IMPACT OF HEAT AND NUTRITIONAL STRESS ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF BUCKS

by

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(2010-20-112)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Impact of Heat and Nutritional Stress on the Growth and Reproductive Performance of Bucks"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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SEM	Standard Error Mean
SL	Scrotal Length
SLA	Scrotal Length Afternoon
SLM	Scrotal Length Morning
SPSS	Statistical Package for the Social Sciences
SRH	Somatotropin-Releasing Hormone
SSTA	Scrotal Skin Thickness Afternoon
SSTM	Scrotal Skin Thickness Morning
SWL	Seasonal Weight Loss
TE	Tris Ethylenediaminetetraacetic
THI	Temperature Humidity Index
TMB	Tetramethylbenzidine
WAD	West African Dwarf

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SYMBOLS AND ABBREVIATIONS

Abbreviation	Expansion
μ	Overall Mean
AKST	Agricultural Knowledge Science and Technology
BCS	Body Condition Score
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
BW	Body Weight
C	Control
cDNA	Complementary Deoxyribonucleic acid
CS	Combined Stress
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FI	Feed Intake
FSH	Follicle Stimulating Hormone
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDP	Gross Domestic Product
GH	Growth Hormone
GHR	Growth Hormone Receptor
GLM	General Linear Model
GnRH	Gonadotropin-Releasing Hormone
H and E	Haematoxylin and Eosin
hGH	Human Growth Hormone
HPG	Hypothalamo-Pituitary-Gonadal
HRP	Horseradish Peroxidase
HS	Heat Stress
HSP	Heat Shock Protein
IGF	Insulin-like Growth Factor

IU	International Unit
LDH	Lactate dehydrogenase
LH	Luteinizing Hormone
LN_2	Liquid Nitrogen
LTLA	Left Testicular Length Afternoon
LTLM	Left Testicular Length Morning
LTWA	Left Testicular Width Afternoon
LTWM	Left Testicular Width Morning
mRNA	Messenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
NICRA	National Initiative on Climate Resilient Agriculture
ns	Non-Significant
NS	Nutritional Stress
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
qPCR	QuantitativePolymerase Chain Reaction
RH	Relative Humidity
RNA	Ribonucleic acid
rpm	Revolutions per Minute
RTLA	Right Testicular Length Afternoon
RTLM	Right Testicular Length Morning
RT-qPCR	Real Time-QuantitativePolymerase Chain Reaction
RTWA	Right Testicular Width Afternoon
RTWM	Right Testicular Width Morning
SC	Scrotal Circumference
SCA	Scrotal Circumference Afternoon
SCM	Scrotal Circumference Morning
SE	Standard Error

INTRODUCTION

CHAPTER 1
INTRODUCTION

In India, livestock plays a major role in the agricultural sector and it has a crucial role in deciding the economy of the nation. Small ruminants like sheep and goat are integral part of livestock sector and it decides the livelihood of poor and marginal farmers in developing countries. One of the major challenges affecting the livestock production in the present scenario is climate change. While climate change is a global phenomenon, its negative impacts are more severely felt by the poor people in developing countries who rely heavily on the natural resource base for their livelihoods. Rural poor communities rely greatly for their survival on agriculture and livestock, considering that they are amongst the most climate-sensitive economic sectors (Martello *et al*., 2010). Climatic extremes and seasonal fluctuations in herbage amount and quality are considered as imperative source of influence on the well-being of livestock in extensive production systems, which can impair reproduction and production efficiency of grazing animals (Martin *et al*., 2004; Naqvi and Sejian, 2010). Therefore, grazing animals in extensive rearing can face nutritional imbalance during extreme summer months.

Climate change presents a number of challenges, both to the practical responsibilities of keeping animals alive, healthy and productive and ensuring that livestock diversity is maintained over the longer term. Climate change threatens to make livestock keeping livelihoods more insecure, alter the production environments and upset established forms of livestock management (FAO, 2015). The occurrence of extreme weather events further exacerbates the negative impacts of the climate change. In the changing climate scenario thermal stress is one of the major factor which affects livestock production. The predicted effects of climate change include a trend toward higher temperatures over the coming decades. In the tropics and subtropics, a rising temperatures will lead to significant trouble for the livestock production. Heat Stress (HS) affects animals in a number of ways; it affects the production, leads to decline in fertility and increased mortality. The balance of ecosystem changes due to climate change

resulting in the increased occurrence, distribution and abundance of disease vectors which leads to the epidemiology of many crop and livestock diseases. In male animals, HS reduces libido by reducing level of testosterone, sperm output, decreasing sperm motility and by increasing proportion of morphologically abnormal spermatozoa in the ejaculate. It has been reported in both bulls and boars that HS causes an initial decline in circulating concentrations of testosterone (Hansen, 2009). Further, severe HS can compromise LH (Luteinizing Hormone) secretion in males. However, the major site for disruption of reproductive function appears to be the spermatogenic cell lineage in the testis. Temperature and humidity together can create more negative impact on goat production than each affecting individually. Several factors influence the production and reproduction parameters in the animals. Decreased quantity and quality of drinking water for livestock, due to drought and extreme weather events in the present changing climate further aggravates the negative impacts. High temperatures also increase animal's water requirements and reduce their appetites and feed intakes. HS, directly through hyperthermia and indirectly through reduced nutrient intake and behaviour changes affects the metabolic and physiological acclimation of the animals, reducing the productive potential of the animals and making them more susceptible to diseases (Bernabucci *et al*., 2010). Further, during HS, anabolic hormones decline and catabolic hormones increase leading to altering the energy balance in livestock. High temperature stress affects production and reproduction performance by decreasing antioxidant enzyme activity, which increases oxidative damage in the tissues, and by changing carbohydrate, lipid, and protein metabolism.

Climate change is predicted to affect the rainfall patterns. The variations in rainfall pattern due to change in climate affects the vegetation cover which leads to scarcity in pasture availability, ultimately resulting in nutritional stress (NS) to the animals. Many semi-arid areas are expected to experience lower rainfall in the coming decades, with shorter growing periods for plants and more frequent droughts. Climate change is likely to affect feed quality, as high temperatures tend to increase the lignification of plant tissues and thereby make forage less digestible. It also affects the fed supplies by loss of grazing land because of

drought (FAO, 2015). This is likely to increase the risk of prolonging the NS to the grazing livestock.

The Reproductive functions in young animals are more susceptible to dietary restrictions of energy and protein than in the adults and severe feed restriction may even result in permanent damage to gonadal and neural tissues controlling reproductive activities. While the restricted feed intake in adult animals can reduce androgen secretion and semen quality, such effects are temporal as re-feeding the underfed adult animals usually restores reproductive function (Brown, 1994). An adequate plane of nutrition is of vital importance for normal development of young ones and the rate of sexual development is highly dependent on the growth rate of the animal. In general, high energy intake has beneficial effects such as advancement of onset of puberty as a result of enhanced reproductive development and increased testicular size and sperm production in both young and adult animals. However, the excessive intake for a prolonged period can have detrimental effects on reproduction (Brown, 1994). The seasonal fluctuation in the availability of feed in the tropics also leads to seasonal weight gain during the pasture availability period and a seasonal live weight loss during the scarcity period for the animals (Lamy *et al*., 2012).

In tropical countries like India, especially during the summer months, the high ambient temperature and reduced pasture availability as a consequence of climate change is a major constraint for sustaining livestock productivity. In Indian context, heat and nutrition stress are the major stresses affecting production and reproduction of animals. HS and nutrition stress affects the productive and reproductive parameters of livestock such as body weight, scrotal and testicular measurements, sexual behaviour, seminal attributes and reproductive hormone level (Sahoo *et al*., 2013). These environmental stresses occur cumulatively rather than in isolation. Combined effect of HS and NS severely hampers the productive potential of livestock. Studies show that the impacts of these stresses are more severe on the production potential of an animal when they occur together rather than occurring individually. However, no such reports are available for goat species.

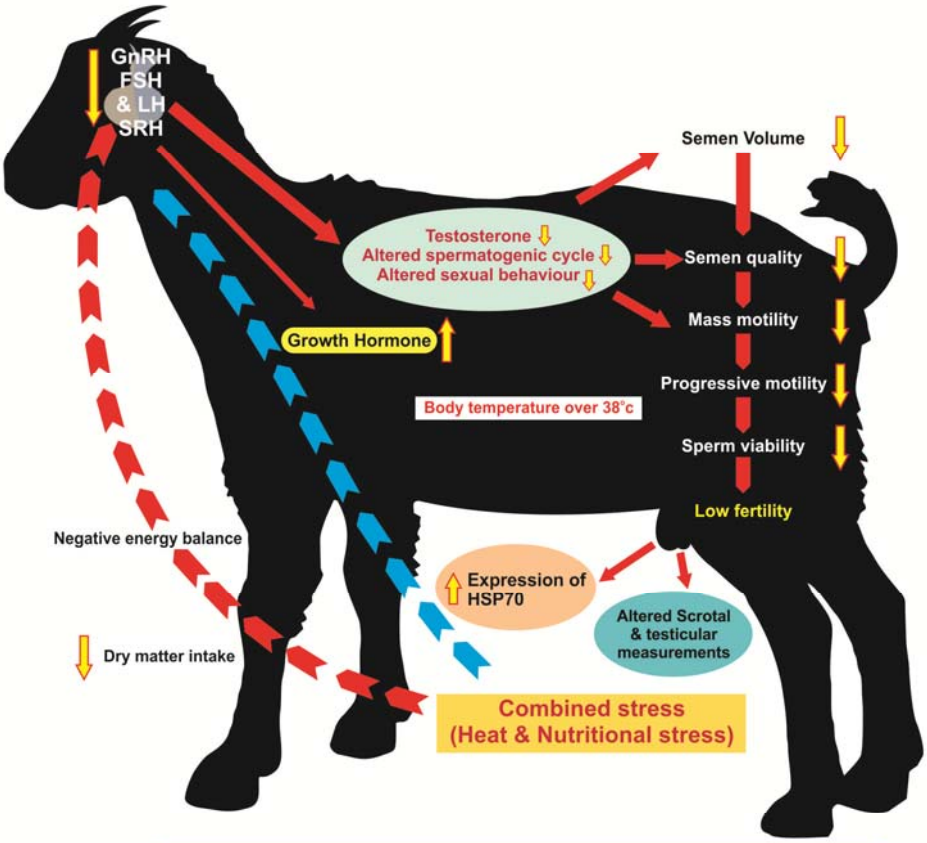
In the coming decades, millions of people whose livelihoods and food security depend on farming, aquaculture, fishing, forestry and livestock keeping, especially in the developing countries, are likely to face unprecedented climatic conditions (FAO, 2015). Hence research efforts are needed to meet the challenges of the changing environment and sustaining livestock productivity, which ensures livelihood security for the poor and marginal farmers. The role of livestock in rural communities is changing rapidly. Goats play a vital role in the livelihoods of small-scale farmers in developing countries (Kumar, 2007). The goat is an important source of milk, meat and fibre for the people throughout the world. Goat has been described as a poor man's cow (or mini-cow) because of its immense contribution to the poor man's economy. They not only supply nutritious and easily digestible milk to their children but are also a regular source of additional income for poor and landless or marginal farmers (Kumar and Deoghare, 2002; Trana *et al*., 2006). Goats are found in many climatic regions of the world ranging from the arctic cold, temperate, deserts and mountains to subtropical and tropical dry and humid zones. In these regions, climatic stressors, limitations of food, water quality and quantity add challenges to the adaptability of goats, in addition to presence of internal and external parasites. Further, the productivity of goats under the prevailing traditional production system is very low (Celi *et al*., 2008). It is because they are maintained under the extensive system on natural vegetation on degraded common grazing lands and tree lopping. Even these degraded grazing resources are shrinking continuously as a result of climate change. Hence research efforts are desirable to identify the suitable goat breed that can withstand low pasture availability.

Though the animals live in a complex world, researchers most often study the influence of only one stress factor at a time. Comprehensive, balanced, and multifactorial experiments are technically difficult to manage, analyze and interpret. When exposed to one stress at a time, animals can effectively counter it based on their stored body reserves and without altering the productive functions. However, if they are exposed to more than one stress at a time, the summated effects of the different stressors might prove detrimental to these animals (Sejian *et al*., 2010a; Sejian *et al*., 2011). Such a response is attributed to animal's

inability to cope with the combined effects of different stressors simultaneously. In such a case, the animal's body reserves are not sufficient to effectively counter multiple environmental stressors. As a result their adaptive capabilities are hampered and the animal struggles to maintain its normal homeothermy. Moberg (2000) described a hypothetical scheme indicating how two stressors can summate together and influence normal functions. He also showed that, when two stressors occur simultaneously, total impact might be severe on biological functions. However the influence of HS on biological functions and productive responses when coupled with long term NS in goat are not available in literature. In addition, despite the general awareness that energy demands vary between different seasons, only few quantitative data exist relating environment, nutrient need and productive efficiency in goat. Hence the present study was conducted to evaluate the influence of two environmental factors, HS and NS simultaneously on the productive and reproductive performance of *Osmanabadi* goats.

The primary objectives of the study are:

1. To study the influence of heat and nutritional stress on growth parameters in bucks.
2. To observe the impact of heat and nutritional stress on the seminal attributes in bucks.
3. To determine the impact of heat and nutritional stress on the plasma testosterone concentration in bucks.
4. To determine the impact of heat and nutritional stress on Heat Shock Protein (HSP) expression in testicles of bucks.



FSH-Follicle Stimulating Hormone, GnRH-Gonadotropin-Releasing Hormone, HSP-Heat Shock Protein, LH-Luteinizing Hormone, SRH-Somatotropin-Releasing Hormone

Fig 1: Concept figure of the present study.

REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

2.1 Importance of livestock to Indian economy

Livestock is an integral part of agriculture sector. The livestock sector contributes to 40 per cent of the world's agriculture Gross Domestic Product (GDP) (Naqvi and Sejian, 2011). It provides employment opportunities to 1.3 billion people, and creates livelihoods for one billion of the world's population living in poverty (FAO, 2006; Naqvi and Sejian, 2011). Globally, livestock contribute 15 per cent of the total food energy and 25 per cent of dietary protein (FAO, 2009; Salem *et al*., 2011). It plays a major role in the economy of nation. India ranks first in livestock population in the world. Many people, especially rural population depend on livestock sector directly or indirectly for their livelihood and it also provides vast employment opportunities. Over 70 per cent of the rural households in India own livestock, and a majority of them are small, marginal and landless households (Ali, 2007). Livestock play a crucial role in food and nutritional security of a nation. It also has importance in environment conservation. Livestock products such as meat, milk and egg and the varieties of food products from these has large demand on daily dietary needs of human life and these provide major part of income to livestock farmers. Exporting these products helps in increasing the international trade and foreign exchange earnings of the nation (Herrero *et al*., 2013). Livestock products provide high quality protein and micronutrients that are required for normal development and good health. Apart from essential protein and nutritious human diet through milk, eggs and meat, livestock also provides products such as manure, hides, skins, blood, bone and fat which provides additional income to the livestock farmers. Livestock is highly interrelated with crop production. The crop fields require large amount of manure for satisfying the nutrient requirements of crops where the livestock sector act as the source for manure and has a major role in fertilizer industry and providing additional economic benefits to farmers. Livestock manure increases soil fertility, soil structure and water-holding capacity. Mixed farming which includes the combining of crops and livestock reduces the risk of the farmer and

provides economic safety (Herrero *et al*., 2009). Livestock provides saving, security, and also allows resource-poor households to increase their assets. Small ruminants like sheep and goat are mainly reared in the arid and semi-arid regions and they provide a wide range of services and products to the farmers. Food security is one of the major issues in developing countries and livestock production has a crucial role in many of these countries (Naqvi and Sejian, 2011). The demand for livestock products will increase continuously since the human population rate is on an increasing trend. During the last three decades, the sustained improvement of incomes and rapid urbanization in parallel to a population growth have prompted a higher demand on meat and other animal products, particularly in developing countries (FAO, 2009; Salem *et al*., 2011). Globally livestock products contribute 17 per cent to kilocalorie consumption and 33 per cent to protein consumption (Rosegrant, 2009). About one-fifth of the global trade of agricultural products are livestock products (Ali, 2007). An in-depth understanding of the dynamics of consumption of animal products for developing economies like India is helpful not only for academic exploration but also for policy formation (Gandhi and Zhou, 2010). Economic losses to livestock farmers are mainly due to decreased growth and reproductive performance, increased mortality, reduced milk and egg production. Increases in livestock productivity in the recent past are mainly due to the developments in animal science and technology, developments in breeding, animal health and as well as nutrition that will continue to contribute to potential production (Thornton, 2010). Livestock sector supports national economy as well as the socio-economic growth of the country and offers great potential and outstanding contribution to the agriculture sector. Animal husbandry practices are a boon for sustaining the livelihood of the poor and marginal farmers who are affected by adverse climatic conditions and national calamities like drought, flood etc. It plays multiple roles in livelihoods of people in developing communities. Insurance sector and banking is associated with the livestock sector to reduce the risk of livestock farmers. Development of livestock sector is therefore essential for the alleviation of poverty and development of the rural economy.

2.2 Significance of rearing small ruminants

Poor and marginal farmers including women depend on small ruminants for their livelihood. Procurement of large ruminants is difficult for poor and marginal farmers hence they prefer small ruminants which includes goat and sheep. Initial investment, feeding and management expenses are less for small ruminants when compared to the large ruminants. Other advantages of small ruminants are, they are prolific breeder's, an asset that can be converted to money (Chowdhury *et al*., 2002). They effectively utilize feed, needs low cost and less space for housing requirements, management practices are easy and labour demand is less. These provide additional economic benefits to small ruminant farmers. Small ruminants play a vital role in securing the livelihood of poor and marginal farmers (Agnihottri and Rajkumar, 2007; Escareno *et al*., 2013).They play a crucial role in economy of the country and provides valuable contribution for stable households in developing countries. The use of livestock and small ruminants in particular provides many benefits to millions of farming community in the semiarid tropics (Naqvi *et al*., 2013). They are critical to the progress of sustainable and environmentally sound production systems (Sejian *et al*., 2014). Small ruminants can be easily integrated into different farming systems due to their small size. Their importance is primarily due to their small size, which is advantageous to the farmer as it favours low investments, risk of economic loss is less and preference over large ruminants for food and reproductive efficiency and available land can be economically utilized (Omoike, 2006; Aphunu, 2011). This small size along with early maturity increases the demand for small ruminants. The importance of small ruminants lies in the formation of an economic and ecological niche in small farm systems and agriculture (Devendra, 2001; Escareno *et al*., 2013). Small ruminants especially sheep and goats have important role in the socio-economic well-being of people in developing countries in the tropics in terms of providing nutrition, income and savings, insurance against emergencies, cultural and ceremonial purposes (Kosgey *et al*., 2008). Recent year trend shows that the importance for small dairy ruminants has increased significantly, especially in developing countries, where they are an important alternative for the supply of dairy products for human dietary demands (Lerias *et al*., 2013). In Asia,

the growth rate of the population of small ruminants (sheep and goat) is 5.3 per cent which is much higher than the large ruminants (cattle and buffalo) which are 1.8 per cent (Chowdhury *et al*., 2002). Population of sheeps and goat in India was 71.5 and 140.5 million in 2007 and in 2010 this was increased to 74.0 and 154.0 million respectively (FAO, 2010; Agrawal *et al.*, 2014). Small ruminants also play a complementary role to other livestock species in the use of available feed resources and provide the opportunity of using vast areas of natural grassland in regions where crop production is not possible (Kosgey, 2008). Small ruminants play an important role in food and nutritional security of nation. They provide nutrient and protein rich products for human dietary needs such as milk and meat. Goat and sheep meat are rich in proteins. These products have high demand and economic value which yields economic profit to the small ruminant farmers. They also provide draught power in the highlands. They have significant role in subsistence agriculture because of their unique ability to adapt and maintain in harsh environments (Debele *et al*., 2013). In rural economy of India, Sheep and goat production is an integral component and serves as important source of economic sustenance for weaker segments of the society (Agrawal *et al.*, 2014). Sheeps and goats can thrive in limited pasture availability and different climatic conditions and they have wide range adaptation strategies to cope up with the environment challenges. Under the changing climate scenario increase in temperature results in farmers shifting from beef cattle, dairy cattle and chickens to goats and sheep in Africa because goats and sheep are more tolerant to heat (Seo and Mendelsohn, 2008). The productive potential of sheep and goat is influenced by the exposure to adverse climatic conditions, which include high ambient temperature and lack of food resources and water scarcity. Goats are generally compacted and large surface per unit weight is exposed in order to dissipate heat (Okoruwa, 2014). The farmers in the arid and semi-arid regions depend on goat and sheep for their livelihood. The surviving ability in harsh environment conditions gives preference to small ruminants like sheep and goat when compared to the large ruminants by the farmers of arid and semi-arid regions. Sheep and goat breeds can survive in water deficit conditions which are common in arid and semi-arid regions.

2.3 Importance of goat rearing

Goat rearing is one of the major sources of income for poor and marginal farmers. Goat rearing is done by the poor and marginal farmers because of lack of money to buy large ruminants and goat rearing requires less expense for feeding, housing requirements and management practices and provides maximum output. This low input and maximum output which increases the family income is the advantage of goat rearing and hence known as poor man's cow. Goats are mainly reared for meat, milk and hides. Goat products, especially meat (Chevon) has high demand in the international market and exporting of these products increases the scope of international trade. Chevon provides protein of high quality and low saturated fats which is good for human health and hence compared with other red meats it is a healthier alternative (Anaeto *et al*., 2010). Chevon which has low cholesterol and goat milk which has medicinal value and easily digestible has high demand across the globe and this gives importance to goat rearing. Goats are browsers and they are reared mainly by allowing them for grazing in pasture lands and feeding crop residues. Goats while grazing help in dispersal of seeds and improvement in vegetation. They play a major role in weed management also. Women play a major role in goat rearing at household level. Goats have major contribution to food, rural employment and GDP. Contribution of goat is large to the livelihoods of livestock keeping households of low- and medium-input farmers who have few resources beyond their smallholdings and livestock (Boyazoglu *et al*., 2005; Escareno *et al*., 2013). As, Goats have low body mass, and low metabolic requirements, an important asset for minimizing their maintenance and water requirements, in areas where water sources are widely distributed and food sources are limited by their quantity and quality. The ability to reduce metabolism allows them to survive even after prolonged periods of food scarcity. Their skilful grazing behaviour and efficient digestive system enable goats to attain maximal food intake and food utilization in a given feeding situation (Silanikove and Koluman, 2014).

2.3.1 Advantages of goat rearing from climate change perspectives

Goats can survive in various agro climatic conditions especially in arid and semi-arid conditions and they are capable of adapting to wide range of environmental challenges. They can survive in drought prone areas, thrive in wide variety of plants, bushes, crop residues and they are disease resistant when compared to other small ruminants. (Froghi and Hosaini, 2012). They are considered to be more tolerant to increased Temperature Humidity Index (THI) values when compared with dairy cows because of their metabolic size and high capacity to conserve water (Silanikove, 2000a; Hamzaoui *et al*., 2013). Several water saving mechanisms are activated in goats during water shortage periods which minimizes their loss of water and hence their capacity to withstand water deficit increases (Silanikove, 2000b).

2.4 Factors influencing goat production and reproduction

Major factors influencing production and reproduction of goat includes climate, diseases, Parasites, Nutrition, age, body weight, breed, growth rate, biomass productivity, photoperiod, geographical location, management practices. Climatic factors which affect goat productivity are temperature, relative humidity (RH) and wind speed. Animal experiences stress to maintain homeothermy when they are in out of comfort zone. This demands extra energy to thermoregulate, so that energy available for production processes are reduced (Nardone *et al*., 2006). Animal compromise their productive potential to adapt to environmental challenges and this creates economic burden to the farmer. Averages daily gain of animals is influenced by available nutrients, hormones and enzymes, as well as, elevated ambient temperatures are considered (Maurya *et al*., 2009). Reproductive performance of goats is directly depended on the genetic potential of the animal, management and environment (Aguiar *et al*., 2013). Increases in maintenance requirements associated with sustaining constant body temperature, and altered feed intake leads to production losses (Mader and Davis, 2004; Indu *et al*.,2013). Increase in environment temperature may affect production through reduced growth, meat, milk, and impaired reproductive performance (Yadav *et al*., 2013). Seasonal weight loss (SWL) is an important factor that affects animal production

in tropics. In dry season reduced availability as well as low quality pasture results in SWL. Reduction in live weight leads to reduction in fertility and animal become more vulnerable to diseases and parasites (Lerias *et al*., 2013). The energy exchange between the animal and the environment is affected by environmental factors, animal factors and thermoregulatory mechanism (Nienaber *et al*., 1999; Maurya *et al*., 2009). The Hypothalamo-Pituitary-Gonadal (HPG) axis is the neuroendocrine pathway regulating reproductive status. HS and NS are the major factors affecting the HPG axis and results in compromised reproduction. Several factors influence the morphological characteristics of spermatozoa including the genetic make-up and physiological stage of the animal, nutrition, season, climatic factors, and disease (Dana *et al*., 2000; Mekasha *et al*., 2007a).

2.4.1 Climate

Animal agriculture is affected by climate in four ways (1) availability of feed-grain and price; (2) pastures and production of forage crop and quality; (3) health, growth and reproduction; and (4) diseases and pests distributions (Rotter and Van de Geijn, 1999; Gaughan *et al*., 2009). Climate affects all productive traits of livestock. Rise in environmental temperatures affects reproductive efficiency and results in reduced sperm production. Combined effect of temperature and humidity or drought will be extremely fatal to production parameters (Nardone *et al*., 2006). The amount of food and water intake, the availability of potential energy in the ingested forage, the animal heat production system, available net energy for productivity and body composition of growing animals are affected by climatic conditions. (Adedeji *et al*., 2011). Perez-razo *et al*. (1998) reported that poor survival of kids born in summer may be partly due to HS and in tropics an important cause of mortality in kids may be HS.

2.4.2 Nutrition

Nutrition is a major factor affecting reproduction. The factors which determine nutrient requirements are age, sex, breed, production system (dairy or meat), body size, climate and physiological stage. Nutrition controls reproductive endocrine functions in many species (Martin *et al*., 2004; Sejian *et al*., 2011). Lack of feed and pasture availability decreases the body weight. High ambient

temperature results in HS which leads to reduced feed intake causing severe NS in goat. Water is the most critical factor and the need is *ad libitum* under hot and humid environmental conditions. In arid and semi-arid regions across the globe lack of adequate year-round feed resources is probably another important factor resulting in decline in animal production (Ben Salem and Smith, 2008; Kawas *et al*., 2010; Sejian *et al*., 2014). Combined effect of nutrition deficiency and HS will severely affect the production parameters. Nutrition has an important impact on the reproductive performance in sheep, but the magnitude of the effect may vary according to the season (Sejian *et al*., 2014). Under extensive management system, level of nutrition may have high influence on reproduction and production of rams (Maurya *et al*., 2012). In males, undernutrition causes hypogonadism and infertility. The responses of male animals to nutritional manipulations can be divided into short-term effects that act mainly on the neuroendocrine system controlling testicular activity and long-term effects that act on testicular growth and sperm production (Maurya *et al*., 2010). In young animals, reproductive functions seems to be more susceptible to energy and protein restrictions than those in adult and severe nutritional restrictions can cause permanent damage to gonad and neural tissues (Martinez *et al*., 2012).

2.4.3 Age

Age is a major factor in production and reproduction. Puberty has a major role in lifetime production and is related with both age and body weight. Hence nutrition has also role in the start of puberty. A study conducted in ossimi rams showed that there exists a relationship between age and testosterone concentration. Age progress and increasing the testicular measurements improved sexual activity and semen characteristics in the ram (Mahmoud, 2013). Age has influence on sexual behaviour of the rams (Snowder *et al*., 2002; Simitzis *et al*., 2005; Mahmoud, 2013). In Black Bengal bucks semen volume and sperm concentration increased with age, but there is decreasing trend percentage of sperm livability with age (Mia *et al*., 2013).

2.4.4 Diseases and parasites

Diseases and parasites are important factors which have major impact on livestock production and productivity and affect the food supplies, trade and commerce, and human health globally (Lamy *et al*., 2012). They cause threat to the life of the animals. Major cause of economic loss in livestock production is due to the occurrence of diseases in the new born and neonatal mortality (Mohri *et al*., 2007). Livestock diseases can cause direct losses and indirect losses. Direct losses includes deaths, stunting, reduced fertility, and changes in herd structure, and indirect losses includes additional costs for drugs and vaccines, increase in labour costs and profit losses due to late access to better markets and use of suboptimal production technology in revenue (Rushton, 2009; Lamy *et al*., 2012). Helminth infestation is one of parasitic diseases which causes continuous serious health problem to West African Dwarf (WAD) goats affecting their productivity (Adedeji *et al*., 2011). Development of gastro-intestinal parasites is favoured by the climatic factor during the period of NS and wet season in the tropical area (Hawlader *et al*., 2002; Dhara, 2011) like India and could negatively affects the reproductive and productive performance of goat and slows down the genetic progress (Dhara, 2011). Gastrointestinal nematodes are important pathogens which negatively affects production of small ruminants. Goat kids are mainly affected by coccidiosis and this negatively affects their growth. Climate change resulting in higher temperatures may increase the rate of development of certain pathogens or parasites that have one or more stages of life cycle outside their host animal. This may shorten generation times and, possibly, the total number of generations per year increases, leading to higher pathogen/parasite population sizes (Harvell *et al*., 2002; Chauhan and Ghosh, 2014). During onset of monsoon parasitic infestation resulting in toxaemia and diarrhoea were found to be most common diseases at farmers field especially which hampers the productive potential of goats (Mishra et al., 2015).

2.4.5 Significance of understanding the impact of climate change on goat production

Scientific evidences and reports show that climate change is already affecting the living world. Temperature changes and increases in frequency of extreme weather cause adverse effects on agriculture and livestock sector. Climate change and global warming cause great threat to entire livestock population across the world. These impacts includes alteration in the productivity of rain-fed crops and forage, decline in water availability, and changing severity and distribution of important livestock diseases (Thornton, 2010) Animal agriculture is one of the climate-sensitive economic sector (Renaudeau *et al*., 2012). Under the changing climate scenario elevated ambient temperature and increase in frequency of extreme weather events cause adverse effects on livestock production. Impact of climate change is not only the health and welfare of animals, but also the more than a billion people who depend on them for their livelihood (Tologbonse *et al*., 2011). Climate change is a major threat to the sustainability of livestock production systems in many parts of the world (Moss *et al*., 2000; Naqvi and Sejian, 2010). Goats are subjected to different kind of stresses under the changing climate scenario. Among the various stresses HS is the most intriguing factor that adversely affects animal production. Available body resources are redistributed due to these stresses at the cost of reduced growth, body condition score (BCS), production and reproduction (Maurya *et al*., 2009; Samad *et al*., 2014). It is important to understand the impact of climate change on goat production since goat production is sensitive to changing climate. Grazing animals will be more affected due to climate change due to lack of pasture availability, water and HS due to increased ambient temperature. Yields of rangelands reduce due to increasing temperatures and decreasing rainfall and this contribute to their degradation. Increased temperatures tend to reduce animal feed intake and lower feed conversion rates (Rowlinson, 2008; Salem *et al*., 2011). Goats are browsers and they are reared mainly by allowing them for grazing in pasture lands and feeding crop residues and this variation in availability of pasture and water due to decreased rainfall and increased temperature will severely affect the goat production. Water scarcity is a major problem faced by farmers in the arid and

semi-arid regions across the globe. Climate change will further intensify the water scarcity problems and it will have adverse impact on the drinking water sources of goat.

2.5 Heat stress impact on growth and reproduction in goat

Under the changing climate scenario, HS is one of the major environmental factor which affect the productive potential of goat. Effect of HS includes reduced dry matter intake, increased water intake, Increase sweating and panting to maintain core body temperature, Energy is shifted from milk production to maintenance, increased mortality and morbidity, increased rectal temperature and respiratory rate and reduced immune function. High ambient temperature along with humidity results in HS and it has adverse impact on growth and reproduction. Reproductive functions are negatively affected by HS(West, 2003). In male animal, reproductive processes are very sensitive to disruption by increase in temperature with the most pronounced consequences being reduced quantity and quality of sperm production and decreased fertility (Chauhan and Ghosh, 2014). Reproductive function is influenced by environmental temperature in the male by alteration in spermatogenesis and reduction in semen quality and male fertility (Marai *et al*., 2008). Reproductive functions are disturbed due to redistribution of blood flow takes place from the body core to the periphery increases for sensible heat loss to regulate the body temperature during HS and reduced dry matter intake due to HS affects metabolic heat production, energy balance and nutrient availability. Assessment of seminal parameters, sexual behaviour, and scrotal attributes can be used as reliable tool for breeding soundness evaluation (Samad *et al*., 2014).

2.5.1 Heat stress impact on body weight:

Growth is defined as the irreversible positive changes in the measured dimensions of the body. The reason for the effects of increased environmental temperature on growth reduction could be due to decrease in anabolic activity and the increase in tissue catabolism (Marai and Habeeb, 1998; Marai *et al*., 1999; Sejian *et al*., 2010). They attributed this increase in tissue catabolism due to

increase in catecholamines and glucocorticoids after exposure to HS. The anabolic decrease, especially metabolizable energy for both body maintenance and weight gain, causes a loss in the production per unit of feed. The tissue catabolism increase occurs mainly in fat depots and/or lean body mass (Kandemir *et al*., 2013). The somatotrophic axis which includes growth hormone (GH), growth hormone receptor (GHR), and insulin-like growth factor(IGF-1) are affected due to HS. Body weight shows decreasing trend due to reduced feed intake under HS (Hooda and Upadhyay, 2014). Overall growth performance of animals which includes growth rate, daily weight gain and live body weight is adversely affected by HS. (Maurya *et al*., 2009).

2.5.2 Heat stress impact on scrotal and testicular measurements:

Testis is the principle organ of male reproductive system and is responsible for production of (male germ cells) spermatozoa and androgens, mainly testosterone (Gofur *et al*., 2014). Testicle size is a good indicator of sperm-producing ability. The increase in testicular temperature is one of the major causes of animal subclinical infertility and sterility. Due to this condition testicular degeneration, spermatogenesis abnormalities and spermatic dysfunction resulting in sperm abnormalities occurs (Saadi *et al*., 2013; Oliveira *et al*., 2014). Seasonal changes have effect on the scrotal circumference (SC), sperm motility and concentration and quantity of sperm defects. (Rege *et al*., 2000; Teodoro *et al*., 2013). SC values were lowest during summer and increased in autumn in rams (Marai *et al*., 2008). Seminal quality and male fertility tend to decrease during the summer months possibly due to the seasonal effects on the hypothalamo-hypophyseal axis, or due to the direct temperature effect on the testicles and epididymis (Teodoro *et al*., 2013). The normal absorptive and secretory function of epididymis is disturbed during increased testicular temperature and induces changes in ions and proteins of cauda epididymis which decreases the progressive motility of sperms (Mohamed *et al*., 2012). A decrease in testicular measurements (testes weight and size) in HS is due to the degeneration of the germinal epithelium and partial atrophy in seminiferous tubules (Marai *et al*., 2008).

2.5.3 Heat stress impact on sexual behaviour:

The expression of sexual behaviour depends on the rate of steroid hormones, level of nutrition nutritional, physical and social environment and social group structure (Benia *et al*., 2013).HS results in reduced libido in animals. In Rams, libido seemed to be affected by ambient temperature (Marai *et al*., 2008). Libido is reduced due to HS by decreasing level of testosterone, sperm output, decreasing sperm motility and by increasing up proportion of morphologically abnormal spermatozoa in the ejaculate (Perez-Crespo *et al*., 2008; Gupta *et al*., 2013). In arid and semi-arid regions high ambient temperature and sudden temperature variations have detrimental effect on sexual behaviour of animals (Maurya *et al*., 2009).

2.5.4 Heat stress impact on seminal attributes:

Environmental stress leads to production of low quality semen in animals (Alejandro *et al*., 2014). They further added that the direct exposure of the testis to high temperatures causes changes in certain critical stages of spermatogenic cycle, which is directly related to the quality of the ejaculate. Bill Epperson, (2002) found that after thermal stress induced changes in semen included alterations in the shape of the sperm cell head and tailpiece. According to Marai *et al*. (2002), high ambient temperature leads to testicular degeneration and reduction in percentage of normal and fertile spermatozoa in the ejaculate. HS adversely affects the spermatogenesis (Salem *et al*., 2011). Increased testicular temperature due to HS results in reduced sperm output, decreased semen quality and sperm motility and increase in percentage of morphologically abnormal and aged spermatozoa in the ejaculate. Poor sperm morphology is an indicator of decreased fertility in many species, including goats (Mekasha *et al*., 2007b). Spermatocyte and spermatids are found to be more affected by HS (Hansen, 2009). Increase in ambient temperature decreases the percentage of live sperms. Changes in temperature and humidity can lead to thermal discomfort, which results in reduced feed intake and interference with spermatogenesis and quality of semen (Kunavongkrit *et al*, 2005;Aguiar *et al*., 2013).Reduced body weight due to reduced feed intake in HS negatively affects the sperm productivity

(Mekasha *et al*., 2007; Akpa *et al*., 2013).HS has negative impact on semen attributes, such as sperm concentration, sperm motility, sperm viability, sperm morphology, and acrosome integrity (Akpa *et al*., 2013).). Apoptosis of germinal epithelium of the seminiferous tubules increased due to elevated testicular temperature (Pei, 2012; Mohamed *et al*., 2012). Under HS the sperm cell concentration was significantly lower than in thermoneutral conditions. (El-Darawany, 1999; Marai *et al*., 2008). In a study conducted on Black Bengal bucks, during winter the highest ejaculate volume was obtained and lowest during summer and intermediate in rainy season (Mia *et al*., 2013). From the histological studies, it has been concluded that pachytene spermatocytes and early spermatids are the cells in the testis which are most susceptible to heat (Setchell, 1998). Marai *et al*. (2008) reported that the semen pH has high correlation with environmental temperature. Studies show that with increase in ambient temperature the sperm motility decreases (Marai *et al*., 2008).

2.5.5 Heat stress impact on reproductive hormone level:

Production of GnRH is reduced and also the sensitivity of the pituitary to GnRH is also reduced by HS which affects the hormonal control of reproduction. (Sheba *et al*., 2012). The hormone responsible for spermatogenesis and sexual behaviour is testosterone, thus the seasonal pattern of testosterone secretion could limit the male reproductive efficiency during some periods of the year (Todini *et al*., 2007; Farshad *et al*., 2012).Testosterone level is decreased during HS. In Ossimi rams the lowest level of serum testosterone was recorded during hot environmental conditions (El-Darawany, 1999). Luteinizing hormone (LH) secretion is reduced under HS. HS on scrotum adversely affects the function of sertoli cells and release of androgen (Maurya *et al*., 2009). In Ossimi rams, the lowest serum testosterone level was recorded during hot environmental conditions (Naqvi *et al*., 2012).

2.6 Nutritional stress impact on goat growth and reproduction

Nutrition is the primary factor which has major role in growth and reproductive performance in livestock. Compromised nutrition reduces reproductive efficiency. Under arid and semi-arid regions low biomass

productivity, high climatic variability, and limited availability of water are major constraints for livestock production. In these regions grazing animals are usually subjected to periods of undernutrition during extreme hot environment due to lack of availability of feed and limited pasture conditions caused by reduced availability of nutrients, which in turn declines productivity (Soren, 2012). HS leads to reduced dry matter intake which leads to NS in livestock. Water is also most critical for livestock, and under hot and dry climatic conditions the need is *ad libitum* for animals. Decreased rainfall and increase in temperature leads to reduction in pasture availability in the current climate change scenario will affect the growth and reproduction in livestock. Occurrence of drought conditions will increase this pasture scarcity problems and this has severe impact on growth and reproduction in animals. Most goats graze on native pasture without supplementary feeding in subtropical Mexico and they are subjected to large seasonal variations in food availability (Delgadillo *et al*., 2004). The ability to produce and maintain functional gametes is related to the ability of the organism to secure and consume energy in the form of food (Maurya *et al*., 2010). Reproductive efficiency is reduced due to compromised nutrition. It is highly related with feed and water availability. Quantity and quality of forage decreases during drought period further increases the impact of NS. Plane of nutrition play a major role on reproductive performance of livestock and hence nutritional management is essential for optimizing reproductive performance in livestock.

2.6.1 Nutritional stress impact on body weight

Nutritional imbalances in animals can result in abrupt changes in body weight and BCS (Jalilian and Moeini, 2013). In a study conducted on kids more stress was experienced by feed restricted and thermal exposed group and the body weight losses incurred in this group could not be compensated during post stress period (Hooda and Upadhyay, 2014). During summer months, low quality forages results in slower growth and reduced body size (West, 2003). Allometric parameters were reduced significantly in non descript Indian bucks while subjected to 50 per cent restricted concentrate and this affect the production parameters of breeding buck (Samad *et al*., 2014). Angora goats are more

vulnerable to NS at the time of unfavourable feed conditions because they store relatively less body fat (Froghi and Hosaini, 2012). Level of nutrition has significant effect of on birth weight in Sudan Nubain goats (Elabid, 2008). During the period of optimum grass growth the bucks were found to restore their body weight and body condition (Kridli *et al*., 2007**)**. Similar reports were documented by Al-Ghalban *et al*., (2004) in goats.

2.6.2 Nutritional stress impact on scrotal and testicular measurements

Nutrition has a major role in testicular growth and testicle size. SC is a good indicator of breeding ability. Hence it can be considered in selection purpose of bucks. Level of nutrition has influence on Sperm production and testicular size in rams (Maurya *et al*., 2010). Changes in protein ingestion may affect testicular size and sperm production even when such changes exceed the maintenance requirements (Fernandez *et al*., 2004; Martinez *et al*., 2012). In male sheep testicular growth and endocrine functions of testes are changed due to severe malnutrition (Martin and Brown, 1995). Lamy *et al*.(2012) reported undernutrition in boer goats reduces testicular volume and SC which ultimately culminated in reduced sperm concentration and increase in sperm abnormality. Nutrition has a major role in development of testicles and spermatozoa production (Martin *et al*., 2010). Reproductive function in young animals seems to be more susceptible to restrictions in dietary energy and protein than the adults and this may lead to permanent histological changes in the testis of small ruminant males (Rekik *et al*., 2007). Moderate changes in plane of nutrition affect the secretion of gonadotrophins. However this effect is for few weeks which is followed by long term growth retardation of testis (Martin *et al*., 2010). Further, they emphasised that variation in nutrition alters the total mass of testicular tissue, as well as the efficacy with which the gametes are produced by the testicular tissue in rams.

2.6.3 Nutritional stress impact on sexual behaviour

Nutrition is the crucial factor which controls sexual behaviour in livestock. Nutritional restriction severely affects the sexual behaviour of rams (Kumar *et al*., 2015).Similar findings was reported by Maurya *et al*.(2010). NS reduces libido of the animal. The decrease in protein resources reduces the circumference of the

scrotum which ultimately reduces the libido and fertility in rams (Kheradmand *et al*., 2006). The sexual behavior of an animal is altered in nutrition deprived animals primarily due to low circulating testosterone concentration. (Maurya *et al*., 2010). However, Martin *et al*., (2010) concluded that unless the NS is of higher magnitude it is very difficult to assess its impact on sexual behaviour in male animals (Martin *et al*., 2010). Further, during the non-breeding season, nutrition induced testicular growth does not seem to be related with gonadotrophin response in mature goats (Martin *et al*., 2010).

2.6.4 Nutritional stress impact on seminal attributes:

Semen volume, sperm concentration, mass motility, individual motility are affected due to nutrition stress. Sperm production is related with scrotal and testicular measurements (Zarazaga *et al*., 2005; Moghaddam *et al*., 2012)**.** Nutrition changes in mature rams and bucks results in profound responses in testicular size and therefore the rate of production of spermatozoa (Xu, 2015). These effects are mainly due to changes in the seminiferous tubules size and in the efficiency of spermatogenesis (Martin and Brown, 1995).In underfed rams spermatogenic activity, sperm content of the epididymis, and other variables were lower than in *ad libitum* fed rams (Kumar *et al*., 2015).Alterations in feed intake seem to have minimum effect on gonadal endocrine function. However such effect may induce profound changes on sperm production in mature male sheep and goat (Martin et al., 2010). This shows the sensitivity of sperm production for even minor changes in nutritional status of an animal. In small ruminants there exists a direct relationship between level of nutrition, testicular mass and the spermatozoa number available for ejaculation (Martin *et al*., 2010). Decrease in plane of nutrition decreases the quality of the semen produced for a period of time greater than the seven week duration of spermatogenesis (Robinson *et al*., 2006). The response of gametogenic tissue to alterations in nutrition is rapid, but the endocrine regions are less affected in the gonads. The alterations in expression of nutritional responses among sexes, breeds and species probably reflect the differences in the role of this nutritional factor as a reproductive function modulator (Martin and Walkden-Brown, 1995). In nutritionally stressed rams,

rapidly motile sperm counts were lower while medium and slow motile spermatozoa counts were higher (Kumar *et al*., 2015). Nutrition also found to be altering the spermatogenic activity in rams. It has been reported that a*d libitum* fed rams showed high spermatogenic activity and sperm content of the epididymis as compared to underfed rams (Kumar *et al*., 2015).

2.6.5 Nutritional stress impact on reproductive hormone level

NS alters the reproductive hormone level. Alterations in GnRH pulse frequency and thus LH and FSH secretion due to changes in nutrient supply (Martin and Brown, 1995). Blood levels of testosterone vary according to breed, plane of nutrition, season and age (Zamiri and Khodaei, 2005; Hashem, 2014).In mature male ruminants, nutritional signals have influential effects on the reproductive system and the responses are partly independent of changes in gonadotrophin secretion (Martin and Walkden-Brown, 1995). The energy balance of animals is controlled by the interactions between metabolites and hormones (Scaramuzzi*et al*., 2006). The energy balance of an animal is closely related to fertility since animal needs large amount of energy for reproduction (Martin *et al*., 2008). Variations in available energy and feed intake alter the endocrine axis and HPG axis. An animal would divert the energy available for reproduction to brain function during energy deficiency which ultimately affects the reproductive endocrinology (Maurya *et al*., 2010). Testosterone levels showed a decreased trend under NS in rams (Kumar *et al*., 2015). The level of testosterone is a good indicator of semen production and fertility.

2.7 Concept of multiple stresses impacting livestock production

Climate change is the biggest environmental crisis faced by the world in the present scenario. Both the direct and indirect effects of climate change adversely affects the livestock productivity. Increased ambient temperature along with high RH causes HS and the reduced feed intake due to HS and reduction in pasture availability results in NS. Along with heat and NS animal has to walk a long distance in search of pasture in hot and humid conditions. Animals are subjected to physical stress while walking in hot semiarid environment and apart from HS, and feed and water scarcity animals are also exposed to walking stress

(Sejian *et al*., 2012). All these events takes place in continuity as a result of climate change and they never occur in isolation. Simultaneous occurrence of these multiple stresses will severely hamper the production and reproduction of livestock. They alter the homeostasis and metabolism of the animal. Under hot and semi-arid environments thermal stress and feed scarcity are the major factors for the low productivity of sheep in (Martin *et al*, 2004; Sejian *et al*, 2010). Reports show that in tropics only 30 per cent of the feed are available during summer months thus putting the animals under severe feed deprived conditions (Sejian *et al*., 2011). HS and NS, when combined can cause severe infertility in farm animals. Under arid and semi-arid environments goats are subjected to multiple stresses simultaneously (Sejian *et al*., 2011;Hooda and Upadhyay, 2014). Apart from these the low biomass productivity, high climatic variability, and scarcity of water are the major constraints of arid and semiarid tropical environment affecting livestock productivity (Sejian *et al*., 2012). Occurrence of combined effect of these stresses may create adverse impact on livestock than that would occur with stresses in isolation. Cumulative evidence supports the view that stressful stimulation and a low level of nutrition are two environmental factors, which modulate growth and affect reproduction of animals (Polkowska, 1996). Under arid and semi-arid environments goats are subjected to multiple stresses simultaneously and apart from HS and physical strain of grazing activity, the sheep and goats are subjected to shortage in feed (Hooda and Upadhyay, 2014). When goats are subjected to different stresses, physiological changes occur but paramount and important effects are weight loss, loss of performance and sometimes even loss of life (Hooda and Upadhyay, 2014). Marai *et al*., (2007) stated that growth, the increase in the live body mass or cell multiplication, is controlled genetically and environmentally. Elevated ambient temperature is considered to be one of the environmental factors influencing average daily gain (Sahoo *et al*., 2013). Similar findings were documented by Ismail *et al*., (1995). Sejian *et al*. (2010) also reported the reduction of BCS in sheep in both NS group and CS group. The reason for this could be due to insufficient fat storage as a result of restricted feeding. Similar findings of low BCS in undernourished ewes were reported by Sejian *et al*. (2010). The reduced feed intake as a result of

thermal stress in the ewes of thermal stress group could be the reason for reduced BCS as compared with C group.Sejian *et al*. (2014) reported that Multiple stresses leads to significant weight reduction in body weight in the *Malpura* ewes .The reason for this is attributed to the feed restriction and also the walking stress (Sejian *et al*., 2014b). It is a well-established fact that HS alone did not influence bodyweight when fed *adlibitum* (Sejian *et al*., 2010). This emphasis the fact that proper nutrition during thermal stress is more important to maintain body weight. Similar findings were reported by Karim and Patnayak, (1998) in sheep.

2.8 Closing remarks

HS and NS are the major environmental stresses which adversely affects growth and reproduction in goat. Due to this economic output of the goat farms will be severely affected. To reduce the economic burden on goat farmers as a result of HS and NS strategies need to be developed with multidisciplinary approach to reduce the adverse effects of these stresses negatively impacting growth and reproductive performance. Nutrition management strategies and modern technologies in reproductive management should be effectively utilized to reduce the adverse effects of HS and nutrition stress on growth and reproduction in goat.

MATERIALS AND METHODS

CHAPTER 3
MATERIALS AND METHODS

3.1 Location

The experiment was carried out at the National Institute of Animal Nutrition and Physiology experimental livestock farm, Bengaluru, India which is located in southern Deccan plateau of the country at longitude 77º 38´E and the latitude of 12º 58´N and at altitude of 920 m above mean sea level. The average annual maximum and minimum ambient temperature ranges between 15 to 36ºC. The mean annual RH ranges between 20 and 85 per cent. The annual rainfall in this area ranges from 200 to 970 mm with an erratic distribution throughout the year. The average annual minimum and maximum temperature ranges between 15-22 and 27-34 ºC respectively. The average annual RH ranges between 40-85 per cent.. The experiment was carried out during April-May. The temperature and RH variations during the study period (April-May) ranged between 24-38 and 30-38 per cent respectively under hot semi-arid environment. The average meteorological data for the entire study period both inside the shed as well as outside are given in table 1 and table 2 respectively. 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 between75-78 are considered stressful and THI above 78 considered Extreme distress.

3.2 Animals

Osmanabadi is a dual purpose (meat and milk) hardy goat breed, which originated in the semi-arid areas of central tropical India. The *Osmanabadi* breed derives its name from its habitat and distributed in Ahmednagar, Solapur and Osmanabad districts in Maharashtra (Motghare *et al*., 2005; Deokar *et al*., 2006). It has spread over a wide range of agro-climatic conditions in Maharashtra and adjoining parts of Karnataka and Andhra Pradesh. The goats are large in size. Coat colour varies, but mostly it is black (73 per cent) and the rest are white, brown or spotted. The average body weights of adult male and female animals are 34 kg and 30 kg

respectively. The breed is considered useful both for meat and milk. Average daily yield varies from 0.5 to 1.5 kg for a lactation length of about 4 months. In favourable conditions the does will breed regularly twice a year and twinning is common in this breed. The study was conducted in 24 (one year old) *Osmanabadi* bucks weighing between 15 to 20 kg. The animals were housed in well-ventilated sheds made up of asbestos roofing at the height 2.4 m and open from side and maintained under proper hygienic conditions. Prophylactic measures against goat diseases like goat pox, peste des petits ruminants, enterotoxaemia, endo and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

3.3 Technical programme

The study was conducted for a period of 45 days. Twenty four adult bucks were used in the study. They were randomly allocated into four groups of six animals each viz., C (n=6; control), HS (n=6; Heat Stress), NS (n=6; Nutritional Stress) and CS (n=6; Combined Stress). The animals were stall fed with a diet consisting of 60 per cent roughage (Hybrid Napier) and 40 per cent concentrate (maize 36 kg, wheat bran 37 kg, soya bean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5kg/100 kg of feed). C and NS bucks were maintained in the shed in thermo-neutral condition while HS and CS bucks were exposed outside to summer HS between 10:00 h to 16:00 h. C and HS bucks were provided with *ad libitum* feeding while NS and CS bucks were provided with restricted feed (30 per cent of intake of *ad libitum*) to induce NS. All four group animals were fed and watered individually throughout the study period. All cardinal weather parameters were recorded both inside and outside the shed. Semen samples and blood samples were collected at fortnightly interval. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to both HS and NS and for slaughtering the animals for collection of organs for histopathological section and gene expression.

Table 1: Ingredients and chemical composition of concentrate mixture and hybrid napier hay fed to goats

Attribute	Concentrate mixture (kg/100 kg)	Napier hay (*Pennisetum purpureum*)
Ingredients		
Maize	36	-
Wheat bran	37	-
Soybean meal	25	-
Mineral mixture	1.5	-
Salt	0.5	-
Chemical composition (%)		
Dry matter	92.9±0.079	94.0±0.289
Organic matter	95.9±0.190	95.4±0.298
Crude protein	19.6±0.176	6.21±0.098
Ether extract	1.82±0.183	1.49±0.026
Total ash	4.10±0.190	4.64±0.298
Fibre fractions (%)		
Neutral detergent fibre	40.4±1.400	82. 9±0.881
Acid detergent fibre	11.1±0.239	64.6±1.950
Acid detergent lignin	2.14±0.029	12.3±0.651
Nutritive value		
Total digestible nutrients %*	72.2	55.0
Digestible energy (kJ/kg) *	13.3	10.1
Metabolizable energy (kJ/kg) *	10.9	8.28

*Calculated values

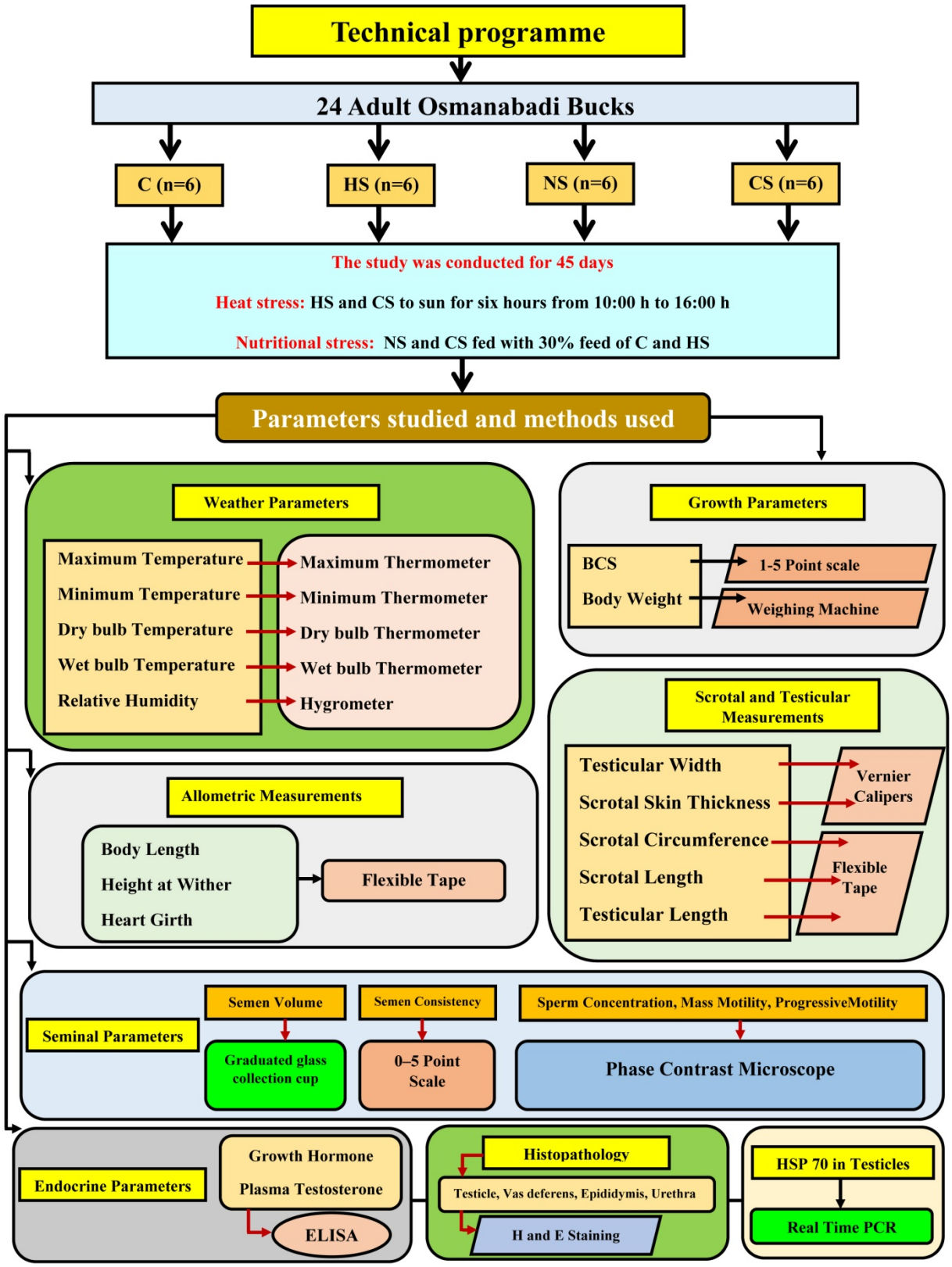


Fig. 2 Technical programme – Flow chart

Plate 1: Animal handling

Plate 2: Measuring of feed for the animals

3.4 Weather parameters recording (Procedure)

The weather parameters were recorded twice daily (8:00 h and 14:00 h). The maximum, minimum, wet bulb and dry bulb temperatures were recorded using maximum thermometer, minimum thermometer, wet bulb thermometer and dry bulb thermometer respectively. The humidity was recorded using hygrometer.

3.5 Growth Parameters (Procedure)

3.5.1 Body weight recording

Body weight was measured using weighing machine (Essae-Teraoka Limited, India,) in kg.

3.5.2 BCS

The bucks were condition scored in the scale of 1–5 where, '1' indicates completely emaciated while '5' indicate very fatty bucks. BCS of the bucks was assessed by careful palpation of the spinous and transverse process in the loin area, immediately behind the last rib as described by Russel *et al*., (1969). The scoring pattern followed for BCS calculation is as follows:

Score	**Assessment**
1	Emaciated
2	Thin
3	Average
4	Fat
5	Obese

Plate 3: Feeding of animals

Plate 4: Recording of weather parameters inside the shed

Plate 5: Recording of weather parameters outside the shed

Plate 6: Body weight recording

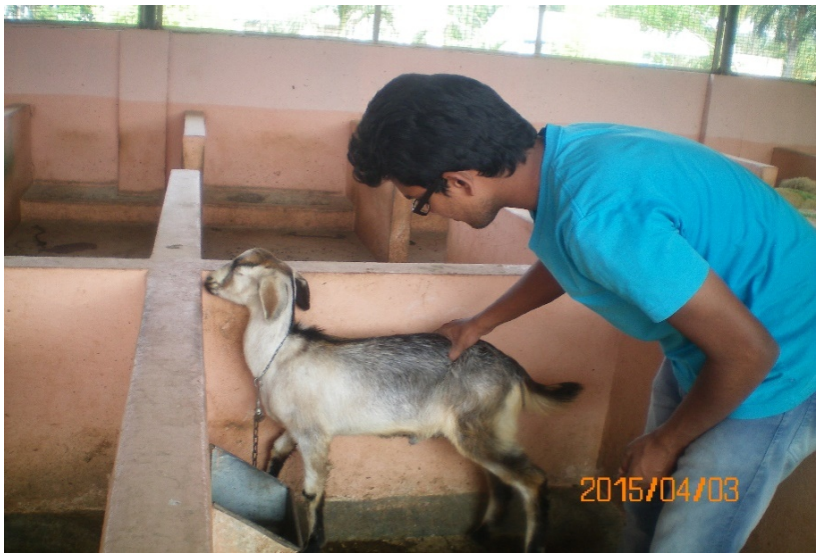


Plate 7: BCS recording

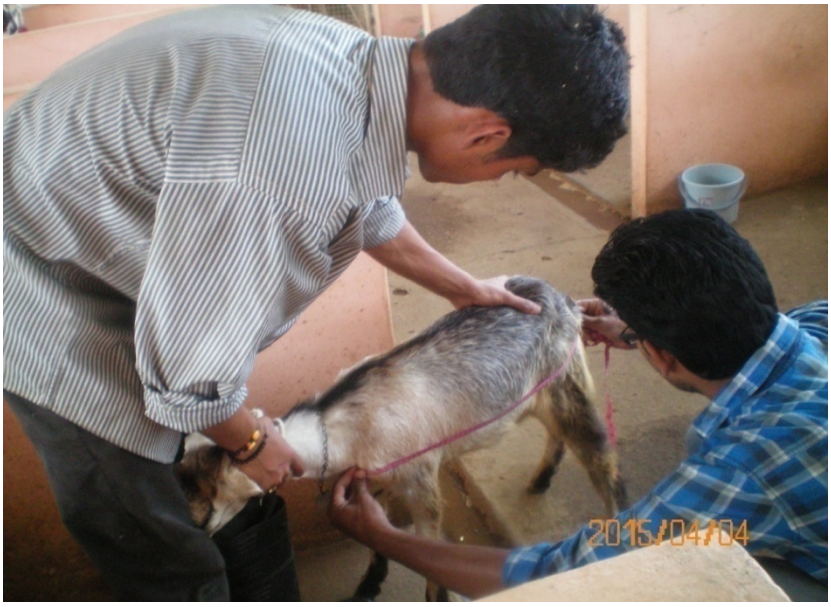


Plate 8: Measurements of body length using flexible tape

3.5.3 Allometric measurements

Allometric measurements were measured using a flexible tape in cm. This includes:

5.3.1 Body length: This was measured from the point of shoulder to the pin bone point.

5.3.2 Height at withers: This was measured from wither to tip of fore leg

5.3.3 Heart girth**:** This is the circumference of the body at a point immediately behind the fore limbs and perpendicular to the body axis.

3.6 Scrotal and testicular measurements

3.6.1 Testicular width, Scrotal skin thickness (SST): Measured using Vernier calipers in centimeters.

3.6.2 SC, Scrotal length (SL), Testicular length: Measured using flexible tape in centimeters.

3.7 Seminal parameters (Principle, methodology and calculation)

Semen samples were collected from the bucks by electro ejaculation method. A battery-operated electro-ejaculator (Bailey ejaculator, Western instrument company, Colorado) and a series of short electrical stimuli (approximately 5 sec) were administered at 20 sec intervals (Al-Ghalban *et al.*, 2004). After ejaculation, the semen samples were transferred immediately to the laboratory in a thawing jar containing water at 37°C and assessed for:

3.7.1 Semen volume:

Measured directly to the nearest 0.1 ml using a graduated glass collection cup.

3.7.2 Semen consistency:

Graded on 0–5 point scale. The scoring pattern followed for semen consistency calculation is as follows:

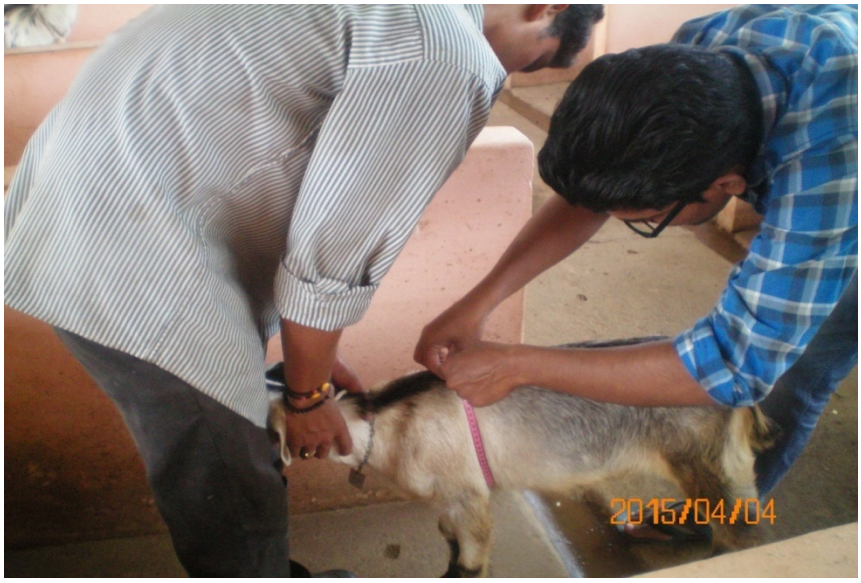


Plate 9: Measuring the heart girth using flexible tape

Plate 10: Measurements of scrotal length using flexible tape

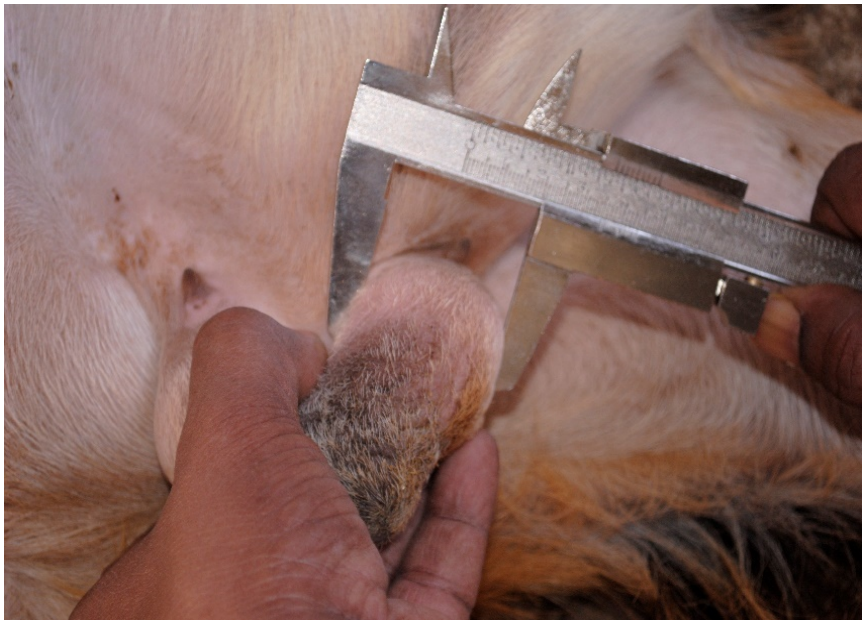


Plate 11: Measurements of scrotal width using Vernier callipers

Plate 12: Electro ejaculation method of semen collection

Score	Assessment
<1	Watery
1-1.5	Thin
2-2.5	Thick
2.5-3	Very thick

3.7.3 Sperm concentration:

Determined using phase contrast microscope fitted with stage warmer in $\times 10^6$/ml.

3.7.4 Mass motility:

The semen was kept in the warm water bath (37°C) and the mass motility was graded on 0–5 point scale within 30 min of the ejaculation. The mass activity was evaluated on a small drop of freshly collected neat semen kept on a clean pre warmed glass slide at 37°C and examined under low power magnification (10X) in phase contrast microscope fitted with thermostatically controlled warm stage. The mass activity of semen was graded based on the presence and intensity of waves and the samples were scored on a scale of 0 (no swirl waves and eddies) to 5 (very fast waves and eddies).

3.7.5 Progressive motility:

The semen was kept in the warm water bath (37°C) and individual progressive forward motility (%) was assessed within 30 min of the ejaculation. An aliquot (5 µL) of the diluted semen samples in phosphate buffered saline was placed on a pre-warmed glass slide (37°C), covered with a cover slip and per cent progressive forward motile spermatozoa was recorded using phase contrast microscope by two independent observers and the values were averaged for each ejaculate.

Plate 13: Semen analysis using Phase contrast microscope

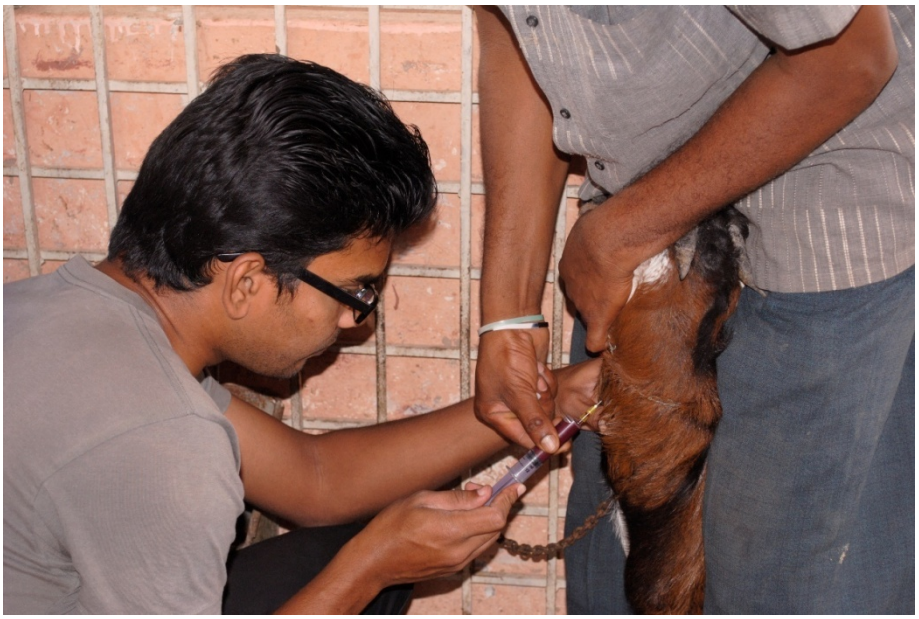


Plate 14: Blood collection

3.8 Blood collection (Procedure)

Three ml of blood samples were collected fortnightly from all four groups simultaneously at 14:00 h using 20 gauge sterilized needles and plastic syringe from external jugular vein in tubes. Heparin (Sisco Research Laboratories pvt. Ltd, Bombay) was used as the anticoagulant @ 10 IU per ml of blood.

3.8.1 Plasma separation (Procedure)

Plasma was separated from blood by centrifugation at 3,500 rpm at room temperature for 20 min. The plasma was then divided into aliquots in microcentrifuge tubes, and kept frozen at -20°C till further analysis. Plasma samples were used to estimate endocrine parameters.

3.9 Estimation of endocrine parameters (Principle, procedure and calculation)

Endocrine parameters included in the study were GH and plasma testosterone. The parameters GH and plasma testosterone was estimated by ELISA (LDN, Nordhorn, Germany).

3.9.1 Plasma testosterone

Principle

The principle of the enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and samples) and an enzyme– labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in samples and controls can be directly read.

Procedure

- It was assured that all reagents reached room temperature before use. Standards, controls and specimen samples were assayed in duplicate. Once the procedure was started, all steps were completed without interruption.
- The working solution of the testosterone -Horseradish Peroxidase (HRP) conjugate and wash buffer were prepared.
- 50 µL of each standard, control and specimen sample were pipetted into correspondingly labelled wells in duplicate.
- 100 µL of the conjugate working solution was pipetted into each well using multichannel pipette.
- The plate was incubated on a plate shaker (approximately 200 rpm) for 1 h at room temperature.
- The wells were washed 3 times with prepared wash buffer (300 µL/ well for each wash) and the plate was taped firmly against absorbent paper to ensure that it is dry.
- 150 µL of tetramethylbenzidine (TMB) substrate was pipetted into each well at timed intervals.
- The plate was incubated on a plate shaker at room temperature for 10-15 min (or until calibrator A attained a dark blue colour for desired OD)
- 50 µL of stopping solution into was added into each well at the same timed intervals as done for pipetting TMB substrate.
- The plate was read on a microwell plate reader (Thermo Scientific, MULTISCAN GO, Finland) at 450 nm within 20 min of addition of the stopping solution.

Calculations

1. The mean optical density of each standard duplicate was calculated.
2. A standard curve was drawn on semi- log paper with the mean optical densities on the Y- axis and the standard concentrations on the X-axis.
3. The mean optical density of each unknown duplicate was calculated.
4. Value of the unknowns were read directly of each unknown duplicate.

3.9.2 Growth hormone

Principle

The principle of the enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for hGH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of hGH is conjugated to horse radish peroxidase (HRP). hGH from the sample and standards are allowed to bind HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of hGH in the sample. A set of standards is used to plot a standard curve from which the amount of hGH in samples and controls can be directly read.

Procedure

- It was assured that all reagents reached room temperature before use. Calibrators, controls and specimen samples were assayed in duplicate. Once the procedure was started, all steps were completed without interruption.
- Prepared working solution of the anti-hGH-HRP-Conjugate and wash buffer.
- 25 µL of each calibrator, control and specimen sample were pipetted into correspondingly labelled wells in duplicate.
- 100 µL of the conjugate working solution was added into each well using multichannel pipette.
- The plate was incubated on a plate shaker (approximately 200 rpm) for 1 h at room temperature.

- The wells were washed 3 times with prepared wash buffer (300 µL/ well for each wash) and the plate was taped firmly against absorbent paper to ensure that it is dry.
- 100 µL of tetramethylbenzidine (TMB) substrate was pipetted into each well at timed intervals.
- The plate was incubated on a plate shaker at room temperature for 10-15 min (or until calibrator A attained a dark blue colour for desired OD)
- 50 µL of stopping solution was added into each well at the same timed intervals as done for pipetting TMB substrate.
- The plate was read on a microwell plate reader (Thermo Scientific, MULTISCAN GO, Finland) at 450 nm within 20 min of addition of the stopping solution.

Calculations

- The mean optical density of each calibrator duplicate was calculated.
- The mean optical density of each unknown duplicate was calculated.
- The mean absorbance value of the "0" calibrator was subtracted from the mean absorbance values of the calibrators, controls and specimen samples.
- A calibrator curve was drawn on log paper with the mean optical densities on the Y- axis and the calibrator concentrations on the X-axis.
- The value of the unknowns were read directly of the calibrator curve.

3.10 Histopathology of male reproductive tract (Principle, methodology)

Histopathological observations in different groups of animals subjected to different kinds of stress:

The tissues were collected immediately after sacrifice from same site in all the animals from C, HS, NS and CS in buffered 10 per cent formalin. The fixed tissues were processed routinely to get Haematoxylin and Eosin (H&E) stained sections (Luna, 1968). The organs collected for the study includes testicle, vas deferens, epididymis and urethra. The slides were interpreted in comparison with C group and representative lesions were photographed.

Plate 15: Tissue sample collection on the day of slaughter

3.11 Expression of HSP-70 in testicle

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

3.11.1 Sample collection and storage

The testis 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 RNA shield (Zymo Research, USA). All the samples were stored at -80°C till further use.

3.11.2 Sample preparation for RNA isolation

After thawing, the tissues were removed from RNA shield (Zymo Research, USA) 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 it 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.

3.11.3 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).

3.11.4 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 minat 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 25ng/µL with nuclease free water and 2 µL of diluted cDNA was used for each reaction in qPCR.

3.11.5 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/). The preferences were given to the primers binding to the exon-exon junction. The primers were titrated with different concentrations (10, 5, 2.5 and 1 μM) for selecting optimum concentration to be used for qPCR experiments.

3.11.6 Quantitative RT-PCR analysis

The relative expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 20 μL reaction was carried out in duplicates 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 30sec and extension at 72^0C for 30 sec). The melt curve analysis was performed to check the non-specific amplification. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control and the relative expression was analyzed using the formula, $2^{\Delta\Delta CT}$ (Tarif *et al*., 2012). The results were expressed in fold change as compared to untreated control (control=1fold).

3.12 Statistical Analysis

The data was analyzed by general linear model (GLM) repeated measurement analysis of variance (SPSS16.0). Effect of fixed factors namely C, HS, NS and CS was taken as between subject factor and days (longitudinal time over which experiment was carried out; Day0, Day 15, Day 30 and Day 45) were taken as within subject factor and also interaction between group and days was analyzed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests as described by Kramer, (1957).

Table 2: Primers used for HSP70 expression. GAPDH used as reference gene to normalize the gene expression of target genes

Gene ID	Primers	Primer sequence (5”- 3”)	Primer Length (bp)	Product Size (bp)	Accession No
HSP70	F	TGGCTTTCACCGATACCGAG	20	167	NM_001285703.1
	R	GTCGTTGATCACGCGGAAAG	20		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	20	265	AF030943
	R	TCATAAGTCCCTCCACGATG	20		

HSP70 -Heat Shock Protein 70; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase

RESULTS

CHAPTER 4
RESULTS

4.1 Weather parameters

The maximum, minimum, wet and dry bulb temperatures and RH were recorded and the THI was calculated on an average of fortnightly interval. The obtained THI inside during both morning and afternoon are described in table 3. The THI values inside the shed show that the animals were in comfort zone both during morning and afternoon.

The THI outside the shed during both morning and afternoon are described in table 4. The THI values outside the shed shows that during the morning hours the animals were in comfort zone while during afternoon the animals were under extreme distress.

4.2 Body weight, BCS and Allometric measurements changes

The effects of HS, NS and CS on body weight, BCS and Allometric measurements are described in table 5. Body weight recorded showed significant ($P<0.01$) changes for the treatment. There were significant ($P < 0.01$) differences in body weight among the *ad libitum* (C and HS) and non-significant difference between restricted (NS and CS) feeding groups. Both C and HS groups differed significantly ($P < 0.01$) in body weight as compared to restricted feeding groups (NS and CS). Further, experimental days significantly ($P<0.01$) influenced body weight throughout the study period. In addition, there was significant ($P<0.01$) interaction between groups and experimental days for the body weight in the study. BCS recorded also showed significant ($P<0.01$) changes for the treatment. There were significant ($P < 0.01$) differences in BCS among the *ad libitum* (C and HS) and non-significant difference between restricted (NS and CS) feeding groups. Both C and HS groups differed significantly ($P < 0.01$) for BCS as compared to restricted feeding groups (NS and CS). However, experimental days did not influence BCS in the study. But interaction between groups and experimental days differed significantly ($P < 0.01$) for the BCS in the study.

Heart girth recorded also showed significant ($P<0.05$) changes for the treatment. There were only significant ($P < 0.05$) differences in heart girth in CS

Table 3: Mean and SEM of climatological data during the experimental period inside the shed

Time of Recording	Minimum temperature (º C)	Maximum temperature (º C)	Dry bulb temperature (º C)	Wet bulb temperature (º C)	RH (%)	THI
Morning (8:00 hrs)	21.50 ±0.60	32.37 ±0.20	23.80 ±0.21	20.10 ±0.17	78.67 ±4.67	72.21 ±0.25
Afternoon (14:00 hrs)	24.4 ±0.56	35.60 ±0.72	26.47 ±0.28	20.93 ±0.09	45.67 ±7.21	74.73 ±0.22

SEM-Standard Error Mean; RH- Relative Humidity; THI- Temperature Humidity Index

Table 4: Mean and SEM of climatological data during the experimental period in the outside environment

Time of Recording	Minimum temperature (º C)	Maximum temperature (º C)	Dry bulb temperature (º C)	Wet bulb temperature (º C)	RH (%)	THI
Morning (8:00 hrs)	26.93 ±2.87	34.03 ±0.52	23.13 ±0.20	22.97 ±0.66	61.00 ±7.77	73.92 ±0.57
Afternoon (14:00 hrs)	27.23 ±3.46	38.33 ±0.52	29.57 ±0.38	26.53 ±0.71	37.00 ±4.16	80.99 ±0.25

SEM-Standard Error Mean; RH- Relative Humidity; THI- Temperature Humidity Index

Table 5: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on body weight, BCS and allometric measurements in goats

FACTOR	Body Weight (Kg)	BCS (1-5 point Scale)	Heart girth (cm)	Height at wither (cm)	Body length (cm)
μ ± SE	15.29±0.28	2.44±0.05	62.06 ± 0.55	60.08 ± 0.46	55.02 ± 0.48
GROUP	**	**	*	ns	**
C	17.37[a]±0.36	3.02[a]±0.11	63.52[a]± 0.70	62.16[a]± 0.64	57.46[a]± 0.65
HS	16.18[a]±0.27	2.73[b]±0.06	63.36[ab] ± 0.58	60.77[a]± 0.64	56.70[a]± 0.63
NS	13.84[b]±0.43	2.13[c]±0.08	61.84[b]± 0.48	58.80[ab]± 0.49	53.10[b]± 0.77
CS	13.78[b]±0.48	1.90[c]±0.11	59.50[b]± 0.74	58.61[b]± 0.84	52.81[b]± 0.95
DAYS	**	ns	ns	ns	**
0	16.36±0.28	2.42±0.07	62.25± 0.41	60.11 ± 0.50	55.91± 0.56
15	15.33±0.29	2.50±0.07	62.45± 0.61	60.34 ± 0.64	55.75± 0.56
30	14.96±0.28	2.52±0.05	61.94 ± 0.62	61.05 ± 0.50	55.03 ± 0.67
45	14.23±0.30	2.33±0.08	61.58± 0.85	58.83 ± 0.51	53.39 ± 0.67
GROUP * DAYS	**	**	**	**	**
C0	16.37±0.56	2.33±0.13	61.43 ± 0.81	59.72 ± 1.01	57.07 ± 1.11
C15	16.90±0.57	2.92±0.13	63.60 ± 1.22	61.03 ± 1.27	57.33 ± 1.12
C30	18.02±0.57	3.33±0.11	64.03 ± 1.24	63.75 ± 1.01	57.13 ± 1.34
C45	18.42±0.60	3.50±0.16	65.00 ± 1.69	64.13 ± 1.03	58.32 ± 1.34
HS0	16.37±0.56	2.58±0.13	62.25 ± 0.81	59.28 ± 1.01	55.70 ± 1.11
HS15	16.30±0.57	2.67±0.13	63.90 ± 1.22	61.60 ± 1.27	55.73 ± 1.12
HS30	16.10±0.57	2.83±0.11	63.12 ± 1.24	61.48 ± 1.01	56.05 ± 1.34
HS45	15.80±0.60	2.83±0.16	64.17 ± 1.69	60.70 ± 1.03	59.32 ± 1.34
NS0	16.33±0.56	2.33±0.13	63.52 ± 0.81	59.95 ± 1.01	54.65 ± 1.11
NS15	13.63±0.57	2.33±0.13	61.38 ± 1.22	59.40 ± 1.27	54.65 ± 1.12
NS30	12.90±0.57	2.17±0.11	61.77 ± 1.24	59.75 ± 1.01	53.13 ± 1.34
NS45	11.73±0.60	1.67±0.16	60.70 ± 1.69	56.10 ± 1.03	49.97 ± 1.34
CS0	16.37±0.56	2.42±0.13	61.80 ± 0.81	61.48 ± 1.01	56.23 ± 1.11
CS15	14.47±0.57	2.08±0.13	60.90 ± 1.22	59.33 ± 1.27	55.28 ± 1.12
CS30	12.83±0.57	1.75±0.11	58.85 ± 1.24	59.23 ± 1.01	53.78 ± 1.34
CS45	10.98±0.60	1.33±0.16	56.47 ± 1.69	54.38 ± 1.03	45.95 ± 1.34

BCS-Body Condition Score; C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

bucks as compared to other groups (C, HS and NS).However; experimental days did not influence heart girth in the study. But interaction between groups and experimental days differed significantly ($P < 0.01$) for the heart girth in the study. Both treatment and experimental days did not influence height at withers in the study. However, interaction between groups and experimental days differed significantly ($P < 0.01$) for the height at withers in the study. Length of the animal recorded between point of shoulder to pin bone showed significant ($P<0.01$) changes for the treatment. There were no significant differences in length among the *ad libitum* (C and HS) and restricted (NS and CS) feeding groups. However, length differed significantly ($P<0.01$) between *ad libitum* and restricted fed groups. Further, experimental days significantly ($P<0.01$) influenced length of the animals throughout the study period. In addition, there was significant ($P<0.01$) interaction between groups and experimental days for the length of the animal.

4.3 Scrotal measurements

The effects of HS, NS and CS on scrotal measurements are described in table 6. Among the scrotal measurements, SCA and SLA differed significantly ($P<0.05$) between the groups. However, SCM, SLM, SSTM and SSTA did not differ between the groups. Further, the experimental days in the study significantly ($P<0.01$) influenced SCM, SSTM and SSTA. In addition, interaction between groups and experimental days influenced significantly ($P<0.01$) SCM and SCA in the study.

4.3 Testicular measurements

The effects of HS, NS and CS on testicular measurements are described in both table 5 and table 7. The different treatment significantly influenced LTLA ($P<0.01$), LTWM ($P<0.05$) and LTWA ($P<0.01$) parameters but did not influence LTLM. The experimental days in the study highly significantly ($P<0.01$) influenced all the left testicular parameters (table 3). Further, the interaction (table 3) between groups and experimental days significantly influenced LTLM ($P<0.05$), LTLA ($P<0.01$), LTWM ($P<0.05$) and LTWA ($P<0.01$). The different treatment in the study significantly influenced only RTLA ($P<0.05$).The experimental days in the study highly significantly ($P<0.01$) influenced RTLM,

Table 6: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on scrotal measurements in goats

FACTOR	SCM (cm)	SCA (cm)	SLM (cm)	SLA (cm)	SSTM (cm)	SSTA (cm)
μ ± SE	16.11 ± 0.18	16.62 ± 0.16	10.72 ± 0.15	11.23 ± 0.19	0.33 ± 0.01	0.28 ± 0.007
GROUP	ns	*	ns	*	ns	ns
C	16.18^{a}± 0.30	16.48^{b}± 0.30	10.87^{a}± 0.26	10.92^{bc}± 0.24	0.34^{a}± 0.01	0.30^{a}± 0.01
HS	16.65^{a}± 0.13	17.55^{a}± 0.15	11.15^{a}± 0.20	12.08^{a}± 0.23	0.33^{a}± 0.01	0.28^{a}± 0.01
NS	15.78^{a}± 0.15	16.00^{b}± 0.16	10.30^{a}± 0.19	10.38^{c}± 0.28	0.33^{a}± 0.01	0.28^{a}± 0.01
CS	15.84^{a}± 0.15	16.45^{b}± 0.09	10.54^{a}± 0.16	11.56^{ab}± 0.19	0.31^{a}± 0.01	0.26^{a}± 0.01
DAYS	**	ns	ns	ns	**	**
0	16.35 ± 0.20	16.52 ± 0.23	10.89 ± 0.17	11.25 ± 0.19	0.35 ± 0.02	0.33± 0.01
15	16.17 ± 0.20	16.64 ± 0.19	10.67 ± 0.27	11.51 ± 0.28	0.33 ± 0.01	0.29 ± 0.01
30	15.81 ± 0.15	16.44± 0.18	10.70 ± 0.18	11.25 ± 0.20	0.33 ± 0.01	0.25 ± 0.01
45	16.13 ± 0.22	16.86 ± 0.15	10.60 ± 0.22	10.93 ± 0.27	0.29 ± 0.01	0.24 ± 0.01
GROUP * DAYS	**	**	ns	ns	ns	ns
C0	15.78 ± 0.40	15.85 ± 0.46	10.70 ± 0.34	11.03 ± 0.38	0.34 ± 0.03	0.33 ± 0.01
C15	16.48 ± 0.40	16.80 ± 0.37	10.72 ± 0.53	11.03 ± 0.56	0.35 ± 0.02	0.32 ± 0.02
C30	16.08 ± 0.30	16.50 ± 0.37	10.83 ± 0.36	10.80± 0.41	0.35 ± 0.02	0.30 ± 0.02
C45	16.37 ± 0.43	16.77 ± 0.29	11.23 ± 0.44	10.80 ± 0.54	0.32 ± 0.02	0.25 ± 0.02
HS0	16.83 ± 0.40	16.95 ± 0.46	11.05 ± 0.34	11.43 ± 0.38	0.34 ± 0.03	0.33 ± 0.01
HS15	16.67 ± 0.40	17.72 ± 0.37	11.42 ± 0.53	12.95 ± 0.56	0.33 ± 0.02	0.29 ± 0.02
HS30	16.23 ± 0.30	17.27 ± 0.37	11.00 ± 0.36	12.25 ± 0.41	0.33 ± 0.02	0.25 ± 0.02
HS45	16.88 ± 0.43	18.25 ± 0.29	11.12 ± 0.44	11.68 ± 0.54	0.30 ± 0.02	0.24 ± 0.02
NS0	16.10 ± 0.40	16.57 ± 0.46	10.82 ± 0.34	11.12 ± 0.38	0.38 ± 0.03	0.33 ± 0.01
NS15	15.82 ± 0.40	15.65 ± 0.37	10.20 ± 0.53	10.23 ± 0.56	0.31 ± 0.02	0.31 ± 0.02
NS30	15.65 ± 0.30	15.80 ± 0.37	10.38 ± 0.36	10.17 ± 0.41	0.33 ± 0.02	0.26 ± 0.02
NS45	15.55 ± 0.43	15.97 ± 0.29	09.82± 0.44	10.00 ± 0.54	0.29 ± 0.02	0.23 ± 0.02
CS0	16.68 ± 0.40	16.72 ± 0.46	10.98 ± 0.34	11.42 ± 0.38	0.32 ± 0.03	0.33 ± 0.01
CS15	15.70 ± 0.40	16.40 ± 0.37	10.35 ± 0.53	11.82 ± 0.56	0.34 ± 0.02	0.26 ± 0.02
CS30	15.25 ± 0.30	16.20 ± 0.37	10.57 ± 0.36	11.77 ± 0.41	0.31 ± 0.02	0.21 ± 0.02
CS45	15.73 ± 0.43	16.47 ± 0.29	10.25 ± 0.44	11.23 ± 0.54	0.26 ± 0.02	0.24 ± 0.02

SCM-Scrotal Circumference Morning; SCA- Scrotal Circumference Afternoon; SLM- Scrotal Length Morning; SLA- Scrotal Length Afternoon; SSTM- Scrotal Skin Thickness Morning; SSTA- Scrotal Skin Thickness Afternoon;C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Table 7: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on testicular measurements in goats

FACTOR	LTLM (cm)	LTLA (cm)	LTWM (cm)	LTWA (cm)
μ ± SE	10.48±0.21	10.99±0.18	3.21±0.09	3.08±0.08
GROUP	ns	**	*	**
C	10.58^{a}±0.29	10.87^{b} ±0.25	3.28^{ab}±0.15	3.15^{ab}±0.14
HS	11.33^{a}±0.21	12.20^{a} ±0.21	3.68^{a}±0.09	3.53^{a}±0.07
NS	10.03^{a}±0.38	10.20^{b} ±0.34	3.02^{b}±0.11	2.90^{b}±0.11
CS	09.98^{a}±0.28	10.69^{b} ±0.26	2.85^{b}±0.12	2.75^{b}±0.11
DAYS	**	**	**	**
0	11.24±0.34	11.48±0.31	3.26±0.12	3.16±0.08
15	10.53±0.21	11.17±0.23	3.46±0.12	3.30±0.10
30	10.35±0.32	10.95±0.24	3.32±0.11	3.22±0.11
45	09.80±0.22	10.36±0.21	2.76±0.07	2.66±0.08
GROUP * DAYS	*	**	*	**
C0	10.57±0.67	10.68±0.62	2.98±0.23	2.90±0.15
C15	10.35±0.42	10.78±0.45	3.58±0.23	3.50±0.20
C30	10.55±0.64	10.87±0.48	3.40±0.23	3.20±0.22
C45	10.85±0.44	11.13±0.42	3.15±0.14	3.02±0.16
HS0	11.63±0.67	11.95±0.62	3.62±0.23	3.50±0.15
HS15	11.37±0.42	12.48±0.45	4.12±0.23	3.68±0.20
HS30	11.67±0.64	12.72±0.48	3.70±0.23	3.55±0.22
HS45	10.65±0.44	11.63±0.42	3.28±0.14	3.37±0.16
NS0	11.23±0.67	11.48±0.62	3.20±0.23	3.12±0.15
NS15	10.27±0.42	10.35±0.45	3.20±0.23	3.08±0.20
NS30	09.68±0.64	09.77±0.48	3.25±0.23	3.20±0.22
NS45	08.95±0.44	09.20±0.42	2.43±0.14	2.22±0.16
CS0	11.53±0.67	11.78±0.62	3.30±0.23	3.13±0.15
CS15	10.13±0.42	11.07±0.45	3.00±0.23	2.93±0.20
CS30	09.52±0.64	10.45±0.48	2.93±0.23	2.92±0.22
CS45	08.75±0.44	09.47±0.42	2.17±0.14	2.03±0.16

LTLM-Left Testicular Length Morning; LTLA- Left Testicular Length Afternoon; LTWM- Left Testicular Width Morning; LTWA- Left Testicular Width Afternoon; C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns - Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

RTLA and RTWM while significantly (P<0.05) influenced RTWA (table 8). Further, the interaction between groups and experimental days significantly influenced only RTWM (P<0.01) and RTWA (P<0.01).

4.4 Seminal attributes

The effects of HS, NS and CS on seminal attributes are described in table 9. Semen volume of C group differed significantly (P<0.01) as compared to HS, NS and CS bucks. The highest semen volume was recorded in C group bucks. The mass motility and progressive motility showed similar trend between the groups. The significantly (P<0.05) higher mass motility and progressive motility was recorded in C group bucks. However, both mass motility and progressive motility did not differ between the stress groups (HS, NS and CS). However, sperm concentration and semen consistency did not differ between the groups. The experimental days significantly influenced all the seminal attributes in the study. The experimental days significantly influenced sperm concentration (P<0.05), semen volume (P<0.01), semen consistency (P<0.05), mass motility (P<0.01) and progressive motility (P<0.01). However, the interaction between groups and experimental days significantly influenced only semen volume (P<0.01) and semen consistency (P<0.05) in the study.

4.5 Endocrine parameters

The effects of HS, NS and CS on plasma endocrine profile are described in table 10. Plasma GH level showed significant (P<0.01) variation between the groups. The highest plasma GH was recorded in CS group and the lowest in rest all the groups (C, HS and NS). Further, the experimental days (P<0.05) and interaction between treatment and experimental days (P<0.01) also influenced plasma GH concentration. Plasma testosterone level also showed significant (P<0.01) variation between the groups. The highest plasma testosterone level was recorded in C group and the lowest in rest all groups (HS, NS and CS). Further, experimental days also significantly (P<0.01) influenced plasma testosterone level. However, interaction between treatment and experimental days did not influence plasma testosterone concentration.

Table 8: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on testicular measurements in goats

FACTOR	RTLM (cm)	RTLA (cm)	RTWM (cm)	RTWA (cm)
μ ± SE	11.00±0.21	11.58±0.19	3.30±0.08	3.24±0.08
GROUP	ns	*	ns	ns
C	11.16^{a}±0.38	11.45^{ab}±0.33	3.31^{ab}±0.12	3.29^{ab}±0.13
HS	11.64^{a}±0.23	12.43^{a}±0.20	3.59^{a}±0.08	3.58^{a}±0.06
NS	10.42^{a}±0.28	10.81^{b}±0.24	3.09^{b}±0.10	3.00^{b}±0.09
CS	10.76^{a}±0.23	11.64^{ab}±0.15	3.22^{ab}±0.13	3.08^{b}±0.12
DAYS	**	**	**	*
0	12.04±0.28	12.34±0.26	3.52±0.09	3.39±0.08
15	10.74±0.30	11.47±0.24	3.32±0.12	3.27±0.11
30	10.83±0.27	11.48±0.21	3.32±0.1	3.15±0.11
45	10.38±0.23	11.05±0.20	3.05±0.07	3.13±0.09
GROUP * DAYS	ns	ns	**	**
C0	12.22±0.56	12.43±0.52	3.14±0.18	3.20±0.15
C15	10.68±0.60	11.27±0.48	3.50±0.24	3.32±0.22
C30	11.27±0.54	11.33±0.42	3.25±0.20	3.35±0.21
C45	10.48±0.45	10.77±0.41	3.33±0.14	3.28±0.19
HS0	12.43±0.56	12.73±0.52	3.75±0.18	3.65±0.15
HS15	11.45±0.60	12.62±0.48	3.38±0.24	3.60±0.22
HS30	11.95±0.54	12.57±0.42	3.73±0.20	3.33±0.21
HS45	10.72±0.45	11.80±0.41	3.48±0.14	3.72±0.19
NS0	11.47±0.56	11.75±0.52	3.26±0.18	3.00±0.15
NS15	10.33±0.60	10.55±0.48	3.03±0.24	3.08±0.22
NS30	09.92±0.54	10.62±0.42	3.32±020	3.18±0.21
NS45	09.97±0.45	10.32±0.41	2.77±0.14	2.75±0.19
CS0	12.03±0.56	12.43±0.52	3.92±0.18	3.70±0.15
CS15	10.48±0.60	11.43±0.48	3.37±0.24	3.07±0.22
CS30	10.20±0.54	11.38±0.42	2.98±0.20	2.75±0.21
CS45	10.33±0.45	11.32±0.41	2.62±0.14	2.78±0.19

RTLM-Right Testicular Length Morning; RTLA- Right Testicular Length Afternoon; RTWM- Right Testicular Width Morning; RTWA- Right Testicular Width Afternoon;C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns- Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other

Table 9: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on sperm parameters in goats

FACTOR	Sperm Concentration ($\times 10^6$/ml)	Semen Volume (ml)	Semen Consistency (1-3 point Scale)	Mass Motility (1-5 point Scale)	Progressive Motility (%)
μ ± SE	10.72±0.70	0.43±0.02	1.76±0.16	3.2±0.15	64.3±2.840
GROUP	ns	**	ns	*	*
C	13.24^{a}±0.8	0.57^{a}±0.02	1.75^{a}±0.26	4.1^{a}±0.17	79.2^{a}±2.90
HS	11.06^{ab}±1.2	0.41^{b}±0.04	1.98^{a}±0.17	3.2^{b}±0.26	65.8^{ab}±5.24
NS	07.84^{b}±1.3	0.37^{b}±0.05	1.56^{a}±0.22	2.7^{b}±0.34	56.9^{b}±6.53
CS	10.74^{ab}±1.2	0.37^{b}±0.04	1.75^{a}±0.15	2.8^{b}±0.31	55.2^{b}±5.49
DAYS	*	**	*	**	**
0	11.50±1.05	0.43±0.03	2.00±0.19	3.7±0.23	74.4±3.79
15	12.79±0.87	0.53±0.04	1.83±0.21	3.5±0.26	71.5±3.86
30	10.36±1.16	0.39±0.03	1.79±0.21	3.3±0.24	64.0±4.78
45	08.23±1.31	0.35±0.03	1.42±0.21	2.3±0.29	47.3±5.82
GROUP * DAYS	ns	**	*	ns	ns
C0	11.75±2.10	0.47±0.06	1.83±0.39	3.8±0.47	75.0±7.59
C15	13.10±1.74	0.68±0.07	1.67±0.42	4.3±0.52	83.3±7.71
C30	14.03±2.33	0.52±0.07	1.75±0.41	4.3±0.47	81.7±9.56
C45	14.07±2.62	0.60±0.07	1.75±0.42	3.8±0.57	76.7±11.63
HS0	11.77±2.10	0.37±0.06	2.00±0.39	3.8±0.47	80.8±7.59
HS15	12.25±1.74	0.55±0.07	1.67±0.42	3.3±0.52	67.5±7.71
HS30	12.02±2.33	0.28±0.07	2.08±0.41	2.8±0.47	55.8±9.56
HS45	08.22±2.62	0.43±0.07	2.17±0.42	2.8±0.57	59.2±11.63
NS0	10.57±2.10	0.48±0.06	2.25±0.39	3.3±0.47	70.0±7.59
NS15	10.67±1.74	0.50±0.07	2.00±0.42	3.5±0.52	76.7±7.71
NS30	6.07±2.33	0.38±0.07	1.50±0.41	3.0±0.47	60.8±9.59
NS45	4.050±2.62	0.10±0.07	0.50±0.42	1.0±0.57	20.0±11.63
CS0	11.92±2.10	0.42±0.06	1.92±0.39	3.7±0.47	71.7±7.59
CS15	15.15±1.74	0.40±0.07	2.00±0.42	3.0±0.52	58.3±7.71
CS30	09.32±2.33	0.38±0.07	1.83±0.41	2.8±0.47	57.5±9.56
CS45	06.57±2.62	0.27±0.07	1.25±0.42	1.7±0.57	33.3±11.63

C-Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns- Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

Table 10: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional on growth hormone and plasma testosterone.

FACTOR	Growth Hormone (ng/ml)	Plasma Testosterone (ng/ml)
μ ± SE	1.94±0.074	4.44±0.26
GROUP	**	**
C	1.44^{b}±0.12	6.04^{a}±0.33
HS	1.65^{b}±0.15	4.59^{ab}±0.61
NS	1.89^{b}±0.16	3.53^{b}±0.59
CS	2.77^{a}±0.24	3.51^{b}±0.71
DAYS	*	**
0	1.61±0.15	7.08±0.61
15	1.91±0.17	3.38±0.37
30	2.00±0.14	3.87±0.51
45	2.24±0.16	3.43±0.42
GROUP * DAYS	**	ns
C0	1.61±0.21	6.64±1.23
C15	1.64±0.34	5.64±0.75
C30	1.39±0.28	5.56±1.01
C45	1.14±0.32	6.33±0.83
HS0	1.46±0.21	7.65±1.23
HS15	1.80±0.34	3.42±0.75
HS30	1.57±0.21	4.09±1.02
HS45	1.77±0.32	3.22±0.83
NS0	1.76±0.21	6.75±1.23
NS15	1.84±0.34	2.36±0.75
NS30	1.63±0.28	2.79±1.02
NS45	2.31±0.32	2.22±0.83
CS0	1.59±0.21	7.21±1.23
CS15	2.34±0.34	2.11±0.75
CS30	3.49±0.21	3.04±1.02
CS45	3.75±0.32	1.95±0.83

Control; HS-Heat Stress; NS-Nutritional Stress; CS-Combined Stress; μ- Overall Mean; SE- Standard Error**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$; ns- Indicates non-significant; Values bearing different superscripts within a column differ significantly with each other.

4.7 Histopathology

The HE staining results of different organs studied revealed that only testicle section showed significant changes between the groups. However, vas deferens, epididymis, and urethra did not showed significant changes for different treatments in the study. Testicular section showed significant changes for different stresses. These changes in different stress groups (HS, NS and CS) were compared with C group. The highest loss of spermatid density with decreased spermatogenesis was recorded in CS followed by HS and NS groups compared to C group.

4.8 Gene expression

Testicular HSP70 expression

Testicular HSP70 mRNA transcript expression between the C, HS, NS and CS groups of goats are described in plate 17. The results revealed that testicular HSP70 mRNA expression was evident in C (1 fold), HS (1.95 fold), NS (1.51 fold) and CS (1.02 fold). On comparative basis, the higher expression of testicular HSP70 mRNA was recorded in HS goats (plate 17). Within the stress groups, the highest testicular HSP70 mRNA expression was reported in HS group followed by NS and CS groups.

DISCUSSION

CHAPTER 5
DISCUSSION

The current experiment is the first of its kind to assess the impact of cumulative stress effects in livestock which is a common occurrence in the changing climate scenario rather than individual stresses. Research efforts are therefore needed to assess the cumulative effects of the environmental stresses on livestock. In that line, this is the first experiment which attempted to establish the cumulative impact of both HS and NS simultaneously on goat production. With goat considered an important species for securing the socio-economic aspect of poor and marginal farmers, such research efforts would be very valuable if one attempts to improve goat production in the changing climate scenario.

5.1 Growth Parameters

The body weight reduced significantly in CS and NS groups as compared to both C and HS groups. Generally it is a well-established fact in small ruminants that the animals try to reduce their feed intake when subjected to HS in an attempt to reduce their metabolic heat production (Marai *et al*., 2007; Okoruwa, 2014). This may lead to reduction in their body weights. In the present experiment also the different nutritional treatment reflected on the body weight changes. The significantly lower body weight in CS as compared to HS group could be attributed to the differences in their feed intake. This shows that the feeding schedule followed was able to successfully induce nutritional insufficiency in the present study. The CS and NS showed significant variations in body weight whereas HS did not influence body weight significantly as compared to C bucks. However, the same HS when coupled with restricted feeding was very detrimental to body weight. In a similar study on *Malpura* ewes, Sejian *et al*. (2011) reported that HS alone did not influence body weight changes. However, both nutritional restriction and combination of heat and nutritional restriction was found to influence body weight significantly. This shows that proper nutrition during thermal stress is more important to maintain body weight without much variation. Karim and Patnayak, (1998) were also of same opinion that when sheep under HS

Fig.3: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on body weight, BCS and allometric measurements in goats. (a)-Body weight, (b)-BCS, (c)-Length, (d)-Height, (e)-Heart girth

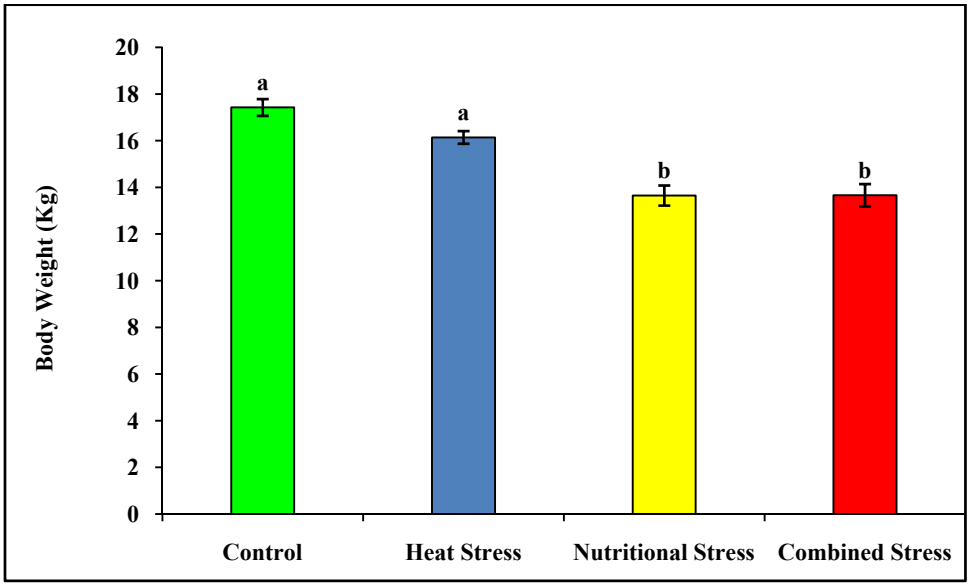


Fig.3 (a): Body Weight

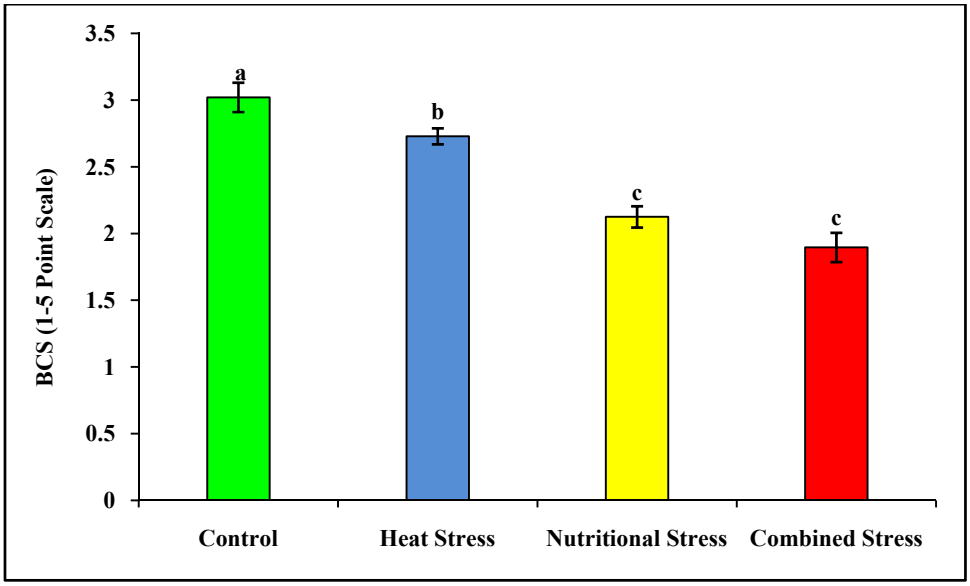


Fig.3 (b): BCS

were fed adequately they could maintain normal body weights. In addition, both experimental days and interactions between treatment and experimental days also significantly influenced body weight. This shows that the relationship between the groups changed over time for body weight indicating perhaps the groups differed in their responses at some time points but not others. This indicates the animals are trying to adapt to the existing conditions to maintain their body weight.

BCS showed significant variations for all treatments in the study. Both HS and NS significantly reduced the BCS in the study. This is evident from the significant variation in BCS between C, HS and NS groups. However, the BCS did not differ between NS and CS groups. This shows the severity of nutritional restriction in inducing changes in BCS. In a study conducted on *Malpura* sheep, Sejian *et al*. (2010) and Maurya *et al*. (2010) reported the sensitivity of nutritional status in inducing changes in BCS under hot semi-arid environment. However, BCS differed significantly between HS and CS groups. The significantly lower BCS in CS as compared to HS group suggests the detrimental effects of cumulative effects of both HS and NS in CS group.

The allometric measurements are body measurements which reflect the growth potential of an animal. Rana *et al*., (2014) reported non-significant influence of HS on heart girth and length of the sheep. Similarly in current study also HS alone did not influence both heart girth and length of the animals. However, when both HS and NS are coupled they were able to bring significant changes in all allometric measurements in CS group. This shows the sensitivity of body measurement changes to nutrition level. Further, McManus *et al*., (2009) reported significant influence of shoulder height, body length, and heart girth to reflect the adaptive capabilities of different breeds of dairy cattle. Blome *et al*., (2003) and Bartlett *et al*., (2006) are also of the same opinion that nutritional status of an animal is the crucial factor influencing allometric measurements in cattle during HS condition. Further, the significant influence of interactions between treatments and experimental days on allometric measurements shows that the relationship between the groups changed over time indicating perhaps the groups differed in their responses at some time points but not others. This

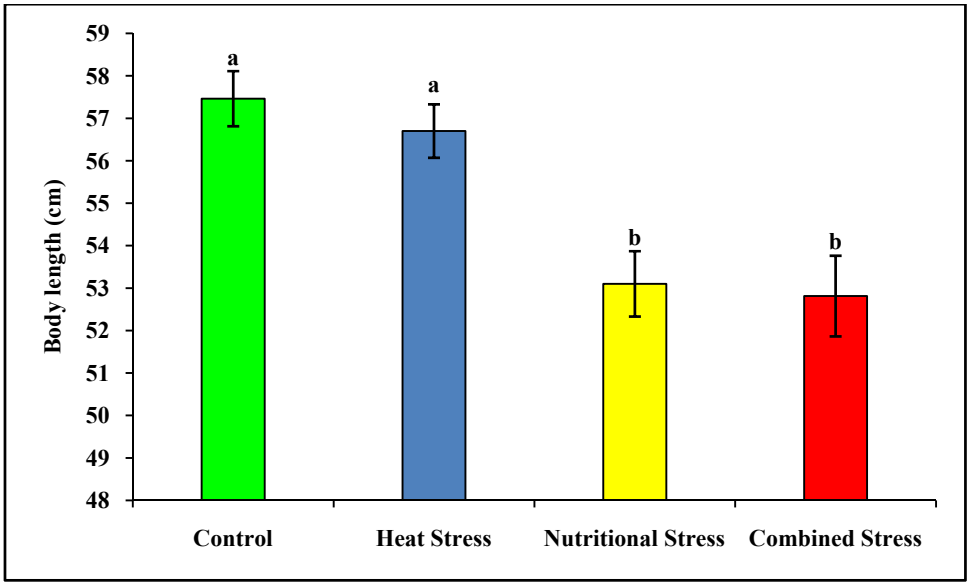


Fig.3 (c): Body length

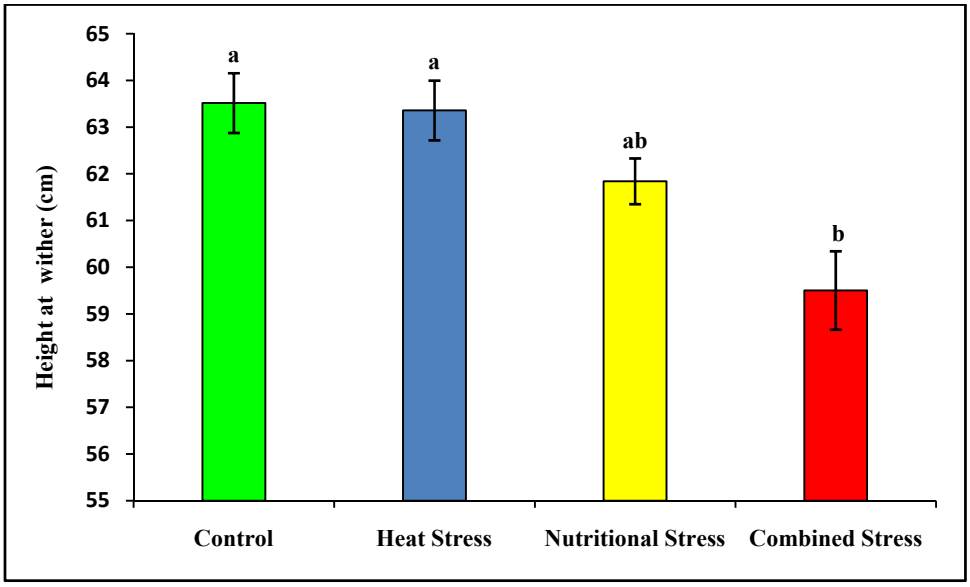


Fig.3 (d): Height at wither

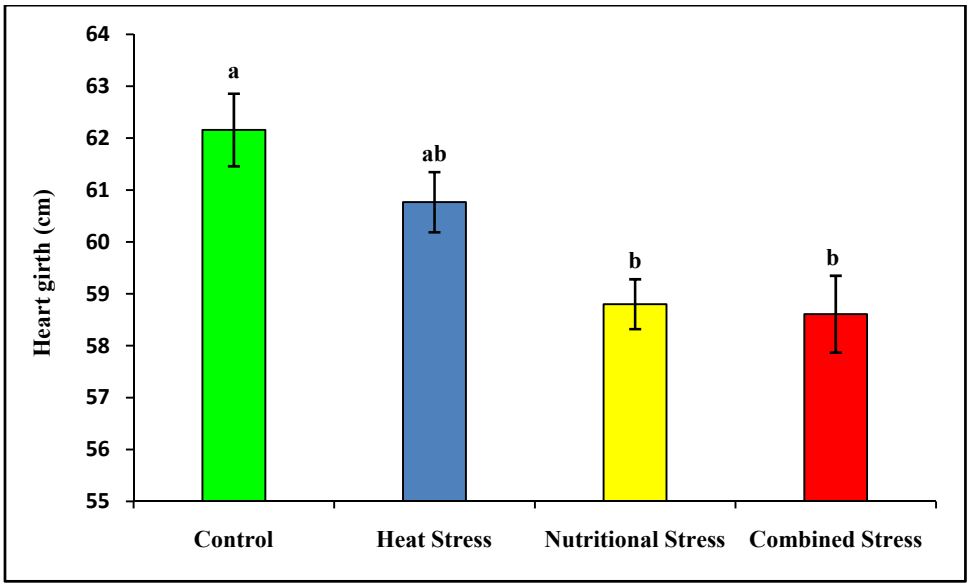


Fig.3 (e): Heart girth

indicates the animals are trying to adapt to the existing nutrition level to maintain their body measurements.

5.2 Scrotal and testicular measurements

Among the scrotal measurements, only SCA and SLA differed significantly between the groups. The highest SCA and SLA was recorded in HS group. Scrotal measurements are generally considered good reliable indicators for reproductive efficiency in livestock species (Maurya *et al*., 2010; Kafi *et al*., 2004). The SC significantly increased in afternoon in HS group. However, Yarny *et al.* (1990) reported reverse finding of decreased SC in dairy cattle when subjected to HS. SC and testicular measurements have been widely used in predicting the reproductive capacity of male domestic animals (Sarder, 2005; Maurya *et al*., 2010). Scrotal variables are highly heritable traits and are considered as an excellent index of sperm production in the ram (Kafi *et al*., 2004). Further, level of nutrition and daily body growth influences SC (Cameron *et al*., 1988). Further, El-Darawany, (1999) and Marai *et al*., (2006) reported significant increase in SL during HS condition in sheep. This finding coincided with our finding of increased SL during afternoon in HS group in the current study. The increased SL during afternoon could be attributed to the extension of tunica dartos muscle in scrotum. However, the SLA was significantly lower in CS group as compared to HS group. This reduction in SLA could be attributed to the lower feed intake in CS group. Further, between NS and CS groups, the SLA was significantly higher. This difference could be attributed to the HS in CS group. In addition to the effect of treatment, the experimental days influenced SCM and the interactions between treatment and experimental days also significantly influenced SC both during morning and afternoon. This shows SCA can be a good indicator for studying the impact of environmental stresses on livestock reproduction.

Generally testicular measurements are considered to be excellent indicators of sperm production capacity and spermatogenic functions during hot summer conditions (Marai *et al*., 2009; Akpa *et al*., 2013). LTLA showed significant variations between the groups. The LTLA was highest in HS group.

Fig.4: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on scrotal measurements in goats. (a)-SCM, Fig (b)-SCA, (c)-SLM, (d)-SLA, (e)-SSTM, (f)-SSTA

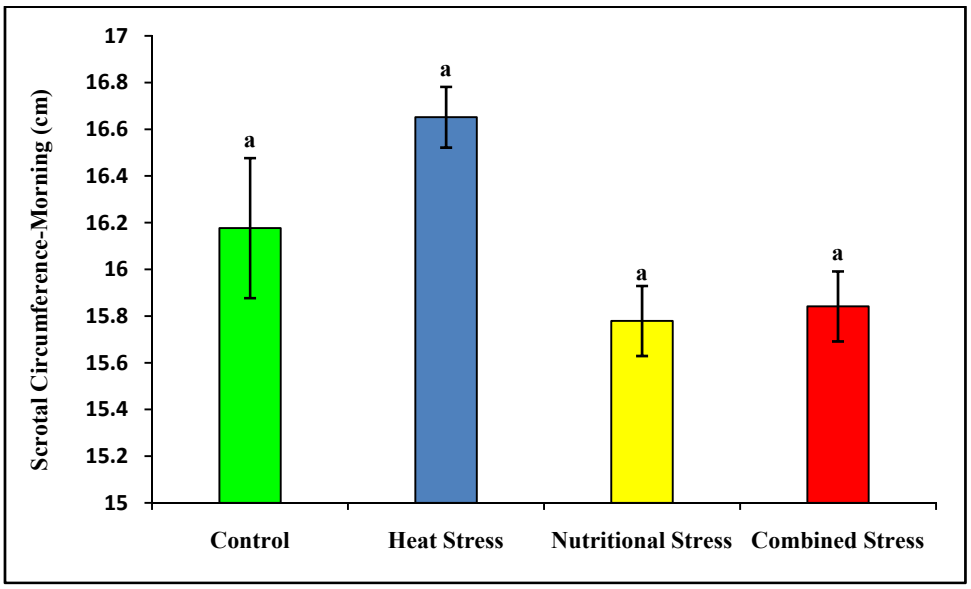


Fig.4 (a): Scrotal Circumference-Morning

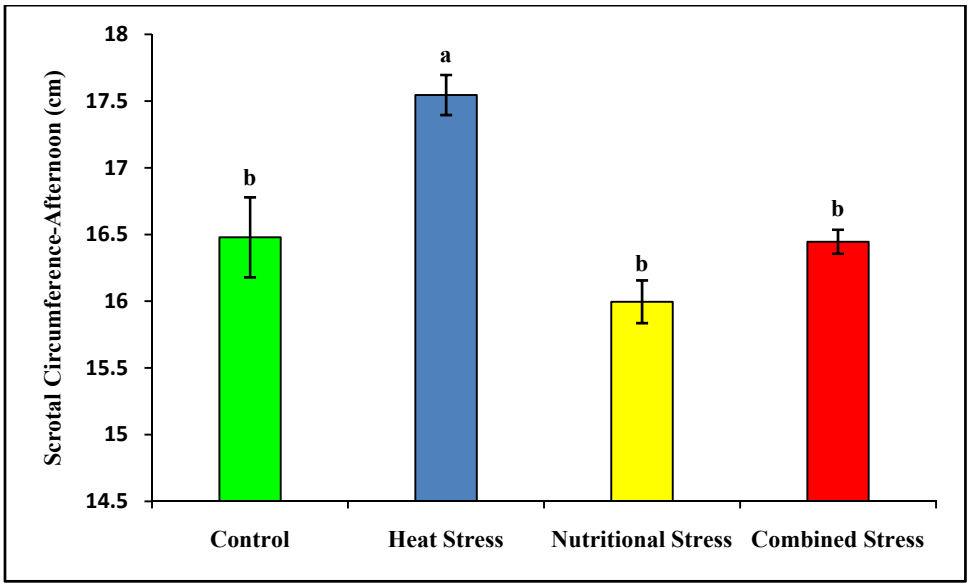


Fig.4 (b): Scrotal Circumference-Afternoon

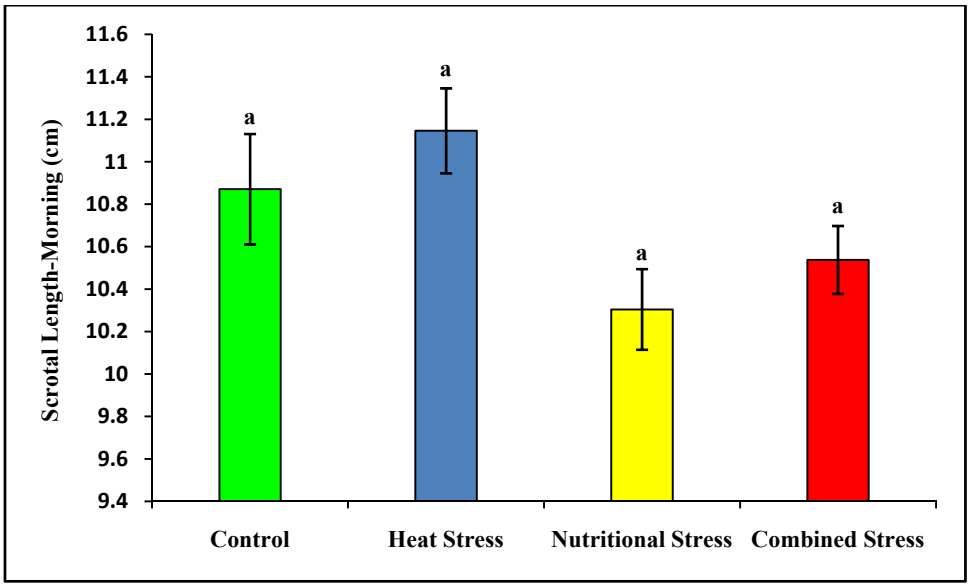


Fig.4 (c): Scrotal Length-Morning

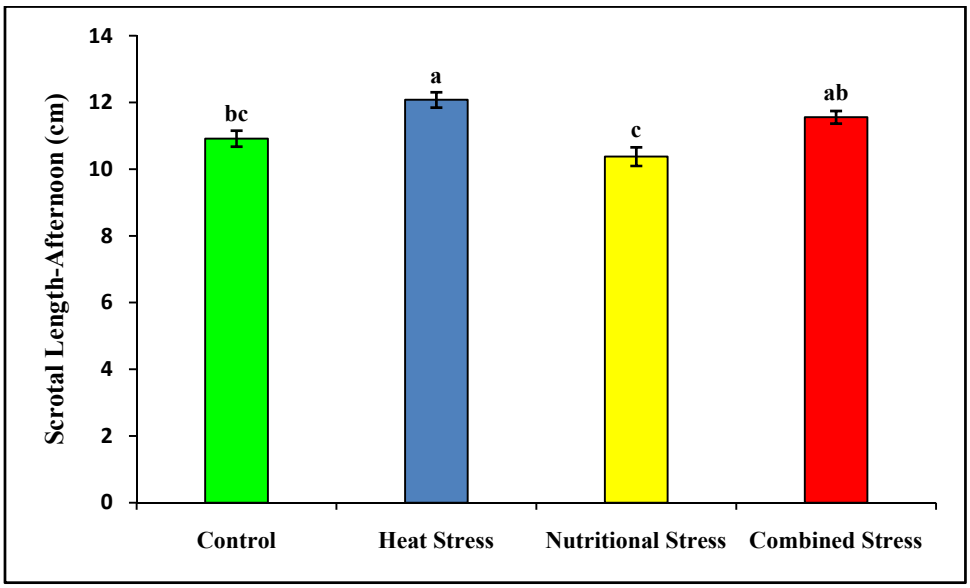


Fig.4 (d): Scrotal Length-Afternoon

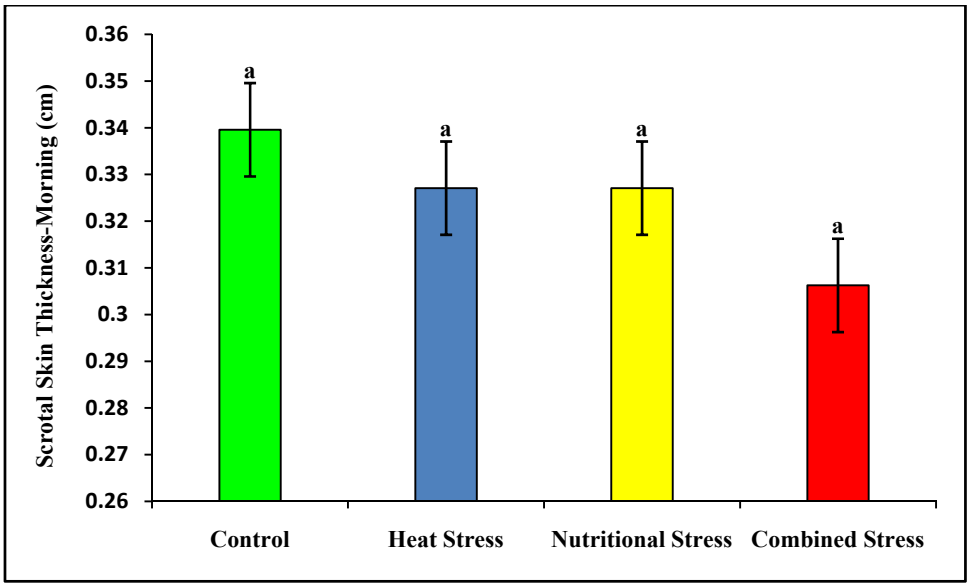


Fig.4 (e): Scrotal Skin Thickness-Morning

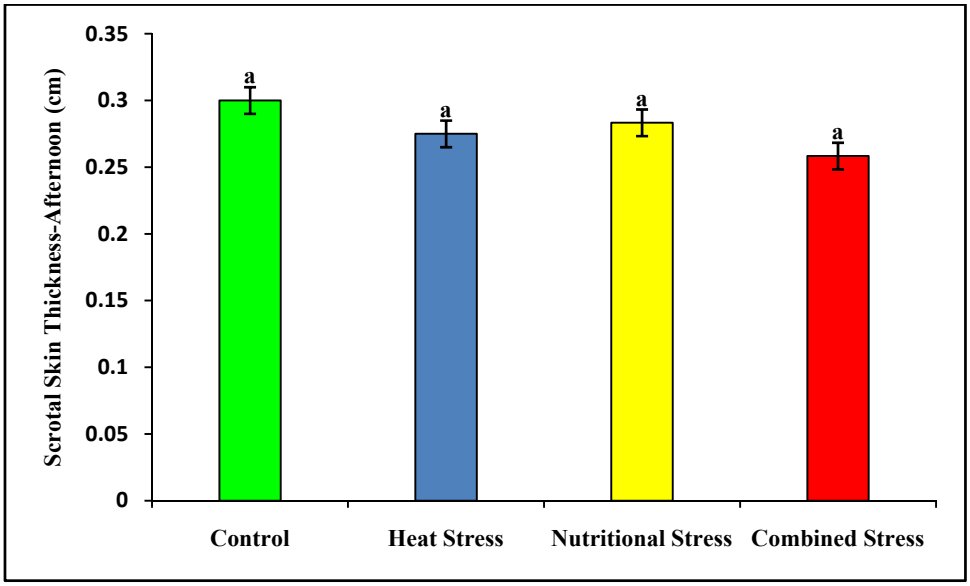


Fig.4 (f): Scrotal Skin Thickness-Afternoon

Also both CS and NS groups LTLA are significantly different with HS group. Further, experimental days significantly influenced all left testicular parameters. In addition, interactions between treatment and experimental days also significantly influenced left testicular measurements. This shows that the relationship between the groups changed over time for left testicular measurements indicating perhaps the groups differed in their responses at some time points but not others. This indicates the animals are trying to adapt to the existing conditions in terms of maintaining the left testicular measurements. RTLA showed significant variation only between HS and NS groups. This shows that testicular parameters are sensitive to both HS and NS. The highest RTLA was recorded in HS groups. It has been reported that nutritional status and temperature changes are considered most important factors influencing testicular measurements in goats (Coelho *et al*., 2006; Almeida *et al*., 2007; Delgadillo *et al*., 2007). Further, experimental days significantly influenced all right testicular parameters. This showed that all right testicular parameters behaved differently during the study period. Raji *et al*. (2008) reported that there is a linear relationship between body weight and testicular measurements in rams. Research has shown that improving nutritional status can increase testicle size and subsequent sperm production substantially in sheep (Maurya *et al*., 2010).

5.3 Seminal attributes

Sperm concentration differed significantly only between C and NS group bucks. Surprisingly sperm concentration in both HS and CS group bucks did not differ both with C and NS groups. This shows the adaptive nature of both HS and CS group bucks to HS condition. Leon *et al.* (1991) and Akpa *et al*. (2013) reported that sperm concentration might vary according to feeding regime and climatic condition. Although CS bucks are subjected to both HS and NS, still the sperm concentration value was almost comparable to both C and HS groups. This shows the extreme adaptive capability of *Osmanabadi* bucks to the cumulative effects of both HS and NS. Further, the experimental days also significantly influenced sperm concentration. This shows the animals trying to adapt to the existing climatic condition. However, the interactions between treatment and

Fig.5: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on testicular measurements in goats. (a)-RTLM, (b)-RTLA, (c)-RTWM, (d)-RTWA, (e)-LTLM, (f)-LTLA, (g)-LTWM, (h)-LTWA

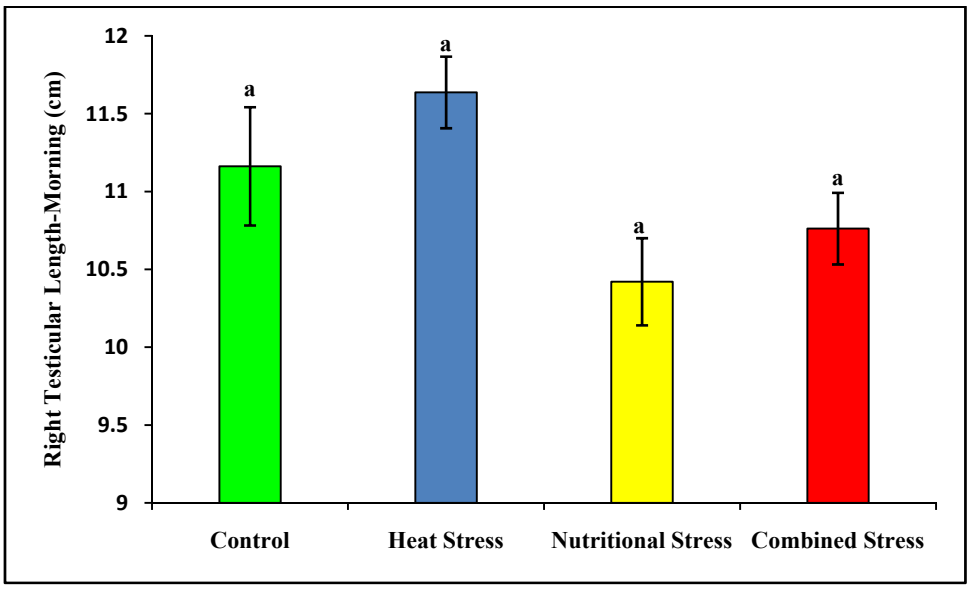


Fig.5 (a): Right Testicular Length-Morning

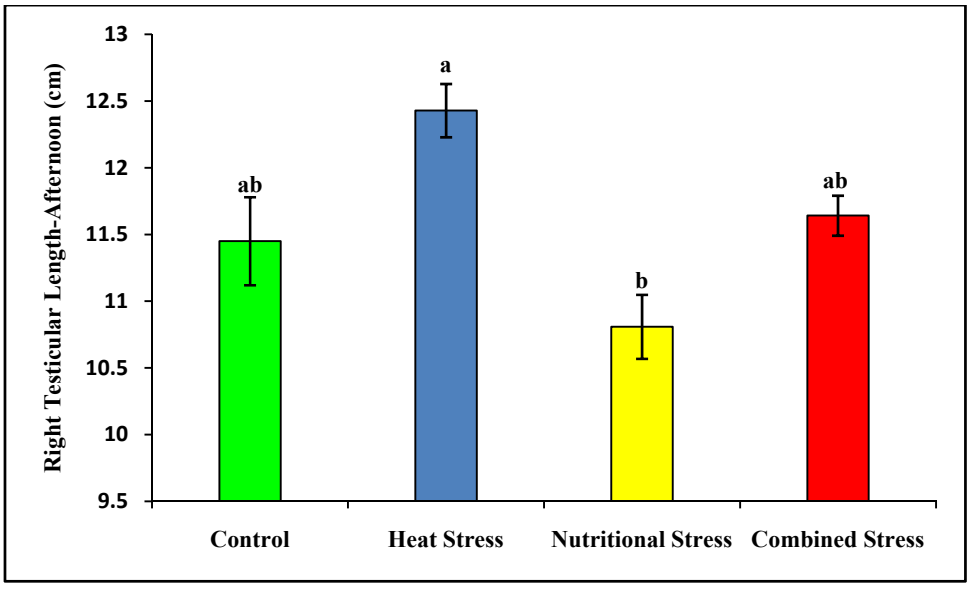


Fig.5 (b): Right Testicular Length-Afternoon

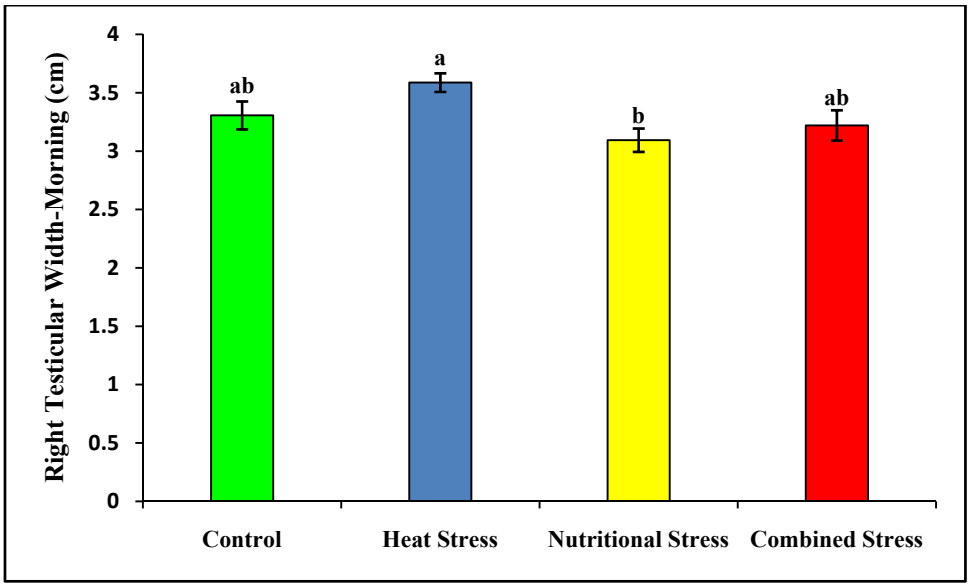


Fig.5 (c): Right Testicular Width-Morning

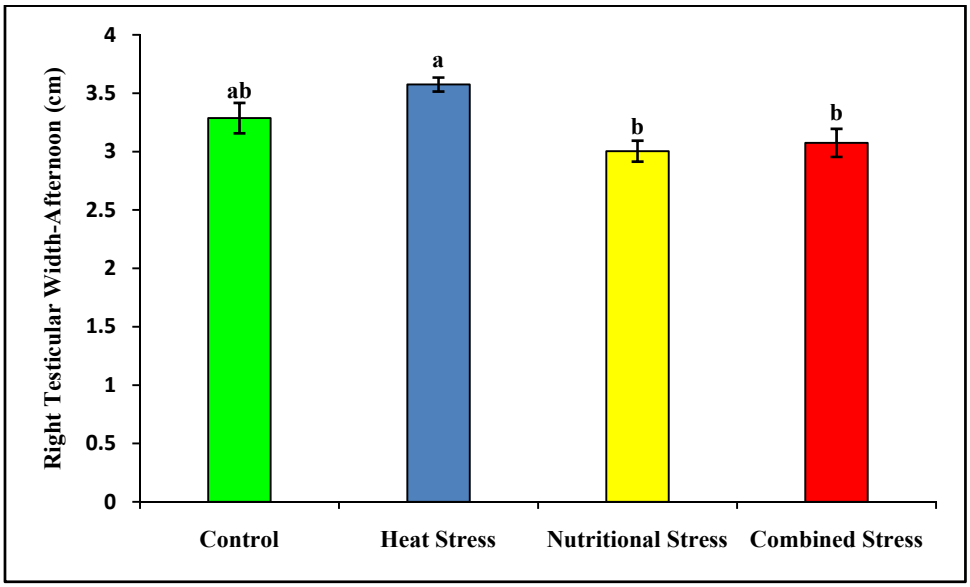


Fig.5 (d): Right Testicular Width-Afternoon

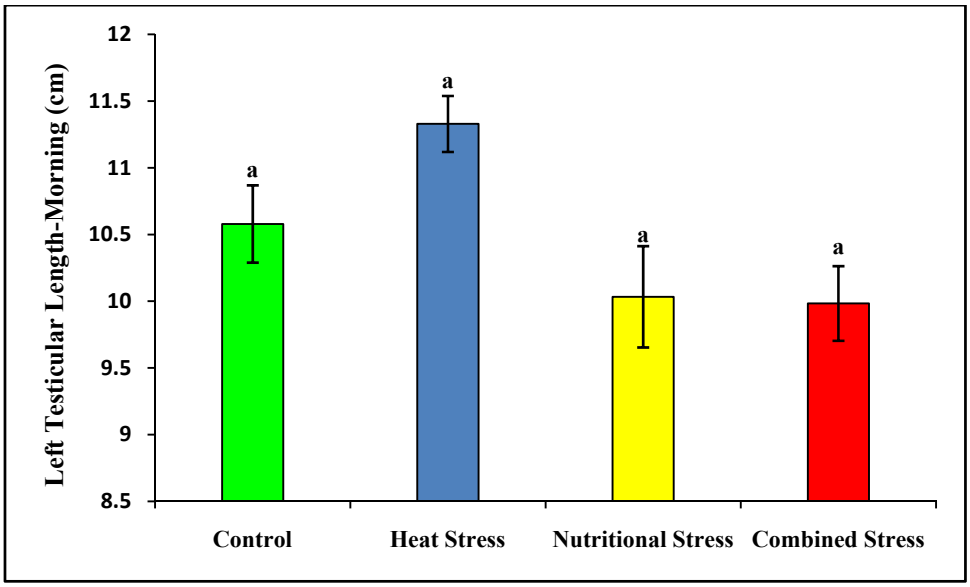


Fig.5 (e): Left Testicular Length-Morning

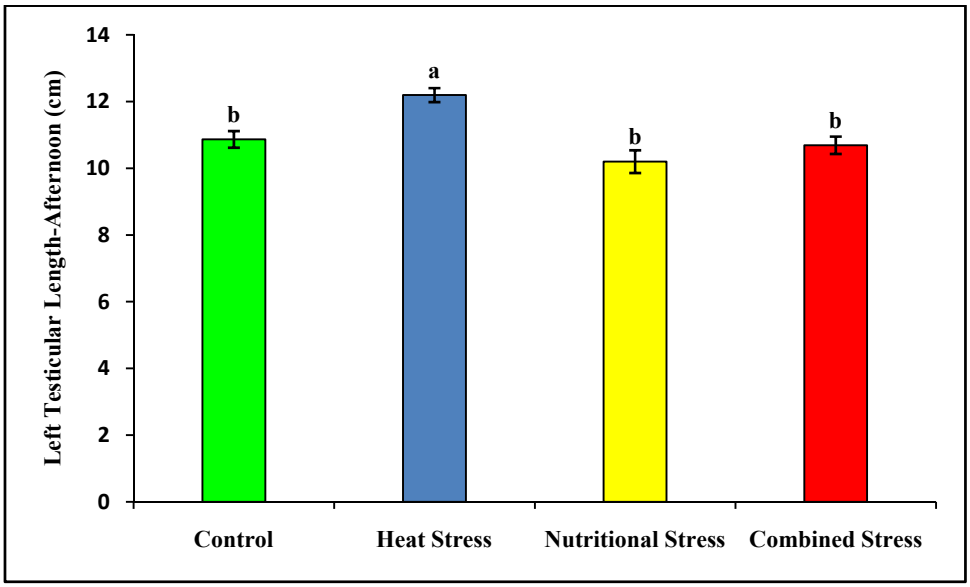


Fig.5 (f): Left Testicular Length-Afternoon

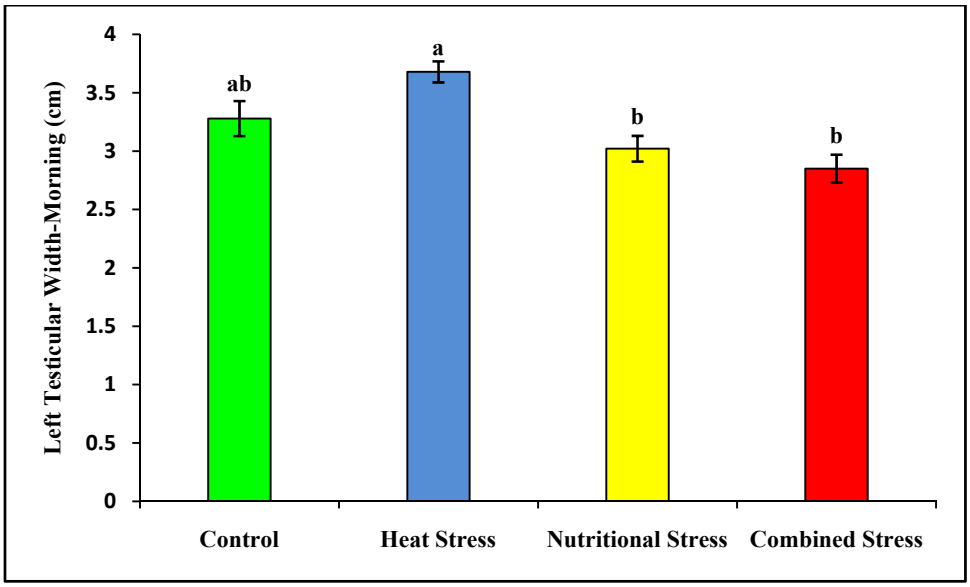


Fig.5 (g): Left Testicular Width-Morning

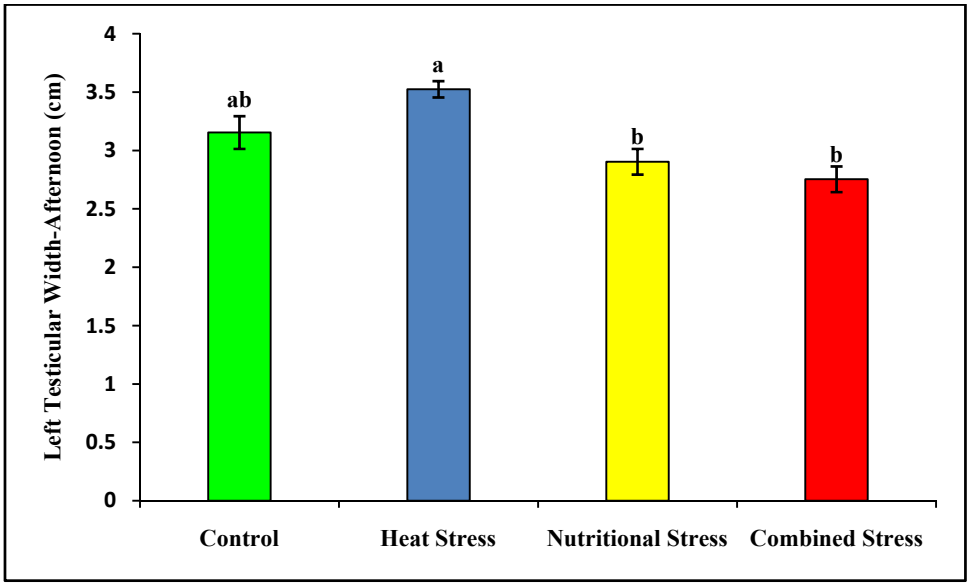


Fig.5 (h): Left Testicular Width-Afternoon

Fig.6: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional) on sperm parameters in goats. (a)-Progressive motility, (b)-Semen consistency, (c)-Sperm concentration, (d)-Semen volume, (e)-Mass motility

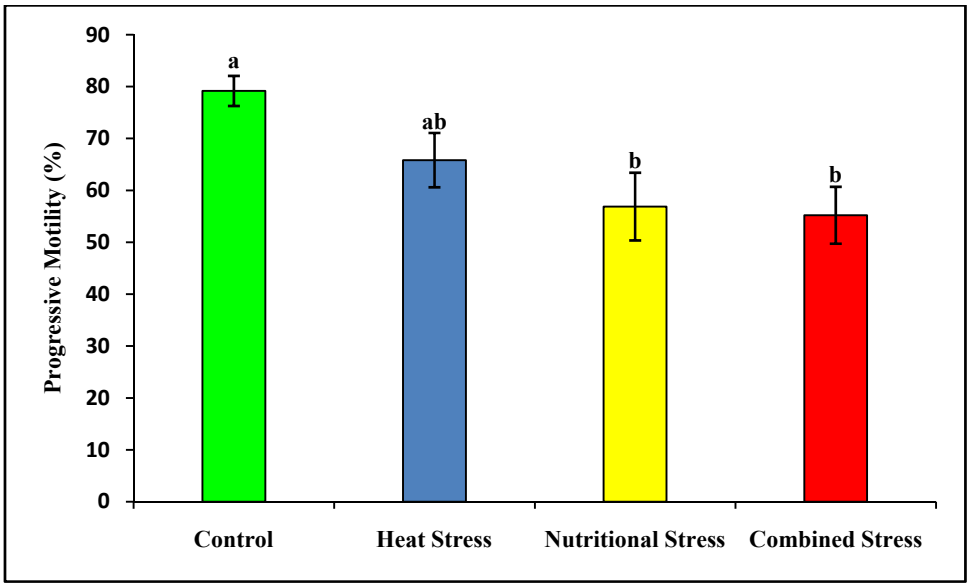


Fig.6 (a): Progressive Motility

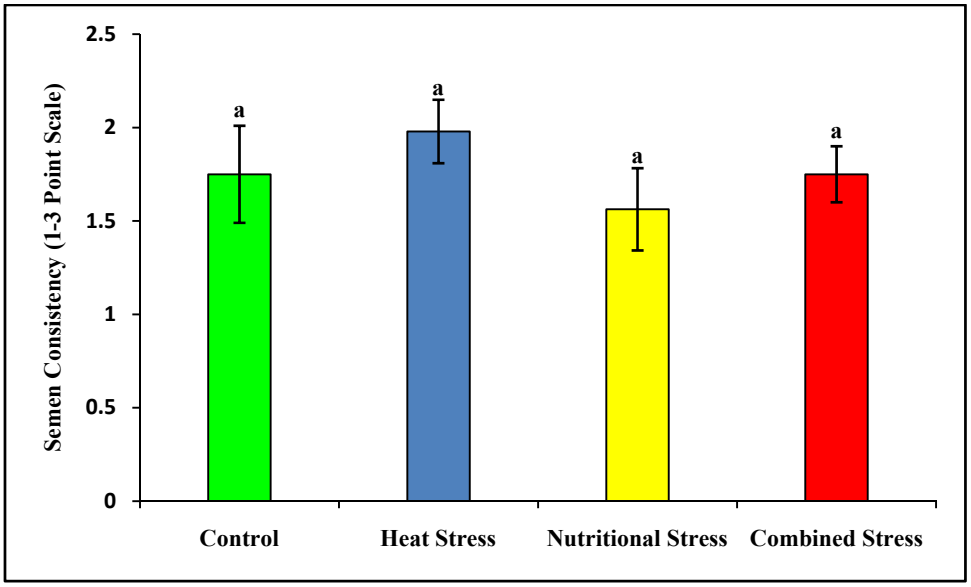


Fig.6 (b): Semen Consistency

experimental days also did not influence sperm concentration suggesting that the animal response was the same across the study.

Semen volume showed highly significant variations to the different stresses. All three stress groups HS, NS and CS group bucks showed significant reduction to semen volume in the present study as compared to C group. This shows the sensitivity of seminal volume parameter to environmental stresses. There are reports suggesting the significant influence of both HS and NS on semen volume in goats (Soderquist *et al*., 1992; Akpa *et al.,* 2013). There are also reports suggesting the seasonal variation in quality and quantity of goat semen proving the sensitivity of semen production to air temperature variations (Hammoudi *et al*., 2010; Farshad *et al*., 2012). Further, the semen volume did not differ with each other among the stress groups (HS, NS and CS groups) suggesting the additional stress in CS group did not brought significant detrimental effects on semen production. This again shows the extreme adaptive capability of CS group bucks to alter their physiological functions to maintain the reproductive activity. The non-significant difference between HS, NS and CS group bucks for semen volume showed the superior adaptive capability of *Osmanabadi* bucks to CS. This is evident from the fact that the *ad libitum* feeding of HS group did not brought significant increase in semen production as compared to both NS and CS groups. Further, the highly significant influence of experimental days and interactions between treatment and experimental days shows that the relationship between the groups changed over time for semen volume indicating perhaps the groups differed in their responses at some time points. This shows that the animals are trying to adapt to both heat and nutritional stress by altering their responses to maintain semen production.

Semen consistency did not differ between the groups. However, both experimental days and interactions between treatment and experimental days significantly influenced semen consistency. This shows that the relationship between the groups changed over time for semen consistency indicating perhaps the groups differed at some time points but not others. Mass motility differed significantly between the groups. However, this difference was the same in all

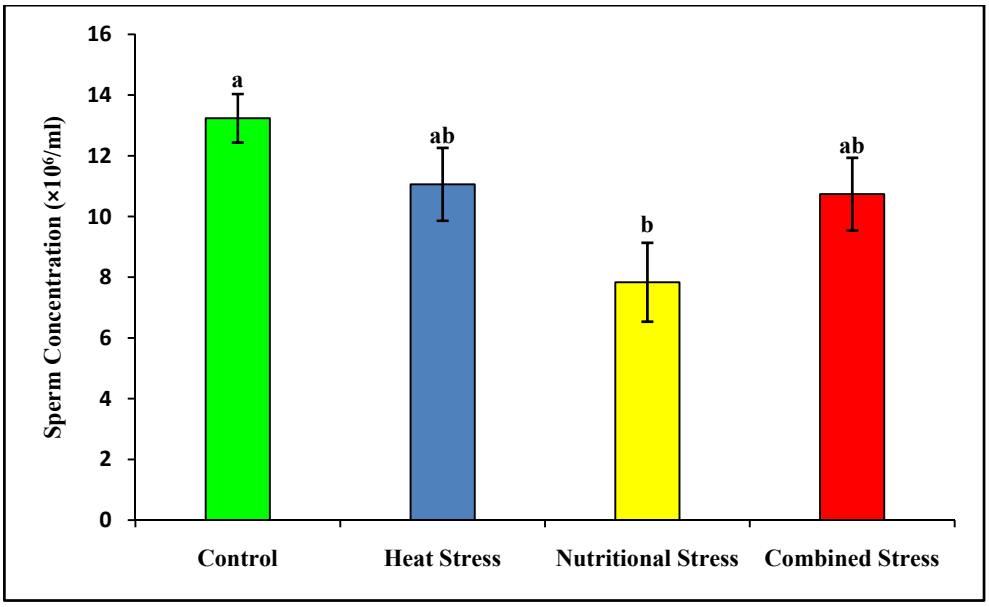


Fig.6 (c): Sperm Concentration

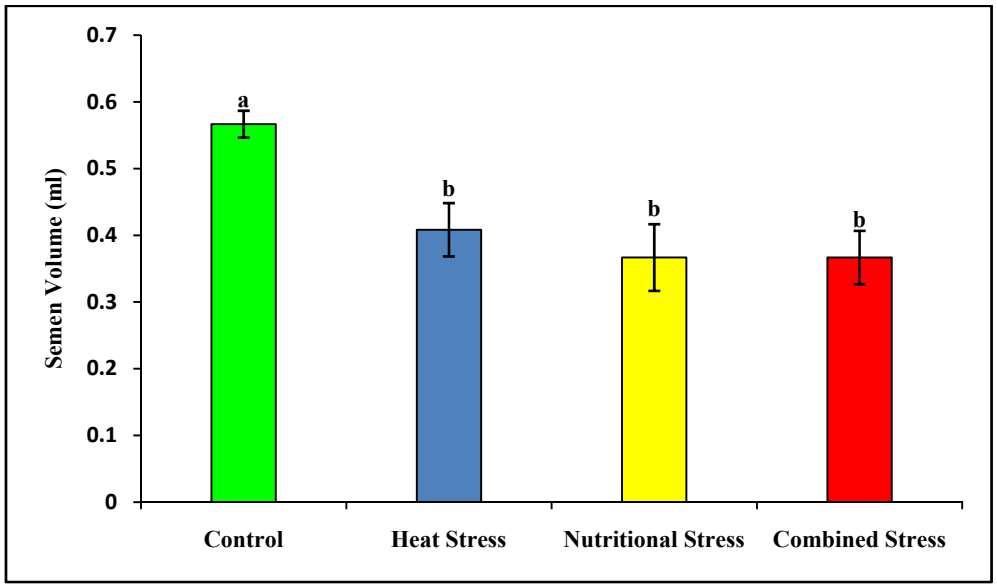


Fig.6 (d): Semen Volume

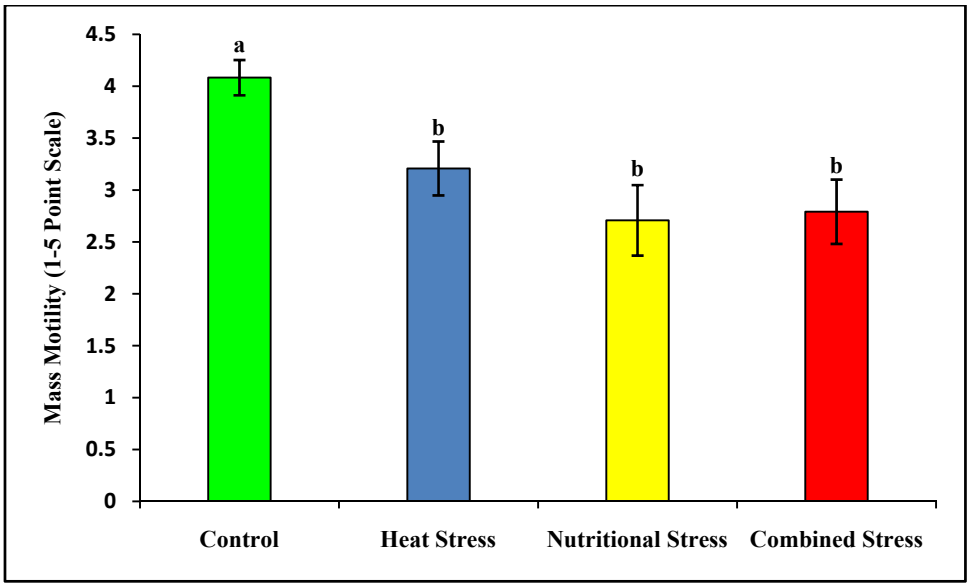


Fig.6 (e): Mass Motility

stress groups (HS, NS and CS) as compared to C group. Similarly the experimental days also significantly influenced mass motility. However, the interactions between treatment and experimental days did not influence mass motility suggesting that the animal response was the same across the study. Progressive motility differed significantly between the groups. However, this difference was the same in all stress groups (HS, NS and CS) as compared to C group. The significantly lower levels of mass motility and progressive motility in stress groups (HS, NS and CS) bucks as compared to C group could be ascribed to the collective effects of reduction in spermatogenesis (Cataldo *et al*., 1997), depressed pituitary activity (Teodoro *et al*., 2013), decline in sex hormone binding globulin (Karagiannidis *et al*., 2000) and sperm cell death in seminiferous tubules (Setchell, 2006). Similarly the experimental days also significantly influenced progressive motility. However, the interactions between treatment and experimental days did not influence progressive motility suggesting that the animal response was the same across the study and the effect of treatment and experimental days on progressive motility persisted over time.

5.4 Endocrine parameters

The highest plasma GH concentration was recorded in CS group and this concentration was significantly different from other groups (C, HS and NS). Genetic and environmental factors are largely translated in hormonal signals affecting growth processes involving a complex sequence of interactions between different hormones (Todini, 2007; Kafi *et al*., 2012). Nutrition is one of the environmental cues that affect the somatic growth of ruminants (Thorn *et al*., 2006). In addition, nutrition is considered to be one of the prime regulators of the levels of GH and IGF-1 in livestock (Lee *et al*., 2006). Plasma level of GH is inversely correlated with level of feed intake in sheep (Sejian *et al*., 2014). The present study also proved this hypothesis in goat with high level of plasma GH recorded in nutritional restricted animals. The significantly high level of plasma GH in CS in this study indicated that these animals suffered with severe nutrition deficiency. The underlying mechanisms for this increased GH in combined stress goats could be due to a marked reduction in GH receptors, or a decrease in the

Fig.7: Effect of heat stress, nutritional stress and combined stresses (heat and nutritional on endocrine parameters in goat. (a)-Growth hormone, (b)-Plasma testosterone.

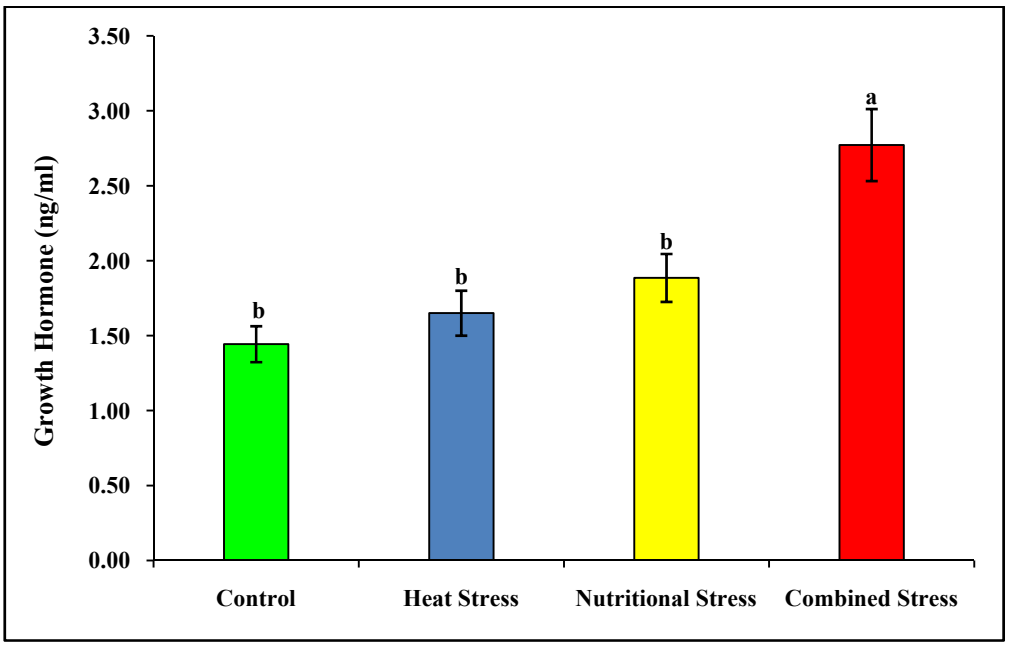


Fig.7 (a): Growth Hormone

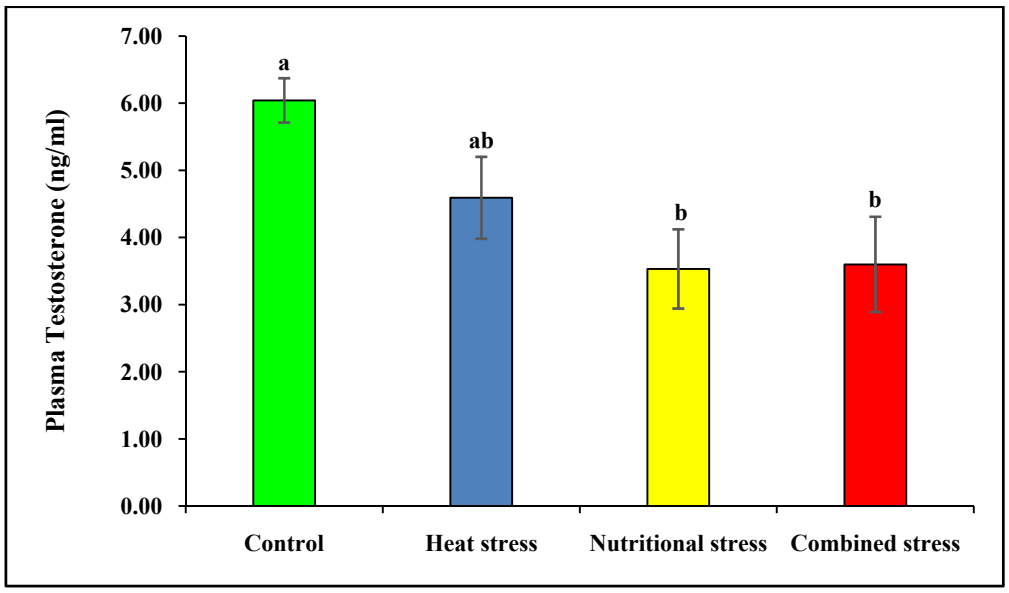


Fig.7 (b): Plasma Testosterone

binding of GH to its receptor, or post-receptor phenomena (Pulina *et al*., 2012). The significant influence of experimental days and interaction between treatment and experimental days on plasma GH concentration shows that the level of GH was significant at some point whereas at some other time it was non-significant between the groups. This show the animals of different groups were trying to adapt to the existing stressful conditions of different magnitude.

Testosterone levels are good markers for semen quality and production. There are reports which suggest strong influence of both HS (Hafez, 1993) and nutritional status (Maurya et al., 2010) on plasma testosterone concentration in rams. The reason for the reduced testosterone concentration in stress groups could be due to lower level of GnRH from the hypothalamus as a result of both HS and NS (Hafez, 1993; Maurya *et al*., 2010). It has been reported that both the production of GnRH as well as the sensitivity of pituitary to GnRH are reduced as a result of HS ultimately leading to reduced testosterone production (Sheba *et al*. 2012). The reduction in luteinizing hormone during HS from pituitary gland plays a significant role in reducing the testosterone production (Sheba *et al*. 2012; Naqvi *et al*., 2012). Among the stress groups, the level of testosterone did not differed between the groups. This shows the severity of both HS and NS on plasma testosterone concentration in goat. The non-significant influence of interaction between treatment and experimental days on plasma testosterone further shows that the effect of different stress conditions persisted over the period of time. This shows that the stress group (HS, NS and CS) animals were trying to deviate the available nutrient resources to adaptation process by compromising their testosterone production which is the principle hormone governing the reproductive performance in bucks.

5.5 Testicular HSP70 gene expression

HSP70 is one of the most abundant and best characterized HSP family that consists of highly conserved stress proteins, expressed in response to stress, and plays crucial roles in environmental stress tolerance and adaptation in goat (Gupta *et al*., 2013; Banergee *et al*., 2014; Mohanarao *et al*., 2014). These authors observed that the expression patterns of HSP70 genes in livestock are both species

Fig.8. Testis HSP70 mRNA transcript expression between the control, heat stress, nutritional stress, combined stress (heat & nutritional) groups of goats

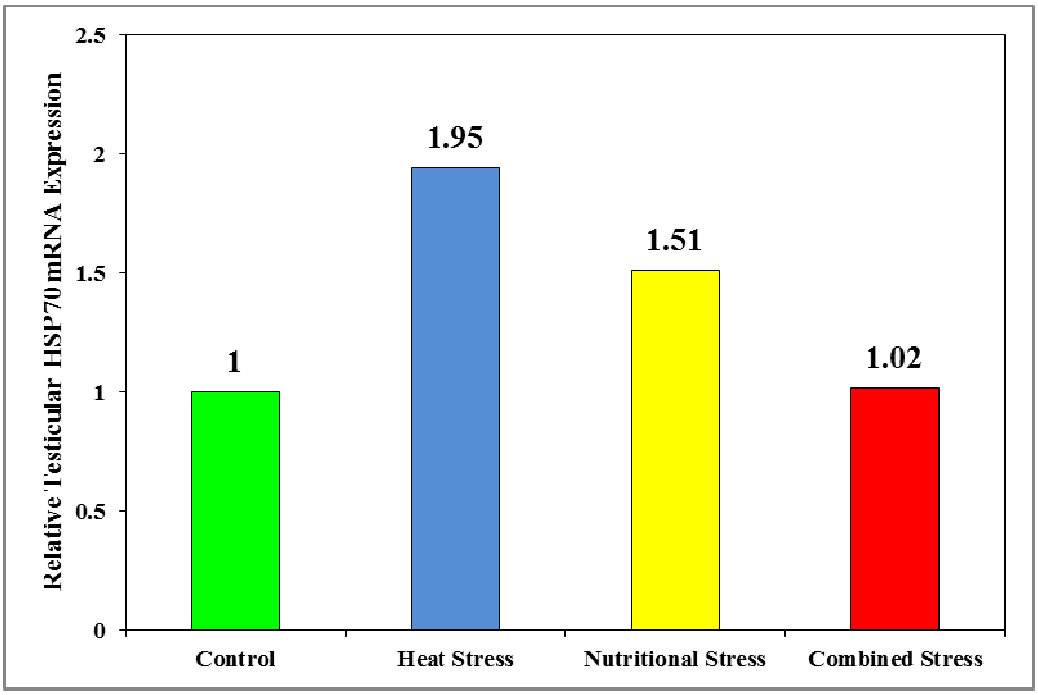


Fig. 8: Relative Testicular HSP70 mRNA Expression

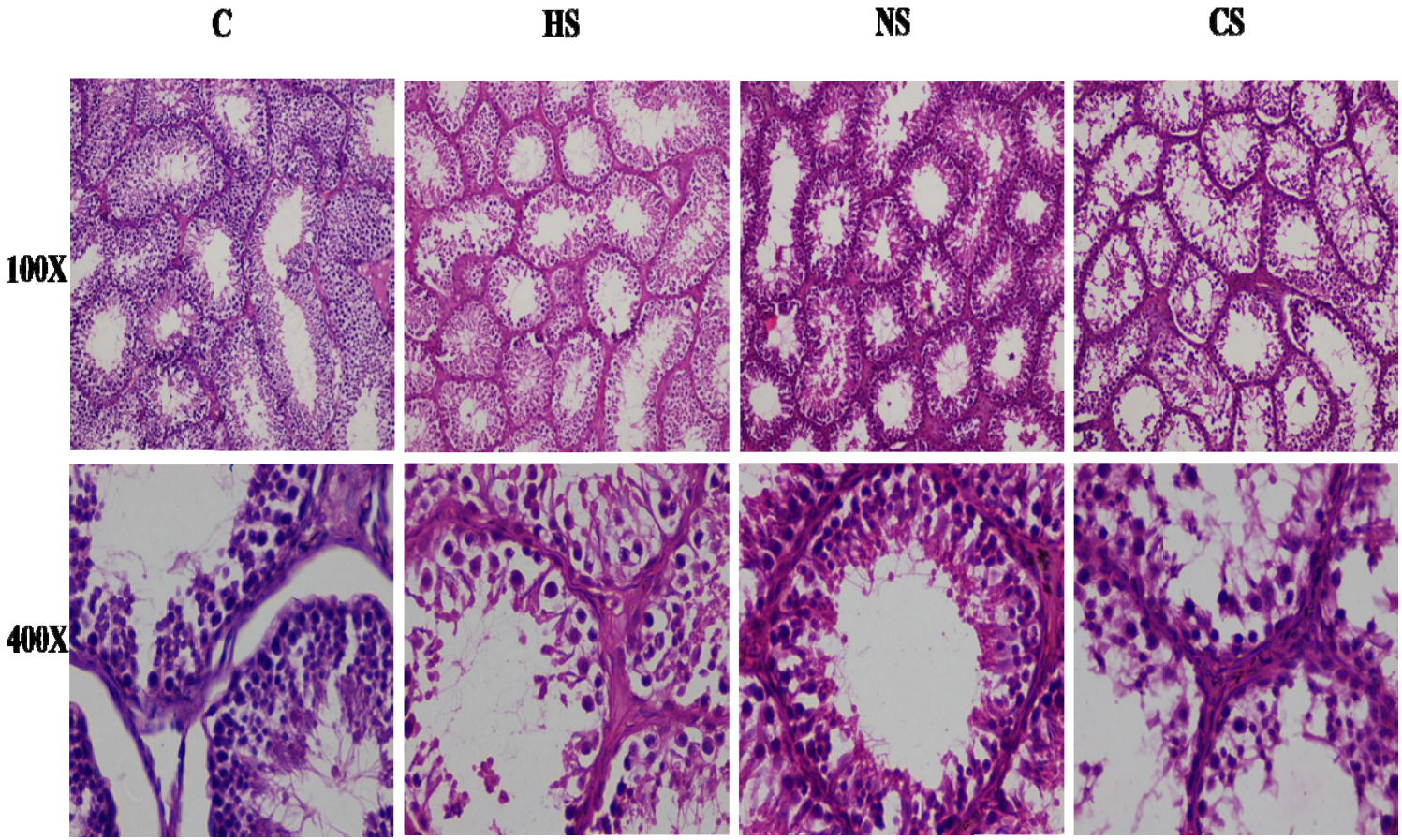


Plate 17: Histopathological changes in H&E stained sections from testicles of C, HS, NS and CS goats subjected to different kinds of stress. There was highest loss of spermatid density with decreased spermatogenesis in CS followed by HS and NS groups compared to C group

and breed specific. They attributed this difference to the variations in thermal tolerance and adaptation to different climatic conditions. The highest testicular HSP70 Messenger Ribonucleic Acid (mRNA) expression was recorded in HS followed by NS and CS in the current study. The significantly lower HSP70 mRNA expression in NS and CS group as compared to HS group could be attributed to the severe nutritional restriction in these groups. Enhanced HSP70 expression may be a response to stressful environments and may improve cell survival by protecting proteins from degradation and facilitating their refolding (Dangi *et al*., 2014). The body responds to a variety of insults that damage intracellular proteins by producing HSPs. Exposure to heat results in redistribution of blood to the periphery and compensatory reduction in the blood supply to the testicles as generally the reproductive activities are compromised during HS condition. This lack of blood supply to testicles could have damaged the cells lining the testicles, permitting endotoxin to accumulate leading to increased HSP expression in HS group bucks. There are reports suggesting that the exposure to HS induces production of HSP70 in the gut, liver and other tissues (Hotchkiss *et al*., 1993; Flanagan *et al*., 1995). The finding that the greatest increase in HSP concentrations following exposure to HS in testicles of HS group provides additional support for the contention that damage to the cells of the testicles is central to the adverse effects of heat load. The reduced reproductive performance in NS and CS group bucks could be attributed to the lower ability of these bucks to induce sufficient HSP70 expression as a result of severe NS in these bucks. This again signifies the importance of optimum nutrition for sufficient HSP70 expression to prevent damage to the reproductive tissues and to maintain the reproductive efficiency in bucks.

5.6 Histopathology

The HE staining results of different organs studied revealed that only testicle section showed significant changes between the groups but did not influence the vas deferens, epididymis and urethra. This show the environmental stresses bring about reduced reproductive function in bucks by hampering testicular function. Testicular section showed significant changes for different

stresses. The highest loss of spermatid density with decreased spermatogenesis was recorded in CS group followed by HS and NS groups compared to C group. This shows that both HS and NS proved very detrimental to testicular activity of CS group bucks. Further, the significantly lower plasma testosterone concentration and reduced seminal volume, mass motility and progressive motility in CS group bucks as compared to C and HS groups justifies this observation.

SUMMARY AND CONCLUSION

CHAPTER 6
SUMMARY AND CONCLUSION

Though the animals live in a complex world, researchers most often study the influence of only one stress factor at a time. Comprehensive, balanced, and multifactorial experiments are technically difficult to manage, analyze and interpret. However, in the changing climate scenario environmental stresses impact animal production in combination rather than individually. Hence, the present study was conducted to evaluate the influence of two environmental factors, HS and NS simultaneously on the productive and reproductive performance of *Osmanabadi* goats.

Twenty four adult *Osmanabadi* bucks (average BW 16.0 kg) were used in the present study. The bucks were divided into four groups viz., C (n=6), HS (n=6), NS (n=6) and CS (n=6). The study was conducted for a period of 45 days. The animals were stall fed with a diet consisting of 60 per cent roughage (Hybrid Napier) and 40 per cent concentrate (maize 36 kg, wheat bran 37 kg, soya bean meal 25 kg, mineral mixture 1.5 kg and common salt 0.5kg/100 kg of feed). C and HS bucks had *ad libitum* access to their feed while NS and CS bucks were under restricted feed (30 per cent intake of C bucks) to induce nutritional stress. The HS and CS bucks were exposed to solar radiation for six hours a day between 10:00 h to 16:00 h to induce heat stress. Growth parameters, scrotal and testicular measurements, seminal attributes and plasma endocrine parameters were recorded at fortnightly interval. After slaughter different organs were collected for histopathological observations and for testicular HSP70 gene expression. The data was analyzed using repeated measures analysis of variance.

Both C and HS groups showed significantly higher ($P<0.01$) body weight and body condition scoring (BCS) as compared to restricted feeding groups (NS and CS). The allometric measurements also were significantly ($P < 0.01$) lower in restricted fed groups (NS and CS) as compared to *ad libitum* fed groups in CS bucks as compared to other groups (C and HS). The significantly lower body weight in CS as compared to HS group could be attributed to the differences in their feed intake. Both CS and NS showed significant variations in body weight

whereas HS did not influence body weight significantly as compared to control bucks. However, the same heat stress when coupled with restricted feeding was very detrimental to body weight. Both heat and nutritional stress significantly reduced the BCS in the study. This is evident from the significant variation in BCS between C, HS and NS groups. Similarly in current study also heat stress alone did not influence both heart girth and length of the animals. However, when coupled with nutritional stress, heat stress was able to bring significant changes in all allometric measurements in CS group. The interaction between treatment and experimental days on BW, BCS and allometric measurements indicates that the animals are trying to adapt to the existing conditions to maintain their body measurements.

Among the scrotal measurements, SCA and SLA differed significantly ($P<0.05$) between the groups. The highest SCA and SLA was recorded in HS group. However, the SLA was significantly lower in CS group as compared to HS group. This reduction in SLA could be attributed to the lower feed intake in CS group. This shows SCA can be a good indicator for studying the impact of environmental stresses on livestock reproduction. LTLA showed significant variations between the groups. The LTLA was highest in HS group. Also both CS and NS groups LTLA are significantly different with HS group. RTLA showed significant variation only between HS and NS groups. This shows that testicular parameters are sensitive to both heat stress and nutritional stress. The interactions between treatment and experimental days also significantly influenced SC, left testicular length, left testicular width and right testicular width both during morning and afternoon. This indicates the animals are trying to adapt to the existing conditions in terms of maintaining the scrotal and testicular measurements.

Sperm concentration differed significantly only between C and NS group bucks. Surprisingly sperm concentration in both HS and CS group bucks did not differ both with C and NS groups. The highest semen volume ($P<0.01$) was recorded in C group bucks as compared to other groups. Semen consistency did not differ between the groups. The significantly ($P<0.05$) higher mass motility and

progressive motility was recorded in C group bucks. However, both mass motility and progressive motility did not differ between the stress groups (HS, NS and CS). The significant interactions between treatment and experimental days for semen volume shows that the relationship between the groups changed over time indicating perhaps the groups differed in their responses at some time points. This shows that the animals are trying to adapt by altering their responses to maintain semen production.

The highest plasma GH ($P<0.01$) was recorded in CS group and the lowest in rest all the groups (C, HS and NS). The significant influence of experimental days and interaction between treatment and experimental days on plasma GH concentration shows that the level of GH was significant at some point whereas at some other time it was non-significant between the groups. This show the animals of different groups were trying to adapt to the existing conditions of different nutrition level. The highest plasma testosterone level was recorded in C group and the lowest in rest all groups (HS, NS and CS). Among the stress groups, the level of testosterone did not differ between the groups. This shows the severity of both heat and nutritional stress on plasma testosterone concentration in goat. The non-significant influence of interaction between treatment and experimental days on plasma testosterone shows that the effect of different stress conditions persisted over the period of time.

The higher expression of testicular HSP70 mRNA was reported in HS goats. Within the stress groups, the highest testicular HSP70 mRNA expression was reported in HS group followed by NS and CS groups. The HE staining results of different organs studied revealed that only testicle section showed significant changes between the groups. However, vas deferens, epididymis, and urethra did not showed significant changes for different treatments in the study. Testicular section showed significant changes for different stresses. The highest loss of spermatid density indicating decreased spermatogenesis was recorded in CS followed by HS and NS groups compared to C group.

The present study reveals that heat stress did not brought significant variations for most of the growth and reproductive parameters studied as

compared to control group. This indicates that when nutrition is not compromised, the animals were able to withstand heat stress impact on growth and reproductive performance. Further, there were no much significant difference between nutrition stress and combined stress groups on majority of the growth and reproductive parameters studied. This indicates the extreme adaptive capability of combined stress group bucks for maintaining growth and reproductive performance even when they were subjected to two stresses simultaneously.

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ABSTRACT

IMPACT OF HEAT AND NUTRITIONAL STRESS ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF BUCKS

by

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to assess the combined effect of heat stress and nutritional restriction on growth and reproductive performances in *Osmanabadi* Bucks. Twenty four adult *Osmanabadi* bucks (average body weight (BW) 16.0 kg) were used in the present study. The bucks were divided into four groups viz., C (n=6; control), HS (n=6; heat stress), NS (n=6; nutritional stress) and CS (n=6; combined stress). The study was conducted for a period of 45 days. C and HS bucks had *ad libitum* access to their feed while NS and CS bucks were under restricted feed (30% intake of C bucks) to induce nutritional stress. The HS and CS bucks were exposed to solar radiation for six hours a day between 10:00 h to 16:00 h to induce heat stress. The data was analyzed using repeated measures analysis of variance. Both C and HS groups showed significantly higher ($P<0.01$) body weight and body condition scoring (BCS) as compared to restricted feeding groups (NS and CS). The allometric measurements also were significantly ($P < 0.01$) lower in restricted fed groups (NS and CS) as compared to *ad libitum* fed groups in CS bucks as compared to other groups (C and HS). Among the scrotal measurements, Scrotal circumference afternoon (SCA) and scrotal length afternoon (SLA) differed significantly ($P<0.05$) between the groups. The highest semen volume ($P<0.01$) was recorded in C group bucks as compared to other groups. The significantly ($P<0.05$) higher mass motility and progressive motility was recorded in C group bucks. However, both mass motility and progressive motility did not differ between the stress groups (HS, NS and CS). The highest plasma GH ($P<0.01$) was recorded in CS group and the lowest in rest all the groups (C, HS and NS). The highest plasma testosterone level was recorded in C group and the lowest in rest all groups (HS, NS and CS). The interaction between treatment and experimental days significantly ($P<0.01$) influenced body weight, BCS, allometric measurements, scrotal circumference, left testicular length and width, right testicular width, semen volume and growth hormone concentration. The higher expression of testicular Heat Shock Protein 70 (HSP70) Messenger Ribonucleic Acid(mRNA) was reported in HS goats. Testicular section showed significant changes for different stresses. The highest loss of spermatid density indicating decreased spermatogenesis was recorded in CS followed by HS and NS

groups compared to C group. It can be concluded from this study that when nutrition is not compromised *Osmanabadi* bucks were able to withstand heat stress. This is evident from the non-significant difference on various growth and reproductive parameters studied between C and HS groups. Further, the study also revealed that *Osmanabadi* bucks possessed superior adaptive capability to combined stresses simultaneously. This is evident from the significant interaction of treatment and experimental days on majority of the parameters studied.

Key words: Combined stress, Goat, Growth, *Osmanabadi* bucks, Heat stress, Nutritional stress, Seminal traits, Testosterone