-like receptors are the initial molecules which determine the quality of innate immune response. Therefore elucidating the role of different toll- like receptors during heat stress exposure in livestock may help to bridge the knowledge gap to a greater extent. Further, such efforts in goat which has been projected as ideal animal model in the changing

climate scenario may help a lot to understand the biological mechanisms by which heat stress modulates the immune response in these animals. These are all the type of research which can have a greater impact in ensuring livestock related food security to feed the growing human population (Myers et al., 2017).

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 temperature-humidity index (THI) during the study period (45 days) are listed in figure 1. THI was calculated by the formula described by McDowell (1972).

3.2. Animals

A total of 12 ten months to one-year old Malabari female goats weighing between 12-19 kg were used. Malabari goat breed is a meat purpose animal originated in the humid tropical region of southern India. The animals were brought from different locality and acclimatized to the current experimental location for a period of 45 days. These animals were maintained in well ventilated sheds following standard farm management procedures. Plate 3.1and Plate 3.2 describes experimental goats both inside and outside the shed respectively.

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Plate3.1. Pictorial Representation of control group Malabari breed goat kept inside the shed

Plate 3.2: Pictorial description of Female Malabari Goat exposed to heat stress in outside environment

3.4. Experimental design

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

3.5. Sample collection and storage

The MLN samples were collected from all the animals in each group immediately after slaughter. The samples were cut into small pieces, washed in Phosphate Buffered Saline and immersed in RNA shield (Zymo Research, USA) and snap chilled in LN2. Then samples were shifted and stored at –80 °C till further use.

3.6. Sample preparation for RNA isolation

After thawing, the tissues were removed from the RNA shield (Zymo Research, USA) and immediately processed for RNA isolation. The total RNA was isolated from tissues using the GeneJET RNA Purification Kit (Thermo Scientific, Lithuania) and the procedure was done as per manufacturer's

protocol. Approximately, to 25 mg of tissues were pulverized and homogenized with a Cole-Parmer LabGEN DTH homogenizer in lysis buffer and liquid nitrogen (–196°C). After homogenization, 300 µL of lysis buffer supplemented with β-mercaptoethanol (10 µL/ml) in 2.0 ml DNase, RNase free microcentrifuge tube. To the lysate, 10 µL of proteinase K in 590 µL of Tris Ethylenediaminetetraacetic Acid buffer was added, then vortexed and incubated at 15–25 °C for 10 min. Then, the contents were centrifuged for 8 min at 12,000 g and the supernatant was transferred into a new RNase-free micro centrifuge tube. 450 µL of ethanol was added and mixed well by pipette. Then 700 µL of lysate was transferred to a spin column in a 2 ml collection tube and centrifuged for 1 min at 12,000 g. The process was repeated till all the lysate was allowed to pass through the column. After discarding the flow through, 700 µL of wash buffer 1 was added and centrifuged for 1 min at 12,000 g followed by two time washing with 600 and 250 µL of wash buffer 2 followed by centrifugation at 12,000 g for 1 and 2 min, respectively. About 30 µL of warm nuclease-free water was added to the membrane and incubated for 2 minutes and centrifuged at 10,000 g for 1 min to elute RNA. The purified RNA samples were stored at –80 °C until cDNA synthesis.

3.7. DNase treatment

Total RNA isolated from different tissues was treated with DNase (TURBO DNA-free, Ambion, USA) in order to eliminate the genomic DNA contamination in total RNA. During and after DNase treatment, 1 µL of RNase inhibitor (20 U/µL, Invitrogen, USA) was added. After DNase treatment quality and quantity of the isolated RNA was analysed using Nanodrop spectrophotometer. Integrity of the total RNA was checked using denaturing agarose gel (1%) electrophoresis and visualization under UV light. Two intact bands of 28 s and 18 s indicated good quality and intactness of RNA.

3.8. cDNA synthesis

The total RNA was reverse transcribed into cDNA using Maximafirst strand cDNA synthesis kit for Real Time quantitative polymerase chain reaction (RTqPCR)(Thermo Scientific, Lithuania). The procedure was performed as per manufacturer's protocol with modifications are as follows: 4 µL of 5 x Reaction Mix, 2 µL Maxima Enzyme Mix, 1.5 µg of Template RNA was used for mesenteric lymph node sample and 20 µL of nuclease-free water were added into a sterile, RNAase-free tube. Then the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 min at 25 °C, followed by 20 min at 50 °C and the reaction was terminated by heating at 85 °C for 5 min). The product of the first strand cDNA synthesis was diluted to a final concentration of 25 ng/ µL with nuclease-free water and 2 µL of diluted cDNA was used foreach reaction in qPCR.

3.9. Primer design and synthesis.TLR1-10 primers

TLR 1-10 Primers Sequences of the primers used for amplifying the target regions of TLR genes have been published by Paul *et al.* (2015). The primer sequences are described in Table 3.1.

3.10. Real time—qPCR Real-time PCR

Real time—qPCR Real-time PCR (ABI StepOnePlus Real-time PCR System, Foster City, CA, USA) was performed using SYBR green chemistry in a 20L reaction using Maxima SYBR Green/ROX qPCR master mix (Lithuania, EU). The reaction mixture consists of Maxima SYBR Green/ROX qPCR master mix (2X) −10L, Forward primer 5 M, Reverse primer 5 M, template DNA 50 ng, nuclease-free water to make up 20L. The reaction conditions were as follows: enzyme activation at 95°C for 10 min and 40 amplification cycles consisting of initial denaturation at 95° C for 15 s, annealing at 61° C for 30 s and extension at 72° C for 30 s. Melt curve analysis was performed to check the non-specific amplification. GAPDH gene (Glyceraldehyde 3 Phosphate Dehydrogenase) was used as an internal control. The relative gene expression was calculated using the formula 2 CT (Livak and Schmittgen,

2001). The results were expressed in fold change as compared to untreated control (control = 1fold)

3.11. Statistical analysis

The changes in relative expression of different genes in relation to the reference gene were analysed using SPSS (16.0) software using one-way analysis of variance (ANOVA). The significance level was set at $P<0.05$. Mesenteric lymph node histological data also were analysed by one-way analysis of variance (ANOVA). Further, the correlation coefficient between the THI and all genotypic traits were established by Pearson's correlation coefficient test using SPSS (version 18.0) software. The R^2 values were used to establish the correlation association between THI and various genetic traits with two levels of statistical significance set at $P<0.01$ and $P<0.05$.

Table 3.1: Primer sequences for different TLR genes and GAPDH reference gene

Gene ID	Primers	Primer Sequence	Annealing Temp	Product Size	Accession No.
TLR-1	F	5-ACTTGGAATTCCTTCATTACGA-3	60	176	HQ263209.1
	R	5-GAAGACTGAACACATCATGGA-3			
TLR-2	F	5-TTCCGTCTCTTTGATGAG-3	60	114	JQ911706.1
	R	5-CTTGGTGTTCATGATCTTC-3			
TLR-3	F	5-GATGTATCGCCGTGCAAAGACA-3	60	195	HQ263210.1
	R	5-TGCATATTCAAACTGCTCTGCT-3			
TLR-4	F	5-CTTGCGTCCAGGTTGTTCCTAA-3	60	153	JF825527.1
	R	5-CTGGGAACCTGGAGAAGTTATG-3			
TLR-5	F	5-CCTCCTGCTCAGCTTCAACTAT-3	60	172	FJ659852.1
	R	5-TATCTGACTTCCACCCAGGTC-3			
TLR-6	F	5-CCTTGTCTTTCACCCAAATAGC-3	60	150	HQ263211.1
	R	5-GTTGGTCTTCCAGTGAGT-3			
TLR-7	F	5-TCTTGAAGGAAAGGACTGGTTA-3	60	205	HQ263216.1
	R	5-AAGGGGCTTCTCAAGGAATATC-3			
TLR-8	F	5-CGCACCGTCTAGGATTTATT-3	60	209	JF825528.1
	R	5-AAGCCGGGTCAGATTGGT-3			
TLR-9	F	5-CTGACACCTTCAGCCACCTGAG-3	60	156	HQ263217.1
	R	5-TGGTGGTCTTGGTGATGTAGTC-3			
TLR-10	F	5-ATGGTGCCATTATGAACCCTAC-3	60	248	HQ263213.1
	R	5-CACATGTCCCTGTGGTGTCTAA-3			
GAPDH	F	5-GGTGATGCTGGTGCTGAGTA-3	60	265	AF030943
	R	5-TCATAAGTCCCTCCACGATG-3			

TLR-Toll-like receptor; GAPDH used as reference gene to normalize the gene expression of target genes; F-Forward Primer; R-Reverse Primer

RESULTS

Chapter 4

RESULTS

4.1. The THI index

The THI values to depict the level of heat stress are depicted in Fig. 4.1. The THI values both inside and outside the shed in the morning are 69.9 and 73.5 respectively while in the afternoon the values were 74.9 and 86.5 respectively. The THI index inside shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P<0.01$). The THI values 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 are considered extreme distress.

4.2. Melt Curve

Amplification plot showed distinct variation of log (ΔRn) for different genes against PCR cycle number (Fig.4.2a). Multicomponent plot also showed the difference between the amplified and the non-amplified genes based on the graph pattern using the SYBR green dye (Fig.4.2b). Different genes (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10) showed different Tm in the melt curve graph (Fig.4.2c). 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: Description of the average temperature-humidity-index between both inside and outside the shed during the experimental period

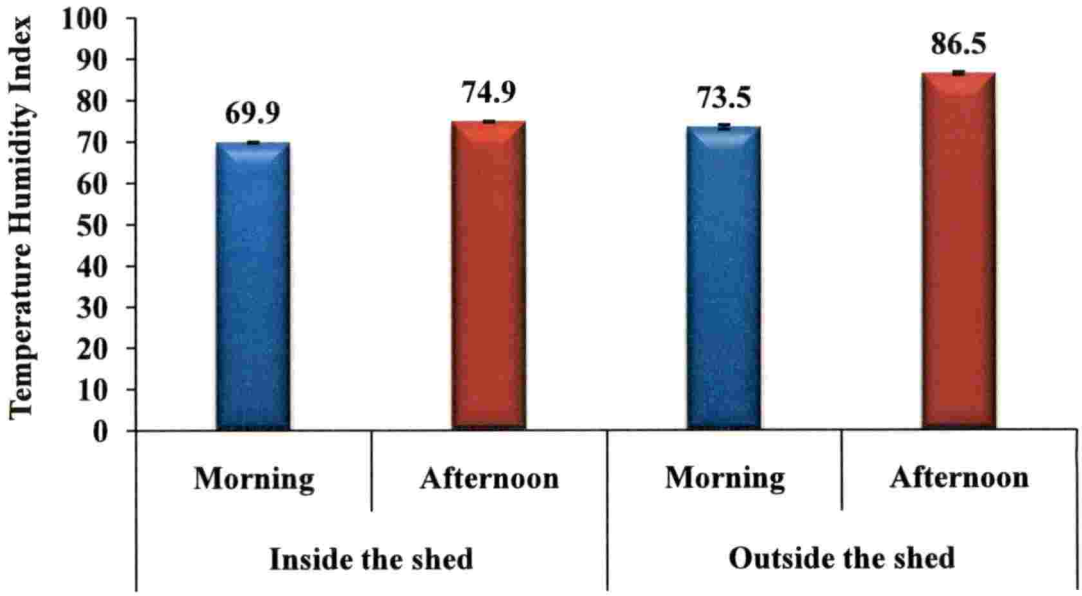


Fig.4.2a Description of amplification plot showing distinct variation of log (ΔRn) for different genes against PCR cycle number

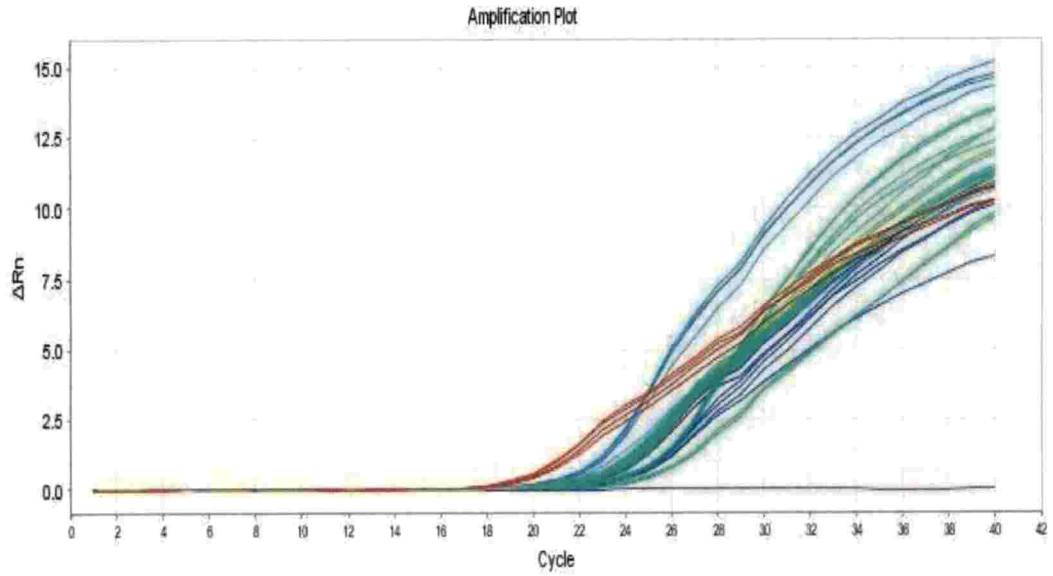


Fig. 4.2b Description of multicomponent plot also showing the difference between the amplified and the non-amplified genes based on the graph pattern using the SYBR green dye

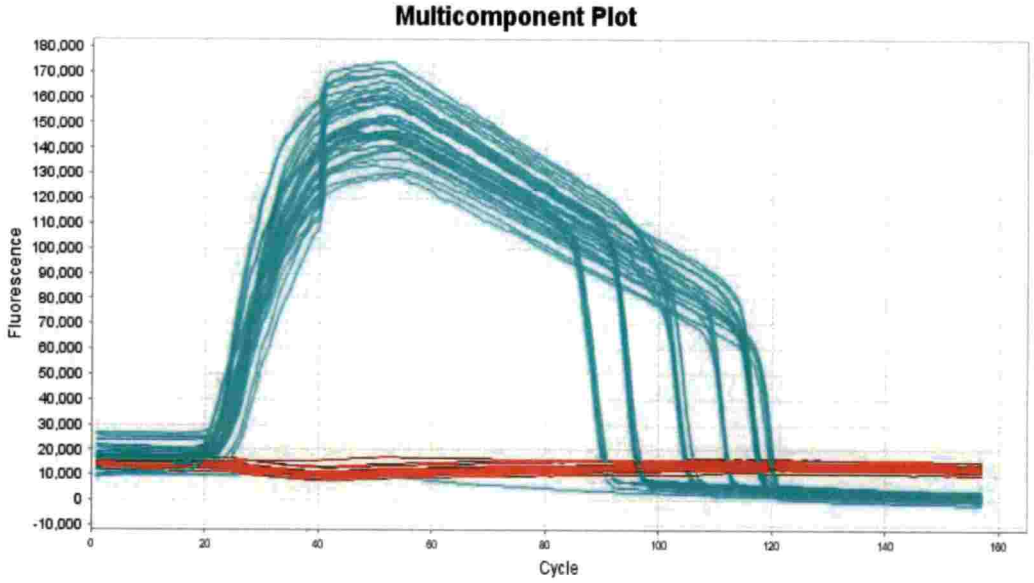
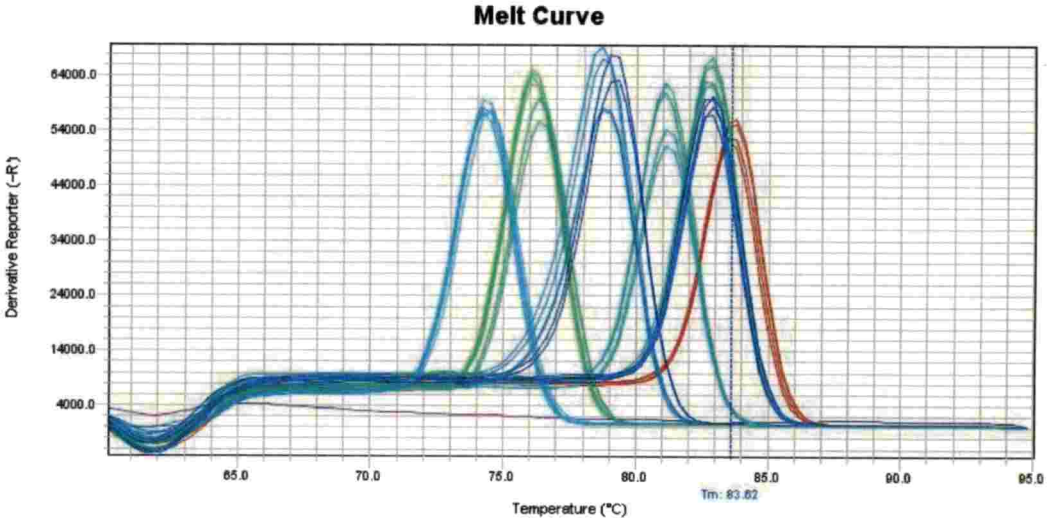


Fig.4.2c Description of different TLRs genes showing different Tm in the melt curve



4.3. Mesenteric lymph node TLR1 mRNA expression

Relative lymph node TLR1 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.3. The fold changes of expression patterns of TLR1 gene between control and heat stress groups are 1.0 and 0.6 respectively. The expression patterns of TLR1 gene significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Further a strong negative correlation ($P <0.01$) was established between THI and TLR1 gene expression pattern (Table 4.1).

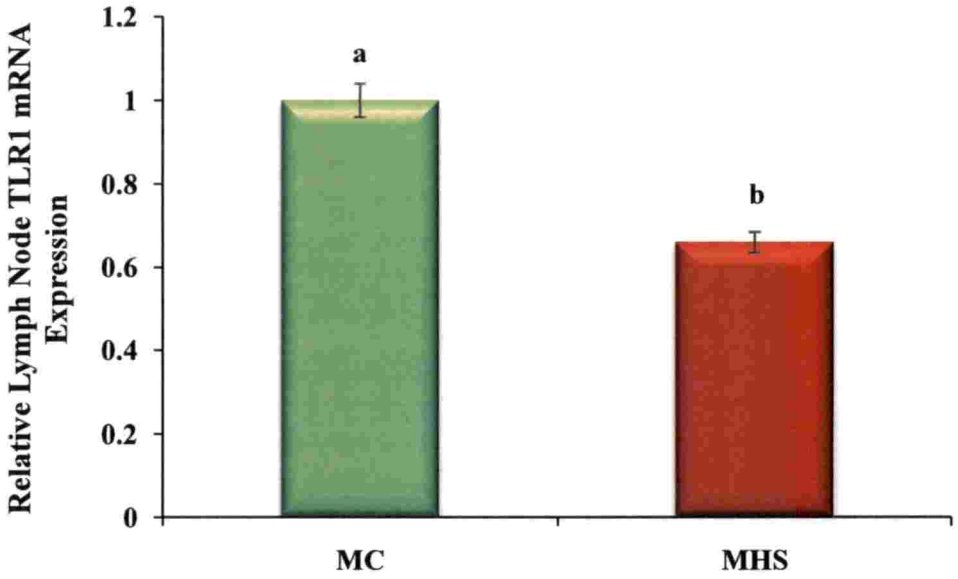
4.4. Mesenteric lymph node TLR2 mRNA expression

Relative lymph node TLR2 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.4. The fold changes of expression patterns of TLR2 gene between control and heat stress groups are 1.0 and 2.2 respectively. The expression patterns of TLR2 gene significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong positive correlation ($P<0.01$) was established between THI and TLR2 gene expression pattern (Table 4.1).

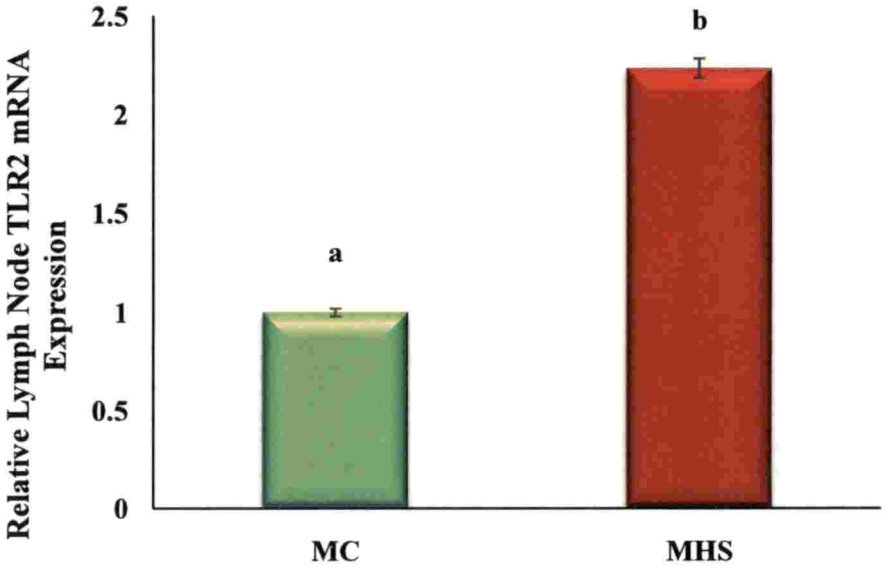
4.5. Mesenteric lymph node TLR3 mRNA expression

Relative lymph node TLR3 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.5. The fold changes of expression patterns of TLR3 gene between control and heat stress groups are 1.0 and 0.7 respectively. Although the expression patterns of TLR3 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. Further, the correlation between THI and TLR3gene expression pattern was non-significant (Table 4.1).

4.3. Relative lymph node TLR1 expression patterns between control and heat stressed Malabari goats



4.4. Relative lymph node TLR2 expression patterns between control and heat stressed Malabari goats



4.6. Mesenteric lymph node TLR4 mRNA expression

Relative lymph node TLR4 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.6. The fold changes of expression patterns of TLR4 gene between control and heat stress groups are 1.0 and 0.6 respectively. The expression patterns of TLR4 gene in heat stress group showed down regulation ($P<0.05$) trend as compared to control group animals. Further a strong negative correlation ($P<0.01$) was established between THI and TLR4 gene expression pattern (Table 4.1).

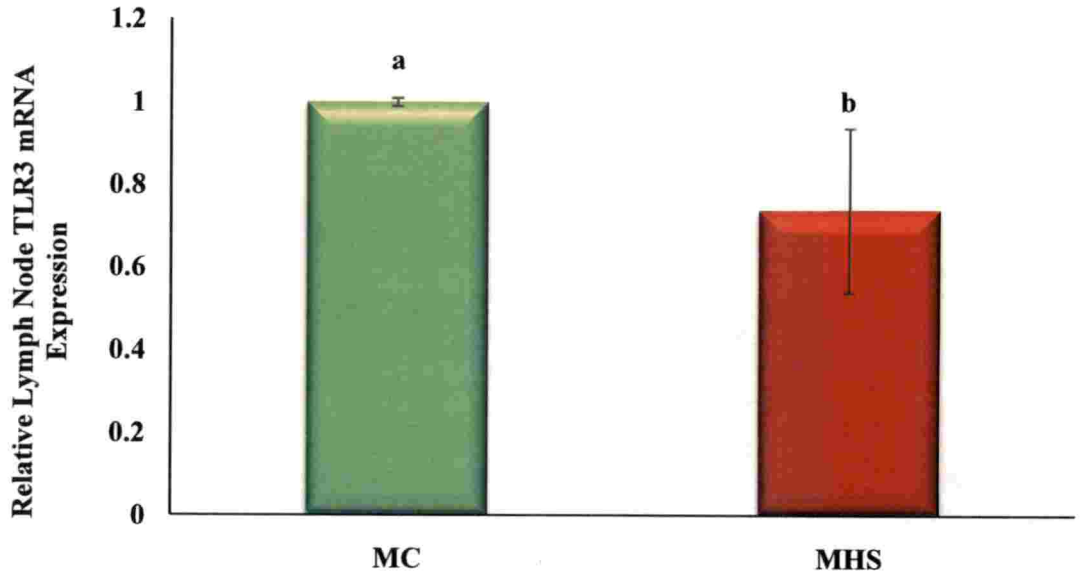
4.7. Mesenteric lymph node TLR5 mRNA expression

Relative lymph node TLR5 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.7. The fold changes of expression patterns of TLR5 gene between control and heat stress groups are 1.0 and 0.7 respectively. However, this down regulation of TLR5 in heat stress group was not statistically significant. Further a strong negative correlation ($P<0.01$) was established between THI and TLR5 gene expression pattern (Table 4.1).

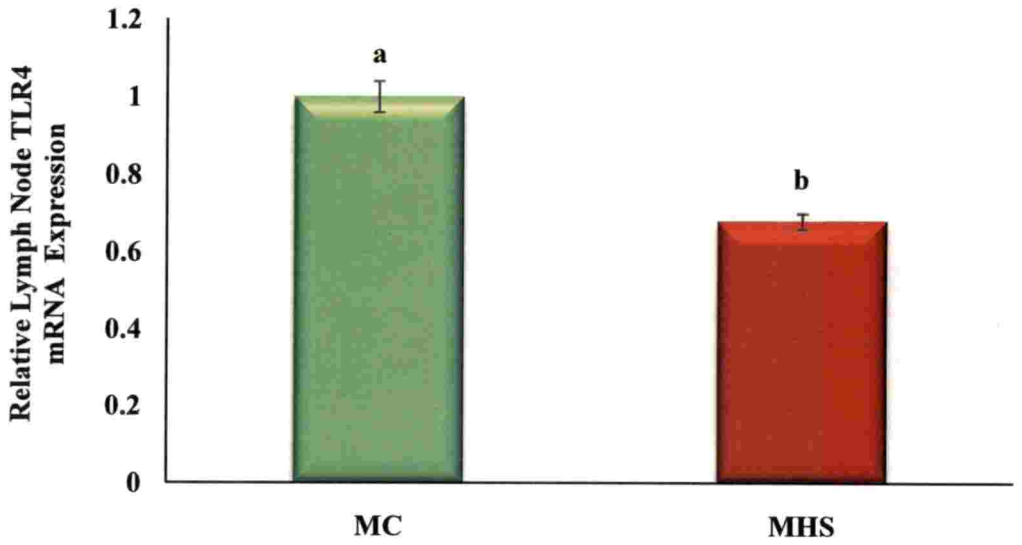
4.8. Mesenteric lymph node TLR6 mRNA expression

Relative lymph node TLR6 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.8. The fold changes of expression patterns of TLR6 gene between control and heat stress groups are 1.0 and 0.6 respectively. The expression patterns of TLR6 gene significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Further, there was no significance obtained between the THI and TLR6 gene expression pattern. (Table 4.1)

4.5. Relative lymph node TLR3 expression patterns between control and heat stressed Malabari goats



4.6. Relative lymph node TLR4 expression patterns between control and heat stressed Malabari goats



4.9. Mesenteric lymph node TLR7 mRNA expression

Relative lymph node TLR7 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.9. The fold changes of expression patterns of TLR7 gene between control and heat stress groups are 1.0 and 1.07 respectively. Although the expression patterns of TLR7 gene in heat stress group showed up regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. Further, there were no significance obtained between the THI and TLR7 gene expression pattern (Table 4.1).

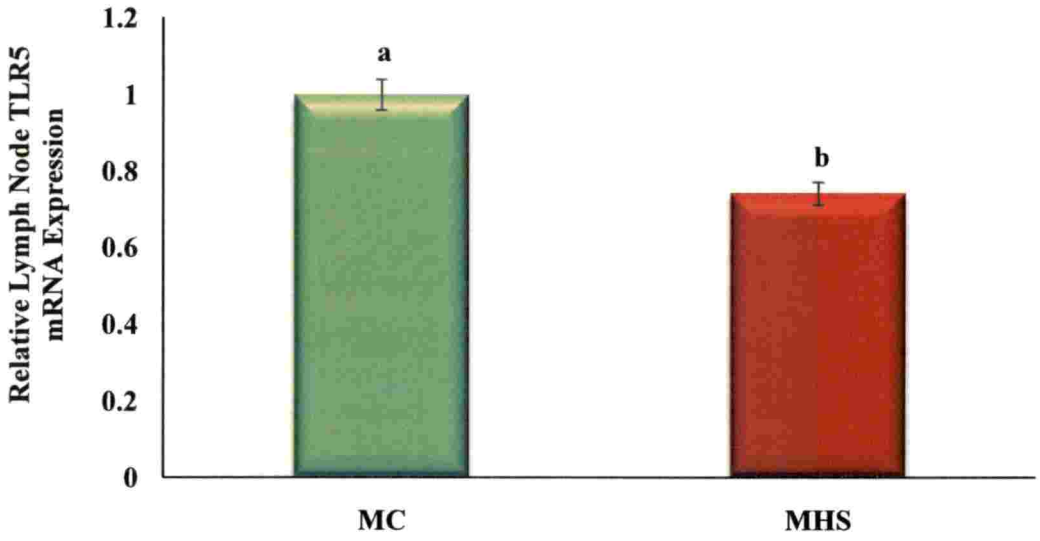
4.10. Mesenteric lymph node TLR8 mRNA expression

Relative lymph node TLR8 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.10. The fold changes of expression patterns of TLR8 gene between control and heat stress groups are 1.0 and 0.7 respectively. Although the expression patterns of TLR8 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. Further a strong negative correlation ($P<0.01$) was established between THI and TLR8 gene expression pattern (Table 4.1).

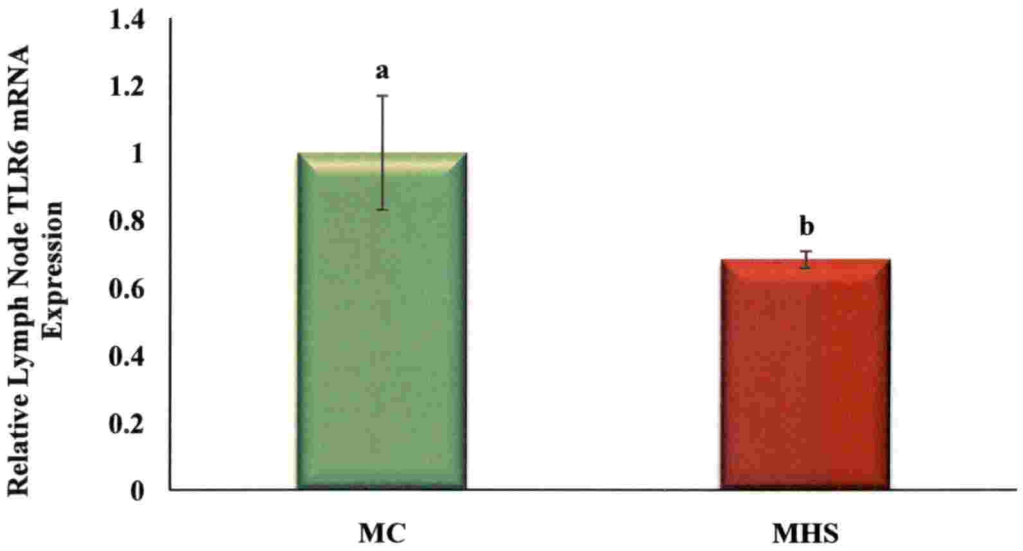
4.11. Mesenteric lymph node TLR9 mRNA expression

Relative lymph node TLR9 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.11. The fold changes of expression patterns of TLR9 gene between control and heat stress groups are 1.0 and 0.7 respectively. Although the expression patterns of TLR9 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. Further a strong negative correlation ($P<0.01$) was established between THI and TLR9 gene expression pattern (Table 4.1).

4.7. Relative lymph node TLR5 expression patterns between control and heat stressed Malabari goats



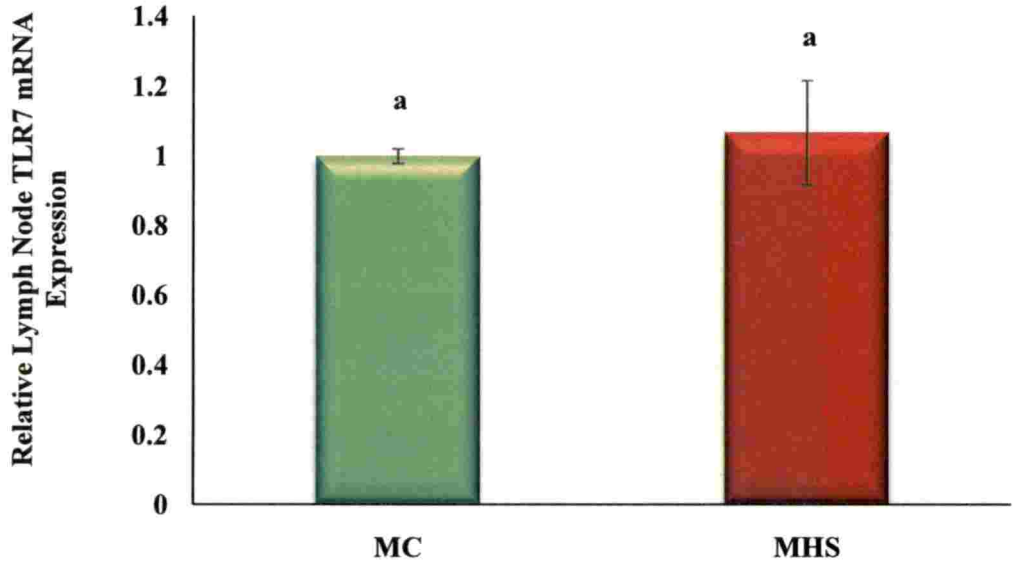
4.8. Relative lymph node TLR6 expression patterns between control and heat stressed Malabari goats



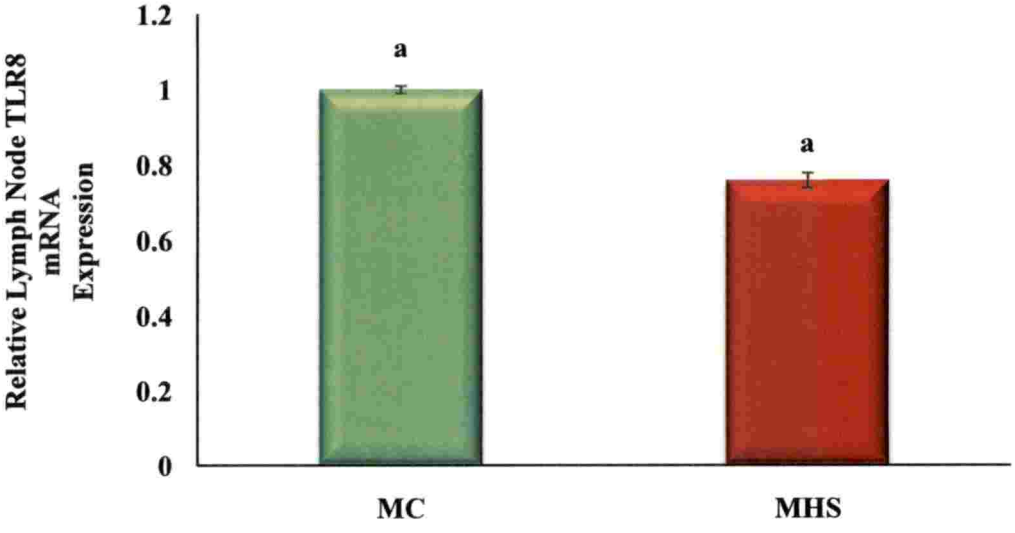
4.12. Mesenteric lymph node TLR10 mRNA expression

Relative lymph node TLR10 mRNA expression pattern of Malabari goats under control and heat stress group are depicted in the fig 4.12. The fold changes of expression patterns of TLR10 gene between control and heat stress groups are 1.0 and 0.9 respectively. Although the expression patterns of TLR10 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. Further, there were no significance obtained between the THI and TLR10 gene expression pattern (Table 4.1).

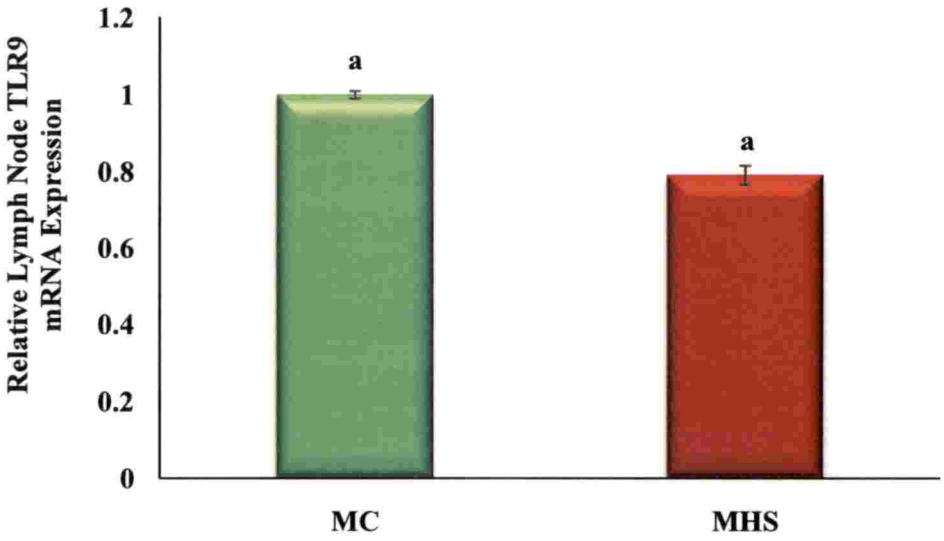
4.9. Relative lymph node TLR7 expression patterns between control and heat stressed Malabari goats



4.10. Relative lymph node TLR8 expression patterns between control and heat stressed Malabari goats



4.11. Relative lymph node TLR9 expression patterns between control and heat stressed Malabari goats



4.12. Relative lymph node TLR10 expression patterns between control and heat stressed Malabari goats

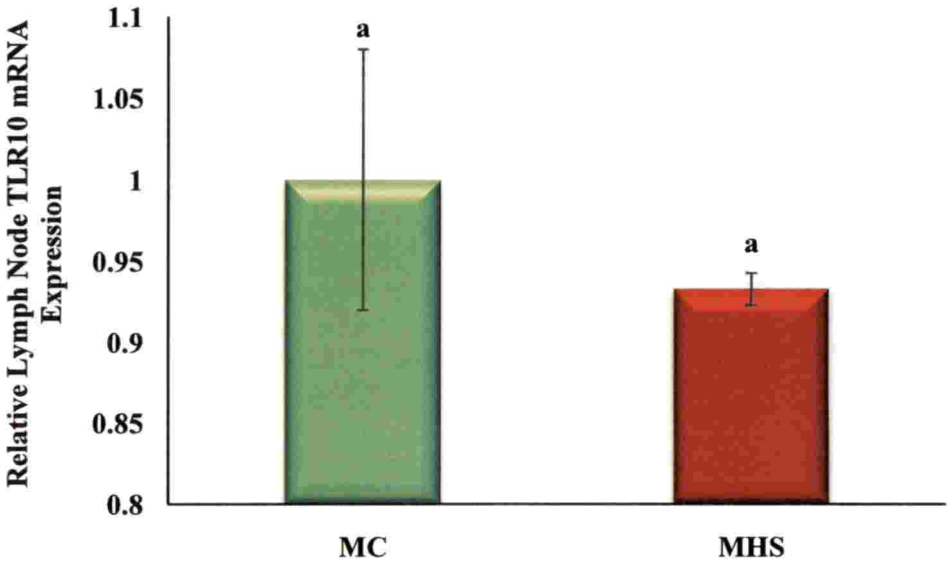


Table 4.1: Correlation association between THI and TLR gene expressions

	THI	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10
THI	1										
TLR1	-0.97**	1									
TLR2	0.97**	-0.91*	1								
TLR3	-0.54	0.61	-0.34	1							
TLR4	-0.96**	0.99**	-0.90*	0.61	1						
TLR5	-0.95**	0.95**	-0.95**	0.36	0.95**	1					
TLR6	-0.80*	0.91*	-0.72	0.53	0.92**	0.88*	1				
TLR7	0.19	-0.12	0.40	0.69	-0.12	-0.41	-0.14	1			
TLR8	-0.99**	0.94**	-0.99**	0.44	0.93**	0.95**	0.75	-0.30	1		
TLR9	-0.94**	0.88*	-0.99**	0.26	0.87*	0.94**	0.70	-0.48	0.98**	1	
TLR10	-0.39	0.57	-0.30	0.32	0.60	0.57	0.85*	-0.09	0.33	0.28	1

THI- Temperature humidity index; TLR- Toll-Like Receptors

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

DISCUSSION

Chapter 5

DISCUSSION

The study is one of its kinds to establish the impact of heat stress on different toll like receptor expression pattern in the Malabari goats. The study is of higher relevance given the fact that climate change associated immune compromise was found to be one of the most crucial factors hampering livestock production negatively. As the scientific community battles against identifying resilient livestock species to cope up to climate change challenges and with the limited information available pointing towards goat being the most resilient species research efforts are needed to identify climate resilient goat breeds to sustain livestock production in the changing climate scenario. In this line, the current study provides some useful basic information in establishing heats stress immune system relationship in goats. This research finding from the study may be helpful for the livestock community to identify goat breed with better thermo- tolerant ability.

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

Heat stress was found to reduce the expression pattern in TLR1 in MHS group as compared to MC group. However, there are reports that contradict this statement; in a study conducted by Sophia *et al.* (2016b) TLR1 showed higher expression in the Osmanabadi goats when exposed to heat stress. Further, Tirumurugaan *et al.* (2010) reported that TLR1 has over expressed in organs such as uterus, skin, lymph node, PBMC and lungs in heat stress induced Kanni breed goats. In addition, Paul *et al.* (2015) concluded in his

study that TLR1 was highly expressed during summer season in Black Bengal goats and they attributed this to the immune suppressive effect of heat stress in these goats. This shows that there are breed variation in the expression pattern of TLR1. Further, a strong negative correlation of THI with TLR1 supports this argument of extreme adaptive nature of Malabari breed.

Higher expression of TLR2 was established in MHS group as compared MC group. Similar heat stress induced increase in expression pattern of TLR2 was reported by Ju *et al.* (2014) and they established role of TLR2 in heat regulation in pigs. Paul *et al.* (2015) also established similar finding of heat stress induced increase in TLR2 expression in Black Bengal goats. Further, Paul *et al.* (2015) attributed this heat stress induced TLR2 expression to increased innate immune response to PAMPs by differentially mediated immune response by promoting both TLR expression and signalling in immune cellsduring summer season. In addition, Bharati *et al.* (2017) established the role of TLR2 as immune-modulators in acquisition of thermo-tolerance in Tharparkar cattle to long term heat stress exposure. Similarly, the increased TLR2 expression in heat stressed Malabari goats could be attributed to its immune-modulatory effects to impart thermo-tolerance to this breed. However, Sophia *et al.* (2016b) did not observed any influence of heat stress on the expression pattern of TLR2 in Osmanabadi goats and they attributed this no difference in expression to the extremely adaptive nature of this breed to heat stress. A strong positive heat stress correlation of THI with TLR2 also indicated the sensitivity of this TLR for heat stress and reflects the compromised immune response.

The TLR3 did not differ between MC and MHS groups. Further, the non-significant correlation between THI and TLR3 indicates the non-sensitivity of this gene to heat stress. However, there are reports suggesting significantly higher TLR3 expression in other heat stressed indigenous goats (Paul *et al.*, 2015; Sophia *et al.*, 2016b). The non-significant influence of heat stress in inducing changes in TLR3 expression pattern in the current study could be

attributed to the higher thermo-tolerance of Malabari goats. Further, these differences in expression pattern of TLR3 during heat stress exposure in different indigenous breeds suggests that there could be breed differences in expression pattern of TLR during heat stress exposure in goats.

The TLR4 expression was lower in MHS group as compared to MC group. Similarly, Sophia *et al.* (2017) also reported significantly lower expression of TLR4 in Osmanabadi goats and they attributed this to the immune-suppressive effects of heat stress in this breed. Moreover, the expression of TLR4 in the current study in Malabari goats and the study by Sophia *et al.* (2017) in Osmanabadi goats were in MLN and spleen respectively. Since both LN and spleen are primary lymphoid organs the significantly lower expression of TLR4 expression in these organs across the studies indicates the sensitivity of expression pattern of TLR4 to heat stress in goats. This indicates that TLR4 may be considered as an immunological marker reflecting the immune-suppressive effects of heat stress in goats. However, there are also contrasting reports of higher expression of TLR4 in response to heat stress in different species (Ju *et al.*, 2014; Paul *et al.*, 2015; Bharati *et al.*, 2017). This difference between these studies could be attributed to the difference in the magnitude of heat stress as well as the difference in the adaptive capacity of different livestock species. Further, this difference in expression pattern across the studies could also be attributed to the difference in organ of such expression as all the studies reporting higher expression pattern of TLR4 in response to heat stress was in PBMC while those reporting lower expression was in either LN or spleen. In addition, a strong negative correlation of TLR4 with THI indicates the sensitivity of this gene to heat stress reflecting the compromised immune status in Malabari breed.

The TRL5 expression did not varied between the groups. However, in a similar study conducted in Osmanabadi goats Sophia *et al.* (2017) reported significantly higher TLR5 expression in spleen of Osmanabadi goats. This difference between the studies indicates the breed differences in eliciting

immune response in response to heat stress. Further, Sophia *et al.* (2016b) contrasted their previous finding and reported no variation in hepatic expression pattern of TLR5 between Osmanabadi control and heat stress groups. This difference could be attributed to the fact that liver is considered a secondary lymphoid organ and heat stress was not able to induce changes in TLR5 expression pattern in their study. Similarly, Paul *et al* (2015) reported no variation in TLR expression pattern between the control and heat stress groups of Black Bengal goats. These differences across the study clearly indicate the differences in thermo-tolerance even in indigenous breeds of goats. Further, a strong negative correlation between THI and TLR5 indicates the compromised immune response in Malabari breed.

The TLR6 expression pattern also showed the same trend as that of TLR4. This finding was in contrast to several other findings wherein heat stress was found to up-regulate the TLR6 expression pattern in other species (Paul *et al.*, 2017; Sophia *et al.*, 2017; Plain *et al.*, 2010; Srikanth *et al.*, 2017). This indicates the uniqueness of Malabari breed as compared to other breeds that they were not able to maintain the immune response even during exposure to extreme climatic conditions. This is evident from the significantly lower TLR4, and TLR6 in heat stress groups as compared to control group reflecting that the animals were not able to mount appropriate immune response. This indicates that heat stress was found to be playing a detrimental role in maintaining immune response in this breed which is well known for its survival in harsh hot and humid tropical environmental conditions. Also, a negative correlation of TLR6 with THI proves the sensitivity of this TLR for heat stress heat stress in Malabari goats.

The TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 genes did not showed any variation in their expression patterns between the MC and MHS group. Although non-significant for the treatment effect, still the correlation effect was significant between THI and TLR8 and TLR9 indicating the sensitivity of these genes to heat stress. Most of the studies in farm animals reported

significantly higher level of expression pattern of TLR7 as compared to the control group (Paul *et al.*, 2015; Sophia *et al.*, 2016b and Sophia *et al.*, 2017). Similarly, TLR8 expression pattern also was found to be significantly higher in other breeds of goats (Paul *et al.*, 2015; Sophia *et al.*, 2016b and Sophia *et al.*, 2017). TLR9 has been shown to be necessary in the recognition of CpG motifs and plays a critical role in CpG ODN-mediated activation of immune responses (Takeshita *et al.*, 2000). There are reports suggesting the up-regulation of TLR9 in heat stressed animals (Takeshita *et al.*, 2000; Paul *et al.*, 2015). The non-significant influence of heat stress on the expression pattern of TLR9 in Malabari breed in this study was similar to the finding in Osmanabadi breed by Sophia *et al.* (2016b; 2017). These findings again indicate breed differences with respect to maintaining immune response during heat stress exposure. However, no variation in expression pattern of TLR10 in heat stressed group as compared to control group in the current study was in contrast to findings in other indigenous goat breeds (Paul *et al.*, 2015; Sophia *et al.*, 2016b; Sophia *et al.*, 2017). The non-significant influence of heat stress on TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 in the current study could indicate the non-sensitivity of these variables to heat stress and may reflect the thermo-tolerance ability of Malabari breed to maintain the immune-modulatory effects of these TLRs. Also, there is a possibility that the heat stress in the current study may not be of higher magnitude to induce changes in these TLRs as this breed is well known for its survival in hot humid tropical environment.

The study is the first of its kind to establish the impact of heat stress on the expression patterns of different TLRs in Malabari goat. Malabari breed showed extreme resilience to cope with heat stress in terms of maintaining the innate immune response and this was evident from the no effect of heat stress on the expression patterns of TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 genes. Further, the study also indicated that TLR1, TLR4, and TLR6 genes were sensitive to the heat stress effects and the significantly lower expression of these genes in MHS group as compared to MC group indicates the partly

compromised immune status in this breed. A negative correlation ($P<0.01$) was also established between THI and different TLRs except TLR3, TLR7 and TLR10. In addition, the significantly higher expression of TLR2 in the heat stress group indicates the reliability of this gene in assessing the immune status of this breed during heat stress exposure. Therefore, TLR2 could serve as ideal immunological marker for establishing the superior thermo-tolerance ability of Malabari breed in terms of maintaining the immune status during heat stress challenges.

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Toll-like Receptors are one among them and widely been studied to identify specific signature molecules in microbes. These receptors in important organs and its expression pattern in response to particular stimuli is one of the factors determining the disease resistance capability of an animal. Research efforts are needed to quantify immune responses to environmental stresses in different indigenous goats and these efforts will be of practical relevance to identify a animal with better immune potential in the changing climate scenario. Hence the present study was designed to establish the impact of heat stress on different toll-like-receptors (TLR) genes expression in lymph node of Malabari goats. Such an attempt may yield fruitful results and can establish the impact of climate change on livestock immune response. The study was conducted with the primary objective of establishing the expression pattern of different cell surface and intra cellular TLRs in Malabari goats subjected to heat stress and also to establish the correlation between THI index and expression patterns of both cell surface and intracellular TLRs in Malabari goats subjected to heat stress.

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 heat stress (n=6; heat stress). The C animals were maintained in the shed in comfort condition while heat stress 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 heat stress 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 THI values for the entire study duration during morning were not stressful to the animals kept both inside and outside the shed. However, the obtained THI values ($P<0.01$) during afternoon indicated that the animals inside

the shed were not stressed while the animals kept outside the shed were under extreme distress.

The fold changes of expression patterns of TLR1 gene between control and heat stress groups are 1.0 and 0.6 respectively. The expression patterns of TLR1 gene significantly ($P<0.05$) down regulated in heat stress group as compared to the control group animals. Further a strong negative correlation ($P<0.01$) was established between THI and TLR1 gene expression pattern. The TLR2 expression pattern showed reverse trend as that of TLR1 in the current study. The fold changes of expression patterns of TLR2 gene between control and heat stress groups are 1.0 and 2.2 respectively. The expression patterns of TLR2 gene significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. The increased TLR2 expression in heat stressed Malabari goats could be attributed to its immune-modulatory effects to impart thermo-tolerance to this breed. A strong positive correlation of THI with TLR2 also indicated the sensitivity of this TLR for heat stress and reflects the compromised immune response.

The fold changes of expression patterns of TLR3 gene between control and heat stress groups are 1.0 and 0.7 respectively. Although the expression patterns of TLR3 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the groups were not statistically significant. The non-significant influence of heat stress in inducing changes in TLR3 expression pattern in the current study could be attributed to the higher thermo-tolerance of Malabari goats. Further, the non-significant correlation between THI and TLR3 indicates the non-sensitivity of this gene to heat stress.

The fold changes of expression patterns of TLR4 gene between control and heat stress groups are 1.0 and 0.6 respectively. Although the expression patterns of TLR4 gene in heat stress group showed down regulation trend as compared to control group, still the difference in expression patterns between the

groups were not statistically significant. The significantly lower expression of TLR4 expression in MLN indicates the sensitivity of expression pattern of TLR4 to heat stress in goats. This indicates that TLR4 may be considered as an immunological marker reflecting the immune-suppressive effects of heat stress in goats. In addition, a strong negative correlation of TLR4 with THI indicates the sensitivity of this gene to heat stress reflecting the compromised immune status in Malabari breed.

The fold changes of expression patterns of TLR5 gene between control and heat stress groups are 1.0 and 0.7 respectively. The expression patterns of TLR5 gene did not differed between the groups. Further, a strong negative correlation between THI and TLR5 indicates the compromised immune response in Malabari breed.

The expression patterns of TLR6 gene significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. This finding was in contrast to several other findings wherein heat stress was found to up-regulate the TLR6 expression pattern in other species. This indicates the uniqueness of Malabari breed as compared to other breeds that they were not able to maintain the immune response even during exposure to extreme climatic conditions. This is evident from the significantly lower TLR1, TLR4, and TLR6 in heat stress groups as compared to control group reflecting that the animals were not able to mount appropriate immune response. This indicates that heat stress was found to be playing a detrimental role in maintaining immune response in this breed which is well known for its survival in harsh hot and humid tropical environmental conditions. Also, a negative correlation of TLR6 with THI proves the sensitivity of this TLR for heat stress in Malabari goats.

The TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 genes did not showed any variation in their expression patterns between the MC and MHS group. Although non-significant for the treatment effect, still the correlation effect was significant between THI and TLR8 and TLR9 indicating the sensitivity of these

genes to heat stress in Malabari goats. The non-significant influence of heat stress on TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 in the current study could indicate the non-sensitivity of these variables to heat stress and may reflect the thermo-tolerance ability of Malabari breed to maintain the immune-modulatory effects of these TLRs. Also, there is a possibility that the heat stress in the current study may not be of higher magnitude to induce changes in these TLRs as this breed is well known for its survival in hot humid tropical environment.

Malabari breed showed extreme resilience to cope with heat stress in terms of maintaining the innate immune response and this was evident from the no effect of heat stress on the expression patterns of TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 genes. Further, the study also indicated that TLR1, TLR4, and TLR6 genes were sensitive to the heat stress effects and the significantly lower expression of these genes in MHS group as compared to MC group indicates the partly compromised immune status in this breed. In addition, the significantly higher expression of TLR2 in the heat stress group indicates the reliability of this gene in assessing the immune status of this breed during heat stress exposure.

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ABSTRACT

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IMPACT OF HEAT STRESS ON DIFFERENT TOLL LIKE RECEPTORS GENE EXPRESSION IN MALABARI GOATS

by

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(2013-20-119)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

BSc-MSc (Integrated) CLIMATE CHANGE ADAPTATION

FACULTY OF AGRICULTURE
Kerala Agricultural University

ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH

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KERALA, INDIA

2018

ABSTRACT

Research efforts are needed to quantify immune responses to environmental stresses in different indigenous goats and these efforts will be of practical relevance to identify an animal with better immune potential in the changing climate scenario. Hence, a study was conducted to establish the impact of heat stress on different toll-like-receptors (TLR) genes expression in lymph node of Malabari goats. The study was conducted with the primary objective of establishing the impact of heat stress on the expression pattern of different toll-like-receptors (TLRs) in Malabari goats. The study was conducted for a period of 45 days using twelve Malabari goats. The goats were randomly allocated into two groups: MC (n=6; Malabari control) and MHS (n=6; Malabari heat stress). At the end of study, all 12 animals were slaughtered and their mesenteric lymph node (MLN) tissues were collected for gene expression. Heat stress significantly ($P<0.05$) down regulated TLR1, TLR4, and TLR6 and significantly increased ($P<0.05$) TLR2 expression pattern. A negative correlation ($P<0.01$) was also established between THI and different TLRs except TLR3, TLR7 and TLR10. Further a strong positive correlation was obtained between THI and TLR2. The results from the study established that Malabari goat breed showed extreme resilience to cope with heat stress in terms of maintaining the innate immune response and this was evident from the non-significant influence of heat stress on the expression patterns of TLR3, TLR5, TLR7, TLR8, TLR9 and TLR10 genes. Further, the study also indicated that TLR1, TLR4 and TLR6 genes were sensitive to the heat stress effects and the significantly lower expression of these genes in MHS group as compared to MC group indicates the partly compromised immune status in this breed. In addition, the significantly higher expression of TLR2 in the heat stress group indicates the reliability of this gene in assessing the immune status of this breed during heat stress exposure.

Keywords: Climate change; Goat; Heat stress; Immune response; TLRs