IMPACT OF HEAT STRESS ON THE MEAT PRODUCTION CHARACTERISTICS AND MEAT QUALITY RELATED GENE EXPRESSION PATTERNS IN INDIGENOUS KODI AADU GOAT BREED

By

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DECLARATION

I, hereby declare that this thesis entitled **"Impact of Heat Stress on the Meat Production Characteristics and Meat quality related Gene Expression Patterns in Indigenous Kodi Aadu Goat Breed"** 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.

Bangalore — Devapriya A

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SYMBOLS AND ABBREVATIONS

% - Percentage

µL – Microlitre

µm – Micrometre

µM – Micromolar

a* - Redness

AHD- Animal Husbandry and Dairying

ALRs- AIM2-like receptors

AM - Ante Meridiem

AOAC- Association of Official Analytical Chemists

AT – Ambient Temperature

b* - Yellowness

BAHS- Basic Animal Husbandry Statistics

BC – Buttock Circumference

BMI- body mass index

bp – base pair

°C - Degree Celsius

CAPN1 – Calpain 1

CAPN2 – Calpain 2

CAST – Capastatin

CC – Chest Circumference

CCRAAS -Centre for Climate Resilient Animal Adaptation Studies

CD – Chest Depth

cDNA - complementary DNA

CH_4- methane

CL – Cooking Loss

CL- corpus luteum

CLRs- C-type lectin receptors

cm – Centimetre

cm^2 – Centimetre Square

CRM – Collagen Content of Raw Meat

CRYAb - αB-crystallin

CS- Combined Stress

CW – Chest Width

DBT– Dry Bulb Temperature

DFD- dark, firm, dry

DGAT- Diacylglycerol Acyltransferase

DL– Drip Loss

DMEB- Dimethyl Amino Benzaldehyde

DNA– Deoxyribo Nucleic Acid

DP– Dressing Percentage

E– East

ECL– External Carcass Length

F– Forward

FAO- Food and Agricultural Organisation

FS– Fore Saddle

FSH- follicle-stimulating hormone

g– gram

GAPDH- Glyceraldehyde 3-phosphate dehydrogenase

GDP- gross domestic product

GH– Growth Hormone

GHR– Growth Hormone Receptor

GOI- Government of India

h– Hour

HCL– Hydrochloric Acid

HCW– Hot Carcass Weight

HLPE- High Level Panel of Experts

HPA axis- hypothalamic–pituitary–adrenal axis

HPRT- Hypoxanthine Phosphoribosyl Transferase

hrs– hours

HS– Hind Saddle

HSF– Heat Shock Factor

HSP– Heat Shock Protein

ICAR- Indian Council of Agricultural Research

ICL– Internal Carcass Length

IFAD- International Fund for Agricultural Development

IFN-β- Interferon-β

IGF1– Insulin like Growth Factor 1

IL-18- Interleukin 18

IMD– The India Meteorological Department

IPCC- Intergovernmental Panel on Climate Change

ISI– Indian Standards Institution

KC– Kodi Aadu Control

kg– Kilogram

KHS- Kodi Aadu Heat Stress

L*- Brightness

LEA– Loin Eye Area

LEP- leptin

LEPR - leptin receptor

LH- luteinizing hormone

LL– Leg Length

LN_2- Liquid Nitrogen

LTL- Longissimus Thoracis et Lumborum

LW– Leg Width

LW– Live Weight

m– Meter

M– Molar

MaxT– Maximum Temperature

Met-Mb– Met Myoglobin

MFD– Muscle Fibre Diameter

MFI– Myofibrillar Fragmentation Index

MG– Mammary Gland

mg– Milligram

min– Minute

Min T– Minimum Temperature

ml– Millilitre

mM– Millimolar

mRNA- messenger RNA

MSTN– Myostatin

N– Normal

N– North

n– Number

NaOH– Sodium Hydroxide

ng– Nanogram

NLRs- NOD-like receptors

nm– Nanometre

No.– Number

NOS1- nitrous oxide synthase 1

NS– Non- Significant

NS- Nutritional stress

NT- Not Transported

PBS- Phosphate Buffered Saline

pH– Potential of Hydrogen

PM– Post Meridiem

PSE - pale, soft, exudative

PST– Pen Surface Temperature

PSW– Pre- Slaughter Weight

R– Reverse

RH– Relative Humidity

RLRs- RIG-I like receptors

RNA– Ribo Nucleic Acid

RP– Reproductive Organ

Rpm– Revolutions Per Minute

RT- qPCR– Real Time- quantitative Polymerase Chain Reaction

RV– Rigor Value

s– seconds

SAM axis - sympathetic-adrenal–medullary

SC– Shoulder Circumference

SE– Standard Error

SL– Sarcomere Length

SOD- super oxide dismutase

T3- triiodothyronine

T4- thyroxine

Ta- annealing temperature

T_{db}– Dry Bulb Temperature

TE- Tris Ethylenediaminetetraacetic Acid (Tris EDTA)

THI– Temperature Humidity Index

TLRs - Toll-like receptors

TNF-α - Tumour Necrosis Factor-α

TNZ– Thermo Neutral Zone

TS- Transportation Stress

T_{wb}– Wet Bulb Temperature

UNICEF- United Nations International Children's Emergency Fund

USA- United States of America

UV- Ultra Violet

WBT– Wet Bulb Temperature

WFP- World Food Programme

WHC– Water Holding Capacity

WHO- World Health Organisation

ZH- Zilpaterol Hydrochloride

INTRODUCTION

CHAPTER 1

INTRODUCTION

Climate change poses a major risk to the existence of livestock as well as the sustainability of livestock systems across the globe (Thornton, 2010). Any changes in the climatic variables such as temperature, humidity, rainfall, wind speed and solar radiation of a given region over a longer period of time is known as climate change (IPCC, 2007). Greenhouse gases are the main causes of climate change. Greenhouse gases are increased in the atmosphere due to natural causes and anthropogenic activities that leads to rise in the global temperature. There is a prediction that earth's temperature will rise by 1.5 degree Celsius to 2 degree Celsius by the end of this century (Thronton, 2010). Livestock farming system plays a vital role in socio-economic development of small and marginal farmers in India as well as around the world. It is widely projected that due to global warming, there will be more fluctuations in earth's microclimate and weather patterns and have a severe consequence on ecosystem, human and livestock production system (Thornton et al., 2014). Increases in the frequency of heat stress, flooding, drought events are projected and that can significantly affect the livestock production system since it adversely affects the quality and quantity of feed and fodder crops, water availability, growth, milk production, reproduction, health and biodiversity (Rojas-Downing et al., 2017).

Extreme climatic events affect the livestock production directly as well indirectly by reducing in the feed and water resources, and upsurge in thermal and

cold stress, disease outbreak (Hopkins and Del Prado, 2007). Among the climatic factors, heat stress is considered as a most important stressors making livestock production more challenging worldwide (Koubkova et al., 2002). Recent climate models predicted that average global temperature will increase substantially and there will be more frequent and intense heat stress events in future (Bernabucci et al. 2010). So, the adverse impact of heat stress on livestock system will also increase substantially. However, heat stress events are more common in tropical, subtropical and arid regions whereas in temperate regions the frequency of heat waves and heat stress conditions may intense due to climate change (Johnson, 2018). High ambient temperature combined with relative humidity ultimately decrease the livestock productive performance since the heat stressed animals are unable to dissipate body heat to maintain homeothermy. However, livestock species have adaptive physiological mechanisms to withstand heat stress. Mainly these adaptive mechanisms favour the animal survival by diverting the energy away from the productive process to manage body temperature (Johnson et al., 2015). Further, the heat stressed animals reduce their feed intake and digestibility in an effort to reduce metabolic heat production to maintain homeothermy. Both reduced energy consumption and increased energy costs for adaptive mechanisms eventually leads to negative energy balance resulting in decline of production, reproduction and health status of livestock (Johnson et al., 2015). The impact of heat stress on livestock varies between species, breed, age, genetic background and nutritional status of an individual animal (Thornton et al., 2009).

Among small ruminants, especially goats are found as more tolerant animal to extreme climate change (Darcan and Silanikove, 2018). Small ruminants are important source of income for rural community. Higher populations of goats are located in arid and semi-arid regions of tropical and sub-tropical countries reflecting its adaptability to harsh tropical environment (Kumar and Roy, 2013). Behavioural, physiological, and morphological adaptive mechanisms and genetic background support goat to thrive in the changing environment (Berihulay et al., 2019). Goats can efficiently utilize poor quality feedstuffs and convert into products and have the ability to diminish their metabolic rate in efforts to save energy during scarcity condition (Silanikove, 2000). Goats have the ability to perform better in harsh environment because of their potential to utilize feed stuffs which cannot be consumed by other ruminants (Silanikove, 1997). Goats have superior thermoregulatory mechanism and have the ability to cope with water scarcity and emit less methane (Silanikove, 2000).

Indigenous goats are more adaptive to the changing environment than the exotic and cross bred goats due to their higher capacity to withstand harsh environment through behavioural, physiological and genetic adaptations (Rojas-Downing et al., 2017; Rashamol and Sejian, 2018). Goats indigenous to the hot arid, tropical, and sub-tropical areas are well acclimate to the rising ambient temperature, lack of feed and fodder, water scarcity and disease, without compromising their productivity (Sejian et al., 2018). In contrast, exotic and cross bred goats are more prone to heat stress condition that causes reduction of growth, impaired reproduction and health status and this is attributed to their high energy requirement

for their maintenance and productive purpose. Therefore, in the context of changing climate, it is crucial to recognize the indigenous goat breeds that can withstand the adverse environmental condition and produce optimally. Research inputs are also equally required to improve the resilience capacity as well as the productivity of indigenous goat breeds Therefore, strengthening and improving the resilience capacity of the goat breeds to extreme climatic events is utmost necessary in order to sustain the production performance of the indigenous goats (Rashamol and Sejian, 2018).

Environmental variables, predominantly the increase in temperature and relative humidity due to climate change not only causes behavioural, physiological and metabolic changes in animals but also impacts severely the meat industry in terms of affecting the meat carcass traits, structure and quality attributes (Zhang et al., 2020). Heat stress influence the meat quality related variables in many ways through different physiological phenomena. Acute heat stress before slaughter causes release of catecholamines that stimulates peripheral vasodilation and increase of glycogenolysis and lactic acid concentration resulting in rapid decrease of muscle pH in early postmortem and leads to pale, soft exudative meat published in pig, poultry, and cattle (Adzitey & Nurul, 2011; Warner et al., 2014; Freitas et al., 2017). During chronic heat stress exposure, the meat of ruminant animals becomes dark, firm dry due to consumed muscle glycogen reserve and production of fewer lactic acid that leads to high ultimate pH (pHu) and darker meat (Adzitey & Nurul,2011; Gregory, 2010). Heat stress causes dehydration in animals that makes dark colour meat due to the contraction of myofibrils (Jacob et al., 2006).

Increased Protein and lipid oxidation takes place in the heat stressed animal resulting in decreased meat safety and shelf life (Wang et al., 2009).

It has been observed that both psoas major and minor muscles were found to be significantly influenced by high ambient temperature in both sheep and goat during summer season in Oman (Kadim et al., 2008). He also reported a significant increase in myofibrillar fragmentation index, meat ultimate pH and decrease in drip loss due to thermal stress (Kadim et al., 2006). Authors stated that the meat darkness was increased in both Omani and Somali goats during hot season as compared to cold season. Effect of hot environment on meat characteristics of Osmanabadi and Salem Black goats by Archana et al. (2018) and they detected that the pH of the longissimus thoracis muscle was lower only in Salem Black goats suggesting the better thermo tolerance ability. (Archana et al., 2018).

Heat shock protein (HSP) plays a significant role in farm animals during the heat stress condition which enhances cell survival, prevents cellular damage and protein degradation (Sejian et al., 2018). Significantly higher expression of HSP 70 mRNA was observed in longissimus thoracis muscles of two indigenous goats breed exposed to thermal stress (Archana et al., 2018). increased expression of HSP27 was also observed in samples of less tender meat of cattle (Carvalho et al., 2014). This indicates that upregulation of HSPs in skeletal muscles could serve as potential biomarkers to reflect meat quality (Cassar-Malek and Picard, 2016). Apart from the classical molecular chaperones there are several other genes which determine the quality of the meat (Lonergan et al. (2010). Calpain system is the most well-known proteolytic system which affects meat tenderness (Huff-Lonergan

and Lonergan, 1999). Increased activity of calpastatin causes a decrease in calpain activity by stopping the calpain proteolytic initiation, and membrane binding the expression of catalytic activity that leads to decreased meat tenderness (Lonergan et al. (2010). Diacylglycerol acyltransferases 1 (DGAT1) is another gene involved in fatty acid metabolism and are observed as an important gene for meat tenderness (Cases et al., 1998). However, not much information is available connecting the real time quantification data of these genes with thermal stress exposure in farm animals.

With this background, a study was proposed with an intention to understand the influence of heat stress on the major carcass characteristics and meat quality parameters in indigenous Kodi Aadu goat breed. The primary objectives of the study were as follows:

1. To establish the influence of heat stress on the carcass characteristics in Kodi Aadu goat breed.
2. To ascertain the impact of heat stress on the meat quality parameters in Kodi Aadu goat breed.
3. To determine the effect of heat stress on the selective meat quality gene expression patterns in Kodi Aadu goat breed

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

2.1. Introduction

Global warming is considered as one among the utmost serious issues of all the time, having its impact on both global and local scales. Among the environmental stress, thermal stress is considered as the most critical issue faced by livestock today. The performance and productivity of livestock are hampered by climate both directly and indirectly having a deleterious impact on the livelihood of rural farmers. Direct effects of heat stress include temperature, photo period, rainfall whereas the indirect effects include limited pasture and water availability, disease outbreak etc. (Sejian et al., 2015a). In developing countries like India, a major proportion of the small scale and poor farmers depend primarily on livestock sector for their source of income and livelihood (Agarwal et al., 2014). The economic returns obtained from the livestock sector are chiefly via milk, meat, manure, skin and wool production, however the current climate change scenario has severe implications on these outputs too. (Thornton et al., 2010).

Thermal stress is one of the primary reasons limiting Animal health and production that adversely affect the food security of the developing countries. Livestock exposed to heat stress experience severe decline in milk production, reproduction, slower growth, meat production and reduced conception rates (Rust and Rust, 2013; Sejian et al., 2015a;). Considering other ruminants, goats have the

superior capability to survive well in most of the agroecosystems mainly because of their unique characteristics like excellent browsing potential, hardy and sturdy nature (Feleke et al., 2016). Additionally, goats can effectively utilize even the limited feed, require less investment, need less area for shelter, low labour demand and are easier to manage (Maitra et al., 2014). Further goats also have an advantage due to their small body size, skillful browsing behavior, high feed conversion ability, high disease tolerance capacity and drought tolerance are crucial qualities to survive efficiently in the current climate change perspective (Shilja et al., 2016). Hence, goat rearing can be projected as a potential source of human nutrition and would also aid to maintain the stable economy and livelihood for farmers (Kumar and Roy, 2013).

Breed differences were observed with respect to the resilient potential of goats to thermal stress. When compared to exotic and crossbred goat breeds, indigenous goat breed possesses superior genetic adaptive strategy to combat heat stress, which further adds on to their thermo-tolerance ability (Nyamushamba et al., 2017). However, indigenous goat breeds also show variations in their adaptive responses to heat stress which needs to be assessed in order to identify specific agro ecological zone goat breeds. Usage of such breeds would ensure the sustain production from goats amidst the alarmingly rising climate change concern. This chapter would address in details the significance of goat in the changing climate scenario and how their production performance gets altered when exposed to adverse environmental condition. Special emphasis would be given to review in

detail how the meat production characteristics get altered when goats are exposed to heat stress.

2.2. Heat stress associated with changing climate

On the global front, global warming is posing to be a great challenge in the 21st century having deleterious impact on all life forms (Sejian, 2013). The developing countries are foreseen to be the maximum affected due to climate change because of their high dependency on natural resources, limited institutional developments, financial crunch and high poverty levels (Sejian, 2013). Climate change does not cause only a single stress, rather it leads to a series issue due to its associated changes like drought, flood, changing temperature, ozone (O3), increased CO2, water logging, soil erosion and salinity affect the fodder accessibility to livestock (Dawson et al., 2014). The predicted effects of climate change drastically affect the fodder growth and production. Thus, the nutrient availability to livestock is reduced (Gaughan et al., 2009). Heat Stress alters both the quantity and quality of pastures thereby leading to reduced livestock production (Gaughan et al., 2009). Heat stress associated reduction in pastureland/grazing land, the animals have to walk a long distance in search for food and water (Sejian and Indu, 2011). These locomotory actions also add tremendous stress to livestock species (Sejian and Indu, 2011). Additionally, water resources, its availability and requirement are negatively impacted by climate change in most countries, through shifting rainfall pattern which later govern the rangeland and grassland ecosystems (Assan 2014). Moreover, intense rainfall cause flood and enhance the moisture content in the atmosphere these two are the favourable condition for the

proliferation of pathogen ticks, mosquitoes and flies. Thus, increase the disease susceptibility in animals and also these pathogens act as vectors for the transmission of deadly diseases to human and animals (Brooks and Hoberg 2007). These direct and indirect environmental impact on livestock affect overall productivity and reduce the production of animal.

Among the environmental stress, thermal stress is considered to be a serious issue due to its deleterious impact on livestock production (Sejian, 2013). Heat stress hampers both the productive and reproductive efficiency in animals and this downfall in production is brought about both by direct and indirect effects of changing climate (Sejian, 2013). In a tropical country like India, livestock are reared predominantly under hot semiarid environment, exposed to high ambient temperatures thereby making them more susceptible to heat stress. Further the relative humidity will also influence the degree of heat stress at a particular ambient temperature (Sejian, 2013). Heat stress causes a series of physiological and behavioural alteration in livestock like increased panting score, pulse rate, skin temperature, water intake and decline in feed consumption. Further, nutritional imbalance, nutritional deficiency and water deprivation may also enhance the influence of heat stress in livestock (Marai et al. 2007). The scale of heat load on animals primarily depends on ambient temperature, relative humidity, species, genetics, nutrition, life stage, breed and health status. Generally, animals close to poles are more affected by higher temperature than animals located near equator, because animals near equator possess better adaption to drought and heat waves (Thornton et al., 2009). In general, thermal stress alters the physiological,

behavioural, endocrinal and metabolic responses that results in limited intake of feed, reduced animal production, reproduction and animal health (Rojas Downing et al., 2017).

The Inter-governmental panel on climate change (IPCC) has predicted earth surface temperature to rise in the range of 2.6°C to 4.8°C by 2100 creating alarming situation to the planet, population and economies (IPCC 2013). This rise in surface temperature is a major cause of worry especially in the tropical countries where the impact is predicted to be more severe (Sejian, 2013). Therefore, it is necessary to take up all the possible mitigative and ameliorative procedures to combat the negative impact of climate change and especially heat stress so as to ensure sustainable farming, economic returns to the poor and food security.

2.3. Contribution of livestock sector to global economy

India holds major livestock populations in the globe. It consists of 56.7% buffaloes, 12.5% cattle, and 20.4% small ruminants (Ratwan et al., 2017). Among all the agriculture sectors, livestock is considered as the major subsector that plays a pivotal role by contributing to the India GDP and ensuring the well-being of the marginal farmers in India (AHD, 2007). Small ruminants plays a greater role to maintain the national income, especially among the rural and marginal farmers. It is estimated that developing countries holds 80% of the total goat population (Homann et al., 2007). Presently, livestock sector is the fastest growing agricultural sectors in developing countries. This sector has contributed nearly 33 per cent to the agricultural gross domestic product (GDP) which continues to increase

(Delgado, 2005 and Thornton, 2010). Additionally, the global demand for animal production is increasing with the rising population, thereby increasing the concern for food security. It is the livestock production that ensures the nation food supply chain through meeting the protein requirements, in addition with increasing incomes in developing countries (Delgado, 2005 and Thornton, 2010).

The livestock population plays a major part in the socio-economic welfare of the nation, particularly in lower middle-class countries. In the developing countries livestock sector alone cares around 600 million livelihoods (HLPE, 2016). This sector contributes the primary and secondary source of livelihood in rural and urban areas. It was calculated that animal-derived foods contributes around 18% of the total global kilocalorie consumption and 34% of total protein intake (FAOSTAT, 2016). Moreover, Animal source food resources are an excellent source of top-quality proteins and essential bioavailable vitamins, minerals and several micro nutrients. Globally livestock dairy production is projected to rise 1077 million tonnes and meat production will be doubled from 258 to 455 million tonnes by 2050 (Alexandratos and Bruinsma, 2012).

Livestock also acts as a powerful protection tool for the poor, predominantly women and pastoralist category. Meanwhile, livestock production is badly affected by climate change, degradation of land, reduced fodder production and competition for water (Thornton., 2010). The chronically undernourished and malnourished people in the world was assessed 821 million in 2018 (FAO, IFAD, UNICEF, WFP and WHO, 2018). Various types of malnourishments exist between nations, regions and households. Timely management of livestock can play a vital role in dealing

malnourishment risks (FAO, 2020). Primary incorporation between the nutrition and livestock sectors is essential to guarantee animal source foods and livestock livelihoods contributes to fighting malnourishment (FAO, 2020). Hence, most of the developing countries depend primarily on livestock commodity products to maintain their economy level and as a source of nutrition (FAO, 2020). Therefore, efforts are needed to identify indigenous breeds that have shown climate resilience capacity.

2.4. Importance of goat in the changing climate

The livestock sector is considered to be unique among all the agricultural sectors for its dual role, of contributing towards to climate change (via greenhouse gas emission) and getting affected by the same (Sejian et al., 2015b). The livestock sector contributes 18% of the global greenhouse gas emissions wherein the livestock related greenhouse gases are CH4, CO2 and N2O (Sejian et al., 2016). Heat stress, has a deleterious effect on livestock causing severe reduction in body weight, growth, milk yield, meat production, reproduction and immune potential (Rojas-Downing et al., 2017). In comparison with other growth factors, body mass is the first and primary variable which is compromised due to heat stress. In a study conducted by Habibu et al. (2016) observed significant reduction in body mass index in heat stressed Sahel and Red Sokoto goats. One of the chief factors for decreased production in livestock industry is the heat stress and its associated vulnerabilities. In United States, livestock industry was reported to have a yearly economic loss of 2.36 billion US dollars due to heat waves (St-Pierre et al., 2003). In case of meat production, heat stress may lead to reduction in body size, carcass

weight and fat thickness in beef cattle (Nardone et al., 2010). Reproduction efficiency of both sexes of livestock have been reported to be affected due to heat stress (Jordan, 2003; Kunavongkrita et al., 2005). In cows, heat stress impairs oocyte quality, embryo development, pregnancy rate, follicle suppression and progesterone level (Jordan, 2003). While in bulls, Kunavongkrita et al. (2005) obseved lower sperm concentration and libido. However, the research on assessing the impacts of climate change on livestock production and vice versa are still limited (IPCC, 2014). Hence, intensified studies including efficient extension programs need to be enforced so as to ensure sustainable livestock production (FAO, 2011).

In the current climate change perspective, among all the livestock species, goats are tip to be playing a vital role for ensuring the livelihood of tropical poor and marginal farmers (Archana et al., 2018). In arid and semi-arid regions of India, goats are capable of managing the feed and water scarcity arising as the product of climatic stresses (Archana et al., 2018). As per the 20th livestock census, (GOI, 2019) India has huge livestock population of 535.78 million, showing a growth of 4.6% over Livestock Census 2012 which mainly includes cattle, buffaloes, goats, sheep and pigs. In the 20th livestock census total goat population was assessed 148.88 million and it has increased by 10.14% and over 27.8% of the total livestock system is contributed by goats. According to Basic Animal Husbandry Statistics (BAHS), (GOI, 2019) total dairy production in the country is 187.75 million tonnes which has improved by 6.5% over past years. Goat contributes 3% of the total milk production. Total meat production in 2018-19 was 8.1 million tonnes when

compared to 6.2 million tonnes in 2013-14. Goats contribute nearly 14% of the total meat production of the country. Hence, goats can be identified as the most important species from the climate change point of view for ensuring the livelihood and food security of the poor and marginal farmers.

Considering other ruminants, goats have the superior ability to withstand in any agroecosystems because of the unique browsing potential (Feleke et al., 2016). Further having the anatomical benefits of the upper lips, goats can survive well with less pasture, especially in the tropical regions wherein, they serve as a main source of income for the poor and rural farmers (Yami and Merkel, 2008). Moreover, goats can effectively utilize feed, require less investment, need less area for shelter, low labour demand and are easy to manage (Maitra et al., 2014). In addition to these features their small body size, skillful browsing ability, higher resistance to disease, higher feed conversion ability and drought tolerance make goats a vital species from the current climate change view (Shilja et al., 2016). Another advantage of goat over other ruminant species, is their excellent thermo-tolerance ability (Silanikove, 2000). Goats are well adapted to extensive environmental circumstances extending from dry arid to hot humid situations. Because of their smaller body size, their metabolic needs are significantly low. Moreover, they have the ability to reduce metabolism and their loose skin helps in easy dissipation of body heat to surroundings (Amankwah et al., 2012). In comparison to other ruminants, goats have the capability to survive well in drought affected regions because of their reduced water needs and unique browsing method. Goats emits very less enteric methane (CH4) in comparison with other ruminant animals per unit body weight

(Koluman et al.,2017). Goats have better digestibility compared to all other domestic ruminant animals because of their smaller body size, they consume less feed (Silanikove, 2000). All these factors together favour towards less CH4 production (Silanikove, 2000). Furthermore, from the production point of view, goat meat possesses less fat content wherein the lower saturated fat content in it would ensure stabilized blood cholesterol level and cardiac performance of the consumer. Goat meat contains the high-quality omega-3 fatty acids, vitamin B, B12 and generally has lower cholesterol and calories than the meat from other livestock (Archana et al., 2018). Additionally, goat milk is more nutritious than other livestock species milk and the crucial source of vitamin A, thiamin, niacin, pantotheanate, and ribofavin (Getaneh et al., 2016). Less investment, minimum feed and small area are required for goat production are economically profit for goat farmers (Feleke et al., 2016). Therefore, goat farming is a potential source of human nutrition and also helps to maintain stable economy for farmers by providing meat, milk and manure as main source of livelihood income (Kumar and Roy, 2013). Breed differences were observed with respect to the thermo tolerant potential of indigenous goats to heat stress. When compared to exotic and crossbred goat breeds, indigenous goat breed possesses superior genetic adaptive strategy to combat heat stress, which further adds on to their thermo-tolerance ability (Nyamushamba et al., 2017). However, indigenous goat breeds also show variations in their adaptive responses to heat stress which needs to be assessed in order to identify specific agro-ecological zone related goat breeds. Usage of such breeds

would ensure the sustain production from goats amidst the alarmingly rising climate change concern.

2.5. Effect of heat stress on goat production

Climatic variables such as temperature, humidity, solar radiation, and wind speed alters pasture and water availability, and increase the incidence of disease occurrence, with growth being the most potent in optimal environmental conditions (Baumgard et al., 2012). Among these climatic variables, temperature fluctuations have the most deleterious effect on livestock production and health. Animal exposed to heat stress reduces the growth, milk production, reproduction and meat production (Sejian et al., 2017). Thermal stress influences the behavioral (Shilja et al., 2016), haematological, physiological, biochemical (Sharma and Kataria, 2011), neuroendocrine (Pragna et al., 2018a), productive and reproductive (Niyas, 2015 and Salles 2020) responses of goat. Subsequently, heat stress creates economic loses to goat production system, highlighting the importance to assess their well-being. Although goats are adapted to thermal stress to a far extent they get impacted when exposed beyond thermo neutral zone range (Agossou et al., 2017).

2.5.1. Impact of thermal stress on growth

Growth, the increase in the live body weight, is controlled internally and externally by well-balanced hormones, enzymes, and nutrients as well as ambient temperatures (Bar and Radde 2009). Heat stress adversely impacts the growth rate, average daily weight gain, live body weight in goats (Gad, 2013), and body condition scoring (Pragna et al., 2018a). Among the growth variables, body weight

is considered as an important variable determining the adaptive nature of goats. Bagath et al. (2016) observed significantly lower body weight in heat-stressed Osmanabadi goats which was attributed primarily to the decreased feed intake during heat stress condition. The key reason for the growth retardation could be diminished anabolic activity attributed to the reduced intake of essential nutrients, mainly metabolizable energy for both maintenance and weight gain and improved tissue catabolism (Daramola et al., 2012). Moreover, the growth reduction varies between different goat breeds, which was revealed in a study led by Pragna et.al, (2018b). In their study, the body weight reduction during heat stress was elevated in Osmanabadi goats when compared to Salem Black and Malabari goat breeds. Additionally, the body condition score in goats also got reduced when exposed to thermal stress observed that increased rate of fat mobilization to support life-saving activities (Pragna et al., 2018b).

Growth is regulated by various hormones and growth factors. Among these glucocorticoids, thyroid hormones, sex steroids, and several locally formed growth factors play the crucial role (Haque et al., 2012). Thyroid hormones play vital roles in animal adaptation to adverse environmental challenges by general metabolism and regulation (Haque et al., 2012). Triiodothyronine (T3) and thyroxine (T4) play an important role in animal growth and metabolic adaptation, which has been reported to be reduced in heat-stressed goats (Aleena et al., 2016). The somatotrophic axis is considered as one of the most significant axes, due to their wide range of effects and critical role in growth (Jaquiery et al., 2012). Nutrition is one among the prime environmental signs that affect the somatic function of

ruminants (Thorn et al., 2006) Nutrition is the major regulators of the levels of IGF-1 and GH in animals (Lee et al., 2005). In a recent study Pragna et al. (2018a) analyzed the expression of IGF -1 gene and Plasma GH were reported to played as ideal biomarkers for establishing the impact of heat stress on growth performance in goats.

2.5.2. Effect of heat stress on meat production

Among the climatic cues, heat stress is recognized as the major issue adversely affecting livestock meat production potential (Nardone et al., 2010). High ambient temperature decreases the quality and yield of meat and leads to decreased carcass weight, dressing percentage, and tenderness of meat (Archana et al., 2018). High temperature may also influence meat carcass traits and the sensory quality of meat from both goat and sheep (Kadim et al., 2006). Meat and its quality traits are controlled by numerous intrinsic and extrinsic variables (Guerrero et al., 2013). The intrinsic variables that influence the carcass quality in ruminants include age, breed, species, gender, and slaughter weight. Likewise, the extrinsic variables influencing the meat quality comprise of ecological, handling and transportation stress, diet etc. (Guerrero et al., 2013). Archana et al. (2018) compared the effect of heat stress on body mass and carcass traits of Osmanabadi (OS) and Salem Black goats (SB). Authors observed that the primal cut such as loin, shank, flank, hind saddle, and rack was higher in the OS control goats in comparison with the SB control goats. Whereas, breast, leg, and fore saddle are higher in the SB control group in comparison to the OS control group. In edible offals, liver weight was reduced in the SB heat stressed group as compared to the SB control. All the quality variables

such as appearance, flavor, juiciness, etc are compromised in heat-stressed groups. Heat Shock Protein 70 (HSP70) expression levels are increased and Myostatin mRNA expression levels were decreased in both heats stressed groups. Interestingly, meat color was not at all influenced by heat stress in both goat breeds.

2.5.3. Consequences of heat stress on reproduction

Heat stress affects the reproductive ability of animals in both sexes through compromised functions of the hormonal imbalance, reproductive tract, nutritional deficiency, and altered reproductive development (Rojas-Downing et al., 2017). In females' high environmental temperature decreases oocyte quality, embryo development, and embryo survival leading to poor-quality oocyte and embryo (Rojas-Downing et al., 2017). As a consequence of all these reduced conception rate with more day's open has been reported especially in the sub-tropical and tropical areas during the hot summer (Gaughan and Smith, 2015). The gonadotropins, follicle-stimulating hormone, and luteinizing hormone play essential roles in the functioning of ovaries, comprising the maintenance of follicular growth, ovulation process, and corpus luteum formation. Female goats are subjected to heat stress, follicular growth to ovulation compromises, escorted by diminished follicular estradiol synthesis activity and LH receptor level (Ozawa et al., 2005). Hence, it leads to deterioration of the follicles before ovulation process. Estradiol is the major hormone responsible for the ovarian function (Sejian et al., 2013; 2015a). Potential adverse effects of low estradiol secretion include suppresses signs of estrus, weakened estrus intensity and duration, gonadotropin surge, transport of gametes which, in sequence, might reduce events related with

development of ovarian cysts, ovulation, and alteration of corpus luteum functioning, linked with decreased progesterone formation and ultimately reduced fertilization (Wolfenson, Roth, and Meidan, 2000). Thermal stress reduces the blood flow to the uterus and enhance the temperature of body. Subsequently, compromised fertilization rate impairs the embryonic formation and exaggerate early embryonic mortalities (De Rensis et al., 2002). Early embryonic mortality is one of the prime reasons for low conception. The existence of intial-stage embryos is associated to normal luteal progesterone production process (Robinson et al., 2008). During initial organogenesis and fetal formation, heat load leads to various jeopardies that are extreme evident throughout the early phases of development process (Demetrio et al., 2007). Fetal malnourishment and ultimately fetal growth retardation during heat stress were also observed (Tao and Dahl, 2013).

2.5.4. Heat stress influence on immunity

Heat stress alters the immune potential and makes animals more vulnerable to diseases and pathogenic infection. There are two types of immunity, innate and adaptive (Lamy et al., 2012). The innate and the adaptive immune systems are controlled by the combined activities of hypothalamic–pituitary–adrenal and the sympathetic-adrenal–medullary axis. stressor proteins like Toll-like receptors (TLRs), heat shock proteins (HSPs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), AIM2-like receptors (ALRs), and RIG-I like receptors (RLRs), cytokines play a crucial role in immune system functioning (Vidya et al., 2018). The stress signs act on the hypothalamic–pituitary–adrenal axis and its end-product glucocorticoids changes the immune system responses in animals (Sophia et al.,

2016a). Furthermore, IFN-γ which is another important element of innate immune response also gets reduced during summer (Ju et al., 2014). Rashamol et al. (2019), observed mRNA expression of L-18 gene, IFN-γ and TNF-α gene were down-regulated in heat stressed Malabari goats when compared to their control groups.

Therefore, all these reports highlight the deleterious effects of heat stress on goat production which needs to be addressed at the earliest so as to ensure sustainable farming in the future.

2.6. Significance of studying the heat stress impact on meat production in small ruminants

Developing countries constitute a significant small ruminant production and they contributed to the major part of the national income (Agarwal et al., 2014). Among small ruminants, goats can withstand extreme heat stress, survive and produce optimally in diversified agro-ecological zones in the resource poor tropical regions of the world (Skapetas and Bampidis,2016). Thus, goats contribute significantly towards safeguarding the livelihood of millions of rural and marginal farmers across the world (Dubeuf et al., 2004). Additionally, goats convert less quality feed into high quality animal protein through its high feed conversion efficiency (Dubeuf et al.,2004). In the last six decades, goat population increased gradually among the weaker socio-economic sections of Asia and Africa and they adapted this as a approach to cope with the vulnerabilities of global warming (FAOSTAT,2020). The world goat population is 1045.916 million in 2018 showing an increase of 200 percent over 60 years (FAOSTAT,2020). Almost 50% of the

small ruminants are found in the tropical regions of the ecosphere, indicating their superior adaptability and thermo-tolerance capacity to survive in tropical climate (Gowane et al., 2017).

In developing countries, protein consumption by humans is primarily dependent on animal source food, because it is cheaper and easily available (Smith et al., 2012). The world meat production has reached 335 million tons in 2018 (FAO, 2018). Out of which, 5.977 million tons were contributed by goats (Hegde, 2020). Goat meat, generally known as chevon, has greater demand in continents such as Asia and Africa (Hegde, 2020). In comparison to beef, chevon has lower fat, B12, folate components, saturated fatty acids and cholesterol with higher calcium, magnesium, and potassium (Skapetas and Bampidis, 2016). Hence, goat meat is considered healthier than other red meat (Ivanovic et al., 2016). Rearing small ruminants offers economic security for poor and marginal farmers because of their less investments, lower feed needs, and easy management (Oluwatayo and Oluwatayo, 2012). The financial investment of buying one buffalo is nearly similar to purchasing 10 goats. In addition, the economic return obtained from 10 goats is 25% more than one buffalo could be due to its lower feed necessities and high feed conversion efficiency (Haenlein and Abdellatif, 2004). Therefore, goat farming is considered as a better option for enhancing economic security of rural farmers in the changing climate scenario.

Hot environments, coupled with compromised nutrition affect the productive potential of ruminants (Sevi and Caroprese, 2012). Both goat meat quantity and quality are impacted by climatic and genetic factors (Goetsch et al.,

2011). Heat stress is the major predisposing factor affecting meat production through alternate the carcass traits, and sensory attributes, which are critical parameters for consumer acceptance (Song and King, 2015). Furthermore, increased shear force and Ultimate pH of meat from heat-stressed goats results in decreased water holding capacity and increased cooking loss, and toughness (Archana et al., 2018) which ultimately lead to drastic loss in meat industry. Besides, reduced weight of edible and inedible components has also been noticed in goats exposed to heat stress (Archana et al., 2018). Consequently, production and processing of good quality meat is a challenge in the changing climate scenario. Hence, it is necessary to identify suitable amelioration strategies, which would minimize stresses.

2.7. Economic importance of studying heat stress impact on indigenous breeds

Climate change is a threat not only to the agricultural sector but also to livestock. To sustain rural livelihoods, it is crucial to recognize livestock breeds that are climate-resilient (Chanamuto and Hall, 2015). In comparison with exotic and crossbred breeds, local breeds are well adapted to sudden changes in the environment and occurrence of diseases (Alamer, 2003). Indigenous breeds possess various unique adaptive mechanisms that help them to thrive well in harsh environments. Morphological advantages of indigenous breeds comprise short and thin hair, lightly pigmented skin, light hair color, small body, higher density of sweat glands, less subcutaneous fat, and slender legs all of which taken together helps them to survive in harsh conditions (Fanta, 2017). Resistance to disease and parasitic infestation are another advantage of indigenous breeds over exotic and

crossbred breeds. In a study conducted in Tanzania, Wambura et al. (1998) analyzed the tick fighting in pure zebu cattle and their crosses. They indicated that pure zebu cattle are less infested with ticks when compared to their crosses. Further, Amaral et al. (2009) conducted a study on Santa Ines, Texel, and Ile de France ewes based on their physiological parameters in Brazil and reported that native breeds were more adapted to high temperature than exotic breeds. Therefore, looking all these considerations the excellent genetic potential of local animals in tolerating to environmental challenges as compared to exotic and crossbred animals could make them be the upcoming animal's generation to guarantee sustainable farming and economic returns.

In developing countries, a larger proportion of the small-scale and poor farmers depend primarily on livestock products for their daily source of income and livelihood (Agarwal et al., 2014). Lemke et al. (2006) analysed the productive adaptability of improved pig breed reared in the semi-intensive system to indigenous pig breed reared in extensive system in Vietnam. They reported that the annual benefit to annual cost ratio was higher in indigenous breeds reared in the extensive system over the improved breeds (2.7:1.0 and 3.0:1.0 for indigenous and 1.2:1 and 2.1:1 for improved breed). Further, Mangi et al. (2015) conducted a comparative study on the seroprevalence of brucellosis (zoonotic disease) in indigenous and exotic cattle breeds. The authors reported higher seroprevalence of brucellosis in exotic breeds, indicating their susceptibility to diseases. In addition, Patel et al. (2020) conducted a survey on the preferences of dairy farmers towards indigenous or exotic cattle breeds for farming in Gujarat. As per the survey report

majority of farmers preferred indigenous cattle breeds because of their higher stability in maintaining milk production, better income and advantage of the dual-purpose ability of indigenous cattle when compared to exotic. Therefore, indigenous livestock may be considered as an ideal source of revenue generation for rural and small farmers in the changing climate change scenario.

Heat stress hampers both the productive and reproductive efficiency in animals, and this downfall in production is brought about both by straight and indirect effects of changing environment (Sejian, 2013). Hempel et al. (2019) projected heat stress risk in European dairy farmers using various climate models. It was observed that milk yield may reduce by about 2.8 % compared to the present milk yield, and the economic loss incurred by farmers during summer may reach about 5.4%. According to Mauger et al. (2015), the annual milk production loss attributed to heat stress in the United States of America could be three times the current scenario which could exceed US$2 billion by the end of the 21stcentury. Further, individual variations in as a result to heat load have also been established in livestock. In a study conducted in Japan using several statistical approaches, Yano et al. (2014) reported the varied responses of individual cows to heat stress wherein high producing cows were found to be more susceptible to heat stress. In another study Ogundeji et al. (2021) analyzed the economic loss of small-scale dairy farmers during heat stress. They stated that absence of any adaptation strategies lead to double-loss in income while inter-mediate mitigation strategies aided to increase the farmer's income. Nesamvuni et al. (2012) analysed the effect of heat stress on dairy cattle reproductive performance and productivity under

expected future climate conditions using the Temperature Humidity Index in South Africa. They advised low-to-high-cost mitigation and adaptation approaches for maintaining production. Therefore, efforts are needed to identify suitable amelioration strategies that can help farmers to sustain livestock production to ensure their economic security.

2.8. Heat stress impact on goat meat production

2.8.1. Impact on carcass characteristics

In developing countries, interest in goat farming for meat production is rising tremendously because it offers a regular source of top-quality animal protein for consumption, contributes to food security and provides a small but regular economic profit for the poor farmers (Thornton, 2010). However, heat stress has an adverse impact on the productive potential especially the meat production and its associated elements in goats (Hashem et al., 2014). The phenotypic traits and production capacity of goats on exposure to heat stress are extremely variable, with impacts on carcass and meat quality (Webb, 2014). Extremes in temperatures impose an adverse effect on animal growth rates and may cause meat carcasses abnormalities like dark firm dry and pale soft exudative meat production (Johnson, 2018).

Livestock carcass characteristics are highly influenced by several internal and external factors (Guerrero et al., 2013). The internal factors include breed, species, age, slaughter weight, and gender while, external factors include heat stress, transportation, handling, and nutrition (Archana et al., 2018). Size of the

animal, is one of the main factors that determine the meat yield and is influenced by body weight at a certain level of maturity (Ozoje and Mgbere, 2002). Alamer, (2009) conducted a study to access the impact of water restriction and high environmental temperature on lactating Aardi goats in Saudi Arabia. The goats were separated into two groups with 50% and 25% drinking water restriction. The author observed that live weight was reduced in both groups with 8% and 6%. In another study conducted by Okoruwa, (2014) to identify the influence of heat stress on thermo-regulatory and live body mass of dwarf goats in Nigeria. The goats were separated in to three treatment groups (T1; confirmed in pens, T2; reared in field between 8am to 1pm, and T3; reared in field between 1pm to 6pm). The author reported that final live bodyweight was significantly lower in T3 (6.40kg) followed by T2 (7.80kg) and T1 (8.66kg). Further, Archana et al. (2018) conducted a study on indigenous Salem Black and Osmanabadi breeds in controlled and heat stressed conditions. The authors observed that Osmanabadi heat stressed group reduced their body weight in comparison with their control group. Whereas, Salem Black heat stressed group did not showed any difference in body weight with their control group.

Another important parameter that influences the carcass and meat quantity is the pre-slaughter weight. During heat stress conditions animals compromise their feed consumption in order to decrease the metabolic body heat production thereby maintain homeothermy, which ultimately results in weight loss (Shilja et al., 2016). Moreover, Kadim et al. (2006) identified that intensified moisture loss through sweating and panting associated with transportation during hot environment is more

likely to increase weight loss in animals. Another study was conducted by Hashem et al. (2013) to evaluate the effect of heat stress on blood variables and carcass quality of Black Bengal goats in Bangladesh. They observed that there was no substantial influence of heat stress on both preslaughter and meat weights.

Hot carcass weight (HCW) is the weight of the animal immediately after slaughter. HCW is the main factor that determines the market quality of carcass and weight loss due to heat stress which could influence this trait to certain extent (Muela et al., 2010). In a recent study led on Osmanabadi and Salem black goats under controlled and heat-stressed conditions, Archana et al. (2018) reported that HCW was significantly reduced in heat stressed Salem black goats as comparison with their control group.

Dressing percentage is specified as the proportion of live weight that is converted into carcass (Coyne et al., 2019). Factors associated with the alternations in dressing percentage include heat load (Archana et al., 2018), diet (Pesonen et al., 2012), age (Rios-Utrera, 2005), gut fill (Coyne et al., 2019), and sex (Owens and Gardner, 2000). Though thermal stress has been reported to significantly influence body weight and carcass weight in goats (Okoruwa, 2014; Archana et al., 2018), its influence on dressing percentage is less established. Macias-Cruz et al. (2010) led a study to understand the effects of Zilpaterol Hydrochloride (ZH) supplementation to heat stressed lambs (Katahdin ×Pelibuey andDorper ×Pelibuey crossbred) in Mexico. Authors observed that a higher dressing percentage in lambs supplemented with ZH than normal heat-stressed groups, indicating its potential heat stress amelioration role. Further, Chaudhary et al. (2015) conducted a study on 60-day old

Sirohi male kids based on the effect of concentrate supplementation on growth performance, carcass, and meat composition under field conditions. The authors observed that sole grazing kids and concentrate supplemented kids showed a dressing percentage of 43% and 46% respectively.

Better loin eye area is the indication of muscular development in animals, which is less in goats compare to other small ruminants (Sen et al., 2004). The loin eye area is typically measured at the interface between the 12th and 13th ribs on both sides of the carcass (Archana et al., 2018). Archana et al. (2018) organised a study to assess the heat stress-induced changes in carcass quality and skeletal muscle gene expression patterns between two indigenous goat breeds. They reported that both heat stress groups showed a significantly lower loin eye area when compared to their control.

The physical and chemical characteristics of fat impact the sensory attributes and meat shelf life (Webb et al., 2005). The comparatively little meat fat content in goats and fat build-up occurs far delay in the growth stages in comparison to other ruminants (Webb, 2014). Carcass yield, composition, and fat content are affected by breed, sex, age, body mass, and nutritional status (Webb, 2014). Shadman et al. (2020) conducted a study on Afshari lambs based on the effect of heat load on body composition. They observed that separable fat content was higher in heat-stressed groups when compared to their control. Further, Archana et al. (2018) conducted a study on Osmanabadi and Salem Black goats in controlled and heat stressed conditions. They reported that Osmanabadi heat stressed group showed a reduced separable fat content in comparison with their control group. In

addition, a lower fat score was reported in thermotolerant breeds (Archana et al., 2018). There are little data available on the heat stress impact on separable fat and fat score, but it is very relevant in terms of consumer preference for selecting good quality meat.

2.8.2. Impact on primal/wholesale cuts

In a recent study, Archana et al. (2018) assessed the impact of heat stress on primal cuts in two South Indian goats, Osmanabadi, and Salem Black. The authors stated that breed effect was noticeable in all wholesale cuts cuts excluding shoulder and neck. The breast and fore saddle weight were significantly lesser in Salem Black heat stressed group when compared to their control group. Similarly, rack weight was lesser in Osmanabadi heat stressed group in comparison with their control group. There are limited data on effect of heat stress on wholesale cuts in goats. In poultry, minimal studies have been organized to evaluate the negative impact of heat stress on the quantity of thighs and breasts (Lu et al., 2007; Zhang et al., 2012). Mello et al. (2015) conducted a study to analyse the effect of acute heat load on broilers growth and carcass quality. They reported that heat stress conditions not compromised the primal cut yields in broilers.

2.8.3. Thermal stress impact on linear carcass measurements in goats

There is minimal literature available in line with the impact of heat stress on linear carcass measurements in goats. Archana et al. (2018) assessed the summer heat stress effects on carcass traits in two indigenous breeds of goats in South India. The authors reported that heat stress had no substantial impact on majority of the

variables documented. But the leg length and chest depth were reduced in both heat stressed breeds. There are scarce reports in pigs judging the impact of heat stress on linear carcass dimensions. Stahly and Cromwell, (1979) evaluated the impact of heat load on carcass characteristics in pigs. They reported a positive result of heat stress on carcass length in pigs.

2.8.4. Impact on non-carcass components and offals

A study was conducted by Hashem et al. (2013) to evaluate the impact of heat stress on carcass and meat quality parameters of Black Bengal goats in Bangladesh. The goats were equally separated into three treatment groups (T0; control group, T4; reared in a field between 9 am to 1 pm, T8; reared in a field between 9 am to 5 pm). The authors observed that non- carcass components such as spleen, pluck, kidney, and blood were improved in the T8 group in comparison with T0 and T4 groups. Further, Archana et al. (2018) led a study in Osmanabadi and Salem Black goats to assess the effects of carcass traits under summer heat stress conditions. They stated head weight and lung with trachea weight were increased and liver weight was decreased in the Salem Black heat stressed group when compared to their control group. In addition, Sen et al. (2004) conducted a study to measure the carcass yield and meat quality of goats and sheep in hot-dry conditions. The authors found no effect of high temperature on non-carcass components in goats and they claimed this to the thermotolerance ability of goats to the warmer climate.

Rana et al. (2014) done a study to analyze the impact of heat stress on meat traits and quality of local sheep. The sheep were equally distributed into 3 treatment groups (T0; no exposure, T4; four-hour exposure to direct sunlight, T8; eight-hour exposure to direct sunlight). They reported heart and lungs with trachea were significantly lower in the T8 group than the T0 group. Additionally, kidney and pluck were significantly higher in the T4 group than the T8 group. In addition, Macias-Cruz et al. (2010) carried out a study to estimate the effects of Zilpaterol Hydrochloride (ZH) supplementation to lambs under hot environment conditions in the north-western region of Mexico. They described head (3.6 v. 3.8 ± 0.058%) and peritoneum (1.7 v. 1.9± 0.13%) weights were increased in ZH supplemented heat stressed group than the normal heat stressed group.

2.8.5. Impact on physico-chemical attributes

Kadim et al. (2006) coordinated a study to evaluate the impact of road transportation on carcass and meat quality parameters of the 3 goats from Oman (Batina, Dhofari, and Jabal Akhdar) during hot season. The goats were separated into 2 groups, 2hr transportation-stress (TS); not-transported (NT). Authors observed that meat from TS goats had pointedly enhance shear force, expressed juice, ultimate pH, cooking loss % in comparison with NT goats. Further, Hashem et al. (2013) formulated a study to evaluate the effect of heat stress on Black Bengal goats' meat and carcass quality. The goats were casually separated in to three treatment groups (T0- 0hr heat exposure; T4- 4hr heat exposure; T8- 8hr heat exposure). They reported pH was significantly higher in T8 (6.30a ±0.045) in comparison with T4 (6.18b ± 0.016) and T0 (6.16b±0.014) groups. Similarly,

cooking loss was highly significant in T8 (42.89a ±0.35) group than those of T4 (41.41a ± 0.55) and T0 (35.77b ±0.79) groups. In a recent study Archana et al. (2018) evaluated the heat stress influence on carcass traits and gene expression patterns between two goat breeds, Osmanabadi and Salem Black, in controlled and heat stress conditions. The authors stated that ultimate pH (pH24hrs) was significantly higher in Osmanabadi heat stressed group than their control group. In addition, cooking loss was reported higher in Salem Black heat stressed group in comparison with their control group. Further, shear force was significantly higher in both heats stressed group than their control.

In another study, Kadim et al. (2008) analyzed the effect of hot and cold season on carcass characteristics of goats (Omani, Somali) and sheep (Omani, Somali, Merino) in Oman. They documented higher value of pH in summer than in cold season in Merino and Somali sheep. In addition, reduced water holding capacity (WHC) was observed during hot season in both goats and sheep. Sen et al. (2004) compared the carcass composition and meat quality parameters of goats and sheep under hot environment. The authors observed that shear force value was significantly higher in goats (7.42) in comparison with sheep (3.74). Further, Rana et al. (2014) conducted a study in Bangladesh to evaluate the effect of heat stress on carcass characteristics and meat quality of sheep. The authors randomly distributed the sheep in to three treatment groups (T0- zero-hr heat exposure; T4- four-hr heat exposure; T8- eight-hr heat exposure). Through their study, it was observed that heat stress did not have a significant effect on cooking loss and pH in sheep. Whereas, drip loss was significantly higher in T0 group than T8 group.

Chulayo and Muchenje (2013) organized a study to evaluate the effects of different season on mutton quality from different sheep breeds (Dormer, South African Mutton Merino, Dorper, and Blackhead Persian) in South Africa. The South African Mutton Merino was recorded to have higher pH and shear force while Blackhead Persian had higher values for cooking loss (CL) than other breeds. Therefore, pH, shear force and CL are generally interconnected to determine the meat quality in goats (Archana et al., 2018). Liu et al. (2012) led a study to evaluate the effect of shade on carcass quality of rearing sheep (Ujumqin wool sheep) under hot environment in China. The sheep were randomly separated in to shaded and un-shaded treatment pens. The authors observed that pH24hr and cooking loss were significantly higher in un-shaded treatment pens than shaded.

2.8.6. Impact on proximate composition

A study was arranged by Archana et al. (2018) to evaluate the effect of heat stress on two indigenous goat breeds (Salem Black and Osmanabadi) during summer condition in South India. The goats were randomly separated in to controlled and heat stressed groups. Through their study, it was observed that no heat stress effect on fat, protein, ash content between the controlled and heat stressed group of two breeds. However, based on relative analysis protein stored in Osmanabadi heat stressed group was higher than the Salem Black heat stressed group. Hashem et al. (2013) organized a study in Black Bengal goats to evaluate the impact of heat stress on carcass characteristics in Bangladesh. The authors randomly distributed the goats in to three treatment groups (T0- 0-hour heat exposure; T4- 4-hour heat exposure; T8- 8-hour heat exposure). They observed that

meat proximate composition such as, crude protein, dry matter, and ash had no difference observed between the treatment groups.

Sen et al. (2004) conducted a study to compare the meat quality parameters and traits of carcass of goats and sheep under hot environment. They observed that goat meat (74.23±0.53) had higher moisture content in comparison with mutton (68.85±0.73). Whereas, less fat content was found in goat meat (3.16±0.71) than mutton (8.47±0.79). Rana et al. (2014) evaluated the impact of heat stress on indigenous sheep in Bangladesh. The authors distributed the sheep in to three treatment groups (T0- zero hr group; T4- four hr group; T8- eight hr group). Their study revealed no significant difference in dry matter, ash, and ether extract between the treatment groups. In addition, crude protein was significantly higher in T0 group in comparison with T4 and T8 group.

2.8.7. Impact on sensory attributes

In a recent study Archana et al. (2018) evaluated the effects of heat stress on Osmanabadi and Salem Black goat breeds under controlled and heat stressed condition. The authors observed that sensory attributes such as appearance, texture, and overall acceptability were higher in Salem Black heat stressed group in comparison with Osmanabadi heat stressed group. Whereas, juiciness was not significantly altered between both the heat stressed groups. Sen et al. (2004) organized a study to compare the traits of carcass and meat quality characteristics of goat and sheep under hot environment. They stated that color of the cooked goat meat (4.50) was valued significantly much better than mutton (3.87). Whereas,

mutton (4.25) was rated significantly higher score for tenderness than goat meat (3.37). In addition, no significant difference was observed in odor and juiciness in both species. In another study Kadim et al. (2008) evaluated the effects of hot and cold season on carcass characteristics of goats (Omani, Somali) and sheep (Omani, Somali, Merino) in Oman. They observed that during the hot season, the colour of the goat mean was darker than that during the cold season.

2.8.8. Impact on endocrine variables related to meat production

A study was corelated by Angel et al. (2018) to evaluate the impact of heat stress on the expression patterns of diverse growth- related genes of Malabari goats under controlled and heat stressed conditions. The authors stated that, insulin-like growth factor-1, hepatic growth hormone, growth hormone receptor and leptin receptor and leptin gene expression patterns were reduced in heat exposed group in comparison with the controlled group. In contrast, the growth hormone mRNA expression level was reported to be contrary wisely proportional to the dietary intake in small ruminant animals (Sejian et al.,2014; Bagath et al., 2016). Shilja et al. (2017) evaluated a study to access the effect of hot season induced heat and nutritional stress on adaptive capacity and blood biochemical response in Osmanabadi goat breeds. The goats were separated into four treatment groups (Heat Stress (HS); Control (C); Combined Stress (CS); Nutritional stress (NS)). The authors observed that plasma cortisol level was higher in CS group followed by C, HS, and NS group. Whereas, plasma aldosterone was advanced in CS group and substandard in C group.

Archana et al. (2018) led a study to govern the influence of heat stress on meat characteristics and plasma Leptin profiles in Osmanabadi and Salem Black goats under controlled and heat stressed conditions. The authors observed that plasma Leptin level was significantly increased in Salem Black heat stressed group in comparison with their control group. Leptin is well known for its role in milk and meat production thereby projecting it to be a ideal molecular marker of carcass quality and quantity traits in goats (Maitra et al., 2014). Bagath et al. (2016) led a study to find the effect of dietary intake on endocrinal profile in Osmanabadi goats during summer season. The goats were randomly separated in to two groups based on body weight. They stated that IGF-1, and Leptin concentration were lower in stressed group as compared to control group. Plasma GH was higher in stressed group in comparison with control group.

2.8.9. Impact of gene expression patterns related to meat production and quality

Though there are limited studies on the impact of heat stress on meat production and quality in goats, researchers working on this field have enlisted certain potential molecular markers for the concerned traits in goats. Archana et al. (2018) conducted a study to compare the impact of heat stress on the carcass characteristics, skeletal muscle HSP70 and myostatin gene expression levels between Osmanabadi (OS) and Salem Black (SB) goat under controlled and heat stressed (HS) conditions. Authors stated that Myostatin mRNA expression level was significantly lower in OS heat stress group than their control group. Whereas, HSP70 expression level was significantly higher in both HS groups, irrespective of

the breed. However, the comparatively low level of rise in HSP70 in SB heat stress group indicates the superiority of this breed to maintain meat quality. In another study, Shilja et al. (2017) evaluated the summer season induced nutritional and heat stress on HSP70 and blood biochemical response and expression pattern in Osmanabadi goats. The animals were randomly distributed into four treatment groups (Control (C); Nutritional stress (NS); Heat Stress (HS); Combined Stress (CS)). On comparative analysis, within the stress group, highest adrenal HSP70 mRNA expression level was observed in CS group followed by HS and NS group. Whereas, in case of hepatic HSP70 mRNA expression level was higher in HS group followed by NS and CS group.

Afsal et al. (2019) conducted a study to determine the influence of heat stress on the expression levels of HSP70 in different organs of Malabari goats under controlled and heat stressed conditions. The authors observed that HSP70 expression level was significantly increased in adrenal while lowered in mesenteric lymph node and thyroid. However, heat stress had no significant effect on expression levels of HSP70 in uterus and liver. Madhusoodan et al. (2020) conducted a study to establish the impact of summer heat stress on the expression levels of different classical heat shock response genes in Salem Black goats under controlled and heat stress conditions. The authors stated that HSP70, HSP90, nitrous oxide synthase 1 (NOS1), super oxide dismutase (SOD) genes were significantly reduced in heat stressed group in comparison with control group. A study was conducted by Sophia et al. (2016b) to evaluate the impact of multiple stresses on the expression patterns of toll-like receptors genes in liver of

Osmanabadi goats. The goats were randomly allocated into four treatment groups (Control (C); Combined Stress (CS); Heat Stress (HS); Nutritional stress (NS). Based on their reports, among the TLRs, TLR6, TLR3, TLR1, TLR8, TLR7, and TLR10 mRNA expressions were up regulated in HS group followed by C, NS and CS groups. Rashamol et al. (2019) conducted a study to analyze the impact of hot environment on the quantitative expression levels of diverse cytokine genes in Malabari goats under controlled and heat stressed conditions. The authors observed that the expression patterns of interleukin 18 (IL-18), tumour necrosis factor-α (TNF-α), interferon-β (IFN-β), and IFN-γ were significantly reduced in heat stressed group in comparison with the control group.

2.9. Concluding remarks

Heat stress acts as the major hindering factor which negatively influences livestock production. Heat stress alters the physiological, behavioral, endocrinal, hematological, and metabolic responses of livestock that results in limited production, reproduction, and deteriorates the health of the animal. Heat stress negatively impacts the meat quantity and quality in goats by altering the carcass parameters such as meat pH, proximate composition, shear force, water holding capacity, sensory attributes, and meat color. These carcass parameters play a key role in consumer preferences and acceptance of meat. Furthermore, heat stress also alters the expression patterns of several genes and endocrine variables responsible for cellular and muscular development. This review focuses on the consequences of heat stress on meat carcass production and meat quality variables in indigenous goats. The information synthesized based on available literature on the subject

clearly projects the need to identify ideal biological markers for climate resilient meat production which would help to identify indigenous breeds which can produce optimally and adapt in a given environmental conditions. Such efforts could help to propagate appropriate indigenous breeds which could endure and produce optimally specific to a particular agro-ecological zone.

MATERIALS & METHODS

CHAPTER 3

MATERIALS AND METHODS

The present study was conducted to evaluate the effect of heat stress on carcass and meat quality characteristics of Kodi Aadu goats. The carcass characteristics (dressing percentage, yield of edible and inedible offals, linear carcass measurement) and meat quality characteristics viz., physico-chemical (pH, WHC, colour, protein solubility, and meat color), compositional (proximate composition, collagen content and solubility), structural characteristics (myofibrillar fragmentation index, muscle fibre diameter, sarcomere length, shear force) and sensory characteristics were evaluated in muscle *longissimus thoracis et lumborum* (LTL) in both control and animals exposed to heat stress.

3.1. Location of the study

The experiment was conducted at the Centre for Climate Resilient Animal Adaptation Studies (CCRAAS), ICAR- National Institute of Animal Nutrition and Physiology experimental livestock farm (NIANP), Bengaluru, India which is located at the longitude 77º 38´E and the latitude of 12º 58´N and at altitude of 920 m above mean sea level. The experiment was conducted during the summer months of May-June, 2020 in state-of-the-art facility climate chamber. The control animals were kept in comfort condition with a temperature of 24^0C in thermo-neutral zone (TNZ) chamber and heat stress group was kept inside the heating/ cooling chamber

with a simulated heat stress model from 10:00 h to 16:00 h (Plate 3.1). The parameters related to meat quality characteristics were conducted at Department of Livestock Products Technology, Veterinary College, Hebbal, Bengaluru.

Plate 3.1: Climate chamber: Thermo-neutral Zone Chamber (left) and Heating/ Cooling Chamber (right) outside view

3.2. Animals used in the study

In the present study Kodi Aadu breed of goat was used as they are considered as one of the breeds that thrives on harsh environmental condition. It is an important meat goat breed in the Southern Tamil Nadu, India. Kodi Aadu goats

are distributed in southern coastal regions of Tamil Nadu and are docile, hardy, meat purpose goats with fast growth rate and early maturity. They have a predominant white coat color with scattered black and brown patches. The average body weight of Kodi Aadu female ranges between 25-30 kg. Twelve female animals aged approximately one year were procured from Tirunelveli district of Tamil Nadu and were transported to the experimental facility at ICAR-NIANP, Bengaluru. The animals were kept for acclimatization for a period of 45 days in the experimental farm unit before the start of the experiment.

3.3. Experimental design

The study was conducted for a period of 45 days in controlled climate chamber between May-June, 2020. Twelve female (one year old) Kodi Aadu goats were used in the present study. The animals were randomly allocated into two groups of six animals each, KC (n=6; Control), KHS (n=6; heat stress). The animals were stall fed with a diet consisting of 60 per cent roughage (Hybrid Napier hay) and 40 per cent concentrate (Maize 36 kg, wheat bran 37 kg, soybean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5 kg per 100kg). All animals were individually given access to ad-libitum feed and water. The control (C) goats were placed in the thermo neutral zone (TNZ), *i.e*., control chamber, while the heat stress (HS) goats were exposed to heat stress in heating chamber with a simulated heat stress model between 10.00AM to 4:00PM on all 45 days. Plate 3.2 describes the animals in the heating climate chamber. All cardinal weather parameters were recorded twice daily for the entire duration of the study. The study was conducted after obtaining due approval from the institute ethical committee (IAEC No:

NIANP/IAEC/2/2017). The animals were slaughtered at the end of the study to assess the meat and carcass characteristics. The skeletal muscles were simultaneously collected for gene expression study.

Plate 3.2: Climate Chamber facility of ICAR-NIANP with the experimental animals

3.4. Recording of weather variables

All the weather parameters were recorded twice daily (8:00 h and 14:00 h) for the entire period of experiment. The weather parameters like maximum temperature, minimum temperature, dry bulb temperature and wet bulb temperature were recorded manually using maximum thermometer, minimum thermometer, dry bulb and wet bulb thermometers respectively. Both ambient temperature and relative humidity were recorded using thermo-hygrometer. Additionally, climatic chambers are well equipped with weather sensors, which are connected to a server, from which we can collect the data in no time. The following temperature (Figure 3.1) model was used to generate heat stress in heating chamber. The model has been prepared as per the IMD data of Tamil Nadu region.

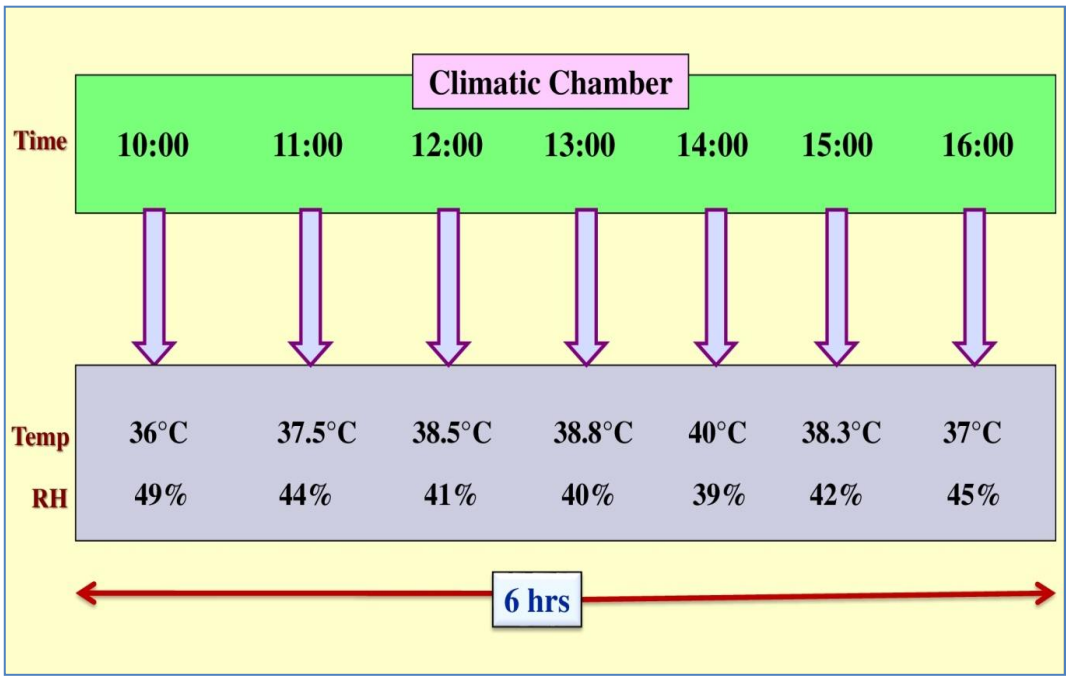


Figure 3.1. Simulated heat stress model used to induce heat stress in the climate chamber

3.5. Calculation of Temperature-Humidity Index (THI)

The temperature humidity index during the current experiment was calculated with the following Mc Dowell (1972) formula:

$$\mathbf{THI = 0.72 (T_{db} + T_{wb}) + 40.6}$$

T_{db}- Dry bulb temperature

T_{wb}- Wet bulb temperature

3.6. Slaughter procedure and carcass evaluation

All twelve animals were fasted for 12 hours overnight with ad libitum access to water before the day of slaughter. The slaughter was conducted following all hygienic procedures in the slaughter house at Experimental Livestock Unit, ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru. The animals were slaughtered by severing their jugular vein and carotid artery. After slaughter, the head was removed at the atlanto-occipital joint, fore and hint limbs were removed at the carpal and tarsal joints respectively. The animals were partially skinned lying on their back on the floor and then suspended to gambrel by the hind leg for further skinning. Sticking, legging, dressing and evisceration were performed as per the procedure described by Gerrard (1964). The animals were made to stand in a relaxed state.

3.7. Major carcass characteristics

3.7.1. Carcass traits

The pre-slaughter weights (PSW) and hot carcass weights (HCW), loin eye area (LEA) was recorded using weighing machine (Essae-Teraoka Limited, India) in kg. Dressing percentage (DP) was calculated on the basis of hot carcass weight (HCW) and live weight (LW) using the following formula

DP= HCW/LW * 100.

Were DP- Dressing Percentage

HCW- Hot Carcass Weight

LW- Live Weight

3.7.2. Non-carcass components and offals

Immediately after slaughter, blood was collected in a trough and weighed in a weighing machine. The edible offals (liver, blood, heart, brain, kidney,) and inedible offals (skin, head, feet, spleen, Reproductive organ, Mammary gland, lung with trachea) were separately weighed.

3.7.3. Carcass primal cuts

The carcass was divided into fore-saddle and hind-saddle at the intersection of 12^{th} and 13^{th} vertebrae and weight was recorded. The cut surface of M. LTL at the interface of 12^{th} and 13^{th} ribs on both side of the carcass was marked on tracing paper and measured by a planimeter and recorded as loin eye area (cm^2). The carcass was cut into different primal cuts *viz*., leg, loin, rack, neck and shoulder, Fore Saddle and Hind Saddle, flank, and breast and fore shank as per specifications of ISI (1995) and were individually weighed and recorded.

3.7.4. Linear carcass measurements

The various linear carcass measurements were recorded on hot carcass using a flexible measuring tape and callipers. The definitions of different carcass measurements are presented as below (Table 3.3)

Table 3.3: Different carcass measurements

Measurements	Description
Carcass length	Measured by a flexible measuring tape from the caudal edge of the last sacral vertebra to the dorso-cranial edge of the atlas (the first cervical vertebra)
Leg length	Measured from the middle of the lump at the proximal end of the tibia to the distal end of the tarsus
Chest depth	The greatest depth, measured by tape for measuring cavities at the horizontal level of the hanging carcass
Chest width	Widest carcass measurement at the ribs using a calliper
Buttock circumference	Measured using a tape held horizontally around the buttocks at the level of the caudal insertion
Carcass internal length	Maximum length between anterior edge symphysis pelvis to the anterior edge of first rib
Shoulder circumference	Maximum width measured at the level of the scapula from one lateral surface to the other

Chest circumference	Measured using a tape held horizontally around the thorax at the level of the caudal portion of the scapula

3.8 Physico-chemical characteristics

3.8.1. Meat pH

The pH of LTL muscle was estimated after 45min and 24hrs of slaughter using a digital pH meter (Labman, LMPH-10, India). Ten grams of muscle sample was taken in a beaker and 100ml of distilled water was added. It was homogenized for 15-45 seconds and then the electrode of the pH meter was inserted into the homogenate. The pH was recorded and the probe was thoroughly rinsed with distilled water before each reading. The pH meter was calibrated using pH buffer solutions of pH 4.0 and 7.0 (Thermo Fisher Scientific, Inc, Singapore) at weekly intervals.

3.8.2. Drip loss

Immediately after death, one complete thigh and one complete thigh muscle was weighed and placed in a polyethylene bag. The samples were kept at +4 °C during 24 h, then wiped and weighed. Drip loss was calculated and expressed as the percentage of the initial weight (Remignon *et al*., 1996).

3.8.3. Cooking loss %

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

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

3.8.4. Water holding capacity (WHC)

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

3.8.5. Shear force

The shear force values were estimated using the protocol described by Wheeler et al. (1996) with certain modifications. The meat samples were sealed in a polythene bag and cooked at 100ºC for 30 minutes in a water bath. The samples were then blotted dry and kept in unsealed bag in refrigerator for 12h. Six cores of meat samples with 1.27cm diameter were taken by cutting parallel to the muscle fibres. Theses cores were then sheared using Warner Bratzler Shear (G. R. Electric

Manufacturing Company, Manhattan, USA). The mean shear force needed to shear through the core was assessed by taking the average of the six readings.

3.8.6. Muscle fiber diameter

Fiber diameter was measured as per the method outlined by Jeremiah and Martin (1977). Five grams of meat sample was cut into small pieces and homogenized into slurry, in a solution containing 0.25M sucrose and 1mM EDTA with a domestic mixer grinder twice for 15 seconds at low speed, interspaced with an interval of five seconds. One or two drops of the slurry were transferred into a microscopic slide and covered with a cover slip. The meat slurry was examined directly under Olympus BX53 phase contrast microscope with image analyzer software under 40X. Muscle fiber diameter was measured as the mean cross-sectional distance between exterior surfaces of Sarcolemma of 20 randomly selected muscle fibers and expressed in micrometer (μm).

3.8.7. Sarcomere length

Sarcomere length of each muscle sample was measured as per the method outlined by Hostetler *et al*. (1972). Five grams of meat sample were blended with 35ml of 0.25M sucrose solution for one minute in a domestic mixer grinder at low speed. Immediately after blending, a few drops of slurry containing the fiber fragments were transferred to a microscopic slide and covered with a cover slip. Sarcomere lengths of 25 randomly selected fiber fragments were measured under Olympus BX53 phase contrast microscope with image analyser software under 100X and is expressed in micrometer (μm).

3.8.8. Collagen content

Hydroxyproline content of the meat sample was estimated based on the procedure of Nueman and Logan (1950) with few modifications as recommended by Naveena et al. (2004). Meat sample (2 grams) was hydrolysed with 40 ml of 6N HCl for 18 hours. The acid hydrolysate was filtered and the volume was adjusted to 50 ml with distilled water. Then 25 ml of hydrolysate was taken and pH was adjusted to 7.0 using 40% NaOH and the volume was again made to 50 ml with distilled water. 1ml of an aliquot from this solution was used for hydroxyl proline estimation. 1ml of aliquot from pH adjusted acid hydrolysate solution was taken in a test tube, added with an equal volume of i.e., 1ml of copper sulphate, 2.5M NaOH and 6% Hydrogen peroxide and incubated at room temperature for 5 minutes then in water bath at 80^0C for 5 minutes with occasional shaking followed by chilling in ice-cold water. The chilled solution was mixed with 4ml of 3N Sulphuric acid and 2ml of 5% Dimethyl Amino Benzaldehyde (DMEB) in n-Propanol and placed in a water bath at 70^0C for 16 minutes. The absorbance was measured at 540 nm using UV-VIS spectrophotometer (Model: Shimadzu UV-1900, Japan) and also the hydroxyproline content was determined by referring to a standard graph. The collagen content was calculated by multiplying hydroxyproline content with 7.14 (Dransfield et al., 1983) and was expressed in mg/g tissue.

3.8.9. Collagen solubility

Collagen solubility was assessed using the method described by Mahendrakar *et al*. (1989). Five grams of meat sample was heated in a water bath to boiling

temperature for 30 minutes in a 250 ml beaker covered with watch-glass. The cooked meat was cut into small pieces and homogenized with 50 ml distilled water at 4 ± 1°C in a homogenizer for 2 minutes. The extract was then centrifuged at 4000 rpm for 30 minutes. Aliquots of cooked out juice and centrifugate were acid hydrolyzed to obtain solubilized HP content. HP content was calculated using the below formula.

% Collagen solubility = 7.14 X % HP solubilized

3.8.10. Myofibrillar fragmentation index

Myofibrillar fragmentation index was estimated by following the method given by Davis et al. (1980) with slight modifications as suggested by Hawkins et al. (1987). Minced meat sample (10 gm) was added to 50 ml of cold 0.24 M sucrose and 0.02M potassium chloride solution in a 150 ml homogenization tube and allowed for 5 minutes. The mixture was blended for 40 seconds at high-speed using tissue homogenizer (Model: Z742486, Bench mark, D1000 Hand held homogenizer, Malaysia). The homogenate obtained was filtered through a pre-weighed muslin cloth (250 μm pore size) into the beaker with constant stirring using a plastic stirring rod to hasten filtration. The gentle and uniform squeezing was made to all the samples in a muslin cloth to drain out the excess moisture present. The MFI was reported as the weight of the residue in grams times one hundred.

3.8.11. Rigor value estimation (R-Value)

R- value was estimated as per the method outlined by (Honikel and Fischer, 1977). Approximately, l-3 g of meat was thoroughly homogenized with 10 ml 1M perchloric acid in a homogenizer (Firma Buhler, Tubingen, Germany) for 30 sec. The homogenate is filtered and 0.1 ml of the filtrate is diluted into 4.9 ml 0.l M phosphate buffer pH 7.0. The absorption at 250 and 260 nm is measured with phosphate buffer as reference using a UV-VIS spectrophotometer (Model: Shimadzu UV-1900, Japan) and the R value is calculated by taking the ratio of IMP by ATP. The R- value of the meat samples were estimated at 2 and 24 hours after slaughter.

3.9. Sensory evaluation

The LTL muscle samples were divided into approximately 2×2×2 cm sub samples which were cooked in a pressure cooker for 30 min. Then the samples were coded and serving sequence was randomized. A sensory panel of eight experienced members evaluated the different sensory attributes of the meat samples. There was a total of three sessions for evaluating the sensory attributes and the same panellists were used in all three sessions. The nature of the experiments, without disclosing the identity of the samples, was explained to the panellists. The panellists rated each sample for appearance, flavour, juiciness, texture and overall acceptability on eight-point descriptive scale (where, 8=extremely desirable, 1=extremely undesirable) (Keeton, 1983). For sensory evaluation, panellists were used as additional fixed factor in the analysis to determine how different were the evaluation among the

panel members and to understand what impact this had on the results. Samples were warmed in a microwave oven for 20 s just before sensory evaluation and coded samples were served at room temperature. Water was served for cleansing the mouth between samples to ensure accuracy in the evaluation.

3.10. Meat colour analysis

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

Principle

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

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

3.10.1. Myoglobin and Met-myoglobin content

Myoglobin was extracted from meat sample using a procedure as detailed by Warris (1979) with slight modifications. Meat sample was blended with 5 volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 seconds in a homogenizer. After incubation for 1 hour at 1 °C, mixture was centrifuged in a refrigerated centrifuge at 3500 rpm for 30 min. The clear filtrate was obtained by filtering through Whatman No.1 filter paper. The absorbance of the filtrate was measured at 525, 572, and 700 nm using a UV-VIS spectrophotometer (Model: Shimadzu UV-1900, Japan). The myoglobin (mg/g tissue) and Met-myoglobin (%) (Met-Mb) were calculated using the formula below (Trout, 1989).

$$\text{Mb (mg/g)} = (A_{525} - A_{700}) \text{ X } 2.303 \text{ X dilution factor}$$

$$\% \text{ Met-Mb} = \{1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})]\} \text{ X } 100$$

3.11. Proximate composition

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

3.12. Soluble protein

Protein extractability was determined according to the procedure given by Joo *et al*. (1999). Sarcoplasmic protein and total protein were extracted from 2g of meat sample using 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2) for sarcoplasmic protein and 40 ml of ice-cold 1.1 M potassium iodide in 0.1M phosphate buffer (pH 7.2) for total protein as extracting solution. The samples were homogenized for and kept overnight at 4 °C with frequent shaking. Later, the

samples were centrifuged at 5000 rpm for 20 minutes (Model: Eppendorf Centrifuge 5430 R, Germany) and the concentration of protein in the supernatant was determined by taking 1ml of aliquot from the supernatant were treated with 4ml of Biuret reagent to it and allowed for color development at room temperature in the dark for 20min. Then the absorbance of developed color was measured using UV-VIS spectrophotometer at 550 nm. The protein concentration was estimated using biuret method using the equation of straight line of BSA standard curve. Myofibrillar protein extractability was obtained by calculating the difference between total protein and sarcoplasmic protein extractability.

3.13. Gene expression

3.13.1. Expression patterns of genes in skeletal muscle

Principle

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

3.13.1.1. Sample collection and storage

The muscle (LTL) samples were collected from all the animals in each group immediately after slaughter. The samples were cut into small pieces, washed in

phosphate buffered saline (PBS) and immersed in **RNALater® Stabilization Solution (Life Technologies** GmbH, Darmstadt, Germany). All the samples were stored at -80°C till further use.

3.13.1.2. Sample preparation for RNA isolation

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

About 30 mg of tissue was homogenized by grinding in Liquid Nitrogen (LN_2) (-196°C) in RNAase ZAP (Ambion, USA) treated mortar and pestle. After homogenization, 300 µL of lysis buffer supplemented with β-mercaptoethanol (10 µL/ml) was added and the content was transferred to 1.5 ml microcentrifuge tube. The lysate was vortexed for 10 sec. To the lysate, 10 µL of proteinase K in 590 µL of Tris Ethylenediaminetetraacetic Acid (TE) buffer was added, then vortexed and incubated at 15-25°C for 10 min. Then, the contents were centrifuged for 8 min at 12000 g and the supernatant was transferred into a new RNase-free micro centrifuge tube. 450 µL of ethanol was added and mixed well by pipette. Then 700 µL of was transferred to a spin column with a 2 ml collection tube and centrifuged for 1 min at 12000 g. After discarding the flow through, 700 µL of wash buffer 1 was added and centrifuged for 1 min at 12000 g followed by two time washing with 600 µL and 250 µL of wash buffer 2 followed by centrifugation at 12000 g for 1 min and 2

min respectively. About 40 µL of warm nuclease free water was added to the membrane, and centrifuged at 10000 g for 1 min to elute RNA. The purified RNA samples were stored at -80°C until complementary DNA (cDNA) synthesis.

3.13.1.3. DNase treatment

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

3.13.1.4. cDNA synthesis

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

4 µL of 5X Reaction Mix, 2 µL Maxima Enzyme Mix, 1µg of Template RNA and 20 µl of nuclease free water were added into a sterile, RNAase-free tube. Then the contents were mixed gently and centrifuged and subjected to reverse transcribing PCR (10 min at 25 ° C, followed by 20 min at 50 °C and the reaction was terminated by heating at 85 °C for 5 min). The product of the first strand cDNA synthesis was diluted to a final concentration of 25 ng/µL with nuclease free water and 2 µL of diluted cDNA was used for each reaction qPCR.

3.13.1.5. Primer design and synthesis

Gene specific primers were designed using online NCBI primer design software (Primer 3, http://bioinfo.ut.ee/primer3/) and specificity was checked using Primer3 and BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) for targeted genes. The preferences were given to the primers binding to the exon-exon junction. Table 3.4 describes the primer sequences for both the targeted genes and housekeeping genes.

3.13.1.6. Quantitative RT-PCR analysis

The relative expression of targeted genes such as myostatin (*MSTN)*, calpain 1 (*CAPN1), CAPN2,* CAST, *DAGT1,* heat shock factor (HSF1), heat shock protein (*HSP10)*, *HSP27*, *HSP40*, αB-crystallin *(CRYAb)*, *HSP60*, *HSP70, HSP90, and HSP110 genes* were studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20 µL reaction was carried out in triplicates using 50 ng of template and 0.5 µM primer concentrations. The real time qPCR reaction conditions were: enzyme activation at 95^0C for 10 min and amplification cycle (40 cycles; initial denaturation at 95^0C for 15 sec, annealing at 60°C for 30 sec and extension at 72^0C for 30 sec). The melt curve analysis was done to check the non-specific amplification. Both Hypoxanthine Phosphoribosyl transferase (*HPRT*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) genes were used as an internal control and the relative expression was analyzed using the formula, $2^{\Delta\Delta CT}$ method (Shilja et al., 2016). The results were expressed in fold change as compared to untreated control (control=1fold).

3.14. Statistical analysis

The data was analysed by one- way analysis of variance (ANOVA) SPSS (version 18.0) software. The changes in relative expression of all targeted genes in skeletal muscle in relation to *HPRT* and *GAPDH* as the house keeping genes were analyzed by t-test. Further, the correlation coefficient between the THI and all carcass traits and gene expression patterns were established by Pearson's correlation coefficient test using SPSS (version 18.0) software. The R^2 values were used to establish the correlation association between THI and various carcass traits. Results are shown as mean ± standard error (SE) and the significance level was set at $P < 0.05$.

Table 3.4. Details on the primers used for different target and housekeeping genes

Gene ID	Primers	Primer sequence (5'–3')	Ta (°C)	Primer length (bp)	Product size (bp)	Accession no.
HSF1	F	AAAGTGACCAGCGTGTCCA	60	19	115	NM_001314344.2
	R	AGTCCATGCTCTCCTGCTTC		20		
HSP10	F	CCGCCGAAACTGTAACCAAAG	60	21	137	XM_018061298.1
	R	ATCGAAGGATTCATCAGGCCAA		22		
HSP27	F	CCTGGACGTCAACCACTTC	60	19	77	XM_018040903.1
	R	GCTTGCCAGTGATCTCCAC		19		
HSP40	F	AAGAGCCTGACCAACGACTG	60	20	107	XM_005694067.1
	R	AAAGGAGCTCGTCTTGGGAC		20		
HSP60	F	AGGTTGGTGGGACAAGTGATG	60	21	139	XM_018061271.1
	R	AAGGCTGGAATGCACCGAAG		20		
HSP70	F	TGGCTTTCACCGATACCGAG	60	20	167	NM001285703.1
	R	GTCGTTGATCACGCGGAAAG		20		
HSP90	F	AAGAGCCTGACCAACGACTG	60	20	107	XM_005694067.1
	R	AAAGGAGCTCGTCTTGGGAC		20		
HSP110	F	TACCCACGGCATTTTCACCA	60	20	142	XM_018056621.1
	R	CTCATTAGCCTCGGCATCTGG		21		
MSTN	F	ACCAAGCAAACCCAAAGGT	60	20	138	KX171679.1
	R	CACCCACAGCGATCTACTACC		21		
CAPN 1	F	GAGATCGTCGGAGAAGGATGG	60	21	124	XM_015102890.2
	R	TTGATGGCATTTTCATGGCGG		21		
CAPN 2	F	CAGTGGAATGACAACTGCCC	60	20	127	XM_027564533.1
	R	GGCGGGAATAGTGTCTCAGG		20		

Cryab	F	CGCCCCACACTCACCTAAC	60	19	133	XM_027563779.1
	R	AGATCAGACTCCAACAGGTGC		21		
CAST	F	ATGGCATTTGCAAGCTGGTG	60	20	128	XM_027545566.1
	R	GAGAGCTGACTGCTCCCAAG		20		
DGAT 1	F	CCTGATATGGGGCCACTGC	60	19	146	XM_018058729.1
	R	CCCAACCTCCCGCTAAGTTT		20		
HPRT	F	GCCCCAGCGTGGTGATTAG	60	19	145	XM_018044253.1
	R	ACATCTCGAGCCAGTCGTTC		20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	60	20	245	AJ431207.1
	R	TCATAAGTCCCTCCACGATG		20		

HPRT, GAPDH used as reference gene to normalize the gene expression of target genes. Ta- annealing temperature, bp- base pair, F forward; R reverse; HSF-Heat shoch factor-1, HSP- heat shock protein, MSTN- Myostatin, CAPN1- Calpain 1, CAPN2- Calpain 2, Cryab - αB-crystallin, CAST- Calpastatin, DGAT1- Diacylglycerol Acyltransferase1, HPRT- hypoxanthine phosphoribosyl transferase 1, GAPDH- glyceraldehyde 3-phosphate dehydrogenase

RESULTS

CHAPTER 4

RESULTS

The present study was carried out at ICAR-NIANP and Dept. of Livestock Products Technology, Veterinary College, Bengaluru to evaluate the effect of heat stress on carcass and meat quality characteristics of Kodi Aadu goats. The results of the weather variables, carcass, meat quality characteristics and sensory characteristics of muscle *longissimus thoracis et lumborum* (*LTL*) in both control (KC) and animals exposed to heat stress (KHS) are presented in this section.

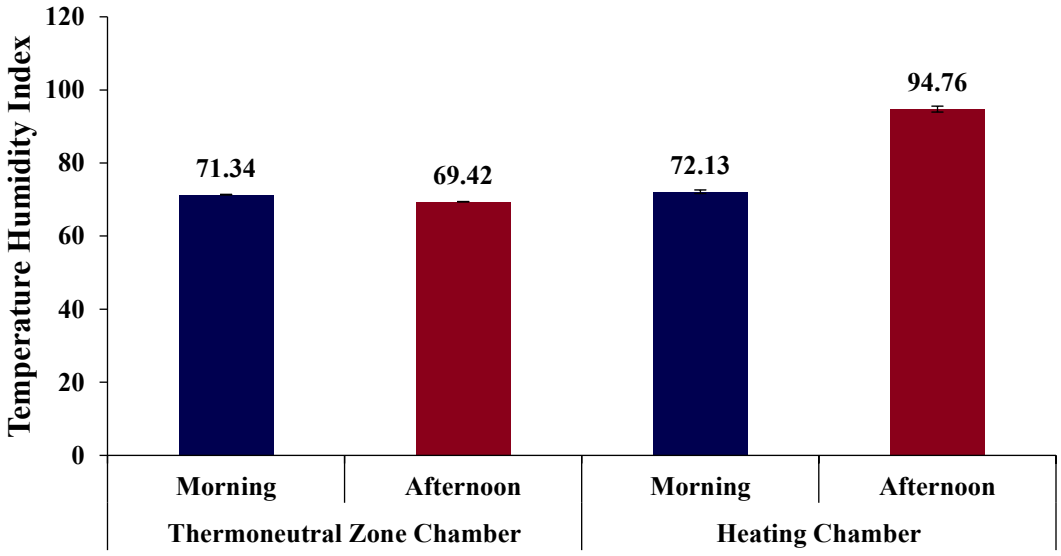
4.1. Weather variables

The average weather variables during the study duration of 45 days period are depicted in Table 4.1.

The cardinal weather variables indicated that there were significant ($P<0.05$) differences in the microclimate between the thermo-neutral chamber and heating climate chamber. The ambient temperature, relative humidity, maximum temperature, minimum temperature, dry bulb temperature, and wet bulb temperature were significantly ($P<0.01$) higher in the heating chamber as compared to thermo-neutral chamber. The Temperature humidity index (THI) values obtained between thermo-neutral and heating climate chambers both during morning and afternoon hours are depicted in Fig. 4.1. The THI calculated based on temperature and relative humidity also

showed similar pattern to other weather variables between the chambers. The THI values obtained both during morning and afternoon in Thermo-neutral zone (TNZ) chamber and heating chambers were 71.34 ± 0.09, 69.42 ± 0.05, 72.13 ± 0.07 and 94.76 ± 0.45, respectively. The THI values were comparable during morning hours in both the climate chambers. However, during afternoon the THI values showed significant ($P<0.01$) variation between the chambers with higher value recorded in the heating chamber.

Fig. 4.1: Average temperature humidity index (THI) for the study period both in Thermo-neutral Chamber and Heating Chamber



The THI values were calculated as per method described by McDowell (1972). Accordingly, the formula used was THI = [0.72 (T_{db} + T_{wb})] + 40.6 where, T_{db} = Dry bulb temperature in ºC; T_{wb} = Wet bulb temperature in ºC. The THI values 72 and less are considered comfortable; THI values between, 75-78 are considered stressful and THI above 78 considered extreme distress. The THI in heating chamber during afternoon is significantly higher than other three values.

Table 4.1: Average weather parameters during the entire study

	Time of Recording	DBT (ºC)	WBT (ºC)	MaxT (ºC)	MinT (ºC)	AT (ºC)	RH (%)	PST (ºC)
Thermo-neutral Zone Chamber	Morning (8:00 h)	25.12 ± 0.09	17.57 ± 0.07	27.34 ± 0.09	24.64 ± 0.14	25.81 ± 0.16	66.34 ± 0.58	26.68 ± 0.13
	Afternoon (14:00 h)	23.35 ± 0.05	16.68 ± 0.05	24.19 ± 0.06	23.68 ± 0.07	24.02 ± 0.07	68.88 ± 0.33	22.33 ± 0.39
Heating Chamber	Morning (8:00 h)	25.42 ± 0.09	18.37 ± 0.04	27.74 ± 0.13	24.70 ± 0.06	25.94 ± 0.05	66.64 ± 0.45	26.56 ± 0.31
	Afternoon (14:00 h)	40.54 ± 0.34	34.69 ± 0.31	41.41 ± 0.13	38.64 ± 0.29	41.03 ± 0.32	55.94 ± 0.79	40.13 ± 0.55

DBT- dry bulb temperature; WBT- wet bulb temperature; MinT- minimum temperature; MaxT- maximum Temperature; RH-relative humidity; AT-Ambient Temperature; PST-Pen Surface Temperature

4.2. Carcass traits

The variation in Mean ± SE of carcass traits *viz.,* pre-slaughter weight, hot carcass weight, dressing percentage and loin eye area (LEA) are depicted in the Table 4.2. The correlation between THI and major carcass traits are described in Fig.4.3.

4.2.1. Pre-slaughter weight (PSW)

The pre-slaughter weight in KC, KHS was 14.11 ± 0.66 and 14.21 ± 0.89 respectively. The Mean ± SE of PSW values showed no significant difference (P>0.05) between control and heat stressed groups.

4.2.2. Hot carcass weight (HCW)

The hot carcass weight in KC, KHS was 6.65 ± 0.32 and 6.67 ± 0.48 respectively. The Mean ± SE of hot carcass weight values revealed no significant difference (P>0.05) between control and heat stressed groups.

4.2.3. Dressing percentage (DP)

The dressing percentage in KC, KHS was 47.14 ± 0.67 and 46.84 ± 1.01, respectively. The Mean ± SE of dressing percentage values showed no significant variation (P>0.05) between control and heat stressed groups of Kodi Aadu breed.

4.2.4. Loin eye area (LEA)

The loin eye area in KC, KHS group was 6.02 ± 0.05 and 5.73 ± 0.09, respectively. The Mean ± SE of loin eye area values showed no significant variation (P>0.05) between control and heat stressed groups of Kodi Aadu breed. However, the THI had a negative correlation (P<0.05) with LEA.

Table 4.2: Effect of heat stress on major carcass traits in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
re-slaughter weight (PSW) (kg)	14.11±0.66	14.21±0.89	NS
ot carcass weight (HCW) (kg)	6.65±0.32	6.67±0.48	NS
ressing Percentage (%)	47.14±0.67	46.84±1.01	NS
oin eye area (LEA) (cm^2)	6.02±0.05	5.73±0.09	NS

NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

Table: 4.3 Correlation coefficient between THI and Major carcass traits of *LTL* muscle

	HI	SW (kg)	CW (kg)	P (%)	EA (cm^2)
HI					
SW (kg)	031				
CW (kg)	014	955**			
P (%)	.076	172	456		
EA (cm^2)	.667*	247	264	178	

THI- Temperature Humidity Index, PSW- Pre- Slaughter Weight, HCW- Hot Carcass Weight, DP- Dressing percentage, LEA- Loin Eye Area; *P<0.05

4.3. Non-carcass components and offals

The Mean ±SE weights of blood, head, skin, feet, heart, liver, kidneys, spleen, lungs with trachea, GIT (gastrointestinal tract), brain, intestine with contents, intestine without contents, omental fat in KC and KHS group are depicted in Table 4.4.

Table: 4.4 Effect of heat stress on non-carcass components and offals in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
lood Wt. (kg)	0.45±0.03	0.45±0.04	NS
ead Wt. (kg)	1.14±0.04	1.14±0.07	NS
kin Wt. (kg)	1.11±0.04	1.16±0.07	NS
eet Wt. (kg)	0.54±0.03	0.54±0.03	NS
eart (kg)	0.09±0.00	0.08±0.01	*
iver (kg)	0.26±0.01	0.25±0.01	NS
idneys (kg)	0.95±0.01	0.10±0.01	NS
pleen (kg)	0.04±0.00	0.04±0.01	NS
ungs (kg)	0.36±0.02	0.36±0.03	NS
rain (kg)	0.09±0.00	0.07±0.01	*
eproductive Organ (kg)	0.02±0.00	0.06±0.04	*
ammary Gland (kg)	0.03±0.01	0.02±0.00	NS

*P<0.05; NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

The effects of heat stress were significant (P<0.01) on some of the offals such as heart (P<0.01), brain (P<0.05) and reproductive organs (P<0.05). Heart weight was observed to have significantly (P<0.05) lower value in the KHS group (0.08 ± 0.01) as compared to the KC (0.09 ± 0.01) animals. Likewise, the brain weight also showed same trend with significantly (P<0.05) lower value in KHS group (0.07 ± 0.01)

as compared to KC group (0.09 ± 0.00) animals. However, reproductive organs showed contrasting result to this, with heat stressed group (0.06 ± 0.04) showing significantly higher ($P<0.05$) value than the control group animals (0.02 ± 0.00). However, no significant difference ($P>0.05$) was observed in blood, head, skin, feet, liver, kidneys, spleen, lungs and mammary gland between KC and KHS groups. Further, there was no any correlation established between THI and non-carcass components (Table 4.5).

4.4. Primal cuts

The Mean ± SE weights of fore saddle, hind saddle, neck, shoulder, rack, loin, flank, shank, leg and breast are described in Table 4.6. Heat stress significantly ($P<0.05$) influenced only loin variable among the primal cuts with significantly lower value in KHS group as compared to KC group. Further, the heat stress did not reveal any significant ($P>0.05$) variation in other primal cuts between KC and KHS group animals. Further, THI also did not showed correlation with any of the primary cuts variables (Table 4.7).

Table: 4.5 Correlation coefficient between THI and Non carcass components and Offals of *LTL* muscle

	THI	Blood	Head	Skin	Feet	Heart	Liver	Kidney	Spleen	Lung	Brain	RP	MG
THI	1												
Blood	0.020	1											
Head	-0.024	0.912**	1										
Skin	0.197	0.785**	0.889**	1									
Feet	-0.040	0.625*	0.659*	0.656*	1								
Heart	-0.065	0.705*	0.831**	0.719**	0.547	1							
Liver	-0.115	0.583*	0.718**	0.729**	0.708**	0.515	1						
Kidney	0.080	0.572	0.642*	0.488	0.504	0.573	0.506	1					
Spleen	-0.265	0.797**	0.845**	0.616*	0.446	0.712**	0.665*	0.414	1				
Lung	0.038	0.510	0.531	0.533	0.418	0.668*	0.658*	0.508	0.481	1			
Brain	0.104	0.634*	0.718**	0.628*	0.399	0.774**	0.329	0.320	0.528	0.496	1		
RP	0.273	0.051	0.248	0.574	0.242	0.394	0.461	0.147	-0.019	0.588*	0.272	1	
MG	-0.252	0.703*	0.802**	0.711**	0.323	0.537	0.593*	0.563	0.649*	0.361	0.521	0.194	1

THI- Temperature Humidity Index, RP- Reproductive Organ, MG- Mammary Gland

Table: 4.6 Effect of heat stress on primal cuts in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
Fore Saddle (Kg)	3.44±0.18	3.42±0.23	NS
Fore Saddle (%)	51.67±0.33	51.35±0.45	NS
Neck (Kg)	0.42±0.02	0.41±0.03	NS
Shoulder (Kg)	1.69±0.09	1.73±0.12	NS
Rack (Kg)	0.66±0.05	0.64±0.07	NS
Breast (Kg)	0.23±0.02	0.25±0.02	NS
Shank (Kg)	0.41±0.01	0.41±0.02	NS
Hind Saddle	3.08±0.16	3.03±0.23	NS
Hind Saddle (%)	46.26±0.47	45.30±0.35	NS
Loin (Kg)	0.53±0.03	0.49±0.05	*
Flank (Kg)	0.24±0.02	0.23±0.02	NS
Leg (Kg)	2.31±0.12	2.30±0.17	NS

*P<0.05; NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

Table 4.7 Correlation coefficient between THI and primal cuts of *LTL* muscle

	THI	FS (kg)	Neck (kg)	Shoulder (kg)	Rack (kg)	Breast (kg)	Shank (kg)	HS (kg)	Loin (kg)	Flank (kg)	Leg (kg)	FS (%)	HS (%)
THI	1												
FS (kg)	-0.020	1											
Neck (kg)	-0.125	0.938**	1										
Shoulder (	0.077	0.970**	0.898**										
Rack (kg)	-0.078	0.924**	0.865**	0.835**	1								
Breast (kg	0.281	0.487	0.437	0.561	0.275	1							
Shank (kg	0.069	0.949**	0.898**	0.900**	0.899**	0.477	1						
HS (kg)	-0.056	0.978**	0.910**	0.966**	0.875**	0.486	0.939**	1					
Loin (kg)	-0.202	0.885**	0.792**	0.841**	0.862**	0.179	0.852**	0.928**	1				
Flank (kg)	-0.047	0.872**	0.757**	0.772**	0.852**	0.423	0.846**	0.827**	0.799**	1			
Leg (kg)	-0.010	0.969**	0.914**	0.977**	0.839**	0.559	0.932**	0.992**	0.878**	0.778**	1		
FS (%)	-0.178	0.012	0.086	-0.168	0.160	-0.093	0.041	-0.135	-0.168	0.226	-0.165	1	
HS (%)	-0.460	0.331	0.325	0.271	0.269	0.093	0.397	0.481	0.565	0.316	0.450	-0.125	1

THI- Temperature Humidity Index, FS- Fore Saddle, HS- Hind Saddle

4.5. Linear carcass measurements

The Mean ± SE measurements of external carcass length, internal carcass length, buttock circumference, leg width, leg length, chest circumference, chest depth, chest width, and shoulder circumference are described in Table 4.8.

The effects of heat stress were significant only on shoulder circumference ($P<0.05$). The values of external carcass length, internal carcass length, leg width, chest circumference, chest depth, chest width and leg length were comparable ($P>0.05$) between KC and KHS groups. Furthermore, no correlation was established between THI and other primal cut variables (Table 4.9).

Table: 4.8 Effect of heat stress on linear carcass measurements in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
External Carcass Length (in)	25.00±0.45	26.00±0.52	NS
Internal Carcass Length (in)	17.33±0.42	18.17±0.40	NS
Buttock Circumference (in)	15.17±0.31	14.58±0.46	NS
Leg Width (in)	3.58±0.15	3.75±0.25	NS
Chest Circumference (in)	22.17±0.60	22.00±0.52	NS
Chest Depth (in)	6.67±0.17	6.83±0.17	NS
Chest Width (in)	1.92±0.08	2.17±0.11	NS
Shoulder Circumference (in)	11.33±0.40	11.67±0.21	*
Leg Length (in)	13.83±0.60	14.50±0.56	NS

*P<0.05; NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

Table: 4.9 Correlation coefficient between THI and Linear carcass measurements of *LTL* muscle

	THI	ECL	ICL	BC	LW	CC	CD	CW	SC	LL
THI	1									
ECL	0.420	1								
ICL	0.412	0.728**	1							
BC	-0.319	0.363	0.236	1						
LW	0.117	0.594*	0.350	0.483	1					
CC	-0.066	0.474	0.411	0.698*	0.399	1				
CD	0.218	0.733**	0.486	0.566	0.347	0.565	1			
CW	0.507	0.355	0.209	0.115	0.299	0.527	0.332	1		
SC	0.226	0.571	0.672*	0.216	0.300	0.631*	0.222	0.230	1	
LL	0.248	0.469	0.399	0.423	0.088	0.535	0.487	0.482	0.379	1

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

4.6. Physico-chemical characteristics

The variation in Mean ± SE of physico-chemical attributes *viz.,* pH, drip loss, cooking loss, water holding capacity, shear force, muscle fiber diameter, sarcomere length, collagen content, Collagen Solubility, myofibrillar fragmentation index and Rigor Value are described in table 4.10.

4.6.1. pH

The Mean ± SE of pH differences of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence ($P>0.05$) both pH_{45min}, and ultimate pH_{24hrs} changes in *LTL* muscle (Table 4.10). However, a strong positive correlation ($P<0.05$) was established between THI and pH_{24hrs} of *LTL* muscle (Table 4.11).

4.6.2. Drip loss (%)

The Mean ± SE of drip loss percentage of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence ($P>0.05$) drip loss and the values were comparable across the groups. Further, THI and drip loss of *LTL* muscle did not show any correlation (Table 4.11).

4.6.3. Cooking loss (%)

The Mean ± SE of cooking loss percentage of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence ($P>0.05$) cooking loss and the values were comparable across the groups. However, a mild positive

correlation (P<0.05) was established between THI and cooking loss of *LTL* muscle (Table 4.11).

4.6.4. Water holding capacity (%)

The Mean ± SE of WHC of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence (P>0.05) WHC and the values were comparable across the groups. Further, THI and WHC of *LTL* muscle did not show any correlation (Table 4.11).

Table: 4.10 Effect of heat stress on physico-chemical attributes in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
pH_{45min}	6.63±0.07	6.76±0.02	NS
pH_{24hrs}	5.63±0.03	6.03±0.05	NS
Drip loss %	1.55±0.21	1.85±0.26	NS
Cooking loss (%)	22.33±1.56	26.43±0.83	NS
Water holding capacity (%)	16.17±2.10	19.17±2.24	NS
Shear force (kg/cm^2)	7.63±0.15	8.03±0.14	NS
Muscle Fiber Diameter (µm)	40.52±1.45	44.82±2.40	NS
Sarcomere Length (µm)	1.73±0.04	1.59±0.09	NS
Collagen Content (mg/g)	4.08±0.17	4.12±0.12	NS
Collagen Solubility (%)	30.29±1.48	27.35±0.38	**
MFI	531.83±17.46	515.17±15.62	NS
Rigor value (Ratio)	1.37±0.01	1.36±0.01	NS

**P<0.01; NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

Table 4.11: Correlation coefficient between THI and physico- chemical attributes of *LTL* muscle

	THI	pH 45min	pH 24 hrs	DL %	CL %	WHC%	RV (Ratio)	MFD	SL	SF (kg/cm^2)	CRM	CS %	MFI
THI	1												
pH 45min	0.487	1											
pH 24 hrs	0.903**	0.392	1										
DL %	0.278	0.228	0.208	1									
CL %	0.592*	0.206	0.519	0.107	1								
WHC%	0.295	-0.134	0.098	0.085	0.336	1							
RV (Ratio)	-0.267	0.162	-0.218	-0.102	-0.052	-0.091	1						
MFD	0.436	0.021	0.419	0.460	0.702*	0.450	-0.338	1					
SL	-0.423	-0.157	-0.316	0.120	-0.453	-0.535	0.228	-0.472	1				
SF (kg/cm^2)	0.526	0.141	0.389	0.174	-0.103	0.287	-0.340	0.153	-0.476	1			
CRM	0.064	0.134	-0.190	-0.275	-0.092	0.069	-0.325	-0.294	0.159	0.177	1		
CS %	-0.518	-0.015	-0.482	-0.239	0.171	-0.143	0.395	0.130	-0.140	-0.550	-0.338	1	
MFI	-0.219	-0.077	-0.218	-0.021	0.073	0.524	0.092	0.362	-0.172	-0.108	-0.075	0.219	1

THI- Temperature Humidity Index, pH_{45}- Potential of Hydrogen at 45min, pH_{24}- Potential of Hydrogen in 24hrs, DL- Drip Loss, CL- Cooking Loss, WHC- Water Holding Capacity, SF-Shear Force, MFD- Muscle Fiber Diameter, SL- Sarcomere Length, CRM- Collagen content of raw meat, MFI-Myofibrillar fragmentation index; **P<0.01; *P<0.05

4.6.5. Shear force

The Mean ± SE of shear force percentage of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence (P>0.05) shear force and the values were comparable across the groups. Further, THI and shear force of *LTL* muscle did not show any correlation (Table 4.11).

4.6.6. Muscle fiber diameter

The Mean ± SE of MFD of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence (P>0.05) MFD and the values were comparable across the groups. Further, THI and MFD of *LTL* muscle did not show any correlation (Table 4.11).

4.6.7. Sarcomere length

The Mean ± SE of sarcomere length of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence (P>0.05) sarcomere length and the values were comparable across the groups. Further, THI and sarcomere length of *LTL* muscle did not show any correlation (Table 4.11).

4.6.8. Collagen content

The Mean ± SE of collagen content of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence (P>0.05) collagen content and the values were comparable across the groups. Further, THI and collagen content of *LTL* muscle did not show any correlation (Table 4.11).

4.6.9. Collagen solubility

The Mean ± SE of collagen solubility percentage of *LTL* muscle in KC and KHS groups were described in Table 4.10. Collagen solubility percentage is the only physico-chemical variable influenced ($P<0.01$) by heat stress across the groups. Further, THI and collagen solubility percentage of *LTL* muscle did not show any correlation (Table 4.11).

4.6.10. Myofibrillar fragmentation index

The Mean ± SE of MFI of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence ($P>0.05$) MFI and the values were comparable across the groups. Further, THI and MFI of *LTL* muscle did not show any correlation (Table 4.11).

4.6.11. Rigor value

The Mean ± SE of rigor value ratio of *LTL* muscle in KC and KHS groups were described in Table 4.10. Heat stress did not influence ($P>0.05$) rigor vale ratio and the values were comparable across the groups. Further, THI and rigor value ratio of *LTL* muscle did not show any correlation (Table 4.11).

4.7. Sensory attributes

The Mean ± SE of sensory attributes of *LTL* muscle in KC and KHS groups were described in Table 4.12. Heat stress did not influence ($P>0.05$) any of the sensory variables such as appearance, flavour, juiciness, tenderness and overall acceptability. Furthermore, THI showed a strong negative correlation ($P<0.01$) with

tenderness and a mild negative correlation (P<0.05) with overall acceptability score of *LTL* muscle (Table 4.13).

Table: 4.12 Effect of heat stress on Sensory attributes in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
Appearance	7.55±0.13	7.35±0.18	NS
Flavour	6.87±0.08	6.85±0.15	NS
Juiciness	6.68±0.08	6.58±0.14	NS
Tenderness	6.68±0.05	6.27±0.08	NS
Over acceptability	7.07±0.08	6.83±0.07	NS

**P<0.01; *P<0.05; NS- Non-Significant, KC- Kodii Aadu Control, KHS- Kodi Aadu Heat Stress

Table: 4.13 Correlation coefficient between THI and Sensory attributes of *LTL* muscle

	THI	Appearance	Flavor	Juiciness	Tenderness	Overall Acceptability
THI	1					
Appearance	-0.273	1				
Flavor	-0.030	-0.335	1			
Juiciness	-0.191	-0.321	0.492	1		
Tenderness	-0.816**	0.298	-0.050	0.274	1	
Overall Acceptability	-0.590*	0.103	-0.268	-0.096	0.437	1

THI-Temperature-humidity-index; **P<0.01; *P<0.05

4.8. Meat color

The Mean ± SE of meat color attributes of *LTL* muscle in KC and KHS groups were described in Table 4.14. Analysis revealed that only yellowness ($P<0.01$) of *LTL* muscle showed significant difference for heat stress treatment (Table 4.14). However, no significant difference could be observed in lightness, redness, myoglobin and met-myoglobin of *LTL* muscle (Table 4.14). Furthermore, THI showed a strong negative correlation ($P<0.01$) with lightness and a mild positive correlation ($P<0.05$) with redness and yellowness of *LTL* muscle (Table 4.15).

Table: 4.14 Effect of heat stress on meat colours in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
Lightness (L*)	38.76±1.95	30.51±1.25	NS
Redness (a*)	11.68±0.39	12.84±0.24	NS
Yellowness (b*)	13.30±0.37	15.16±0.67	**
Myoglobin (mg/g)	2.68±0.10	2.71±0.15	NS
Met-Myoglobin (%)	36.96±2.94	45.76±2.70	NS

**P<0.01; *P<0.05; NS- Non-Significant, KC- Kodii Aadu Control, KHS- Kodi Aadu Heat Stress

Table: 4.15 Correlation coefficient between THI and Meat colours of *LTL* muscle

	THI	L*	a*	b*	Mb (mg/g)	%Met mb
THI	1					
L*	-0.748**	1				
a*	0.624*	-0.748**	1			
b*	0.610*	-0.671*	0.279	1		
Mb (mg/g)	0.054	-0.120	0.429	0.057	1	
%Met mb	0.571	-0.800**	0.696*	0.653*	0.373	1

THI- Temperature Humidity Index, L*- Lightness, a*- Redness, b*- Yellowness

4.9. Soluble proteins

The Mean ± SE of soluble proteins of *LTL* muscle in KC and KHS groups were described in Table 4.16. Analysis revealed heat stress did not influence (P>0.05) any of the soluble proteins such as sarcoplasmic protein, total protein and myofibrillar protein of *LTL* muscle (Table 4.16). Furthermore, THI also did not showed any correlation (P>0.05) with any of the soluble proteins of *LTL* muscle (Table 4.17).

Table: 4.16 Effect of heat stress on soluble protein in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
Sarcoplasmic Protein (mg/g)	33.07±3.42	29.22±3.19	NS
Total Protein (mg/g)	125.09±5.94	134.53±3.41	NS
Myofibrillar Protein (mg/g)	100.45±5.92	108.20±3.30	NS

NS- Non-Significant, KC- Kodii Aadu Control, KHS- Kodi Aadu Heat Stress

Table: 4.17 Correlation coefficient between THI and Soluble Proteins of *LTL* muscle

	THI	Sarcoplasmic Protein (mg/g)	Total protein (mg)	Myofibrillar Protein (mg/g)
THI	1			
Sarcoplasmic Protein (mg/g)	-0.252	1		
Total protein (mg)	0.399	-0.116	1	
Myofibrillar Protein (mg/g)	0.340	-0.570	0.683*	1

THI-Temperature-humidity-index; *P<0.05

4.10. Proximate composition

The Mean ± SE of proximate composition of LTL muscle in KC and KHS groups were described in Table 4.18. Heat stress did not influence (P>0.05) any of the proximate composition such as moisture, fat, ash, and protein percentage of LTL muscle (Table 4.18). Furthermore, THI also did not showed any correlation (P>0.05) with any of the proximate composition of LTL muscle (Table 4.19).

Table: 4.18 Effect of heat stress on proximate composition in Kodi Aadu control and heat stressed groups

Traits	Kodi Aadu		Significance
	KC	KHS	
Moisture (%)	77.68±0.30	76.94±0.39	NS
Protein (%)	17.52±0.27	17.51±0.26	NS
Fat (%)	2.60±0.22	2.61±0.26	NS
Ash (%)	1.80±0.05	1.84±0.11	NS

NS- Non-Significant, KC- Kodi Aadu Control, KHS- Kodi Aadu Heat Stress

Table:4.19 Correlation coefficient between THI and Proximate composition of *LTL* muscle

	THI	Moisture	Protein	Fat	Ash
THI	1				
Moisture	-0.330	1			
Protein	-0.009	-0.285	1		
Fat	0.068	-0.557	0.464	1	
Ash	0.097	-0.002	0.135	-0.274	1

THI- Temperature Humidity Index

4.11. Gene expression patterns in *LTL* muscle

The expression pattern difference in MSTN, CAPN1, CAPN2, CAST, CRYA, DAGT1, HSF1, HSP10, HSP27, HSP40, HSP60, HSP70, HSP90 and HSP110 between KC and KHS groups are described in table 4.20.

Table 4.20 Heat stress influence on the expression patterns of different target genes in relation to the house keeping genes

Gene	Group	Fold change	SE	p-value
MSTN	KC	1	0.22	0.00
	KHS	0.34	0.17	
CAPN1	KC	1	0.18	0.00
	KHS	0.31	0.19	
CAPN2	KC	1	0.23	0.03
	KHS	2.01	0.11	
CAST	KC	1	0.19	0.02
	KHS	2.09	0.18	
CRYA	KC	1	0.39	0.04
	KHS	4.58	0.50	
DAGT1	KC	1	0.18	0.01
	KHS	0.30	0.05	
HSF1	KC	1	0.13	0.12
	KHS	0.69	0.23	
HSP10	KC	1	0.03	0.78
	KHS	0.98	0.10	
HSP27	KC	1	0.36	0.02
	KHS	4.00	0.11	
HSP40	KC	1	0.18	0.02
	KHS	0.47	0.22	
HSP60	KC	1	0.16	0.08
	KHS	0.69	0.04	
HSP70	KC	1	0.23	0.36
	KHS	0.82	0.18	
HSP90	KC	1	0.05	0.04
	KHS	1.95	0.22	
HSP110	KC	1	0.26	0.09
	KHS	0.59	0.10	

MSTN-Myostatin, CAPN1- Calpain 1; CAPN2-Calpain 2; CAST-Calpastatin; CRYA- Crytallin alpha; DAGT1- Diacylglycerol Acyltransferase 1; HSF1-Heat Shock Factor 1; HSP-Heat Shock Protein

4.11.1. *MSTN* mRNA expression

The differences in the *LTL* muscle *MSTN* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.2**.** The *LTL* muscle *MSTN* mRNA expression in KC, and KHS groups were 1.00 ± 0.22, and 0.34 ± 0.17, respectively. The *MSTN* mRNA expression pattern was significantly (P<0.01) lower in KHS group as compared to KC group. Further, the THI had a mild positive correlation (P<0.05) with *MSTN* expression pattern (Table 4.21).

4.11.2. *CAPN1* mRNA expression

The differences in the *LTL* muscle *CAPN1* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.3**.** The *LTL* muscle *CAPN1* mRNA expression in KC, and KHS groups were 1.00 ± 0.18, and 0.31 ± 0.19, respectively. The *CAPN1* mRNA expression pattern was significantly (P<0.01) lower in KHS group as compared to KC group. Further, the THI had a strong positive correlation (P<0.01) with *CAPN1* expression pattern (Table 4.21).

Figure 4.2. Relative mRNA expression of *Myostastin* (*MSTN*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress

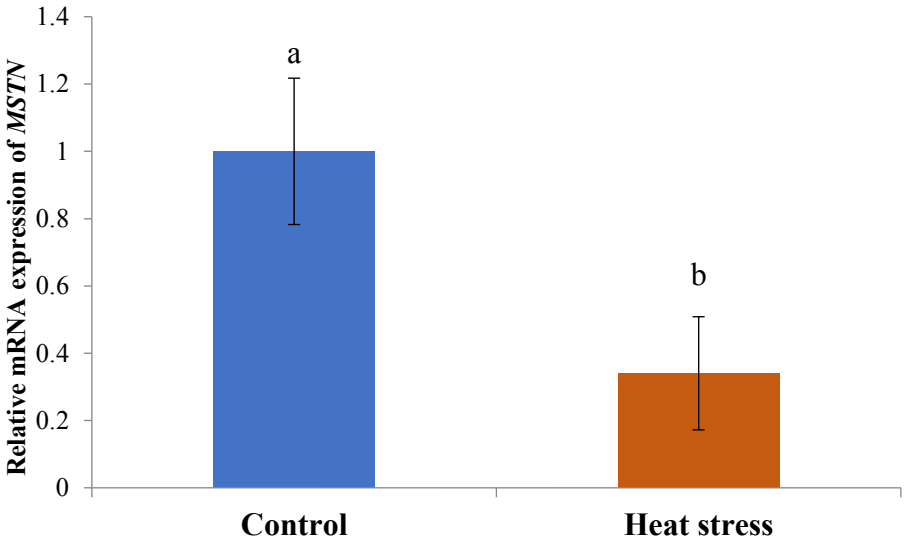
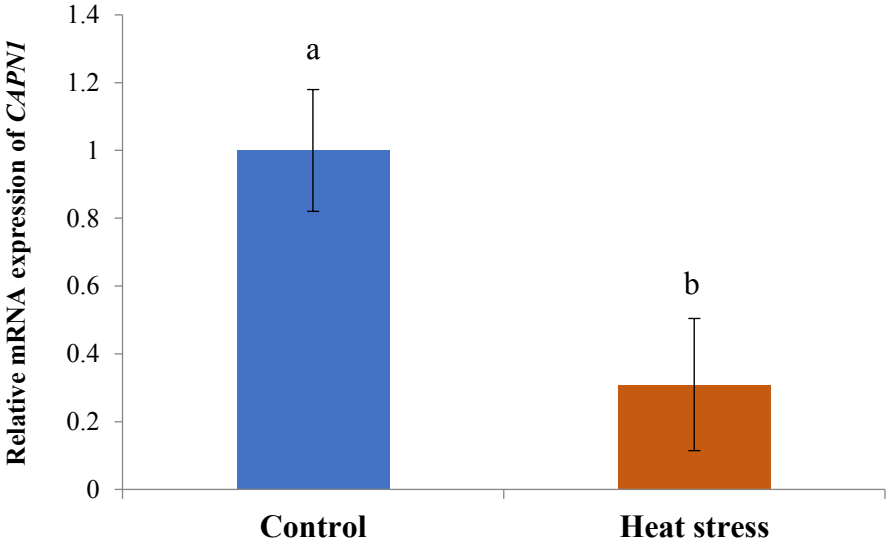


Figure 4.3. Relative mRNA expression of *Calpain 1* (*CAPN1*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress



4.11.3. *CAPN2* mRNA expression

The differences in the *LTL* muscle *CAPN2* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.4**.** The *LTL* muscle *CAPN2* mRNA expression in KC, and KHS groups were 1.00 ± 0.23, and 2.01 ± 0.11, respectively. The *CAPN2* mRNA expression pattern was significantly (P<0.05) higher in KHS group as compared to KC group. Further, the THI had a mild negative correlation (P<0.05) with *CAPN2* expression pattern (Table 4.21).

4.11.4. *CAST* mRNA expression

The differences in the *LTL* muscle *CAST* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.5**.** The *LTL* muscle *CAST* mRNA expression in KC, and KHS groups were 1.00 ± 0.19, and 2.09 ± 0.18, respectively. The *CAST* mRNA expression pattern was significantly (P<0.05) higher in KHS group as compared to KC group. Further, the THI had a mild negative correlation (P<0.05) with *CAST* expression pattern (Table 4.21).

Figure 4.4. Relative mRNA expression of Calpain 2 (CAPN2) in LTL muscle of Kodi Aadu goats subjected to heat stress

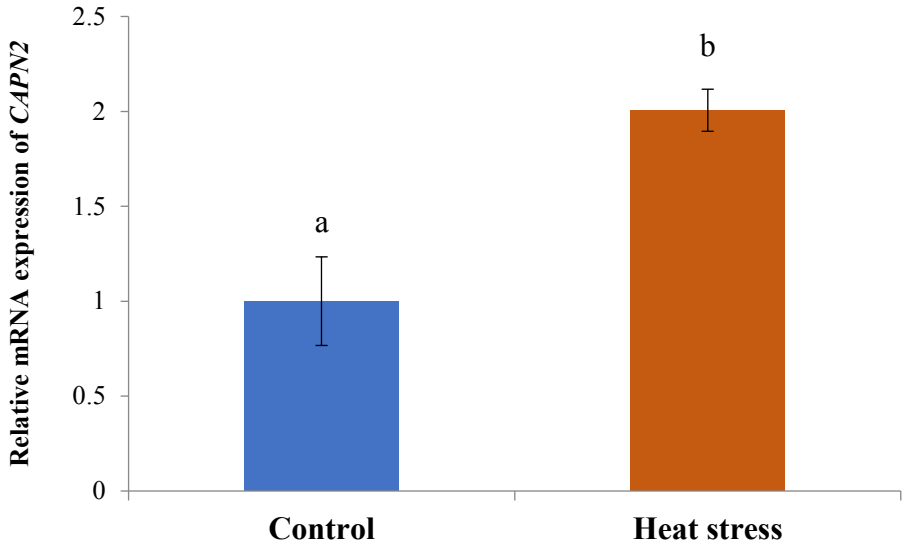
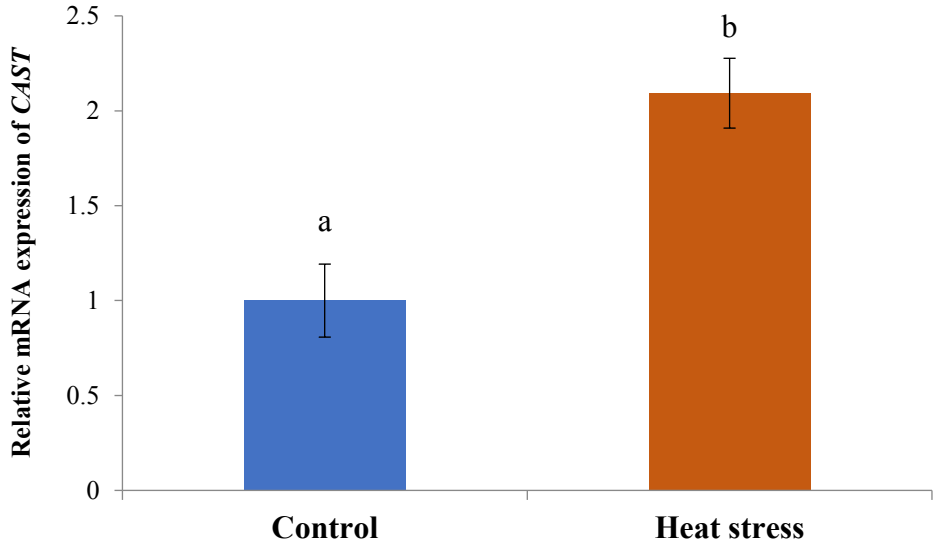


Figure 4.5. Relative mRNA expression of *Calpastatin* (*CAST*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress



4.11.5. *CRYA* mRNA expression

The differences in the *LTL* muscle *CRYA* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.6**.** The *LTL* muscle *CRYA* mRNA expression in KC, and KHS groups were 1.00 ± 0.39, and 4.58 ± 0.50, respectively. The *CRYA* mRNA expression pattern was significantly (P<0.05) higher in KHS group as compared to KC group. Further, the THI had a mild negative correlation (P<0.05) with *CRYA* expression pattern (Table 4.21).

4.11.6. *DGAT1* mRNA expression

The differences in the *LTL* muscle *DGAT1* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.7**.** The *LTL* muscle *DGAT1* mRNA expression in KC, and KHS groups were 1.00 ± 0.18, and 0.30 ± 0.05, respectively. The *DGAT1* mRNA expression pattern was significantly (P<0.01) lower in KHS group as compared to KC group. Further, the THI had a strong positive correlation (P<0.01) with *DGAT1* expression pattern (Table 4.21).

Figure 4.6. Relative mRNA expression of *Crytallin alpha* (*CRYA*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress

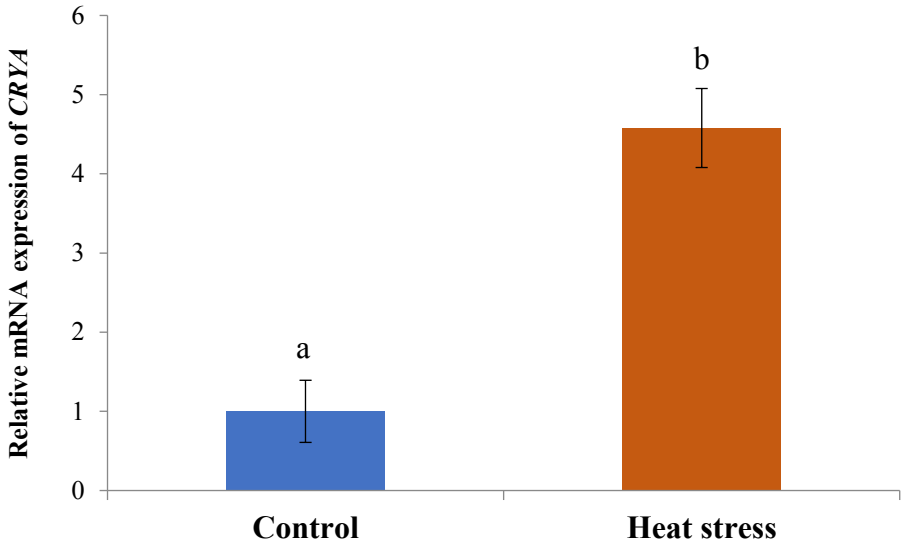
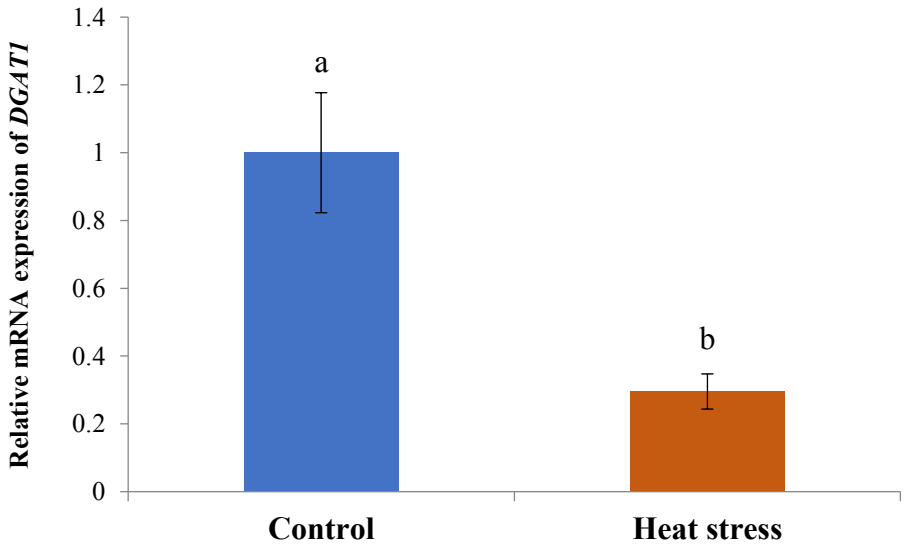


Figure 4.7. Relative mRNA expression of *Diacylglycerol Acyltransferase 1* (*DAGT1*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress



4.11.7. *HSF1* mRNA expression

The differences in the *LTL* muscle *HSF1* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.8**.** The *LTL* muscle *HSF1* mRNA expression in KC, and KHS groups were 1.00 ± 0.13, and 0.69 ± 0.23, respectively. The *HSF1* mRNA expression pattern was comparable (P>0.05) between the KC and KHS groups. Further no significant correlation was established between THI and *HSF1* expression pattern (Table 4.21).

4.11.8. *HSP10* mRNA expression

The differences in the *LTL* muscle *HSP10* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.9**.** The *LTL* muscle *HSP10* mRNA expression in KC, and KHS groups were 1.00 ± 0.03, and 0.98 ± 0.1, respectively. The *HSP10* mRNA expression pattern was comparable (P>0.05) between the KC and KHS groups. Further no significant correlation was established between THI and *HSP10* expression pattern (Table 4.21).

Figure 4.8. Relative mRNA expression of *Heat shock factor 1* (*HSF1*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress

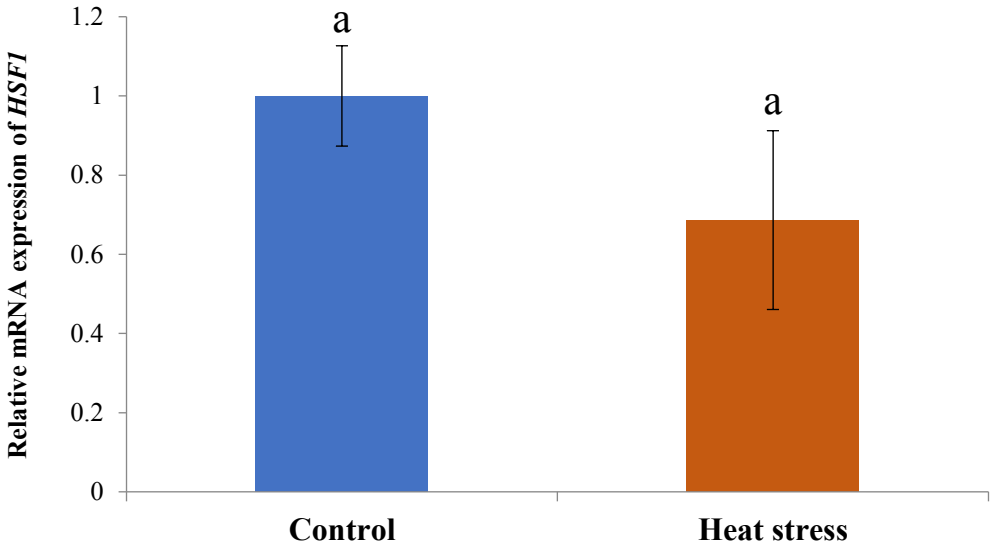
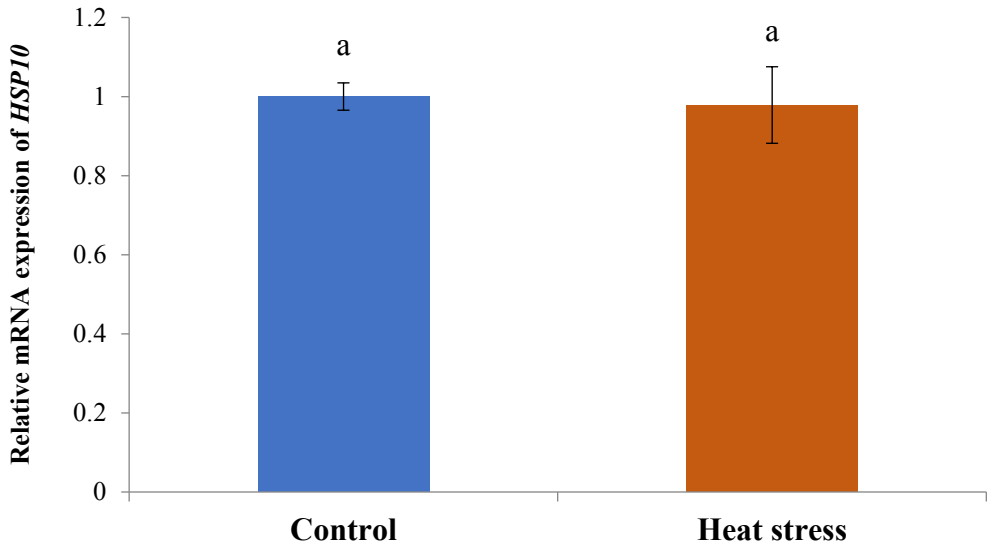


Figure 4.9. Relative mRNA expression of *Heat shock protein 10* (*HSP10*) in *LTL* muscle of Kodi Aadu goats subjected to heat stress



4.11.9. *HSP27* mRNA expression

The differences in the *LTL* muscle *HSP27* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.10**.** The *LTL* muscle *HSP27* mRNA expression in KC, and KHS groups were 1.00 ± 0.36, and 4.00 ± 0.11, respectively. The *HSP27* mRNA expression pattern was significantly (P<0.05) higher in KHS group as compared to KC group. Further, the THI had a strong negative correlation (P<0.01) with *HSP27* expression pattern (Table 4.21).

4.11.10. *HSP40* mRNA expression

The differences in the *LTL* muscle *HSP40* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.11**.** The *LTL* muscle *HSP40* mRNA expression in KC, and KHS groups were 1.00 ± 0.18, and 0.47 ± 0.22, respectively. The *HSP40* mRNA expression pattern was significantly (P<0.05) lower in KHS group as compared to KC group. Further, the THI had a mild positive correlation (P<0.05) with *HSP40* expression pattern (Table 4.21).

Figure 4.10. Relative mRNA expression of *HSP27* in *LTL* muscle of Kodi Aadu goats subjected to heat stress

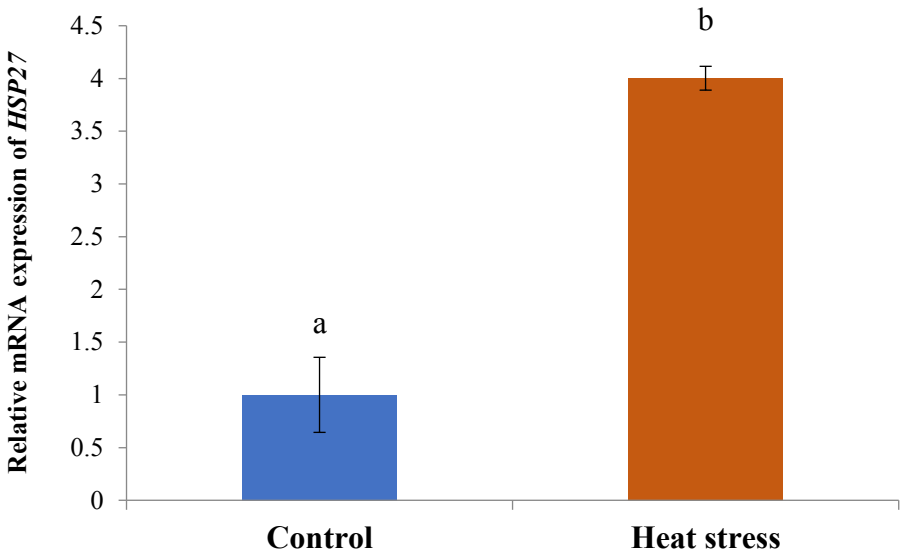
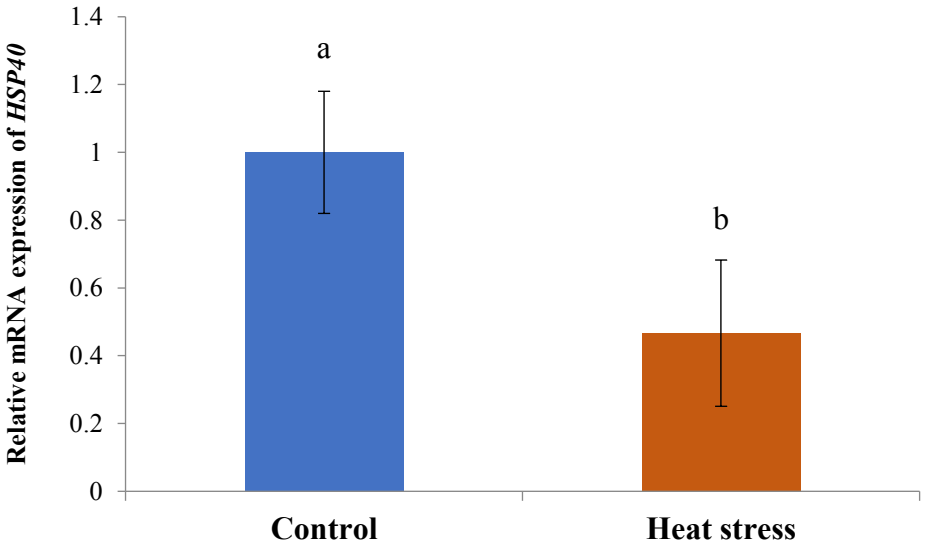


Figure 4.11. Relative mRNA expression of *HSP40* in *LTL* muscle of Kodi Aadu goats subjected to heat stress



4.11.11. *HSP60* mRNA expression

The differences in the *LTL* muscle *HSP60* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.12. The *LTL* muscle *HSP60* mRNA expression in KC, and KHS groups were 1.00 ± 0.16, and 0.69 ± 0.04, respectively. The *HSP60* mRNA expression pattern was comparable ($P>0.05$) between the KC and KHS groups. Further, the THI had a mild positive correlation ($P<0.05$) with *HSP60* expression pattern (Table 4.21).

4.11.12. *HSP70* mRNA expression

The differences in the *LTL* muscle *HSP70* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.13. The *LTL* muscle *HSP70* mRNA expression in KC, and KHS groups were 1.00 ± 0.23, and 0.82 ± 0.18, respectively. The *HSP70* mRNA expression pattern was comparable ($P>0.05$) between the KC and KHS groups. Further no significant correlation was established between THI and *HSP70* expression pattern (Table 4.21).

Figure 4.12. Relative mRNA expression of *HSP60* in *LTL* muscle of Kodi Aadu goats subjected to heat stress

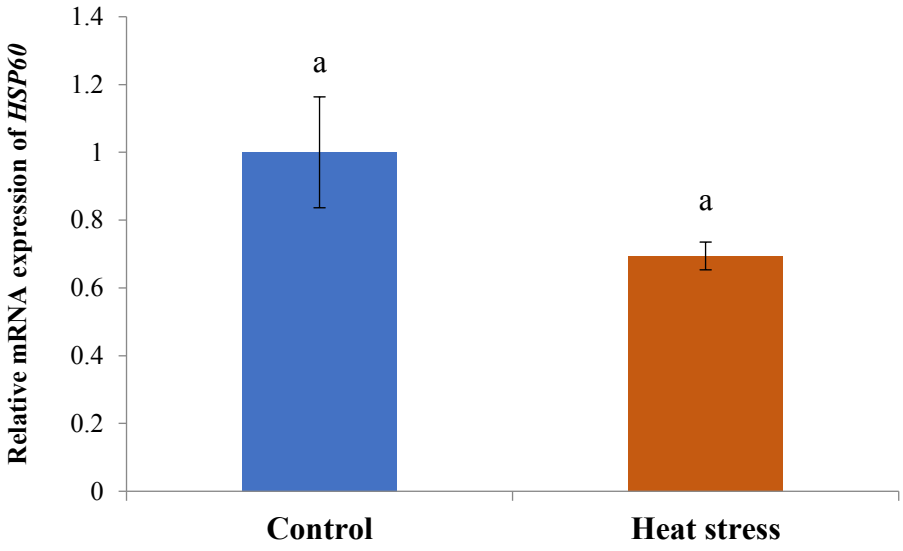
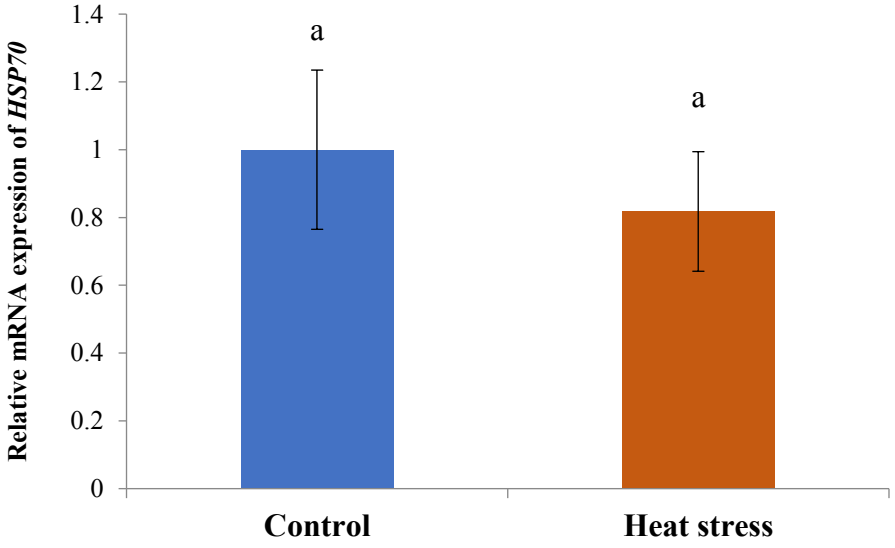


Figure 4.13. Relative mRNA expression of *HSP70* in muscle *LTL* of Kodi Aadu goats subjected to heat stress



4.11.13. *HSP90* mRNA expression

The differences in the *LTL* muscle *HSP90* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.14**.** The *LTL* muscle *HSP90* mRNA expression in KC, and KHS groups were 1.00 ± 0.05, and 1.95 ± 0.22, respectively. The *HSP90* mRNA expression pattern was significantly ($P<0.05$) higher in KHS group as compared to KC group. Further, the THI had a mild negative correlation ($P<0.05$) with *HSP90* expression pattern (Table 4.21).

4.11.14. *HSP110* mRNA expression

The differences in the *LTL* muscle *HSP110* mRNA expression pattern between the control and heat stress groups of Kodi Aadu breed was described in Fig. 4.15. The *LTL* muscle *HSP110* mRNA expression in KC, and KHS groups were 1.00 ± 0.26, and 0.59 ± 0.10, respectively. The *HSP110* mRNA expression pattern was comparable ($P>0.05$) between the KC and KHS groups. Further no significant correlation was established between THI and *HSP110* expression pattern (Table 4.21).

Figure 4.14. Relative mRNA expression of *HSP90* in *LTL* muscle of Kodi Aadu goats subjected to heat stress

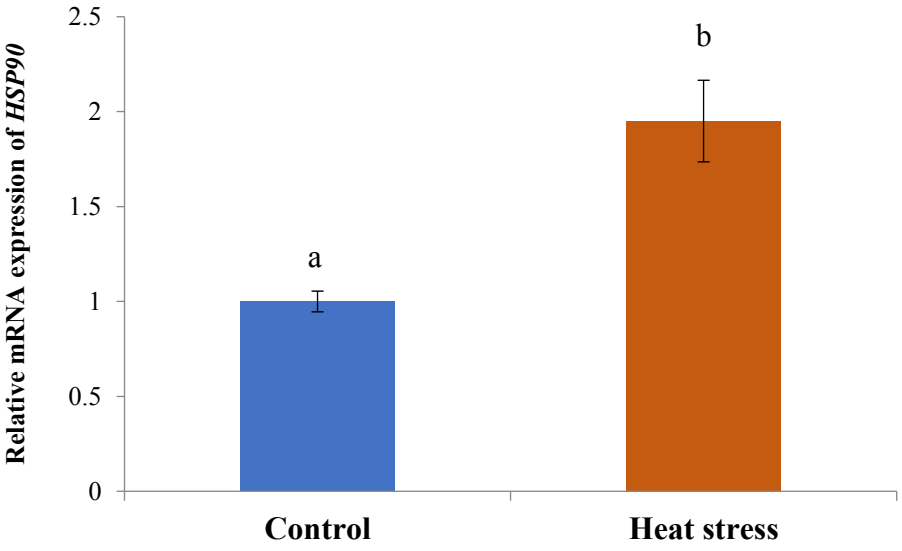


Figure 4.15. Relative mRNA expression of *HSP110* in *LTL* muscle of Kodi Aadu goats subjected to heat stress

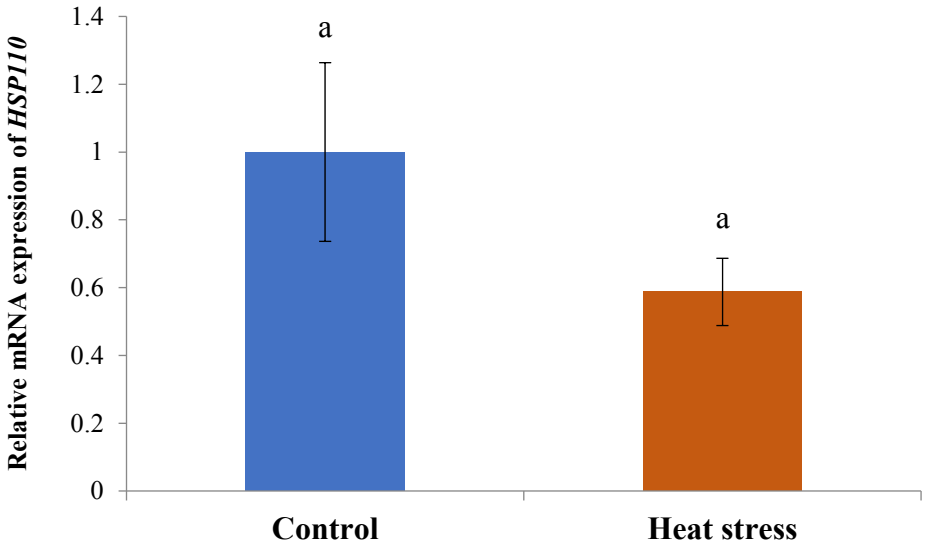


Table 4.21. Correlation between THI and all targeted gene expression patterns

	THI	MSTN	CAPN1	CAPN2	CAST	CRYA	DAGT1	HSF1	HSP10	HSP27	HSP40	HSP60	HSP70	HSP90	HSP110
THI	1														
MSTN	0.900*	1													
CAPN1	0.954**	0.866*	1												
CAPN2	-0.889*	-0.915*	-0.742	1											
CAST	-0.895*	-0.802	-0.870*	0.774	1										
CRYA	-0.832*	-0.748	-0.732	0.836*	0.819*	1									
DAGT1	0.979**	0.952**	0.940**	-0.913*	-0.917*	-0.869*	1								
HSF1	0.724	0.862*	0.775	-0.659	-0.736	-0.400	0.775	1							
HSP10	0.151	0.165	0.141	-0.193	0.051	-0.475	0.192	-0.276	1						
HSP27	-0.937**	-0.947**	-0.839*	0.964**	0.775	0.741	-0.933**	-0.768	-0.101	1					
HSP40	0.890*	0.974**	0.891*	-0.845*	-0.835*	-0.647	0.930**	0.946**	-0.030	-0.917**	1				
HSP60	0.842*	0.742	0.911*	-0.615	-0.921**	-0.796	0.871*	0.659	0.178	-0.645	0.765	1			
HSP70	0.430	0.367	0.545	-0.181	-0.136	0.083	0.321	0.480	-0.114	-0.429	0.452	0.218	1		
HSP90	-0.908*	-0.771	-0.760	0.918**	0.782	0.918**	-0.882*	-0.435	-0.324	0.874*	-0.689	-0.677	-0.158	1	
HSP110	0.806	0.628	0.773	-0.630	-0.576	-0.394	0.686	0.589	-0.143	-0.787	0.682	0.490	0.781	-0.670	1

DISCUSSION

CHAPTER 5
DISCUSSION

This study gains significance as the world is battling to feed the growing human population of 9.6 billion by 2050. Livestock and particularly the small ruminants are tipped to be playing a huge role in ensuring protein availability by 2050. Compared with sheep, goats are better adapted to tropical climate as it is evident from the distribution of goat population in tropical countries. The potential to survive in diversified climatic condition, drought tolerance, ability to survive on limited pasture, ability to walk long distances in search of limited pasture, possessing unique feeding behaviour which imparts them the potential to consume any feed which is not suitable for consumption by any other livestock species makes goats to be the ideal species to survive and produce optimally in the changing climate scenario. However, breed variations were observed in all above traits in goats. Therefore, it is very vital to generate all baseline information pertaining to climate resilience in indigenous goat breeds. Such information would be very vital for identifying and disseminating the ideal goat breed to any specific agro-ecological zone for the marginal farmers to get benefit out of goat farming. This study is one such attempt to elucidate the production potential of Kodi Aadu goat breed in terms of meat production characteristics and quality traits when these animals were subjected to heat stress.

5.1. Weather variables & Temperature Humidity Index

The weather variables recorded and the THI calculated based on the prevailing microclimate clearly demonstrated the respective microclimate proposed for control and heat stress group respectively. The comparable levels of THI in both thermo-neutral and heating chambers during morning hours reflected the common microclimate between the thermo-neutral and heating chambers while the THI during afternoon clearly differentiated the microclimate reflecting the extreme heat stress condition for KHS group while inducing ideal comfort condition for KC group. The THI was calculated as per McDowell (1972) and according to this thermal index any value over 78 are considered extremely stressful while any value above 72 is considered comfortable. Thus, the average THI obtained in this study for thermo-neutral and heating climate chambers were 69.42 and 94.76, respectively. Thus, the hypothesis of inducing heat stress to the KHS group animals was justified as these animals were exposed to the THI which falls in the extreme distress category. Similar observation of inducing heat stress based on McDowell (1972) index was also established by other researchers in goats (Shilja et al., 2016; Aleena et al., 2018). These findings suggests that the heat stress group animals in this study was ensured of subjecting them to extremely severe heat stress while the control group animals were subjected to thermo-neutral condition.

5.2. Heat stress influence on major carcass traits

Heat stress did not alter any of the major carcass traits variables such as pre-slaughter weight, hot carcass weight, dressing percentage and loin eye area (LEA). This shows the extreme climate resilience potential of Kodi Aadu breed as even the very high extremely severe heat stress could not induce any negative impact on the major carcass traits. Animals apart from adapting to the adverse environmental condition, when they maintain their productive responses are considered extremely climate resilient (Sejian et al., 2018). Thus, Kodi Aadu breed could be considered climate resilient breed. However, there were contrasting reports which established negative influence of heat stress either in single or multiple major carcass traits in farm animals (Rout *et al*., 2017; Tadesse *et al*., 2019; Wang *et al*., 2020). Hashem *et al*. (2013) also established similar non-significant influence of heat stress on both PSW and HCW in Black Bengal goats. However, Archana et al. (2018) observed negative influence of heat stress on PSW, HCW and LEA in both Osmanabadi and Salem Black goat breeds. These findings projects that even among indigenous breeds which are considered thermo-tolerant there are differences established in their productive response. Thus, comparatively by keeping intact all major carcass traits, Kodi Aadu breed can be considered a superior thermo-tolerant breed.

5.3. Heat stress influence on non-carcass components and offals

Like major carcass traits, most of the non-carcass components and offals also did not differ between the groups reflecting the coping ability of Kodi Aadu breed to heat stress challenges without compromising these productive variables.

The heart, brain and reproductive organs differed for heat stress treatment. The increased reproductive organ weight in KHS group could reflect the superiority of this breed to maintain the reproductive functions. The significant effect of heat stress on both heart and brain could be to maintain the adaptive behaviour of the heat stressed goats in this study. Additionally, there were no any correlation established between THI and non-carcass variables and offals indicating the extreme adaptive nature of Kodi Aadu breed. Likewise, Sen et al. (2004) also observed no influence of heat stress on non-carcass components in goats attributing this to the extreme adaptive nature of these animals. Similar to our finding Archana et al. (2018) observed no difference in any of the non-carcass traits and offals in Osmanabadi breed but in contrast observed negative influence of heat stress on majority of these variables in Salem Black breed. This diversified finding again establishes the fact that there are breed variations for the productive performance of thermo-tolerant goat breeds.

5.4. Heat stress influence on primal cuts

Except loin cut, rest all primal cut variables remained intact between the KC and KHS groups. This shows that Kodi Aadu breed maintained its productive response irrespective of being exposed to extremely severe heat stress. In a similar study Archana et al. (2018) established significant negative influence of heat stress only on rack cut in Osmanabadi breed and they attributed this to the adaptive nature of this breed being native to the location of the study. However, the same authors observed variations in fore saddle, leg and breast cuts in Salem Black goat breed. These findings across these studies again reflect the breed differences in response

to heat stress in goats. Even almost all linear carcass measurements also remained intact between the KC and KHS groups except shoulder circumference. However, varied results were obtained for the effect of heat stress on linear carcass measurements in sheep and goat (Rana *et al*., 2014; Archana et al., 2018). In the study by Archana et al., it was observed that all the linear carcass measurements remained intact in Osmanabadi breed while in Salem Black breed; heat stress significantly reduced external carcass and leg length. In addition, the non-influence of heat stress on primal cuts and linear carcass measurements was also evident from no correlation established between THI and any of these variables in this study.

5.5. Heat stress influence on physico-chemical attributes

Again, except collagen solubility rest all physic-chemical attributes remained intact between KC and KHS group animals. This again reflects the supreme potential of Kodi Aadu breed to keep intact these vital meat quality variables even after exposure to chronic heat stress of very high magnitude. However, Archana et al. (2018) reported significant influence of heat stress on meat pH and shear force in Osmanabadi breed and cooking loss and shear force in Salem Black breed. The meat pH is usually altered in heat stressed animals as reported in goat (Kadim et al., 2008; Hashem et al., 2013) and cattle (Kadim et al., 2004). Like the ultimate pH, drip loss, the cooking loss, water holding capacity also remained intact reflecting the extreme adaptive nature of Kodi Aadu breed to maintain these vital meat quality variables. However, contradictory reports of significant alteration in all these variables were reported in other indigenous goat breeds (Kadim et al., 2009; Archana et al., 2018; Van Wyk et al., 2020). Shear force is another vital meat

quality variable which determines the meat tenderness and eating quality of meat (Chulayo & Muchenje, 2013). However, there are contradictory reports on heat stress induced changes in shear force value. There are reports which suggested recording higher shear force value in heat stressed animals (Zhang *et al.,* 2012; Goo *et al*. 2019; Archana *et al.,* 2018). Contrary to these findings, Kadim *et al*. (2004) recorded lower shear force values in animals exposed to heat stress. Although the heat stress treatment did not influence meat pH and CL still THI had a positive correlation with both these variables. This reflects the importance of these variables to judge the effect of heat stress on meat quality in goats.

5.6. Heat stress influence on sensory attributes

Heat stress did not influence any of the sensory attributes. This was evident from the no change in appearance, flavour, juiciness, tenderness and overall acceptability scores between KC and KHS groups. However, in a similar study conducted on Osmanabadi and Salem Black goat breeds Archana et al. (2018) reported significant reduction in appearance score in Osmanabadi breed while significantly reduced the scores for sensory attributes in Salem Black breed. Thus, the no effect of heat stress on any of the sensory attributes in this study clearly demonstrates the superiority of Kodi Aadu breed to cope with heat stress as compared to Osmanabadi and Salem Black breed. Further there are other reports which established significant alterations of sensory variables (Zhang et al., 2012; Nikbin et al., 2018), appearance of meat is altered during heat stress condition in animals and this was attributed to the water imbalances occurring in muscle and disturbances in the various physico-chemical properties of meat. Since the heat

stress did not alter any of the physico-chemical attributes in this study and this could have paved way for keeping intact all sensory variables in this study in Kodi Aadu goats. Further, a negative correlation was established between THI tenderness and overall acceptability. Inspite of this negative correlation, Kodi Aadu breed kept intact these vital meat quality variables establishing the climate resilience potential of this breed.

5.7. Heat stress influence on meat color

Among the color variables, only yellowness showed significant variation for heat stress treatment between the groups. Both L* and a* remained intact in Kodi Aadu breed after heat stress exposure. Similar to our findings, Archana et al. (2018) also did not observed any changes in L* and a* after heat stress exposure in two indigenous Osmanabadi and Salem Black goat breeds (Archana *et al*., 2018). This shows the inherent potential of these indigenous breeds to maintain the vital color variables of meat even in extreme stressful condition. In contrast to L* and a*, increased b* was recorded in KHS group as compared to KC group. This was in contrast to the findings of Archana et al. (2018) who did not observe any changes in the b* after heat stress exposure in both Osmanabadi and Salem Black goat breed. This shows the breed variation for heat stress associated changes in b* among the indigenous breeds of goats. However, like L* and a* there was no significant difference in both myoglobin content and met-myoglobin per centage between KC and KHS group again reflecting the potential of Kodi Aadu breed to keep intact these vital color variables. Inspite of no influence of heat stress on L*and a*, still the THI had strong negative correlation with L* and positive correlation with a*

and b*. This shows that these vital color variables could be serve as important indicators of meat quality.

5.6. Heat stress influence on soluble proteins

Again the heat stress did not alter any of the soluble proteins such as the sarcoplasmic, total and myofibrillar proteins remained intact between KC and KHS groups. However, Zhu et al. (2012) observed that meat with low WHC and low pH also exhibited substantially lower sarcoplasmic and total protein than normal meat. Presently not many reports are available lining heat stress with muscle soluble proteins and hence it was not possible to discuss these results further. It is the general observation that the body protein contents are reduced when the animals are subjected to heat stress of both acute and chronic magnitude (Zhang et al., 2012). Further the proximate composition variables also did not show any variations for the heat stress treatment in Kodi Aadu. The moisture, protein, ash, and fat percentages did not vary between KC and KHS groups. This was similar to the findings reported by Archana et al. (2018) in both Osmanabadi and Salem Black breeds wherein they reported no significant influence of heat stress on any of the proximate composition variables. These results indicate that inspite of subjecting the Kodi Aadu breed to high magnitude of heat stress still the nutritional composition of meat remained the same reflecting the excellent climate resilient potential of this breed.

5.7. Gene expression patterns

5.7.1. *MSTN* mRNA expression in *LTL* muscle

The *MSTN* mRNA expression pattern was significantly lower in KHS group as compared to KC. The *MSTN* gene is the growth inhibitor and regulates the muscle development and differentiation (Gabriel *et al*., 2003). The reduced level of *MSTN* expression during heat stress exposure was similar to the finding established by Archana et al. (2018) in Osmanabadi breed. These authors opined that the reduced level of *MSTN* expression could be to avoid further deterioration of meat quality. This shows that heat stressed animals down regulate the expression pattern *MSTN* gene to maintain meat quality. This fact was supported by the no effect of heat stress on majority of meat quality variables such muscle pH, drip loss, WHC and shear force. By down regulating the *MSTN* expression Kodi Aadu goats exhibited their inherent ability to regulate muscle growth. However, Archana et al. (2018) also reported no effect of heat stress on *MSTN* expression pattern in Salem Black breed. This shows even among adapted goat breeds there is variation in the expression pattern of *MSTN* gene.

5.7.2. *CAPN1, CAPN2* and *CAST mRNA* expression in *LTL* muscle

Heat stress significantly reduced the expression pattern of *CAPN1* in *LTL* muscle. Calpain system is the most well-known proteolytic system which affects meat tenderness (Huff-Lonergan and Lonergan, 1999). Both *CAPN1* and *CAPN2* have the capability of degrading myofibrillar and cytoskeletal proteins to maintain tenderness in meat (Koohmaraie and Geesink, 2006; Bhat et al., 2018). The

significant low expression pattern of *CAPN1* in the current study could indicate the compromised meat tenderness after heat stress exposure. However, the increased expression of *CAPN2* could be the indicator of high meat tenderness. These results show that Kodi Aadu breed has the capability to maintain tenderness to certain extent although exposed to very high magnitude of heat stress. Conversely, the high activity of their specific inhibitor, calpastatin, is related to the low degradation of myofibrillar proteins and low meat tenderness (Koohmaraie and Geesink, 2006; Lonergan et al., 2010). The expression pattern of *CAST* was significantly higher in KHS group as compared to KC group. This further indicates that the meat tenderness was getting compromised as a result of exposure to heart stress in Kodi Aadu breed. Two genes (*CAPN2* and *CAST*) out of three targeted in calpain system got over expressed and although such expression pattern was correlated with low meat tenderness still the muscle tenderness was comparable between KC and KHS groups indicating coordinated activities of genes associated with calpain system to maintain tenderness in Kodi Aadu breed.

5.7.3. *DAGT1 mRNA* expression in *LTL* muscle

Heat stress significantly down regulated the expression pattern of *DAGT1*. The *DAGT1* gene is involved in fatty acid metabolism are considered as potential candidate genes for meat tenderness (Cases et al., 1998). Therefore, the increased level of *DAGT1* gene expression is directly proportional to muscle tenderness. However, in this study lower expression of *DAGT1* was established after heat stress exposure in Kodi Aadu breed. But this lower expression may be of very less

magnitude to induce low meat tenderness. This was evident from the no effect of heat stress on meat tenderness in this current study.

5.7.4. *Heat shock factor 1* and different *HSPs* expression in *LTL* muscle

The HSF1 expression pattern was comparable between KC and KHS groups. The regulation of expression of HSF1 is highly complex with many external factors influencing it and no consistent reports are available especially on the level of heat stress that is required to trigger HSF1 expression (Tomanek and Somero., 2002; Madhusoodan et al., 2020). Exposure to heat stress triggers the activation of HSF1 to stimulate the production of HSPs (Das et al., 2016). Therefore, activation of HSF1 is very crucial to induce secretion of HSPs to regulate the cellular functions in heat stressed animals (Madhusoodan et al., 2020). Further, HSF1 was established to be an ideal marker for breeding dairy cattle for thermo-tolerance (Li et al., 2011). Therefore, the comparable level of *HSF1* expression between KC and KHS indicates the better climate resilience capacity of Kodi Aadu breed. Similar to HSF1 expression, the expression pattern of *HSP10, HSP60, HSP70 and HSP110* also was comparable between KC and KHS groups. This could be attributed to the non-significant level of *HSF1* to trigger sufficient heat sock response in Kodi Aadu breed to induce changes in these classical molecular chaperones reflecting the extreme climate resilient potential of this breed.

There are reports which established *HSP70* as marker for reflecting meat quality in livestock (Xing et al., 2017; Archana et al., 2018). HSP70 are molecular chaperones primarily involved in repairing damaged tissue and also helps in

preventing the unfolding and misaggregation of proteins during stressful condition (Chauhan et al., 2014; Parkunan et al., 2017). Thus, quantifying heat stress associated *HSP70* gene expression in skeletal muscle exposure gains significance from meat quality perspectives. The non-significant influence of heat stress on the expression pattern of *HSP70* in *LTL* muscle clearly demonstrates the climate resilience of Kodi Aadu breed. In a similar study in Osmanabadi and Salem Black goat breeds, Archana et al. (2018) established heat stress induced higher expression pattern of *HSP70* in *LTL* muscle and these authors correlated this to the higher adaptive capacity of these two breeds to heat stress. This shows that the non-significant influence of heat stress on the expression pattern of *HSP70* in this study clearly demonstrates the better efficiency of Kodi Aadu breed to cope with heat stress as compared to Osmanabadi and Salem Black breeds. Thus, HSP70 mRNA expression in LTL muscle could serve as ideal biomarker for quantifying the heat stress associated deterioration in meat quality in indigenous goat breeds. However, the expression pattern of *HSP40* was significantly lower in heat stressed animals. Both *HSP70* and *HSP40* similar in function and the main action of *HSP40* were to reduce the oxidative stress in heat stressed animals (Picard et al., 2014; Mullins et al., 2016). Thus, the lower expression of *HSP40* clearly demonstrates the sub threshold level of heat stress attained in the study reflecting the coping ability of Kodi Aadu goats when subjected even to extremely severe heat stress.

The *HSP27* expression pattern was significantly higher in KHS group as compared to KC group. In particular, *HSP27* can interrupt the apoptosis cascade and can stabilize and protect muscle proteins to calpains action (Lomiwes et al.,

2014). Thus, the higher expression of *HSP27* in the current study in Kodi Aadu breed reflects the superior ability of this breed to maintain meat quality. The *HSP27* elicits its action in coordination with the action of other small *HSP*, such as αβ-crystallin (CRYA) (Lomiwes et al., 2014). The *αβ-crystallin* is also referred as *HSPB5* or *CRYA* gene in farm animals. The higher expression of *CRYA* in KHS group supports this argument and thus the similar higher expression of both *HSP27* and *CRYA* could be to act in coordinated way to maintain meat quality in Kodi Aadu breed. Further, the higher expression of *HSP90* in KHS group as compared to KC group clearly demonstrates the thermo-tolerance of Kodi Aadu breed. The *HSP90* was found to be associated with better thermo-tolerance in dairy cattle (Deb et al., 2014) and these authors observed that increased expression of *HSP90* was to regulate effectively the body temperature and to prevent cellular damage in heat stressed indigenous Sahiwal cattle. Thus, the higher expression of *HSP90* in the Kodi Aadu breed could be to maintain thermal balance and prevent cellular damage due to heat exposure. The significant alteration in the expression patterns of *MSTN, CAPN1, CAPN2, HSP27, CRYA* and *HSP90 genes* and the significant correlation of THI with these genes clearly indicates the significance of these genes for reflecting the productive potential of Kodi Aadu goat breed in tropical climate.

Thus, from the study it was clearly evident that Kodi Aadu breed possessed excellent climate resilient potential. This was evident from the non-influence of heat stress of very high magnitude on major carcass traits, non-carcass components and offals, primal cuts, linear carcass measurements, physic-chemical attributes, sensory attributes, meat color and LTL soluble proteins and meat quality associated

gene expression patterns. Most of the diversified variables associated with carcass characteristics and meat quality remained intact in this breed after heat stress exposure clearly demonstrates the very high potential of this breed to produce optimally after coping with the extremely stressful environment. The *MSTN, HSP27, CRYA* and *HSP90* genes could serve as biomarkers for reflecting meat producing capability of Kodi Aadu breed in tropical climate. Hence, the Kodi Aadu breed could be considered ideal meat producing indigenous goat breed to survive in hot tropical climate of Southern India. Further, this breed could be recommended to the poor and marginal farmers of the specific agro-ecological zone of their origin to ensure their livelihood security.

SUMMARY & CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

This study gains significance as the world is battling to feed the growing human population of 9.6 billion by 2050. Livestock and particularly the small ruminants are tipped to be playing a huge role in ensuring protein availability by 2050. Compared with sheep, goats are better adapted to tropical climate as it is evident from the distribution of goat population in tropical countries. The potential to survive in diversified climatic condition, drought tolerance, ability to survive on limited pasture, ability to walk long distances in search of limited pasture, possessing unique feeding behaviour which imparts them the potential to consume any feed which is not suitable for consumption by any other livestock species makes goats to be the ideal species to survive and produce optimally in the changing climate scenario. However, breed variations were observed in all above traits in goats. Therefore, it is very vital to generate all baseline information pertaining to climate resilience in indigenous goat breeds. Such information would be very vital for identifying and disseminating the ideal goat breed to any specific agro-ecological zone for the marginal farmers to get benefit out of goat farming. This study is one such attempt to elucidate the production potential of Kodi Aadu goat breed in terms of meat production characteristics and quality traits when these animals were subjected to heat stress.

The study was conducted for a period of 45 days in controlled climate chamber between May-June, 2020. Twelve female (one year old) Kodi Aadu goats

were used in the present study. The animals were randomly allocated into two groups of six animals each, KC (n=6; Control), KHS (n=6; heat stress). The animals were stall fed with a diet consisting of 60 per cent roughage (Hybrid Napier hay) and 40 per cent concentrate (Maize 36 kg, wheat bran 37 kg, soybean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5 kg per 100kg). All animals were individually given access to ad-libitum feed and water. The control (C) goats were placed in the thermo neutral zone (TNZ), *i.e*., control chamber, while the heat stress (HS) goats were exposed to heat stress in heating chamber with a simulated heat stress model between 10.00AM to 4:00PM on all 45 days. All cardinal weather parameters were recorded twice daily for the entire duration of the study. The animals were slaughtered at the end of the study to assess the meat and carcass characteristics. The muscle *longissimus thoracis et lumborum* (*LTL*) were collected for meat variable analysis gene expression study.

The weather variables recorded and the THI calculated based on the prevailing microclimate clearly demonstrated the respective microclimate proposed for control and heat stress group respectively. The comparable levels of THI in both thermo-neutral and heating chambers during morning hours reflected the common microclimate between the thermo-neutral and heating chambers while the THI during afternoon clearly differentiated the microclimate reflecting the extreme heat stress condition for KHS group while inducing ideal comfort condition for KC group. The THI was calculated as per McDowell (1972) and according to this thermal index any value over 78 are considered extremely stressful while any value above 72 is considered comfortable. Thus the average THI obtained in this study

for thermo-neutral and heating climate chambers were 69.42 and 94.76, respectively. Thus, the hypothesis of inducing heat stress to the KHS group animals was justified as these animals were exposed to the THIS which falls in the extreme distress category.

Heat stress did not alter any of the major carcass traits variables such as pre-slaughter weight, hot carcass weight, dressing percentage and loin eye area (LEA). This shows the extreme climate resilience potential of Kodi Aadu breed as even the very high extremely severe heat stress could not induce any negative impact on the major carcass traits. Thus, Kodi Aadu breed could be considered climate resilient breed.

Like major carcass traits, most of the non-carcass components and offals also did not differ between the groups reflecting the coping ability of Kodi Aadu breed to heat stress challenges without compromising these productive variables. The heart, brain and reproductive organs differed for heat stress treatment. The increased reproductive organ weight in KHS group could reflect the superiority of this breed to maintain the reproductive functions. The significant effect of heat stress on both heart and brain could be to maintain the adaptive behaviour of the heat stressed goats in this study. Additionally, there were no any correlation established between THI and non-carcass variables and offals indicating the extreme adaptive nature of Kodi Aadu breed.

Heat stress significantly influenced only loin variable among the primal cuts with significantly lower value in KHS group as compared to KC group. Further, the

heat stress did not reveal any significant variation in other primal cuts between KC and KHS group animals. Further, THI also did not showed correlation with any of the primary cuts variables This shows that Kodi Aadu breed maintained its productive response irrespective of being exposed to extremely severe heat stress. Even almost all linear carcass measurements also remained intact between the KC and KHS groups except shoulder circumference. In addition, the non-influence of heat stress on primal cuts and linear carcass measurements was also evident from no correlation established between THI and any of these variables in this study.

Again except collagen solubility rest all physico-chemical attributes remained intact between KC and KHS group animals. This again reflects the supreme potential of Kodi Aadu breed to keep intact these vital meat quality variables even after exposure to chronic heat stress of very high magnitude. Like the ultimate pH, drip loss, the cooking loss, water holding capacity also remained intact reflecting the extreme adaptive nature of Kodi Aadu breed to maintain these vital meat quality variables. Although the heat stress treatment did not influence meat pH and CL still THI had a positive correlation with both these variables. This reflects the importance of these variables to judge the effect of heat stress on meat quality in goats.

Heat stress did not influence any of the sensory variables such as appearance, flavour, juiciness, tenderness and overall acceptability. Furthermore, THI showed a strong negative correlation with tenderness and a mild negative correlation with overall acceptability score of *LTL* muscle. Thus, the no effect of heat stress on any of the sensory attributes in this study clearly demonstrates the

superiority of Kodi Aadu breed to cope with heat stress. Since the heat stress did not alter any of the physico-chemical attributes in this study and this could have paved way for keeping intact all sensory variables in this study in Kodi Aadu goats. Further, a negative correlation was established between THI tenderness and overall acceptability. Inspite of this negative correlation, Kodi Aadu breed kept intact these vital meat quality variables establishing the climate resilience potential of this breed.

Among the different color variables, only b* of *LTL* muscle showed significant difference for heat stress treatment. However, no significant difference could be observed in L* and a* of *LTL* muscle. Furthermore, THI showed a strong negative correlation with lightness and a mild positive correlation with redness and yellowness of *Longissimus dorsi* muscle However, like L* and a* there was no significant difference in both myoglobin content and met-myoglobin per centage between KC and KHS group again reflecting the potential of Kodi Aadu breed to keep intact these vital color variables. Inspite of no influence of heat stress on L*, and a* still the THI had strong negative correlation with L* and positive correlation with a* and b*. This shows that these vital color variables could be serve as important indicators of meat quality.

Heat stress did not influence any of the soluble proteins such as sarcoplasmic protein, total protein and myofibrillar protein of *LTL* muscle. Furthermore, THI also did not showed any correlation with any of the soluble proteins of *LTL* muscle. These results indicate that inspite of subjecting the Kodi

Aadu breed to high magnitude of heat stress still the nutritional composition of meat remained the same reflecting the excellent climate resilient potential of this breed.

The *MSTN* mRNA expression pattern was significantly lower in KHS group as compared to KC. By down regulating the *MSTN* expression Kodi Aadu goats exhibited their inherent ability to regulate muscle growth. Heat stress significantly reduced the expression pattern of *CAPN1* in *LTL* muscle. The significant low expression pattern of *CAPN1* in the current study could indicate the compromised meat tenderness after heat stress exposure. However, the increased expression of *CAPN2* could be the indicator of high meat tenderness. These results show that Kodi Aadu breed has the capability to maintain tenderness to certain extent although exposed to very high magnitude of heat stress. Further, the expression pattern of *CAST* was significantly higher in KHS group as compared to KC group. In addition, heat stress significantly down regulated the expression pattern of *DAGT1*. The increased level of *DAGT1* gene expression is directly proportional to muscle tenderness. However, in this study lower expression of *DAGT1* was established after heat stress exposure in Kodi Aadu breed. But this lower expression may be of very less magnitude to induce low meat tenderness. This was evident from the no effect of heat stress on meat tenderness in this current study.

The HSF1 expression pattern was comparable between KC and KHS group. The comparable level of *HSF1* expression between KC and KHS indicates the better climate resilience capacity of Kodi Aadu breed. Similar to HSF1 expression, the expression pattern of *HSP10, HSP60, HSP70 and HSP110* also was comparable between KC and KHS groups. This could be attributed to the non-significant level

of *HSF1* to trigger sufficient heat sock response in Kodi Aadu breed to induce changes in these classical molecular chaperones reflecting the extreme climate resilient potential of this breed. However, the expression pattern of *HSP40* was significantly lower in heat stressed animals. Thus, the lower expression of *HSP40* clearly demonstrates the sub threshold level of heat stress attained in the study reflecting the coping ability of Kodi Aadu goats when subjected even to extremely severe heat stress.

The *HSP27* expression pattern was significantly higher in KHS group as compared to KC group. Further, the *HSP27* elicits its action in coordination with the action of other small *HSP*, such as αβ-crystallin (CRYA). The higher expression of *CRYA* in KHS group supports this argument and thus the similar higher expression of both *HSP27* and *CRYA* could be to act in coordinated way to maintain meat quality in Kodi Aadu breed. Further, the higher expression of *HSP90* in KHS group as compared to KC group clearly demonstrates the thermo-tolerance of Kodi Aadu breed. The higher expression of *HSP90* in the Kodi Aadu breed could be to maintain thermal balance and prevent cellular damage due to heat exposure. The significant alteration in the expression patterns of *MSTN, CAPN1, CAPN2, HSP27, CRYA* and *HSP90 genes* and the significant correlation of THI with these genes clearly indicates the significance of these genes for reflecting the productive potential of Kodi Aadu goat breed in tropical climate.

Thus, from the study it was clearly evident that Kodi Aadu breed possessed excellent climate resilient potential. This was evident from the non-influence of heat stress of very high magnitude on major carcass traits, non-carcass components

and offals, primal cuts, linear carcass measurements, physic-chemical attributes, sensory attributes, meat color and LTL soluble proteins and meat quality associated gene expression patterns. Most of the diversified variables associated with carcass characteristics and meat quality remained intact in this breed after heat stress exposure clearly demonstrates the very high potential of this breed to produce optimally after coping with the extremely stressful environment. The *MSTN, HSP27, CRYA* and *HSP90* genes could serve as biomarkers for reflecting meat producing capability of Kodi Aadu breed in tropical climate. Hence, the Kodi Aadu breed could be considered ideal meat producing indigenous goat breed to survive in hot tropical climate of Southern India. Further, this breed could be recommended to the poor and marginal farmers of the specific agro-ecological zone of their origin to ensure their livelihood security.

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ABSTRACT

IMPACT OF HEAT STRESS ON THE MEAT PRODUCTION CHARACTERISTICS AND MEAT QUALITY RELATED GENE EXPRESSION PATTERNS IN INDIGENOUS KODI AADU GOAT BREED

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THESIS

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ABSTRACT

A study was conducted to assess the impact of heat stress on carcass traits, meat quality variables and various gene expression patterns which governs meat quality in indigenous Kodi Aadu breed. The study was conducted for a period of 45 days in controlled climate chamber. Twelve female (one year old) Kodi Aadu goats were used in the present study. The animals were randomly allocated into two groups of six animals each, KC (n=6; Control), KHS (n=6; heat stress). The control (KC) goats were placed in the thermo neutral zone (TNZ), *i.e*., control chamber, while the heat stress (KHS) goats were exposed to heat stress in heating chamber with a simulated heat stress model between 10.00AM to 4:00PM on all 45 days. At the end of the study all animals were slaughtered and their major carcass traits were recorded. Representative *Longissimus thoracis et lumborum (LTL)* muscles were collected from all animals for meat quality variables analysis and gene expression study. The weather variables recorded and the THI calculated based on the prevailing microclimate clearly demonstrated the respective microclimate proposed for control and heat stress group respectively. Heat stress did not alter any of the major carcass traits and non-carcass components and offals. Heat stress significantly ($P<0.05$) influenced only loin variable among the primal cuts with significantly lower value in KHS group as compared to KC group. Among the linear carcass measurements, only shoulder circumference was significantly ($P<0.05$) altered by heat stress. Except collagen solubility ($P<0.01$), rest all physico-chemical attributes remained intact between KC and KHS group animals. Heat stress did not influence any of the sensory variables and among the different color variables, only yellowness (b*) ($P<0.01$) showed significant difference for heat stress treatment. Further, Heat stress did not influence any of the soluble proteins such as sarcoplasmic protein, total protein and myofibrillar protein of *LTL* muscle. The myostatin (*MSTN),* calpain 1 *(CAPN1)* and Diacylglycerol Acyltransferase 1 (*DGAT1)* mRNA expression patterns were significantly ($P<0.01$) lower in KHS group as compared to KC group. However, the calpain 2 (*CAPN2),* calpastatin (*CAST)* and Crystallin alpha (*CRYA)* mRNA expression pattern was significantly ($P<0.05$) higher in KHS group as compared to KC group. Further, the THI had a mild positive correlation ($P<0.05$) with *MSTN* and strong positive ($P<0.01$) correlation with *CAPN1* and *DGAT1* expression patterns. However, THI had a mild negative correlation ($P<0.05$) with *CAPN2, CAST and CRYA* gene expression patterns. Thus, the study established that *MSTN, HSP27, CRYA* and *HSP90* genes could serve as biomarkers for reflecting meat producing capability of Kodi Aadu breed in tropical climate. Hence the Kodi Aadu breed could be considered ideal meat producing indigenous goat breed to survive in hot tropical climate of Southern India.

Keywords: Calpain system; Carcass; Climate resilience; Goat; Heat stress; HSPs; Meat quality