IMPACT OF STRESS ON ACCOUNT OF SEASONAL VARIATIONS ON THE EXPRESSION PROFILE OF HEAT SHOCK PROTEIN GENES IN MITHUN (*Bos frontalis*)

Thesis

submitted to

NAGALAND UNIVERSITY

In partial fulfilment of requirements for the Degree

of

Doctor of Philosophy

in

LIVESTOCK PRODUCTION AND MANAGEMENT

by

HOMSENG CHOWLU

Admn. No. Ph- 233/17 Regn. No. Ph.D/LPM/00120 (2017-2019)

Department of Livestock Production and Management

School of Agricultural Sciences & Rural Development
Nagaland University,
Medziphema Campus– 797106
Nagaland

2021

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema – 797106, Nagaland

Dr. V. K. Vidyarthi
Professor
Department of Livestock Production and Management

CERTIFICATE – I

This is to certify that the thesis entitled **"Impact of stress on account of seasonal variations on the expression profile of heat shock protein genes in mithun (*Bos frontalis*)"** submitted to Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in **Livestock Production and Management** is the record of research work carried out by Miss **Homseng Chowlu,** Registration No. (Ph.D/LPM/00120) under my personal supervision and guidance.

The result of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Dated:

Place: Medziphema

Dr. V. K. Vidyarthi

Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema – 797106, Nagaland

CERTIFICATE – II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN LIVESTOCK PRODUCTION AND MANAGEMENT

This is to certify that the thesis entitled **"Impact of stress on account of seasonal variations on the expression profile of heat shock protein genes in mithun (*Bos frontalis*)"** submitted by Miss **Homseng Chowlu,** Admission No. Ph-233/17 Registration No. (Ph.D/LPM/00120) to the NAGALAND UNIVERSITY in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Livestock Production and Management has been examined by the Advisory Board and External Examiner on …….

The performance of the student has been found **Satisfactory/Unsatisfactory.**

Member		Signature
1. Prof. V. K. Vidyarthi	Supervisor	.....
2. Prof. D. V. Singh	External examiner	
3. Pro-Vice Chancellor (Dean, NU: SASRD)	Nominee	.....
4. Dr. Sabyasachi Mukherjee	Co-Supervisor	
5. Dr. R. Zuyie	Member	.....
6. Dr. Catherine Rutsa	Member	.....
7. Prof. K. K. Jha	Member	.....
8. Prof. Amod Sharma	Member	.....
9. Head (LPM)	Member	.....

Head
Department of
Livestock production and management

Dean
School of Agricultural Sciences
and Rural Development

DECLARATION

I, Homseng Chowlu hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree to any other Universities/Institute.

This is submitted to SASRD, Nagaland University for the degree of Doctor of Philosophy in Livestock Production and Management.

DATE: (HOMSENG CHOWLU)

PLACE:

Dr. V. K. VIDYARTHI

SUPERVISOR

ACKNOWLEDGEMENTS

With the grace and blessings of the Almighty, I got the courage to complete my study. I kneel down and bow to the Almighty for his constant blessings on me.

I take this privilege to express my heart full thanks and sense of gratitude to the following:

My Supervisor, Prof. V.K. Vidyarthi, Professor, Animal Nutrition, Department of Livestock Production and Management, SASRD, Nagaland University, Medziphema campus, under his guidance and advice I could complete my work successfully.

My Joint supervisor, Dr. S. Mukherjee, Principal Scientist, Department of Animal Genetics and Breeding, ICAR-NDRI, Karnal, who have always encouraged me by extending his help and guidance for completing my work successfully.

My sincere acknowledgements to the advisory committee members Dr. R. Zuyie, Dr. Nizamuddin, Dr. M. Catherine Rutsa, Prof. K.K. Jha and Dr. Amod Sharma for their suggestions and advices.

I extend my thanks to our Dean Dr. Akali Sema and Pro-Vice Chancellor Prof. M. Aleminla Ao, NU-SASRD, Medziphema campus, for granting permission, to carry out the present study.

My sincerely thanks to Dr. Nizamuddin, Head of Department for providing all kind of help during the study.

I convey my heartily gratitude towards The Director, ICAR-NRCM, Jharnapani, Medziphema, Nagaland, for allowing and providing the facilities necessary for the study.

I convey my heartiest acknowledgements to the faulty of the Department and the non-teaching staffs of our department for their help and assistance.

My sincere thanks to Dr. Imsusosang Longkumar, Research Associate, and Mr. Yanger Jamir, JRF, Department of Animal Genetics and Breeding, NRCM, Jharnapani, whose help throughout the course of my work were consistent with their presence and extending out their help and knowledge whenever it was necessary.

Sincere acknowledgements to Dr. Jayanta Chamuah, Scientist, Dr. Kobu Khate, Scientist, Dr. Lozo, Veterinarian, ICAR- NRCM, Jharnapani, who guided and assisted me for the whole study period. I am thankful for their help and guidance due to which I could complete my work on time.

My friends and juniors who always motivated, supported and comforted me during tough times and also encouraged me throughout the course of the study.

I thank my family members for their constant encouragement and support to pursue my study and to have faith on me. Thank you for believing in me. I would have not completed without the blessing of my parents.

Date:

Place: Homseng Chowlu

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
ANOVA	Analysis of variance
Df	Degree of freedom
Fig.	Figure
i.e.	That is
S.S	Sum of square
Viz.	Such as
THI	Temperature Humidity Index
HSP	Heat Shock Protein
kDa	Kilodaltons
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glumatic Pyruvic Transaminase
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
RH	Relative humidity
mg/dl	Milligram per decilitre
g/dl	Grams per decilitre
%	Per cent
@	At the rate of
mmol/L	Millimoles per litre

DEDICATED TO ALL MY LOVED ONES

CONTENTS

ABSTRACT

The present research work was conducted to investigate "**Impact of Stress on Account of Seasonal Variations on the Expression Profile of Heat Shock Protein Genes in Mithun (Bos Frontalis)"** with the aim to study the effect of heat stress on the physiological, haematological parameters and expression of HSP 70 and 90 genes in mithun under different climatic conditions. Six healthy mithuns were grouped as control group (T_1) or non heat stress group in which the observations and blood samples were recorded and collected in the morning hours before the animals were exposed to sunlight. After the observations were recorded the same animals were exposed to sunlight to induce heat stress. The observations were again recorded and collected in the afternoon for the treatment group (T_2) or the heat stress group. The observations were recorded for four different season viz. autumn (August- October), winter (November- January), spring (February- April) and summer (May- July). During the whole study period Temperature humidity index (THI) was recorded. Scrutinization was done using Quantitative Real time PCR to the variation of mRNA expression of HSP70 and HSP90 genes against Beta-Actin which was used as housekeeping gene. Split plot design was used for statistical analysis of the data. Physiological parameters was found to be significantly ($P<0.05$) higher in T_2 group during various seasons. Rectal temperature was found to be highest in summer season. Pulse rate was found to be highest in winter season and respiration rate was highest in spring season. Haematological parameters were found to be significantly ($P<0.05$) higher in T_2 group during various seasons. Glucose levels were found highest in spring followed by winter, summer and autumn season. SGOT was found to be highest in summer followed by spring, autumn and winter season. SGPT was found to be highest in summer and lowest in autumn season. Cortisol level was found to be highest in winter and lowest in autumn season. The expression of HSP70 and 90 genes was found to be significantly different during various seasons. The expression of HSP70 and 90 genes was highest in summer and lowest in winter season. Summer season recorded the highest THI values and winter season with the lowest THI values for the study period. From the study, it could be concluded that heat stress had a significant impact on the physiological and haematological parameters and also on the expression of HSP 70 and 90 genes as a result of thermotolerance against heat stress during various seasons.

INTRODUCTION

Mithun (*Bos frontalis*) a unique, stocky domesticated animal with triangular shaped grey forehead, thick pointed horns and characteristic feature of white stockings in all legs is considered as the pride of North Eastern hilly region of India, reared for socio-economic and cultural life of the local population (Simoons 1984, Mondal and Pal 1999). Mithun is also known as cattle of the mountains as it is found in the hilly altitudes of 300-3000 msl in the four different North Eastern Hilly Region, viz. Arunachal Pradesh, Nagaland, Manipur and Mizoram. The ownership of mithun is considered as a sign of prosperity and superiority of the individual in the tribal society. Mithun is a socio-cultural emblem in the tribal society, playing an important role in the socio-economic and cultural life in the tribal population of the hilly region. This massive bovine species is mainly reared for meat purpose. It is an important inseparable component during various religious festivals, customary feasts and marriages of tribal people. In comparison to the meat of other bovine species, meat of Mithun is rated to be more tender and superior in quality (Mondal *et al.*, 2014).

In India, Mithuns are mainly found in the North Eastern Hilly Region of the four different states namely Arunachal Pradesh, Nagaland, Manipur and Mizoram. As per the quinquennial All India Livestock Census (2019), the population of Mithun in India is estimated to be 386,293 Mithuns. Out of the total population, Arunachal Pradesh had 90.64% that is a population of 350,154 Mithuns, Nagaland had 5.99% (23,123 heads), Manipur had 2.35% (9,059 heads) and Mizoram had 1.02% (3,957 heads). In the census 2012 it was reported that Arunachal Pradesh alone had 83.76% of mithuns, Nagaland had 11.73% followed by Manipur with 3.41% and Mizoram with (1.41%). It can be seen that there is an increase in the population of mithun in Arunachal Pradesh (6.88%),

whereas there is a decline in the population in Nagaland (5.74%), Manipur (1.06%) and Mizoram (0.39%). Over the past census report it has been noted that the Mithun-inhabited States, percent contribution drastically declined except Arunachal Pradesh (Livestock Census of India, 2012).

Mithuns are reared in free range condition in the hilly tropical rain forests of the North Eastern Hilly states of India. Mithun thrives on tree leaves, shrubs, bushes and other natural vegetation in the jungle. Mithuns are reared under traditional free range grazing condition in the forest without any housing and feeding facility.

In the present century, climate change is considered as a major challenge. In country like India, where the seasonal temperature ranges from 10°C to 44°C, livestock industry is always threatened by the effects of climatic stress. Combination of high environmental temperature with extreme weather conditions exerts great influence on the productivity of an animal, making it prone to many diseases (Bhanuprakash *et al.*, 2016). Temperature, humidity and radiations are the climatic variables and variations in these variables could cause potential hazard in the growth and production of all the livestock species (Ganaie *et al.*, 2013). Climate change leads to different environmental stress, affecting productivity, reproductive efficiency and health disorders leading to severe economic losses (St-Pierre *et al.*, 2003 and Sejian *et al.*, 2012). In the pace of time, stress has been defined by several workers. David *et al.* (1990) stated that stress is the representation of body's reaction to conditions that disturbs homeostasis or unbalances the physiological equilibrium, often with detrimental effects. Rosales (1994) stated that stress was the cumulative detrimental condition caused due to various factors on health and performance of animals. Dobson and Smith (2000) defined stress as the condition in which an animal is unable to cope with its environment and the animals fails to achieve their genetic

potential. A sudden change in an animal environment causes condition that is known as stress (Kumar *et al.,* 2011). Animals are exposed to different stressful environmental conditions throughout the year.

Livestock undergo various kinds of stress viz. chemical, physical, nutritional and thermal stress under which an animal faces a sudden change in its environment (Sunil Kumar *et al.,* 2011). Heat stress is one kind of the stress which interferes and is an intriguing factor in livestock production in the present climate change scenario making it challenging for livestock production in any geographical location in the world. Heat stress is a condition in which the animal is unable to dissipate the heat load. The influence of air temperature, humidity, air movement, solar radiation and precipitation contribute to heat stress in animals leading to economic loss (Sejian *et al.*, 2012). These environmental factors along with other indices are used to measure the level of heat stress on the animals (Correa-Calderon *et al.,* 2004). Heat stress negatively affects the productivity of the animals (Niyas *et al.,* 2015). Heat stress is widely recognized as a stressful condition in animals. Responses to heat stress may vary among different species. Response to heat stress varies in physiological, behavioural and hormonal ways in an animal. Slight variations in temperature, wind, relative humidity, precipitation and solar radiation in the animal environment influence variety of physiological responses in animals.

The thermo neutral zone is known as the range of ambient environmental temperatures below or above that thermo neutral range, the animal experiences uncomfortable conditions known as stress. All kind of animals have their own range of ambient environmental temperature where they function comfortably without stressful conditions. Thermo neutral zone is when the animal maintains the normal body temperature required for homeostasis and doesn't have to expend energy to reach physiological equilibrium. Heat stress in animal is

triggered at a point which is known as the upper critical temperature. When the temperatures are above the Upper Critical Temperature, the animals are affected by heat stress and the inability to eliminate the heat load of the animal, which may lead to death. Animal sweats and pants in an attempt to eliminate the load of heat. When the temperature is above the neutral zone, homeotherms maintain body temperature by physiological and behavioural response which helps in thermoregulation. In India, Temperature Humidity Index (THI) has been used for calculating the level of heat stress occurred due to the rise in the ambient temperature of the animal (Upadhyay *et al.,* 2008). Temperature humidity index (THI) is an indicator of heat stress conditions and widely used to describe heat load on animals. THI is used for evaluating the degree of heat stress by combining the outcomes of ambient temperature and relative humidity of an animal.

As per many researchers THI has been categorized in different categories. Armstrong (1994) used THI <71 as a thermal comfort zone, mild heat stress from 72 to 79, moderate heat stress from 80 to 90 and severe heat stress when THI becomes >90. He assumed that the thermo neutral condition of dairy cows does not drop below the thermoneutral zone, which would cause cold stress in the cows. Comparatively, De Rensis *et al.,* (2015) defined THI <68 as thermal comfort zone, THI of 68 to 74 as mild and a THI ≥75 as severe signs of heat stress causing severe decrease in production performance in cows. The main component or parameter required for the management decisions is the THI value. It helps in decision making related to heat stress in animal farms. THI values are provided by the meteorological stations that are closely situated to the farms.

Various kinds of responses that an animal exhibits to cope up due to different kinds of environmental stress include physiological response, blood

biochemical response, neuroendocrine response, molecular and cellular response, metabolic response and behavioural response (Niyas *et al.*, 2015). Physiological responses include changes in the body temperature, rectal temperature, respiration rate, heart rate and skin temperature of an animal. Blood biochemical and endocrine responses are the reactions to the adverse environmental conditions in which the animal try to cope up by changing the concentrations of the blood metabolites, metabolic and stress hormones which are produced under the control of nervous system of an animal. Cellular and molecular responses include the cardinal mechanisms by which the animal survives stressful conditions.

Heat shock proteins (HSPs) are a family of specific proteins that are generated by the cells when exposed to stressful conditions. Heat shock proteins ranges from 10-150 kDa in molecular size. These multi-gene families are found in all major compartments of cells of an organism. They are named according to their molecular weight like HSP70 and HSP90 which have 70 and 90 kilodaltons in size respectively. They play crucial role in permitting the cells to adapt to the changing environment to adapt to the stressful condition and maintain thermal balance. Heat shock proteins are a group of proteins that are synthesized due to the outcome of heat stress and they protect the cells from the severe ill-effects of heat stress and other environment stress. The effects of heat shock proteins are studied and explained in many domestic animals including bovines that exhibit thermoregulatory protective mechanisms when affected by heat stress (Collier *et al.*, 2008 & Kumar *et al.,* 2015). The balance between survival and effective immunity system of an organism is maintained by heat shock proteins, to counterbalance and to acclimatize to the stress caused in the animal. Environmental stress tolerance and thermal adaptation is an important role of HSPs (Sorensen *et al.,* 2003). The major role of HSPs is to function as molecular chaperone which helps the organism to adapt to the stressful condition by

encouraging cell survival and reinstate cellular homeostasis (Collier *et al*., 2008). HSPs permit the cells to resists damage facilitated due to exposure to stressful conditions (Byoung Hwa Roh *et al.,* 2008).

HSPs get easily activated by heat and other environmental stressors, hence are highly conserved proteins, and functions as molecular chaperones resulting thermotolerance and enables cell survival caused by injury and oxidative stress which causes thermotolerance (Yang *et al.,* 2006 and Lindquist and Craig 1988). HSPs are triggered during stressed conditions. They help maintain homeostasis of the cell by hindering the cluster of cytotoxin protein formation as the HSPs react with the denatured proteins in the cell (Mayer *et al.,* 2006). HSP70i (HSP70.1 and HSP70.2) is known to be the most sensitive to temperature among the HSP family members. It is influenced by various physiological, pathological and environmental stressors (Beckham *et al*., 2004). HSP70 and HSP90 among the HSP family are mainly thermo-tolerant developed in the livestock species (Belhadj *et al.,* 2016). HSP 70 among other HSP family has the most important function that is to maintain thermotolerance (Barbe *et al.,* 1998) and survival of the animal from heat stress (King *et al.,* 2002). The level of stress that an organism naturally undergoes leads to the expression of HSPs and is also correlated to stress resistance. Various environmental stressors up-regulates the synthesis of number of HS Proteins, which initiates defence mechanism to maintain homeostasis and cell survival (Feder *et al.,* 1999). The drastic up-regulation in the HSP expression is transcriptionally controlled which is the major role of Heat shock response and is primarily activated by heat shock factor (HSF) (Wu C, 1995).

The main objective for this study was to analyze the expression of heat shock Protein genes in Mithun, since this majestic bovine is reared in the wild under free grazing conditions without any kind of housing and other facilities

under cool hilly ecosystem. It will be interesting to examine the expression profile of heat shock protein genes in mithun under stress conditions owing to seasonal climatic variations under Nagaland conditions. Accordingly, the following objectives are proposed:

OBJECTIVES:

1. To study the physiological and haematological responses to heat stress in mithun in different seasons.

2. To study the effect of thermal stress in the expression of heat shock protein genes (HSP70 and HSP90) in Mithun under *in vitro* condition under different seasons.

3. To estimate the Temperature Humidity Index in Nagaland conditions under various climatic conditions.

REVIEW OF LITERATURE

2.1 PHYSIOLOGICAL AND HAEMOTOLOGICAL PARAMETERS

2.1.1 PHYSIOLOGICAL PARAMETERS

a. RECTAL TEMPERATURE

Hafez (1968) observed that increase in the respiratory activity helps the animal to give off the excess heat load from the body, resulting from the activation of receptors due to stress that signals the hypothalamus.

Pangestu *et al.* (2000) observed that at low elevation with high temperature and humidity the rectal temperature in cattle, sheep and goat were comparatively higher than those at low elevation with low environment temperature.

Adbel-Hafez (2002) found that the inability to maintain heat balance in the animal body results in increased rectal temperature.

Bouraoui *et al.* (2002) stated that during hot weather conditions, different types of stress can contribute to increase in SCC of milking Holstein cows.

Srikandakumar *et al.* (2003) found that in heat stressed animals, the most sensitive indicator for body temperature was rectal temperature.

Mader *et al.* (2006) and Bohmanova *et al.* (2007) observed that THI combines the effects of temperature and humidity and predicts the HS levels in animals.

Swenson and Reece (2006) observed that rectal temperature could be used as the indicator for body temperature in the animals, although throughout the day, there are changes in the body temperature in different parts of the animal

body. They observed an increase in the rectal temperature (38.3 to 40.0°C) in goats.

Gudev *et al.* (2007) found that the lactating buffaloes were sensitive to heat stress when there was a significant increase in rectal temperature at THI of 77.83, resulting due to the inability to maintain their body temperature within the thermo neutral zone.

Sarkar *et al.* (2010) reported that seasonal effect ($P<0.01$) on rectal temperature was observed in yaks with the higher mean in warm season and a negative correlation (-0.42) and (-0.36) between the rectal temperature and respiration rates were observed with elevated plasma corticoid concentration.

Pereira *et al.* (2008); Gaughan *et al.* (2010) and Thompson *et al.* (2011) studied that in continuous or cycling heat exposure resulted in high respiratory rate with low thermal tolerance in *B. taurus*.

Chaurasia *et al.* (2010) found that there was a significant influence of altitude and season on the rectal temperature, respiration rate and pulse rates in mithun. Significant variation between seasons were not observed in rectal temperature and pulse rate at low and high altitudes, but significant ($P<0.05$) differences were observed in respiration rate during summer than winter season. Rectal temperature, pulse and respiration rates were significantly ($P<0.01$) low at high altitude compared to low altitude during summer and winter seasons.

Cowley *et al.* (2015) noticed that heat stressed and thermoneutral paired lactating cows had decreased heart rate.

Nasr *et al.* (2017) disclosed that welfare and economic return can be influenced due to high Temperature Humidity Index, which results in increased SCC and reduction in yield and quality of milk.

Rodrigo *et al.* (2017) reported that there was an increase in rectal temperature due to continuous exposure to intense heat stress which influenced the thermoregulatory mechanisms.

Chowlu *et al.* (2020) found that the group of mithuns that were heat stressed showed significantly higher ($P<0.05$) rectal temperature compared to the group of mithuns that were in the thermoneutral zone.

b. PULSE RATE

Marai *et al.* (2007) noticed increase in heat loss by conduction, convection and radiation and also by diffusion due to increase in pulse rate which escalates the blood flow in the body.

Sejian *et al.* (2010) reported that the mithun under heat stress showed significant increase in the pulse rate compared to the control group. They reported that pulse rate primarily symbolizes the balance between the metabolic status and circulation in the body of an animal.

Chaurasia *et al.* (2010) reported that ambient temperature of 32°C and 83.6% RH did not have much significant influence on rectal temperature but it had negative effect on pulse rate and respiration rate in mithun.

Alam *et al.* (2011) reported higher pulse rate in the heat stressed goats as compared to the goats in the control group.

Popoola (2014) recorded increased pulse rate of West African dwarf goats due to change in values of THI.

Perumal *et al.* (2015) found significant ($P<0.05$) difference in the physiological profiles like RT, RR, PR, HR and ST of mithun in the experimental groups during different weeks of the experimental period and also a significant ($P<0.05$) difference was observed among the experimental weeks.

The stressed animals in the experimental group suffered significantly ($P<0.05$). They concluded that the study helped to understand the effect of walking stress on the productive performance and to maintain the mithun in efficient management condition.

c. RESPIRATION RATE

McDowell *et al.* (1976) reported that about 15% of the total heat loss occurs from the respiratory tract of a heat stressed cattle. They also found that the pronounced increase and considerable variation in RR were not sufficient to prevent hyperthermia in the cows subjected to HS.

Verma (1994) reported that the physiological parameters like Rectal Temperature (99.30 ±0.10°F), Pulse rate (62.11 ±0.50 beats/min), Respiration Rate (27.09 ±0.54 beats/ min) and Skin Temperature (98.50 ±0.45) were significantly higher in mithun.

Sarkar *et al.* (1999) observed in yak that the warm season resulted in elevation ($P<0.01$) of respiration rate for 5 per minute when THI value was increased from 68 to 78.

Gaughan *et al.* (2000) observed that to maintain homeostasis via evaporative cooling, elevated respiration rate showed to be a significant mechanism for thermoregulation. They stated that respiratory rate could be widely used as an indicator of HS in cattle. But they also stated that the decrease in respiration rate might be due to the alteration in respiratory fluctuation related with movement from rapid open-mouth breathing to slow and deep paced open mouth breathing, so respiration rate might not always be an indication of heat coping strategy in an animal.

Srikandakumar *et al.* (2003) reported the fact that when animal is in heat stress, the increased ambient temperature alters the flow of blood and is

redistributed which increases the flow of blood to the skin surface, therefore, increasing the skin temperature of the heat stressed animal.

De Rensis and Scaramuzzi, (2003); West (2003) and Schutz *et al*. (2008) observed that the dairy cows use physiological and behavioural coping strategies to overcome heat stress. Increase in respiration rate, panting, sweating and reduction in milk yield and reproductive performance are the strategies used by the animal to cope up physiologically during heat stress. The heat stressed animal cope up by altering their intake behaviour that is, increased in water and feed intake during cooler period of the day, seeking shade and increase in standing time leading to decrease in movement and activity of the heat stress animal.

Sabuncuoglu (2004) had reported that respiration rate to be the most sensitive physiological characters to the change in climate and physical environment in heat stress cattle calves as previously it was proposed that in heat stress animals, due to the activation of warm receptors in the skin leads to rise in respiration rate.

Berhan *et al.* (2006) and Rahardja *et al.* (2011) reported that measurement of respiration rate helps to estimate the heat production during stress condition. They stated that excessive heat dissipation and maintaining body temperature for achieving homeostasis, it is relevant to experience increase in respiration rate in mithun.

Hafez (2005) described that when domestic animals are exposed to high ambient temperature, warm receptors are activated in the skin, which signal the hypothalamus to accelerate heat dissipation from the body, resulting in increase in respiration rate.

Vupru (2005) revealed from the physiological investigation in mithun that respiration rate (RR), rectal temperature (RT), pulse rate (PR) and heart rate

(HR) increased significantly due to carrying of load from pre work of 18.61± 0.26 to 97.77 ± 1.20, 99.96 ± 0.07° F to 107.27 ± 0.05 °F, 60.33 ± 0.67 to 91.61 ± 0.86 and 78 ± 3.15 to 118.66 ± 6.34 respectively per minute after work.

Gudev *et al.* (2007) reported significant increase in rectal temperature and respiration rate in the lactating buffaloes when THI value was 77.83, which signifies heat stress sensitivity and inability of the animal to maintain thermoneutral conditions. They also observed no significant difference in the rectal temperature when animals were kept in the barn under the same THI value.

Sarker *et al.* (2010) indicated negative correlation (- 0.42 and – 0.036) between rectal temperature and respiration rates with plasma corticoid concentration. They also observed a decline in the corticoids concentration from 13.7 ±1.1 to 4.6 ±0.68 ng/ml during cool to hot seasons.

Renaudeau *et al.* (2012) stated that heat loss through evaporation resulting from increased respiration rate was the principal mechanism to maintain heat stress in animal.

Sejian *et al.* (2012) reported that respiration rate was significantly higher in the stressed animals as to maintain thermoregulation.

Gupta *et al.* (2013) observed that under high ambient temperature, respiration rate of the animals showed elevated respiratory activity.

Ribeiro *et al.* (2014) described that in order to maintain homeostasis and abstain from elevated rectal temperature during hot conditions, respiratory mechanism of the animals helps to cope with heat stress.

Rodrigo (2017) stated that impact of heat stress on the thermoregulatory mechanisms results in an increase in respiration rate followed by elevated rectal temperature due to intense and continuous heat exposure to the animals.

2.1.2 HAEMOTOLOGICAL PARAMETER

a. GLUCOSE

Sano *et al.* (1985) reported that during hot climatic conditions, immediate changes in blood glucose metabolism were less when the goats were exposed to hot environmental conditions, whereas, Marai *et al.* (1992) observed significantly ($P<0.05$) higher levels of blood glucose in mature Ossimi ewes during summer than in winter season.

Patel *et al.* (1991) noticed that when Patanwadi and its crosses with Merino and Rambouillet sheep were exposed to direct sunlight (32.3°C) for 6 hours from the morning till afternoon (37.7°C) for consecutively three days in the month of May, the level of blood glucose was not affected in the sheep.

Habeeb *et al.* (1996) revealed significant reduction in the plasma glucose concentration due to effect of hot summer climate on milk yield and blood biochemistry in Friesian cows during summer compared to winter season.

Thompson (1973) reported that exposure to hot environmental temperature, increases the levels of SGPT and SGOT which might be due to the rise in stimulation of gluconeogenesis caused by corticoid hormones.

Itoh *et al.* (1998) reported that in heat stressed cows, gluconeogenesis and glycogenolysis decreases.

Salem *et al.* (1998) reported that in Chios, Chios crosses with Ossimi rams, there was a significant decrease in the blood glucose levels whereas, in Ossimi x Suffolk insignificant difference was observed due to exposure to high ambient temperature.

Srikandakumar *et al.* (2003) in their study on the effect of heat stress in two breeds of sheep observed significant increase in the plasma glucose in

Merino sheep but significant reduction in the plasma glucose in Omani sheep. In response to the increase pressure due to elevated respiratory activity and reduce feed intake, reserved body fat of Merino sheep attribute to mobilization as compared to the Omani sheep with less fat content.

Srikandakumar and Johnson (2004) found that the effect of heat stress in Holstein, Jersey and Australian Milking Zebu cows revealed significant increase in the plasma glucose concentration in Holstein and Australian Milking Zebu cows but, significant decrease in plasma glucose concentration in Jersey cows during hotter month of the year.

Rasooli *et al.* (2004) recorded significantly lower concentrations of serum glucose during summer than winter in Holstein heifer and further, the serum glucose levels showed a significant negative correlation with mean environmental temperature. Significantly higher serum levels of glucose during winter was ascribed to augmentation of thyroid activity and metabolic rate accompanied with high levels of blood metabolites.

Trana *et al.* (2006) found that the influence of season and nutrition on oxidative status in Red Syrian goats were significantly higher in plasma glucose concentration during summer than during spring season.

Abeni *et al.* (2007) recorded a significant decrease in the plasma glucose concentration in the lactating Holstein cows during the hot season.

Collier *et al.* (2008) observed that lower level of glucose during heat stress may be due to increased glucose oxidation.

Shrikhande *et al.* (2008) reported that the average blood glucose level was higher (47.98 mg/dL) during summer season whereas lowest level (44.20 mg/dL) was recorded during rainy season in lactating cows.

Al-Saeed *et al.* (2009) studied the effect of season on blood biochemical parameters in local cows of Iraq and recorded a significant reduction in the plasma glucose concentration (mg/dL) during summer season (55.09 ±10.0) compared to the concentration during winter season (70.68 ± 15.0). Higher concentration of plasma glucose during winter season could be due to enhanced feed intake during winter compared to summer season.

Bagha *et al.* (2009) reported significant ($P<0.05$) increase in serum glucose during summer season (51.69 ±4.40 mg %) than spring season (38.62 ±4.81 mg %). Body weight gain (gm. /day) was significantly decreased in summer than winter (47.49%). Growth rate was positively correlated with glucose (r=0.440) but negatively with SGPT (r= -0.328) though the differences were significant at 5% level). SGPT were significantly depressed during summer in young crossbred calves.

Avendano-Reyes *et al.* (2010) reported a significantly higher concentration of serum glucose (48.41 mg/dL) in control Holstein cows compared to the corresponding values in animals that were exposed to cooling systems during hot and dry ambient conditions (44.9 mg/dL).

Behl *et al.* (2010) reported that heat stress markedly alters glucose homeostasis in affected cows. Glucose disposal into cells increases in heat stressed cows compared to pair-fed thermal neutral cows.

Hooda *et al.* (2010) found that the glucose concentration decreased significantly ($P<0.05$) in the group-II when two group of buffalo heifers were kept in temperature varied from 8-15°C (Group-I) and relative humidity varied from 59-85% and thermal stress at 40°C (Group-II) for 4 hours daily for a period of 16 days in a Psychrometric chamber.

Cincovic *et al.* (2011) found that the mean values of serum glucose in heat stressed cows were significantly different from the values obtained for the cows in the thermoneutral zone.

The study of Calamari *et al.* (2011) on the effect of selenium supplementation in heat stressed lactating dairy cows showed a significant negative correlation between the THI and the plasma glucose concentration.

Al-Haidary *et al.* (2012) in their study on Najdi rams found a significant increase in the serum concentrations of glucose (mg/dL) in heat stressed rams compared to their counterparts exposed to winter season (95.07 ± 2.16 vs. 71.29 ± 2.80). Further, they observed and opined that due to the secretion of cortisol induced by the stress, it also inhibits glucose uptake and utilization in cells and results in promoting gluconeogenesis, leading to increase in serum glucose concentration.

Kumar *et al.* (2012) reported significant decrease in the plasma glucose concentration during summer stress compared to the corresponding pre-summer values indicating the adverse effects of summer stress. The plasma concentration was significantly higher in heat stressed vitamin C supplemented groups compared to summer stressed vitamin C and E. Se supplemented groups indicating the beneficial effects of vitamin C supplementation.

Pandey *et al.* (2012) showed significant decline and significant increase in serum glucose levels when Marwari goats were exposed to hot and cold ambience, respectively. But, a mean serum glucose concentration of 3.63 ± 0.03 mmol/L was recorded when they were exposed to moderate ambient stress.

Sreedhar *et al.* (2013) recorded the level of serum glucose concentration while adapting to the tropical environmental conditions of Sahiwal heifers (61.90 ± 1.34 to 97.32 ± 0.63 mg/dL), cows (58.61 ± 1.20 to 96.90 ± 0.65 mg/dL) and Jersey × Sahiwal cows (59.26 ± 0.58 to 113.33 ± 0.71 mg/dL). The level of

serum glucose showed an elevated range in the animals while adapting to the tropical conditions.

Chandra *et al*. (2013) supplemented vitamin E at the rate of 1000 IU/day/cow along with zinc at the rate of 60 ppm/day/cow to Sahiwal cows and observed significant ($P<0.05$) increase in plasma glucose concentration during pre-partum, at parturition and postpartum period compared to the control group cows.

Pandey *et al*. (2013) observed significant decline in blood glucose levels in Sahiwal cattle during evening hours compared to morning hours of hot dry season. They opined that the decline in the blood glucose levels during evening hours as a coping strategy of the animal to overcome the stressful conditions arising from high THI and High relative humidity.

Seijan *et al*. (2013) reported that total protein, albumin and globulin showed similar trend of decreases level in 40° C and 42°C temperature groups. But this effect is only significant for total protein and albumin. The significantly reduced total protein in heat stressed group is to support gluconeogenesis to maintain the energy for thermoregulatory process. They also observed plasma glucose level to be significantly ($P<0.01$) differed between the groups and reported concentration of plasma glucose when Malpura ewes (n=28) were exposed to 23°C, 40°C and 42°C of ambient temperature for 21 days and the level of plasma glucose was found to be 54.590, 52.799 and 58.410 respectively and concluded that the highest plasma glucose level at 42°C was due to higher requirement of energy source in the form of glucose to support of physiological mechanism for thermoregulation. They also observed that highest cortisol concentration was recorded in group-3 (34.73 nmol/L) and lowest in group-2 (nmol/L) when Malpura ewes were exposed to three temperature groups at 23, 40 and 42°C, respectively for group-1, group-2 and group-3.

Singh *et al.* (2014) observed a decrease in the level of plasma glucose by 15.69 per cent as the THI score increased from 72 to 84.

Zhang *et al*. (2014) reported that Serum glucose concentration of Holstein cows was significantly lower during moderate THI period and high THI period compared to low THI period. Further, the chromium supplementation did not affect the serum concentrations of glucose during all periods.

Haq *et al*. (2015) observed significantly higher concentration of plasma glucose concentration in crossbred dairy cows when they were exposed to summer season and the supplementation of the diet with ascorbic acid and amla powder reduced the levels of plasma glucose significantly.

Khate (2015) reported that the values of plasma glucose (mg/dl) in mithun were 43.75± 7.74, 43.87± 5.37 and 81.14± 18.08, respectively for C-1 (16°C and 50% RH), C-2 (23°C and 75% RH) and C-3 (37°C and 85% RH) with significantly ($P<0.05$) higher in C-3 but no significant difference between C-1 and C-2.

b. SGOT

Thompson, 1973; Habeeb, 1987; Marai *et al.*, 1995 observed that in heat stressed animals, the level of serum GOT increased which might be caused by the action of corticoid hormones like increase in cortisol, cortisone and adrenocorticotrophic hormone, resulting in increase of gluconeogenesis stimulation.

Marai *et al.* (1992) observed insignificant effect in the levels of SGOT and SGPT caused by different seasons (summer, autumn and winter) in Ossimi x Suffolk under the Egyptian conditions of the year.

Srikandakumar *et al*. (2003) reported a significant decrease in the activity of the plasma AST during heat stress in both Merino sheep from 84.67 to 30.67 IU/l and Omani from 145.00 to 85.83 IU/l and the decrease was within the normal range. Further, they concluded that the decrease in the enzyme concentration is indicative of no damage to the liver tissue but the slowdown of the liver function when the animals were subjected to heat stress.

Srikandakumar and Johnson, (2004) studied the effect of heat stress on blood biochemistry in Holstein, Jersey and Australian Milking Zebu cows reported a significant increase in the plasma aspartate aminotransferase activity (IU/L) during heat stress in all the breeds of cattle.

Rasooli *et al*. (2004) studied the influence of seasonal variation on biochemical parameters and found significantly increased serum AST activity in HF heifers exposed to high ambient temperature.

Study of Al-Saeed *et al*. (2009) showed a significant increase in the plasma AST activity (U/l) during summer (70.9 ± 17.07) as against the winter (61.9 ± 16.6) in local cattle. They concluded that the increase in the plasma AST activity in cattle suggests the cellular damage in the liver. Study of metabolic and hematological profiles in Italian Friesian lactating dairy cows during heat stress showed a significant positive effect of THI on plasma AST activity and the supplementation of selenium did not bear any effect on its activity. Increase in AST indicated slight impairment of tissue as a consequence of oxidative effect (Calamari *et al*., 2011).

Bagha *et al.* (2009) found that the values of SGOT did not vary in different seasons while SGPT was significantly ($P<0.05$) depressed in summer by 53.93 per cent. Non-significant increase in serum glutamic oxaloacetic transaminase activity was observed during spring season (38.68 ± 4.83 Units/ml)

in crossbred calves compared to the enzyme activity during summer (38.44 ± 4.95 Units/ml).

Sharma and Kataria, (2011) reported that the climatic conditions did not bear any significant effect on both liver and serum AST activity in Marwari goats, but the sex of the animal significantly influenced the serum AST activity in both (moderate and extreme) climatic conditions. Though the season did not influence the serum AST enzyme activity significantly in crossbred cows, serum AST activity was significantly increased with advancement of pregnancy during both summer and winter season.

Chandra Bhan *et al.* (2012) recorded a significantly higher AST activity in young and adult Sahiwal cattle during the summer season as compared to the spring season.

Ganie *et al.* (2012) found that the supplementation of 0.2 ppm of selenium did not exert any significant influence on the serum AST activity in buffalo heifers.

Singh *et al.* (2012) observed that the plasma concentration of SGOT increased significantly ($P < 0.05$) due to thermal exposure.

Shiv Pratap Singh *et al.* (2012) reported that exposure to thermal stress resulted in significant ($P < 0.05$) increase the plasma concentration of SGOT and SGPT, whereas the plasma alkaline phosphatase and acid phosphatase concentration was found to decrease. The significant difference in the blood biochemical results would be caused by thermal stress, as the yeast supplemented group of buffalo heifers were found to be in their normal levels.

Ashatsham-ul Haq *et al.* (2013) reported a significant increase in the plasma levels of AST during summer season and the supplementation of the

summer stressed dairy cow with ascorbic acid and amla powder significantly declined the enzyme activity.

Pandey *et al.* (2013) found that the there was a significant increase in the serum AST activity during hot dry conditions in the morning (62.22 IU/ ml) to evening (62.95 IU/ ml) in Sahiwal cows. Though, the activity of the enzyme did not vary during the hot humid condition.

Devi *et al. (*2014) reported a significantly higher serum activity of AST in zinc fed goat kids during their growth phase and higher rate of assimilation of zinc from organic composition might be attributed to higher levels of zinc in the body fluids and higher AST activity.

c. Serum Glumatic Pyruvic Transaminase (SGPT)

Baumgartner and Parnthaner (1994) found that serum GOT level decreased significantly during summer season in Karakul sheep.

Marai *et al.* (1992) observed insignificant effect in the levels of SGOT and SGPT caused by different seasons (summer, autumn and winter) in Ossimi x Suffolk under the Egyptian conditions of the year.

Nazifi *et al.* (2003) who reported that serum activity of serum alanine aminotransferase was significantly higher in sheep exposed to heat stress as compared to the sheep exposed to cold stress.

Al-Saeed *et al.* (2009) found that plasma ALT activity (U/L) showed a significant increase during the summer season (30.8 ± 9.25) compared to its activity during the winter (25.1 ± 10.43) season in White Fulani cows. Further, this increase in the enzyme activity during summer season is indicative of deranged energy metabolism.

Bagha *et al.* (2009) found significantly (P<0.05) lower levels of serum glucose in summer (38.62±4.81 mg %) than in spring (51.69±4.40 mg %) season. They also found that in summer season (10.68±0.96 U/ml) the activity of serum glutamic pyruvic transaminase (SGPT) was significantly low (P<0.05) as compared to spring season (16.44±2.17 U/ml) in the young cross bred calves. There was non-significant (P<0.05) difference in serum alkaline phosphatase during summer (130.43±14.79 KA Units) and spring (119.44±10.81 KA Units). It was concluded that low SGPT activity indicates low amino acid turnover whereas, high level of alkaline phosphatase activity indicates bone resorption or alkalosis and therefore affecting declined growth rate due to summer stress.

Sharma and Kataria (2011) observed a significant increase in the ALT activity in serum (77.68 ± 1.75 vs. 57.41 ± 1.75 IU/L) and in liver tissue (441.31 ± 30.18 vs. 362.88 ± 30.18 IU/L) during extreme climatic conditions compared to moderate climatic conditions in Marwari goats.

Alameen and Abdelatif (2012) recorded numerically higher ALT values during summer compared to winter values indicating no influence of season on ALT activity. However, serum ALT activity significantly increased with advancement of pregnancy during both summer and winter season.

Chandra Bhan *et al.* (2012) recorded a significantly higher ALT activity in young and adult Sahiwal cattle during summer season as compared to the spring season.

Shiv Pratap Singh *et al.* (2012) reported that exposure to thermal stress resulted in significant ($P < 0.05$) increase the plasma concentration of SGOT and SGPT, whereas the plasma alkaline phosphatase and acid phosphatase concentration was found to decrease. The significant difference in the blood biochemical results would be caused by thermal stress, as the yeast supplemented group of buffalo heifers were found to be in their normal levels.

Pandey *et al.* (2013) revealed a significant increase in the serum ALT activity from morning (27.33 IU/ ml) to evening (30.60 IU/ ml) indicating an increased enzyme activity in the liver when the temperature was more in the evening hours.

d. CORTISOL

Scott and Wiersma (1971) stated that the plasma cortisol concentrations have been used as a physiological marker of stress. The level of circulatory cortisol is the indicator of stress response in animal. There is reduction of plasma cortisol during acclimatization that helps the animal in reducing heat production.

Christison *et al.* (1972) reported significant ($P<0.05$) increase in the plasma cortisol levels in cows from 30 to 37 µg/litre exposed for 20 minutes acutely to a moderate heat stress (35°C). After heat exposure for 2 hours the plasma cortisol levels kept elevating and after 4 hours of heat exposure plasma cortisol levels recorded about 43µg/litter. During this period, the rate of cortisol metabolism remained unchanged.

Fuquay *et al.* (1981) reported that serum cortisol concentrations were higher in heat stressed cows compared to cows maintained under cooling.

Kamal *et al.* (1989) found that when polygastric species are exposed to high temperature, there was a decrease in cortisol levels.

Abdel Samee (1991) stated that the effect of ambient temperature or temperature humidity index (THI) did not correlate with the level of cortisol hormone in Hampshire x Suffolk withers.

Marai *et al.* (1992) observed significantly ($P<0.05$) higher cortisol level in the blood plasma of Ossimi x Suffolk ram during summer as compared to winter and autumn seasons under Egyptian conditions.

Koushish *et al.* (1997) found significantly higher levels of cortisol in the blood plasma Beetal goats during hot humid condition as compared to hot dry conditions.

Yousef *et al.* (1997) reported that the cortisol hormone level increased significantly under high ambient temperature.

Lowe *et al.* (2002) reported increase in concentration of plasma cortisol when the Romney cross ewe lambs experienced heat stressed as rectal temperature recorded 40.7^{o} C. When the rectal temperature exceeded 42^{o} C in the heat stressed lambs elevation in the levels of plasma catecholamine was noticed.

Aggarwal and Singh (2008) found that plasma cortisol concentration was higher ($P<0.01$) and varied from 4.80 vs 2.60 ng/ml in Murrah buffaloes when kept in water showers in comparison to water pond buffaloes.

Sarker *et al.* (2010) indicated negative correlation (-0.42 and -0.036) between rectal temperature and respiration rates with plasma corticoid concentration. They also observed a decline in the corticoid's concentration from 13.7 ±1.1 to 4.6 ± 0.68 ng /ml during cool to hot seasons.

Chaurasia *et al.* (2011) found that plasma cortisol (ng/ml) level (4.08± 0.14; 2.89± 0.10 and 3.21± 0.12; 2.70±0.13) were significantly higher ($P<0.05$) at an altitude of 300m MSL and 2100m MSL in different seasons. They reported higher concentration of plasma cortisol levels in the season of March-June (S_1) compared to the season of November-February (S_3).

Seijian *et al.* (2013) reported that the plasma cortisol concentration was highest in the group-3 (34.73 nmol/L) and lowest in group-2 (nmol/L) when Malpura ewes were exposed to 23°C, 40°C and 42°C temperatures for group-1, group-2 and group-3 respectively.

Khate (2015) reported that plasma cortisol level in C-3 group (37°C with 85%RH) was significantly ($P<0.05$) higher compared to C-1 group (16°C with 50%RH) and C-2 group (23°C with 75%RH) which might be due to the hepatic gluconeogenesis to supply more glucose for the respiratory muscular activities to dissipate more heat. He concluded that the mithun were in the adaptive mechanism for thermoregulation in C-3 group as the principal function of cortisol in ruminant species is to favour protein catabolism to supply regular energy for vital body function.

2.1 EXPRESSION OF HSP GENES

Berman *et al.* (1985) studied that when cattle exposed to temperatures higher than their thermal comfort zone (25–26°C) resulted in a marked increase in all the body temperature variables measured.

Lindquist (1986) stated during heat stress HSPs are produced as a response in the cell to adapt and survive. He also stated that biochemical adaptive responses and gene expression are triggered by thermal stresses.

Mosser *et al.* (1987) reported that in Tharparkar and Karan Fries cattle, the upregulation of HSP70 genes acts as adaptive mechanism to cope up with the heat stress caused due to temperature-season change during summer and winter season. Therefore, thermotolerance is maintained by synthesis of HSPs.

De Maio 1999 and Lacetera *et al.* (2006) reported that increased concentration of HSP70 in several types of cells was due to stressful condition in cattle.

Hahn *et al.* (1992) recorded higher mRNA expression of HSP during summer compared to winter season in Tharparkar and Karan Fries cattle. They stated that during the summer season conditions with higher THI value >80 would cause heat stress in the animals.

Kamwanja *et al.* (1994) observed an increase in the HSP70 concentration in the lymphocytes up to 2 to 3 folds when exposed for 1hour at 42°C of different cattle breeds.

King *et al.* (2002) concluded that HSP70 is induced in the heat stressed mice to survive the exposure to 41°C for 30 min. They concluded HSP70 played a significant role in the survival of the heat stressed mice.

Morris (2002) found that expression of HSP70 was expressed constitutively in the skin due to upregulation of heat treatment.

Beckham *et al.* (2004) reported that HSP70 expression is temperature sensitive. They stated that the increase in HSP70 expression in skin during summer and winter season might be induced by heat and hypothermic stress. The unique feature of these molecules is to function physiologically in the skin tissue which is influenced by the expression of HSP70 genes during winter and summer season as the environmental condition changes.

Hansen (2004) found that in heat stress cattle, low expression of HSP70 cause reduction in protein denaturation leading to less harmful effects to the animals.

Byoung Hwa Roh *et al.* (2008) observed that increased HSP expression helps to maintain thermotolerance which enables cells to withstand further damage caused by exposure to stress.

Liu *et al.* (2010) studied expression of heat stress-induced HSP70 in different cells or tissues in bovine lymphocytes.

Patir and Upadhyay (2010) reported a rise in HSP70 expression in the PBMCs of Murrah buffalo lymphocytes due to higher intensity and duration of thermal exposure at 45°C for 2 hours. The rise in the expression of HSP70 due to thermal exposure might be to maintain cellular homeostasis.

Garbuz *et al.* (2011) reported increase in the expression of HSP70 when the temperature in camel increased.

Mishra *et al.* (2011) found an increase *in vitro* cultured lymphocytes by 2.5-fold in the concentration of HSP70 in Murrah buffalo in dry hot condition compared to controlled condition.

Dangi *et al.* (2012) reported significant increase in HSP70 mRNA expression during peak summer season in goats as compared to peak winter season of tropical and temperate region goats.

Gaugharn *et al.* (2013) reported that there was a strong relationship between climatic variables like ambient temperature, relative humidity, solar radiation and wind speed and HSP 70 concentration in adult cattle. The HSP 70 concentration is a reliable indicator of chronic stress but not for a single stressor when animals are exposed to multiple chronic stressors.

Gupta *et al.* (2013) stated that hyperthermia and hypothermia both could cause changes in gene expression. They found increase in expression of many HSPs including HSP32, HSP40, HSP60, HSP70, HSP90 and HSP110 during hypothermic stress. They confirmed that heat shock proteins as indicator of stress marker which is a significant role for cell survival during heat stress in livestock.

NRCM (2013) reported that both HSP-70 and HSP-90 genes have been highly expressed in mithun bull after three hours of ploughing. Moreover, the relative gene expression studies for HSP-70 were highly significant after ploughing in comparison to before ploughing.

Sharma *et al.* (2013) reported that at heat stress the relative expression of Ubiquitin, HSP60, HSP70 and HSP90 increased significantly ($P<0.05$) with the increase in the exposure temperature (25°C, 35°C and 40°C) in the control and

treatment group in goats. They reported significant increase in the mRNA expression of HSP60, HSP90 and Ubiquitin in goats of tropical and temperate region during peak summer as compared with peak winter season.

Banerjee *et al.* (2014) reported that the expression of HSP70 gene (HSPA8, HSPA1A and HSPA6) was highly expressed in blood leukocytes during summer and winter seasons. Higher expression was observed in the heat and cold adapted goat breeds during the summer season.

Dangi *et al.* (2014) reported that during thermal stress elevated HSP expressions could be an indicator for coping up the harmful effects of thermal stress to maintain homeostasis and resist cellular damage.

Deb *et al.* (2014) found that PBMC derived from Sahiwal had significantly higher cell viability than Frieswal. To maintain body temperature and increase survivability of the cell, HSP90 gene expression in Sahiwal was high during induced in-vitro and environmental heat stress conditions. The relative expression of HSP90 peak values are 3.29±0.49 and 2.11±0.38 at 2 hours recovery among Sahiwal and Frieswal, respectively. They also found increase in the mRNA expression of HSP90 as temperature increased from 37°C to 45°C during summer stress. Significantly ($P<0.01$) higher mRNA HSP90 expression between Sahiwal (0.953 ±3.41 at 37°C, 1.83 ±2.93 at 39°C, 2.86 ±2.39 at 41°C and 3.67 ±2.99 at 45°C) and Frieswal (0.874 ±3.85 at 37°C, 1.52 ±2.21 at 39°C, 1.98 ±3.61 at 41°C and 2.98±2.52 at 45°C) samples were recorded.

Rao *et al.* (2014) observed that during summer season (20-40°C) the expression of HSP 70 in Tarai buffalo was significantly ($P<0.01$) higher than winter season (2-17°C). They also found an increase in 2 folds in the relative expression values during summer season and one-fold decrease in relative expression during winter as compared to thermo neutral season (6-24°C).

Rajoriya *et al.* (2014) concluded that the mRNA expression of HSP70 and HSP90 showed no significant difference during the winter and summer season in Tharparkar bull. They also stated that the semen of Tharparkar bull could be cryopreserved throughout the year with the presence of stress resistant spermatozoa in Tharparkar bull semen.

Singh *et al.* (2014) observed that the heat stress at 40 and 44°C the expression of HSP70 genes was moderate to high, but at cold stress at 25°C the expression was suppressed. They reported that at different temperatures in the dermal fibroblast showed an increased level in all the HSP70 genes (HSPA1A, HSPA2, HSPA8) and the relative expression of all the inducible HSP70 genes (HSPA1A and HSPA2) at 40 and 44°C were higher in Karan-Fries than the Tharparkar.

Khate (2015) found that the expression of gene HSP 70 were significantly higher in the mithun maintained at C-3 group (37°C with 85%RH) compared to C-1 group (16°C with 50%RH) and C-2 group (23°C with 75%RH). It was found that HSP70 gene expressed considerably when the mithun group C-3 were at 37°C with 85% RH but no expression was observed in the other mithun group C-1 which were exposed to 16°C with 50%RH and C-2 group which were exposed to 23°C with 75%RH. It was concluded that the level of expression of HSP70 have been correlated with tolerance of thermal heat. The study showed that level of HSP70 gene expression is highly significant in the mithun maintained at 37°C with 85% RH than 16°C with 50% RH and 23°C with 75% RH groups and concluded that there was a strong relationship between HSP70 and heat stress in mithun and HSP70 expression would be used as a useful indicator for body temperature in mithun.

Kumar *et al.* (2015) revealed that during winter and summer seasons, heat shock protein genes pattern was significantly higher ($P < 0.001$), whereas during summer season the magnitude of expression of HSPs was high compared to

winter season. They also reported close similarities of gene sequences of the HSP70 family in *Bos indicus* cattle with other mammalian species.

Manjari *et al.* (2015) observed significantly higher values in the expression of HSP70 during summer season (2.37 ±0.12) as compared to winter (0.29 ±0.04) in buffaloes.

Parmar *et al.* (2015) reported that the relative expression of HSP70 was found to be significantly higher ($P<0.05$) during summer than the corresponding values in winter season in Sahiwal. They found that the elevation in THI triggered substantial upregulation of HSP70 gene in Sahiwal cows which might play a crucial role in providing defence against thermal injury at cellular level.

Maibam *et al.* (2016) described HSP70 genes expression profile as an integral and inducible during thermal stress which response as a protective mechanism in the skin of the cattle during different seasons. They suggested that increase in the expression of HSP70 in the skin of zebu (Tharparkar) and crossbred (Karan-Fries) cattle during winter and summer season might be due to the possible involvement to improve the adverse effect of thermal stress due to environmental stressors to maintain homeostasis and integrity of cells.

Rodrigo *et al.* (2017) summarized thermoregulatory mechanisms to be influenced by exposure to continuous and intense heat stress which leads to increase in respiratory rate and rectal temperature. To maintain homeostasis and maintain the heat load the animal uses adaptive physiological responses like decrease in feed intake in order to induce metabolic shifts, redistribute blood flow and decrease the heart rate.

2.3 TEMPERATURE HUMIDITY INDEX (THI)

Johnson (1980) reported decrease in milk production and feed intake as the THI reaches 72. Decrease in milk yield was reported in the summer season as compared to winter period in Holstein cows.

Yousef *et al*. (1985) stated the combination of air temperature and relative humidity when amalgamated with the level of thermal stress represents a single value as temperature-humidity index (THI). They reported that cattle could tolerate much higher temperatures compared to swine at lower relative humidity due to the fact of absence of sweat glands in swine making them prone to heat stress whereas cattle dissipate heat by sweating.

Du Preez *et al.* (1990) studied that when THI values are higher than 72 it is parallel to temperature at 22°C with 100% humidity, 25°C at 50% humidity or 28°C at 20% humidity which affects the milk production due to heat stress.

Armstrong (1994) reported that thermal stress can be expressed by means of THI having limit value 72. Anything above that value leads to first symptoms of thermal stress. He reported that the first production losses were visible when THI reached the value of 72.

Holter *et al.* (1997) reported that the productivity of dairy cows due to the effect of heat stress could be determined by temperature-humidity index (THI). There was a significant negative correlation between THI and DMI in cows and increase in body temperature mediated the effect of THI on cow performance.

Ravagnolo *et al.* (2000) stated that combined effect of temperature and relative humidity affected the THI values which adversely affected in reduced milk yield. Maximum temperature and minimum relative humidity are the variables responsible for increased heat stress. They found that as THI value exceeded 72, there was 0.2 kg per unit decline in the milk yield and opined that level of heat stress could be estimated using THI.

Pangestu *et al.* (2000) reported at low altitude accompanied by high ambient temperature recorded higher rectal temperature in cattle, sheep and goats as compared to high altitude with low ambient temperature.

Bouraoui *et al.* (2002) found positive correlation of respiration rate, heart rate, rectal temperature and cortisol with THI under the spring period (THI value= 68 ±3.75) vs. summer period (THI value= 78 ±3.23), where spring period was considered as no heat stress and the summer condition as heat stress conditions. They reported with increase in the THI values from 68 to 78, resulted in increased rectal temperature by 0.5°C, increase of heart rate by 6 beats and increase in respiration rate by 5 inspirations per min and also an increase in average cortisol concentration (21.75 nmol/L to 23.5 nmol/L).

Bouraoni *et al.* (2002) and Johnson *et al.* (1963) reported that with THI value of 77, significant decline in the milk yield and DMI was recorded.

Kadzere *et al.* (2002) reported that the temperature-humidity index (THI) is determined by equating air temperature and relative humidity for a particular day. It is used as an indicator of thermal conditions of the environment. The principle of THI was that at any temperature an increase in the relative humidity would make the animal difficult to dissipate heat load from the body.

Broucek *et al.* (2009) and Akyuz *et al.* (2010) distinguished thermal stress as mild stress when THI was 72-79, moderate stress when THI 79-89 and heavy stress when THI <89. They opined THI as a reliable tool to manage livestock under different environmental conditions and could be used as climatic marker to monitor heat stress in the animal physiology and productivity.

Cincovic *et al.* (2010) reported that in hot environment conditions, the most sensitive to thermal stress were the high yielding lactating cows, as high milk production contributed to significant increase in production of metabolic heat.

Gantner *et al.* (2011) found with increase in THI a significant decrease in daily milk yield and daily fat and protein in all the cows were observed. They reported that during spring and summer season, the heat stress conditions recorded a daily THI value >72 whereas in autumn and winter season heat stress conditions were absent.

Rodrigo *et al*. (2017) reported significantly ($P<0.0001$) higher THI values in the heat stressed group (88.7 ±0.4) compared to the thermoneutral group (75.4 ±0.3) of Holstein cows. The THI value was also recorded in the morning (73.66 ±0.07 vs. 87.01 ±0.15) and afternoon (76.59 ±0.06 vs. 89.87 ±0.14) in the thermoneutral and heat stress group of Holstein cows housed in a climate chamber.

Materials and Methods

The experiment was conducted to study the impact of heat stress on the expression profile of heat shock protein genes on account of seasonal variation in Mithun.

3.1 Location of the study:

The experiment was carried out at the Mithun breeding farm, Indian Council of Agricultural Research- National Research Centre on Mithun, Jharnapani, Nagaland, India located between 25°54´30´´ North latitude and 93°44´15´´ East longitude and at altitude range of 250-300 mean sea level.

3.2 Temperature humidity Index:

The ambient temperature and relative humidity were obtained from the meteorology station of ICAR Research Complex, Nagaland Centre located at close proximity for calculation of Temperature Humidity Index. The THI was calculated month wise for one whole year and the months were divided into four different seasons viz. autumn, winter, spring and summer. The THI for the four seasons was calculated for the study.

THI was calculated by using the following formula (Kadzere *et al.*, 2002).

$$\mathbf{THI = 0.72\ (W+D) + 40.6}$$

Where, W stands for wet bulb temperature (°C) and D stands for dry bulb temperature (°C).

3.3 Experimental animals:

Six healthy animals (three male and three female) of 2-5 years with good body condition scorings were selected from the herd of mithuns, who

were originally brought from different hilly tracts of NEH region of India. The animals were identified with ear tag number and chip number. The selected animals were vaccinated and dewormed properly as per the farm protocol. They were maintained under proper and uniform feeding, housing and lighting conditions under proper hygienic conditions.

The experimental animals were grouped as control group (T_1) in which the animals were not exposed to the sunlight in the morning hours and the treated group (T_2) in which the animals were exposed to sunlight in the afternoon hours. The animals were exposed to sunlight to induce heat stress. The physiological and haematological observations were recorded during the morning hours at 0600 hours. After that the same animals were exposed to sunlight and the observations were recorded at 1400 hours. The observations recorded during the morning hours were treated as control group or the non-heat stress group (T_1) and the afternoon ones as the treated group or the heat stress group (T_2).

3.4 Experimental design:

The study was conducted for one whole year to cover the four seasons viz. Autumn (September- November), Winter (December- February), Spring (March- May) and Summer (June- August) to study the effect of heat stress on account of seasonal variation on the expression profile of Heat shock protein genes in Mithun. The animals were grouped as control group (T_1) or non heat stress group in which the observations and blood samples were recorded and collected in the morning hours at 0600 hours before the animals were exposed to sunlight. After the observations were recorded, the same animals were exposed to sunlight to induce heat stress. The observations were again recorded and collected in the afternoon at 1400 hours for the treatment group (T_2) or the heat stress group.

3.5 Housing and Management:

The animals were housed properly in a semi-intensive condition. Proper feeding and management were followed as per the farm protocol. Routine vaccination and deworming were followed. The animals were given feed and water in the feeding trough individually to ensure allotted amount of feed. The shed was cleaned daily before collection of samples. The animals were tied in the neck region to secure collection of blood samples and other physiological readings.

3.6 Blood collection:

Blood samples (7ml) were collected from the jugular vein under aseptic conditions from all the six animals at 0600 hours in the morning before exposure to sunlight and in the afternoon at 1400 hours after exposure to sunlight. The blood samples were collected in heparin tubes for RNA extraction and in serum tubes for serum collection and immediately stored in ice box for transportation. Serum was collected by immediately centrifuged at 7000 rpm for 10 minutes. The serum samples were separated into different aliquots in microcentrifuge tubes and labelled as per the samples and kept frozen at -80°C until further analysis. Serum samples were used to estimate Cortisol, Glucose, SGOT and SGPT.

The blood samples collected in the heparin tubes were used for RNA extraction and stored in Deep Freezer at -80°C for further analysis of Heat Shock Protein gene 70 and 90.

3.7 Parameters studied:

In the present experiment, the parameters studied were:

a. Physiological parameters include rectal temperature, respiration rate and pulse rate.

b. Biochemical parameters include glucose, serum glumatic oxaloacetic Transaminase (SGOT), serum glumatic pyruvate transaminase (SGPT) and cortisol.
c. Expression of HSP gene 70 and 90.
d. Temperature Humidity Index.

3.8 Physiological parameters:

Rectal temperature, pulse rate and respiration rate were recorded as physiological indicators of heat stress. The observations were recorded before exposure to sunlight for the control group in the morning hours at 0600 hours and in the afternoon at 1400 hours after the exposure to sunlight for the treatment group. The animals were tied with causing minimum excitement for precise accuracy. . A digital thermometer was inserted for one minute into the rectum for rectal temperature measurement. Pulse rate was recorded by placing a fingertip on the coccygeal artery at the base of the tail. Respiration rate was recorded by observing the movement of the air from the nostrils.

3.9 Biochemical parameters:

i. Glucose

Glucose was estimated by enzymatic GOD- POD method using Diatek kit. The procedure is described in brief:

Principle: Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidise (Trinder's reaction).

Procedure:

1. 10 µl of standard solution was added in a test tube. The test tube was labelled as standard.
2. 10 µl of sample (serum) was added in another test tube. It was labelled as sample.
3. 1000 µl of reagent was added in both the test tubes.
4. The solutions were pipette gently to mix.
5. The solution was transferred into the cuvette.
6. After 20 seconds, the absorbance (A1) at wavelength of 505 nm using UV Spectrophotometer was read. The value for calculation was recorded.
7. After 40 seconds, again the absorbance (A2) was read, the value was noted.
8. Using the values A1 and A2, calculation were done by the given formulae.

   ΔA = [(A2 – A1) sample or standard]

Calculation

$$\text{Glucose [mg / dl]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Conc. Std [mg / dl]}$$

ii. SGOT/ AST

Serum Glumate Oxaloacetate Transaminase or Aspartate Aminotransferase is an enzyme involved in amino acid metabolism. The SGOT estimation was done by using Diatek kit. The procedures were followed as per the literature provided.

Reagent preparation:

1. Working Reagent was prepared by mixing 4 part of Reagent 1 with 1 part of Reagent 2.

2. The working reagent stays stable up to 28 days at 2-8◦C.
3. Recommendation is to use a fresh Working solution based on its workload.
4. Calculate absorbance change per minute (ΔA/min).

Procedure:

1. In a test tube, 50µl sample with 500µl Working Reagent was mixed.
2. The solution was mixed slowly by pipetting.
3. The initial absorbance was read after 60 seconds at 340nm in a UV Spectrophotometer. The readings for further calculation were noted.
4. The final absorbance after 180 seconds was measured against distilled water at 340 nm by an Analyser.
5. Absorbance change per minute (ΔA/min) was calculated.

Calculation

SGOT (U / L) = ΔAbs / mint X 1746

iii. SGPT/ ALT

Serum Glumate Pyruvate Transaminase or Alanine Aminotransferase is an enzyme involved in amino acid metabolism. Intracellular enzymes are released due to destruction of tissue leading into the circulating blood. The SGPT estimation was done using Diatek kit. The protocol was followed as the literature available.

Reagent preparation

1. Working Reagent was prepared by mixing 4 part of Reagent 1 with 1 part of Reagent 2.
2. The working reagent remains stable upto 28 days at 2-8◦C.
3. It is recommended to use freshly prepared Working solution based on its workload.

PROCEDURE

1. 50 µl Sample with 500 µl Working Reagent was mixed.
2. The solution was mixed properly by pipetting.
3. The initial absorbance was read after 60 seconds and the final absorbance was measured after 180 seconds against distilled water blank at 340 nm by an Analyser.
4. Absorbance change per minute (ΔA/min) was calculate.

Calculation

SGPT (U / L) = ΔAbs / mint X 1746

iv. Cortisol

Cortisol is the major hormone secreted by adrenals. Cortisol was estimated by using Cortisol EIA kit (catalogue no. K2101). The procedure was followed as per the literature provided.

Reagent preparation:

- All reagents should be allowed to each room temperature before use.
- All reagents should be mixed by gently inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

Procedure:

- Put the desired number of microstrips into the frame.

- Allocate 14 wells for the calibrators CAL 1-6 and control samples CONTROL and two wells for each unknown sample.
- Pipette 25µl of CON 1-6, control samples CONTROL and unknown samples into the wells.
- Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape (included into the kit).
- Incubate 60 minutes at 37°C or incubate at +37°C with continuous shaking (ca 600rpm) during 30 min.
- Prepare washing solution by 26X dilution of washing solution concentrate (BUF WASH 26X) by distilled water. Wash the strips 5 times.
- Dispense 100µl of SUBS TMB into the wells.
- Incubate 10-20 minutes at +18.....+25 °C.
- Dispense 100 µl of STOP into the wells.
- Measure OD (optical density) at 450nm.
- Set photometer blank on air.
- Apply lin-log method for data reduction.

Calculation:

- Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.
- Plot a calibration curve on the graph paper: OD versus cortisol concentration.
- Determine the corresponding concentration of cortisol in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on the stage.
- Point-by-point or linear data reduction is recommended due to non linear shape of curve.

4. Expression of HSP gene

i. Blood collection and RNA isolation

Whole blood was collected in the heparinised test tubes from the jugular vein of the mithun under sterilized conditions by trained veterinarian and stored in ice-box for transportation. RNA was isolated by using GCC biotech, GSure blood RNA kit.

RNA extraction protocol:

1. 1 ml of blood was added in a tube and 5X sample volume of RBCL Buffer was added.
2. Mix thoroughly by pipetting and vortexing.
3. The tube was incubated in ice for 15 minutes.
4. During incubation, the tubes were vortex for atleast two times at regular interval.
5. The sample were centrifuged at 400xg for 10 minutes, a whitish cell pellet should form at the bottom of the tube.
6. Remove the supernatant by pipetting. Do not disturb the pellet while removing the supernatant.
7. 2X sample volume of RBCL Buffer were added again in the pelleted cell, mixed by vortexing. While mixing, cell pellet should be dislodged from the tube.
8. Incubate again in ice for another 15 minutes, vortex twice intermittently at regular time interval.
9. Centrifuge again the sample at 400xg for 10 minutes.
10. Remove the supernatant completely and use white colored cell pelleted population as sample for total RNA isolation.
11. Harvested blood cells were re-suspended in 250µl Buffer GRBL1. Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to resuspend the cells.

12. The tubes were incubated at 70◦C for 15 minutes and vortex after every 2 minutes.
13. The samples were centrifuged at maximum speed (10000Xg) for 10 minutes in a table top centrifuge at room temperature.
14. Collect the clear supernatant in a fresh microfuge tube.
15. 250µl GRBL2 was added with the collected flow through and mixed by inverting the tube 4-6 times.
16. 350µl Buffer GRBL3 was added and inverted immediately. The buffer was mixed by inverting only. Do not vortex to mix.
17. Take one pre purification column (white colored) and load the whole solution from previous step on column.
18. Centrifuge for 1 minute at maximum speed (10000Xg) in a table top centrifuge at room temperature.
19. Discard the column, not the flowthrough. This flowthrough contains total RNA population.
20. 600µl isopropanol was added in the collected flowthrough. Mix by inverting the tube several times.
21. Apply the isopropanol-added flowthrough in Gmini Chrom-Column (Column specified for RNA binding) by decanting for pipetting.
22. Centrifuge at 10000Xg for 30-60 seconds. Discard the flowthrough.
23. Wash Gmini Chrom-Column by adding 600 µl Membrane Wash Buffer and centrifuging for 30-60 seconds as previously.
24. Discard the flow-through.
25. Repeat washing step.
26. Discard the flow-through and centrifuge for an additional 2 minutes to remove residual wash buffer from membrane.
27. Place the Gmini Chrom-Column in a clean 1.5 ml microcentrifuge tube.
28. To elute RNA, 50 µl Nuclease free water was added to the center of each Gmini Chrom-Column, let stand for 1 minute, and centrifuge for 1

minute at maximum speed (~8500Xg) on a table top microcentrifuge at room temperature.

29. Discard the column and collect the eluted RNA present in the microcentrifuge tube.
30. The quality of the RNA was analysed in NanoDrop Spectrophotometer (Thermo Scientific, USA).

Absorbance at 260 and 280 nm wavelengths is used against Nuclease Free Water as blank. RNA samples showing the OD 260: OD 280 values were between 1.8-2.0 was expected to contain no protein and was stored at -20°C for further use.

ii. First strand cDNA synthesis

RNA was reverse transcribed using Thermo Scientific, First Strand cDNA Synthesis kit, (Catalogue no. #K1612). An equal concentration of total RNA (900 ng) was used for cDNA synthesis.

cDNA synthesis protocol:

- Thaw, mix and briefly centrifuge the components of the kit. Store on ice.
- Add the reagents into a sterile, nuclease free tube on ice in the indicated manner:
    - Template RNA - 0.1 - 5µg
    - Oligo $(dT)_{18}$ primer or Random hexamer primer - 1 µL
    - Nuclease free water - to 11 µL
- Mix briefly.
- Add the following components in the indicated manner:
    - 5X Reaction buffer - 4 µL
    - RiboLock RNAse Inhibitor -1 µL
    - 10 mM dNTP Mix – 2 µL
    - M-MuLV Reverse Transriptase - 2 µL

- Mix gently and centrifuge.
- For Oligo$(dT)_{18}$ primed cDNA synthesis, incubate for 60 min at 37°C. For Random hexamer primed synthesis, incubate for 5 min at 25°C followed by 60 min at 37°C.
- Terminate the reaction by heating at 70°C for 5 min.
- The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one week.
- For longer storage, -70°C is recommended.

iii. Real Time PCR

Real time PCR (Step One, Applied Biosystems, Foster City, CA, USA) was used to study the expression of HSP 70 and HSP 90 genes in mithun under stress using PowerUp™ SYBR™ Green Master Mix kit (Applied Biosystems). For endogenous control, Beta-actin gene was used. The primers used for Real Time PCR, endogenous and target genes were used from Eurofins Genomics India Pvt. Ltd., Bangalore, Karnataka, India.

Table 3.1: List of primers for the study

Sl. No.	Gene	Primer Sequence	Size (bp)
1.	HSP 70	F:AACATGAAGAGCGCCGTGGAGG R:GTTACACACCTGCTCCAGCTCC	171
2.	HSP 90 Alpha	F:GGAAGATCACTTGGCAGTGAAGCA R:TGATGAACACACGGCGGACATACA	152
3.	Beta-Actin	F:AGTTCGCCATGGATGATGA R:TGCCGGAGCCGTTGT	54

Protocol:

The final reaction volume for RT PCR reactions were 10 µl. SYBR Green Master Mix kit (Applied Biosystems, CA, USA), was used according to the

manufacturer's recommendation. All the reactions were preformed in triplicates. Non Template Control (without cDNA) was also set in the plate.

- Thaw the reagents before use.
- Prepare the appropriate number of reactions:
    - PowerUp SYBR Green Master Mix - 5 µl
    - Forward primer - 0.5 µl
    - Reverse primer - 0.5 µl
    - Nuclease Free water - 2 µl
    - cDNA template - 2 µl
- Mix the components thoroughly and then centrifuge briefly to spin down the contents and eliminate any air bubbles.
- Transfer the appropriate volume of each reaction to each well of an optical plate.
- Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate any air bubbles.
- The plate can be placed in the real-time PCR instrument.

The RT-PCR setup was set at initial denaturation at 95°C for 30 seconds, annealing at 57 °C for 30 seconds and extension at 72°C for 30 seconds for 45 cycles. Relative expression was analyzed using ΔΔCt method (*Livak et. al.,* 2001).

5. STATISTICAL ANALYSIS

The statistical analysis was done using split plot design to study the difference among the seasons and treatment and control groups. Paired t- test was done to analyze the data between the control and treatment groups for every single season.

RESULTS AND DISCUSSIONS

In the present study, six number of mithun were kept under four different seasons viz. autumn (August- October), winter (November- January), spring (February- April) and summer (May- July) of a year. The experimental animals were grouped as control group (T_1) in which the animals were not exposed to the sunlight in the morning hours and the treated group (T_2) in which the animals were exposed to sunlight in the afternoon hours. The animals were exposed to sunlight to induce heat stress. The data on physiological, biochemical parameters and expression of Heat shock protein gene 70 and 90 in mithun (*Bos frontalis*) were collected and analyzed and are presented.

4.1. PHYSIOLOGICAL PARAMETERS

4.1.1. RECTAL TEMPERATURE

The observations of rectal temperature recorded in different treatment groups during various seasons is presented in the Table 4.1.1 and graphically plotted in Fig. 4.1.1. The statistical analysis of the average rectal temperature is given in Appendix 1.

In the autumn season, the average rectal temperature recorded was 99.43± 0.53 vs. 101.25± 0.58 (°F) in T_1 and T_2 group respectively. The values recorded in the T_2 group were significantly ($P<0.05$) higher than T_1 group.

In winter season, the average rectal temperature recorded was 98.88± 0.35 vs. 101.54± 0.16 (°F) in T_1 and T_2 group respectively. The values recorded were significantly ($P<0.05$) higher in T_2 group than T_1 group.

In spring season, the average rectal temperature recorded was 98.90± 0.50 vs. 101.94± 0.32 (°F) in T_1 and T_2 group respectively. The values recorded were significantly ($P<0.05$) higher in T_2 group than T_1 group.

Table 4.1.1: Rectal temperature (°F/min) in mithun under different treatment during various season.

TREATMENT	REPLICATION						AVERAGE
	1st	2nd	3rd	4th	5th	6th	
AUTUMN							
T_1	99.7	98.75	99.85	99.7	98.75	99.85	$99.43^{b} \pm 0.53$
T_2	101.6	100.5	101.65	101.6	100.5	101.65	$101.25^{a} \pm 0.58$
WINTER							
T_1	98.63	98.50	99.40	98.80	98.73	99.20	$98.88^{b} \pm 0.35$
T_2	101.53	101.30	101.77	101.60	101.43	101.60	$101.54^{a} \pm 0.16$
SPRING							
T_1	98.13	99.13	99.50	98.47	99.13	99.03	$98.90^{b} \pm 0.50$
T_2	101.93	101.83	101.77	101.57	102.03	102.50	$101.94^{a} \pm 0.32$
SUMMER							
T_1	99.70	99.23	100.37	99.57	100.50	100.90	$100.04^{b} \pm 0.64$
T_2	102.97	103.33	102.63	104.03	103.47	103.63	$103.34^{a} \pm 0.49$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

In summer season, the average rectal temperature recorded was 100.04± 0.64 vs. 103.34± 0.49 (°F) in T_1 and T_2 group respectively. The values recorded were significantly ($P<0.05$) higher in T_2 group than T_1 group.

Similar reports were made by Srikandakumar *et al.* (2003) who stated that rectal temperature was a sensitive indicator of body temperature in heat stressed animals. Chowlu *et al.* (2020) found that during autumn season, the rectal temperature was significantly higher ($P<0.05$) in the heat stressed group

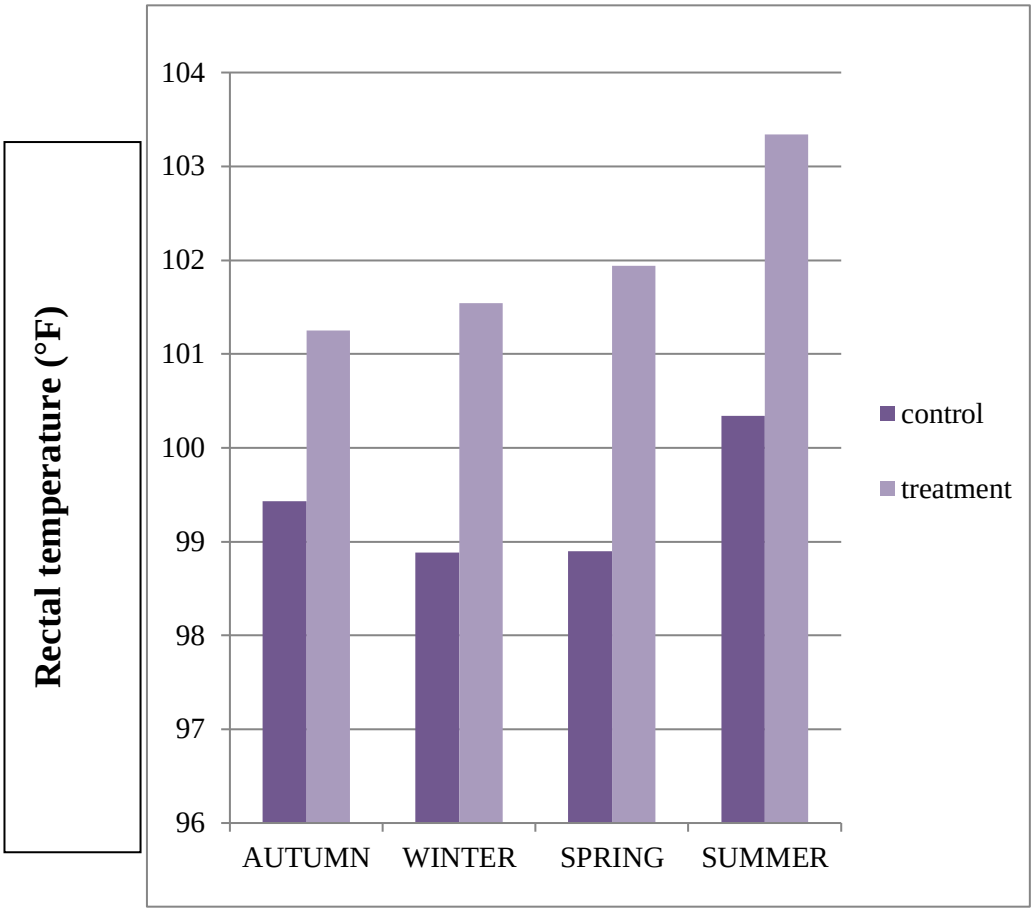

Fig. 4.1.1: Rectal temperature (°F) of mithun under different treatment during various seasons.

of mithuns as compared to the non-heat stressed group of mithuns. Rodrigo *et al.* (2017) found an increase in rectal temperature as thermoregulatory mechanisms influenced by continuous exposure of intense heat stress. Sarkar *et al.* (2010) reported that seasonal effect ($P<0.01$) on rectal temperature was observed in yaks with the higher mean in warm season and the rectal temperature and respiration rates were ($P<0.01$) negatively correlated (-0.42) and (-0.36) with higher plasma corticoid concentration. Gudev *et al.* (2007) found that the lactating buffaloes were sensitive to heat stress and were unable to maintain their core temperature within the thermo neutral zone as there was a significant increase in rectal temperature at THI of 77.83.

However, some researchers also reported contrary result to this Chaurasia *et al.* (2012) reported that there was no significant ($P>0.01$) difference in the values of rectal temperature in growing mithun during different seasons. Khate (2015) reported that there was no significant difference ($P>0.05$) in rectal temperature in two groups with 51.51 and 61.33 THI.

4.1.2. PULSE RATE

The observations of pulse rate (beats/minute) recorded in different treatment groups during various season is presented in the Table 4.1.2 and graphically plotted in Fig. 4.1.2. The statistical analysis of the average pulse rate is given in Appendix 1.

The average pulse rate recorded during autumn season was 34.33± 0.68 vs. 52.17± 0.68 beats/minute in T_1 and T_2 groups, respectively. The rectal temperature recorded for T_2 group was found to be significantly ($P<0.05$) higher compared to T_1 group.

In winter season, the average pulse rate recorded was 43.44 ± 2.18 vs. 60.72 ± 1.93 beats/ minute in T_1 and T_2 group respectively. The pulse rate recorded was significantly ($P<0.05$) higher in T_2 group compared to T_1 group.

Table 4.1.2: Pulse rate (beats/min) in mithun under different treatment during various season.

TREATMENT	REPLICATION						AVERAGE
	R1	R2	R3	R4	R5	R6	
AUTUMN							
T_1	33.5	35	34.5	33.5	35	34.5	$34.33^b \pm 0.68$
T_2	51.5	53	52	51.5	53	52	$52.17^a \pm 0.68$
WINTER							
T_1	42.67	40.67	45.67	43.33	42.00	46.33	$43.44^b \pm 2.18$
T_2	62.33	58.33	59.67	63.33	59.33	61.33	$60.72^a \pm 1.93$
SPRING							
T_1	41.00	43.67	42.67	41.00	40.00	41.00	$41.56^b \pm 1.34$
T_2	61.33	57.67	59.33	60.33	59.33	64.00	$60.33^a \pm 2.17$
SUMMER							
T_1	46.00	42.00	41.00	43.33	38.67	43.67	$42.44^b \pm 2.51$
T_2	60.33	57.00	58.00	59.67	62.00	62.00	$59.83^a \pm 2.05$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

In spring season, the average pulse rate recorded was 41.56 ± 1.34 vs. 60.33 ± 2.17 (beats/minute) in T_1 and T_2 group respectively. The pulse rate recorded was significantly ($P<0.05$) higher in T_2 group compared to T_1 group.

In summer season, the average pulse rate recorded was 42.44± 2.51 vs. 59.83± 2.05 (beats/minute) in T_1 and T_2 group, respectively. The pulse rate recorded was significantly ($P<0.05$) higher in T_2 group compared to T_1 group.

The findings of the current study were similar with the reports made by Sejian *et al.* (2010) who reported that pulse rate of mithun under heat stress

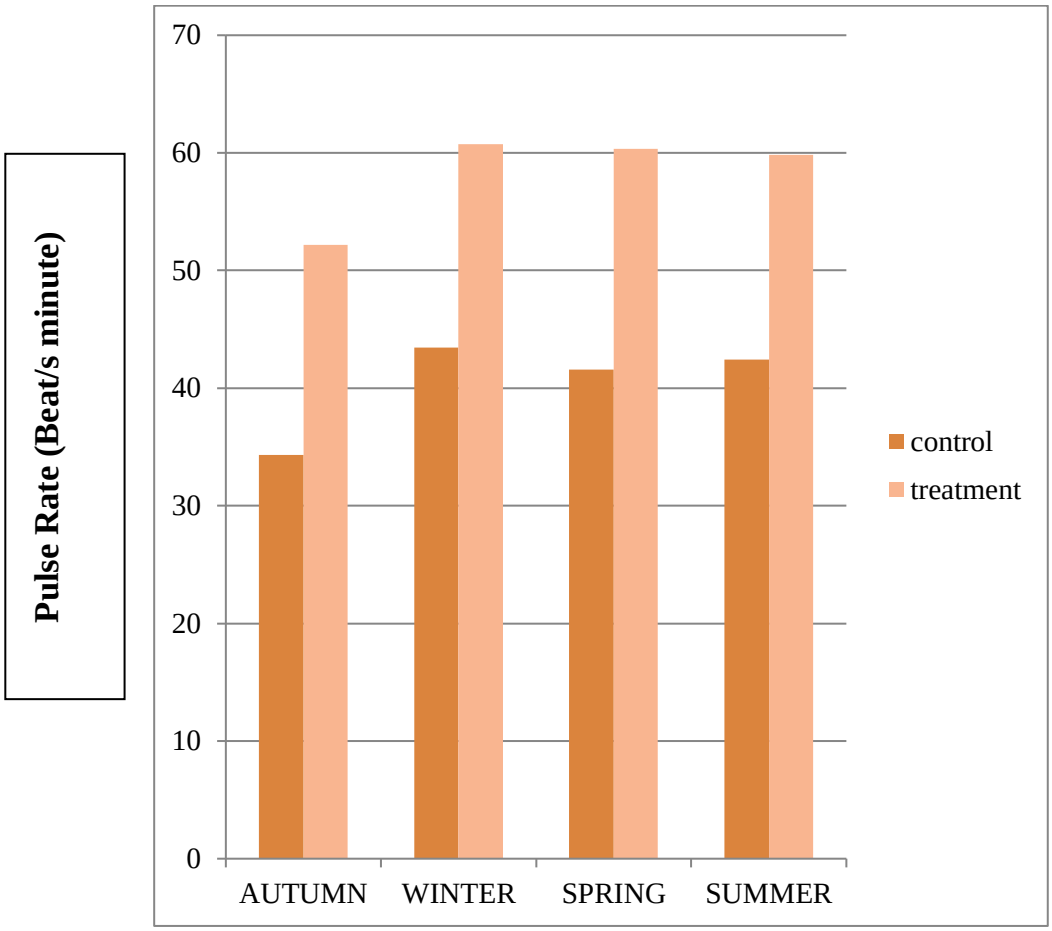

Fig. 4.1.2: Pulse Rate (beats/ min) under different treatments during various seasons.

showed significant increase compared to the control group. They stated that pulse rate primarily reflects the homeostasis of circulation along with the general metabolic status. Hooda *et al.* (2010) reported that the pulse rate increased significantly ($P<0.05$) after exposure to thermal stress at 40°C for 4 hours for 16 days period in a psychrometric chamber. Perumal *et al.* (2015) revealed that there was a significant ($P<0.05$) difference in pulse rate between the experimental groups in different weeks of the experimental period. Chaurasia *et al.* (2010) reported that pulse rate at high altitude was significantly ($P<0.01$) lower compared to low altitude, but pulse rate did not vary between the seasons at both the altitudes. Popoola *et al.* (2014) observed increased pulse rate in animals due to increase in ambient temperature. Gupta *et al.* (2013) also reported increase in pulse rate due to increase in metabolism and muscle activity during the stress condition. Indu *et al.* (2014) reported that in the afternoon the pulse rate recorded was significantly higher in the heat stressed group compared to the non-heat stressed group. Sejian and Srivastava (2010) stated that the there was a significant difference in pulse rate during morning and afternoon between heat stressed group and other groups.

In contradictory, Khate (2015) found no significant difference in pulse rate (61.44 ± 0.67, 63.03 ± 0.70 and 74.17 ± 1.05 per minute) among the groups with different climatic conditions with THI of 51.51, 61.33 and 78.29 respectively. Giasuddin and Islam (2003) also reported that pulse rate in Gayal ranged from 47- 75 beats per minute but did vary with the different age group and season.

4.1.3. RESPIRATION RATE

The observations of respiration rate (breaths/ minute) of mithun in different treatment groups under various seasons is presented in the Table 4.1.3 and graphically represented in Fig. 4.1.3. The statistical analysis of average values of respiration rate is given in the Appendix 1.

Table 4.1.3: Respiration rate (breaths/min) in mithun under different treatment during various season.

TREATMENT	REPLICATION						AVERAGE
	1st	2nd	3rd	4th	5th	6th	
AUTUMN							
T_1	24.5	24	26	24.5	24	26	$24.83^{b} \pm 0.93$
T_2	31	34	32.5	31	34	32.5	$32.50^{a} \pm 1.34$
WINTER							
T_1	25.33	26.00	26.67	26.67	26.00	27.33	$26.33^{b} \pm 0.70$
T_2	33.33	34.33	30.67	33.67	33.00	31.33	$32.72^{a} \pm 1.42$
SPRING							
T_1	26.00	25.00	28.67	28.00	27.33	28.00	$27.17^{b} \pm 1.39$
T_2	35.33	36.00	38.00	36.67	34.00	36.00	$36.00^{a} \pm 1.33$
SUMMER							
T_1	26.00	28.00	24.67	26.67	26.67	28.00	$26.67^{b} \pm 1.26$
T_2	33.33	31.00	32.67	31.33	31.67	36.00	$33.00^{a} \pm 1.81$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

The average values of respiration rate recorded during autumn season are 24.83± 0.93 vs. 32.50± 1.34 (breaths/minute) in T_1 and T_2 group respectively. The values recorded for T_2 group were significantly ($P<0.05$) higher compared to T_1 group.

In the winter season, the average respiration rate recorded for T_1 and T_2 group are 26.33 ± 0.70 and 32.72 ± 1.42 (breaths/minute), respectively. The values recorded for T_2 group were significantly higher ($P<0.05$) compared to the T_1 group during the season.

The average values of respiration rate recorded for spring season were 27.17±39 and 36.00± 1.33 (breaths/minute) for T_1 and T_2 group, respectively.

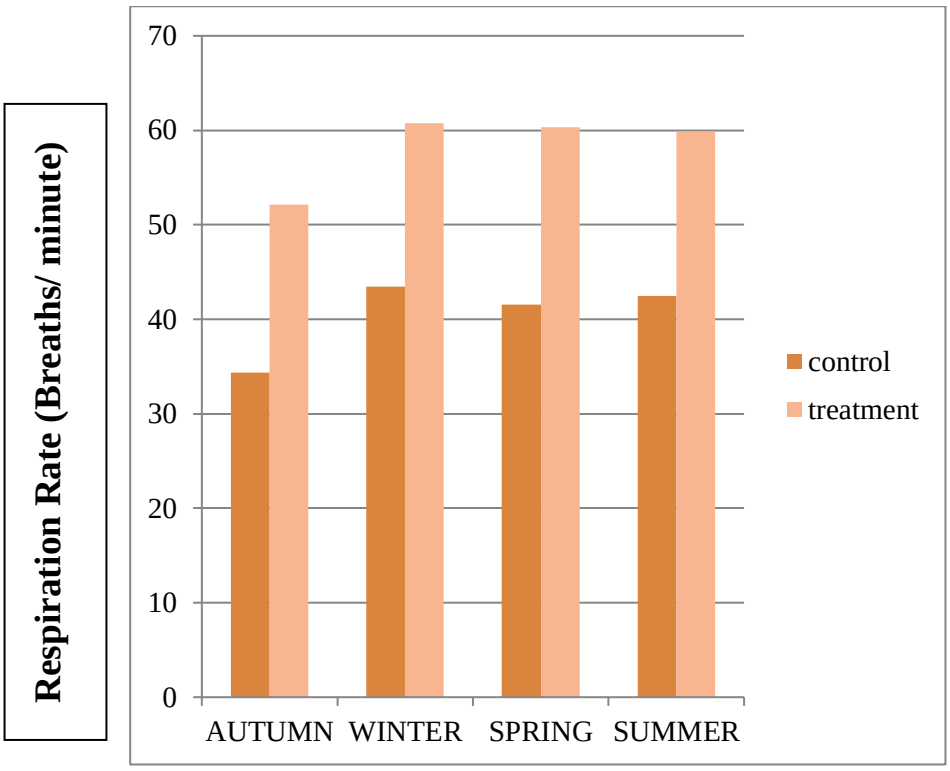

Fig. 4.1.3: Respiration rate (breaths/ min) of mithun under different treatment during various season.

The T_2 group was significantly ($P<0.05$) high compared to T_1 group during the season.

The average respiration rate of mithun during summer was recorded as 26.67± 1.26 and 33.00 ± 1.81 (breaths/minute) in T_1 and T_2 group, respectively. The values recorded for T_2 group were significantly ($P<0.05$) higher than T_1 group in summer season.

Similar results were reported by Rodrigo (2017) who found respiration rate increased due to continuous exposure to intense heat stress influencing thermoregulatory mechanisms. It was reported by Ribeiro *et al.* (2014) that animal uses its respiratory mechanism to avoid increase in rectal temperature and thus maintain homeotherms during hot periods. Renaudeau *et al.* (2012) also stated that the principal mechanism of an animal subjected to heat stress was increase in the respiration rate causing loss of heat through evaporation. Gudev *et al.* (2007) also reported increase in respiration rate at THI of 77.83, showing that the animals are heat stressed and unable to maintain their thermoneutral zone. Indu *et al.* (2014) found that there was an increase in respiration rate during afternoon compared to morning in Malpura ewes. Phulia *et al.* (2010) reported increased respiration rate during afternoon (77.33) compared to morning (43.66) in goats. Chowlu *et al.* (2020) also reported that the values of respiration rate were significantly ($P<0.05$) higher in heat stressed group compared to non-heat stressed group of mithun during autumn season. Sarkar *et al.* (2010) reported that seasonal effect on respiration rates was negatively correlated (-0.43) and (-0.63) with higher plasma corticoid concentration. Gupta *et al.* (2013) reported that the increase in respiration rate in animal is exhibited under high ambient temperature.

In contrary to this results, Khate (2015) reported that respiration rate at THI 51.51 and 61.33 had no significant ($P<0.05$) difference as compared to THI at 78.29 in mithun. Sejian *et al.* (2010) also reported that respiration rate did not vary significantly among the groups during morning in Malpura ewes.

4.2 HAEMOTOLOGICAL PARAMETERS

4.2.1 BLOOD GLUCOSE

The observations of blood glucose (mg/ dL) in mithun in different treatment groups under various seasons is presented in the Table 4.2.1 and graphically represented in Fig. 4.2.1. The statistical analysis of average values of glucose is given in the Appendix 1.

Table 4.2.1: BLOOD GLUCOSE (mg/dL) in mithun under different treatment during various seasons.

TREATMENT	REPLICATION						AVERAGE
	1st	2nd	3rd	4th	5th	6th	
AUTUMN							
T_1	41.73	35.71	40.94	39.07	39.16	40.16	$39.46^{b} \pm 2.11$
T_2	40.51	40.25	43.30	41.51	41.26	41.28	$41.35^{a} \pm 1.07$
WINTER							
T_1	43.37	43.26	46.65	41.98	42.02	42.65	$43.32^{b} \pm 1.73$
T_2	71.67	70.65	74.19	73.17	70.62	70.93	$71.87^{a} \pm 1.57$
SPRING							
T_1	74.97	68.73	69.92	70.70	71.02	68.53	$70.65^{b} \pm 2.35$
T_2	82.34	75.84	70.61	82.42	75.77	73.21	$76.70^{a} \pm 4.80$
SUMMER							
T_1	49.32	53.20	47.45	47.86	51.94	55.16	$50.82^{b} \pm 3.10$
T_2	57.79	62.79	54.99	61.12	58.93	64.91	$60.09^{a} \pm 3.58$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

The average values of blood glucose of mithun during autumn season in T_1 and T_2 group were 39.46 ± 2.11 and 41.35 ± 1.07, respectively. The values recorded for the T_2 group were significantly ($P<0.05$) higher compared to T_1

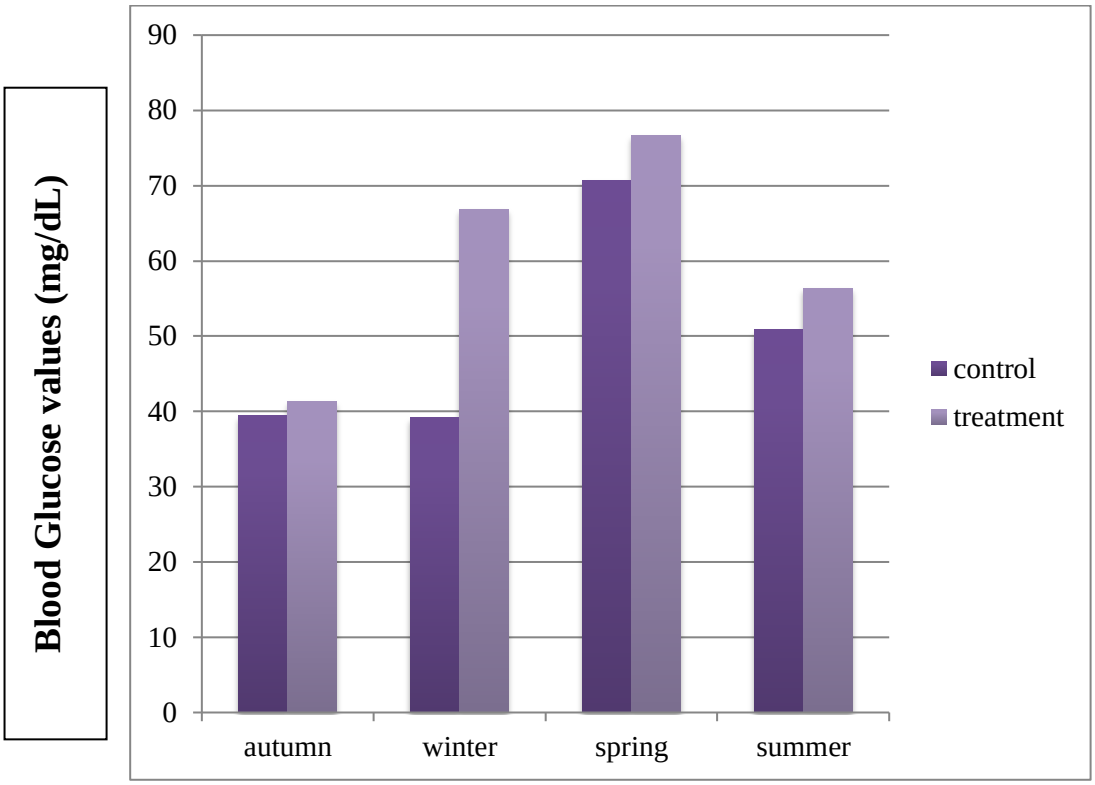

Fig. 4.2.1: Blood Glucose values (mg/dL) of mithun under different treatment during various seasons.

group. An increase in the values of glucose was observed in the heat stress affected (T_2 group) mithuns during autumn season.

The average values of blood glucose during winter seasons in mithun were recorded as 43.32 ± 1.73 and 71.87 ± 1.57 in T_1 and T_2 group, respectively. The T_2 group had significantly ($P<0.05$) higher values compared

to T_1 group. The mithuns were affected by winter heat resulting in increase in glucose level in the heat stressed group (T_2) of mithun compared to the control (T_1) group of mithuns.

The average values of blood glucose during spring season were recorded as 70.65 ± 2.35 and 76.70± 4.80 in T_1 and T_2 group, respectively. The glucose values of T_2 group were significantly ($P<0.05$) higher compared to T_1 group of mithun.

The average values of blood glucose of mithun during summer season were found to be 50.82 ± 3.10 and 60.09 ± 3.58 in T_1 and T_2 group respectively. The glucose values in T_2 group were significantly ($P<0.05$) higher compared to T_1 group of mithun. There was significant effect of summer heat stress on glucose level in mithun.

The above results are supported by Bagha *et al.* (2009) who reported that serum glucose was significantly ($P<0.05$) higher in summer (51.69±4.40 mg %) than spring (38.62±4.81 mg %). Avendano-Reyes *et al.* (2010) also reported a significantly higher concentration of serum glucose (48.41 mg/dL) in control compared to the animals that were exposed to cooling systems during hot and dry ambient conditions (44.9 mg/dL). In heat stressed cows, the mean serum glucose values were significantly different from the values of thermoneutral zone cows (Cincovic *et al.* 2011). Significant increase in serum glucose concentrations (mg/dL) were reported in heat stressed rams compared to winter season (95.07 ± 2.16 vs. 71.29 ± 2.80) (Al-Haidary *et al.* 2012). Chowlu *et al.* (2020) reported significantly higher values in the heat stressed

group compared to the control group of mithuns during autumn season. Calamari *et al.* (2011) found that on the supplementation of selenium to lactating dairy cows during heat stress showed significant negative correlation between THI and plasma concentration. Sejian (2013) observed significantly ($P<0.01$) different concentration of glucose when the Malpura ewes were exposed to different ambient temperatures for 21 days. He reported that the highest glucose requirement was due to the energy requirement in the form of glucose to support physiological mechanism for thermoregulation.

In contrary to the above results, Patel *et al.* (1991) who found that blood glucose was not affected by exposure by direct sunlight in sheep. Habeeb *et al.* (1996) also reported reduction in plasma glucose concentration during summer compared to winter season. Reports were also made by Itoh *et al.* (1998) that during heat stress in cows there was a decrease in gluconeogenesis and glycogenolysis. Similarly, Rasooli *et al.* (2004) also reported significantly lower concentrations of serum glucose during summer than winter in Holstein heifer and further, the serum glucose levels showed a significant negative correlation with mean environmental temperature. Al-Saeed *et al.* (2009) also reported that plasma glucose concentration (mg/dL) reduced significantly during summer season (55.09 ±10.0) compared to winter season (70.68 ± 15.0). Significant decrease in plasma glucose was reported during summer stress compared to corresponding pre-summer values (Kumar *et al.* 2015).

4.2.2. Serum Glumatic Oxaloacetic Transaminase (SGOT)

The observations of SGOT (U/L) in mithun in different treatment groups under various seasons is presented in the Table 4.2.2 and graphically represented in Fig. 4.2.2. The statistical analysis of average values of glucose is given in the Appendix 1.

The average values of SGOT of mithun during autumn season were 40.04± 1.59 and 43.42± 2.28 (U/L) in T_1 and T_2 group, respectively. The

average values of SGOT were significantly (P<0.05) higher in T_2 group compared to T_1 group of mithuns.

Table 4.2.2: SGOT (U/L) in mithun under different treatment during various seasons.

SGOT	REPLICATION						AVERAGE
	R1	R2	R3	R4	R5	R6	
AUTUMN							
T_1	38.94	39.12	40.23	41.60	42.19	38.19	40.04^{b} ±1.59
T_2	39.35	42.49	44.57	43.61	45.72	44.80	43.42^{a} ±2.28
WINTER							
T_1	37.60	39.75	40.57	40.58	40.78	39.41	39.78^{b} ±1.20
T_2	41.85	43.48	42.95	44.16	45.05	43.42	43.48^{a} ±1.08
SPRING							
T_1	44.12	41.09	44.00	45.01	42.47	43.69	43.40^{b} ±1.40
T_2	47.03	44.99	51.97	51.74	48.66	47.65	48.67^{a} ±2.74
SUMMER							
T_1	42.04	43.84	45.41	45.05	44.42	48.67	44.91^{b} ±2.19
T_2	46.54	50.58	52.13	50.88	52.63	54.33	51.18^{a} ±2.64

^{a,b} Means bearing different superscripts in a column within a season differs significantly (P<0.05).

The average values of SGOT during winter season were 39.78± 1.20 and 43.48 ± 1.08 (U/L) respectively in T_1 and T_2 groups. The average values of T_2 group were significantly (P<0.05) higher compared to T_1 group.

The average values of SGOT during spring season were recorded as 43.40 ± 1.40 and 48.67 ± 2.74 (U/L) respectively in T_1 and T_2 groups. The average values were significantly (P<0.05) higher in T_2 group compared to the T_1 group.

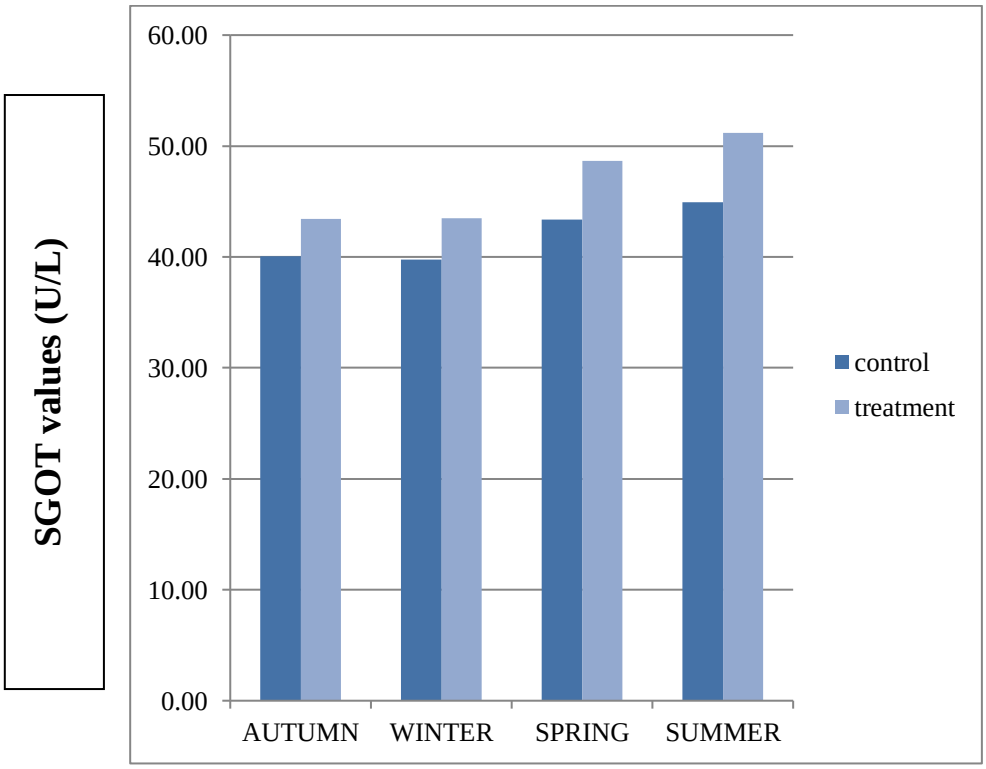

Fig. 4.2.2: SGOT values (U/L) of mithun under different treatment during various seasons.

The average values of SGOT during summer season were recorded as 44.91± 2.19 and 51.18 ± 2.64 (U/L) in control and treatment group respectively. The T_2 group recorded significantly ($P<0.05$) higher values compared to T_1 group.

The present results were in collaboration with Pandey *et al.* (2013) who found that serum AST activity increased significantly from morning (62.22 IU/ ml) to evening (62.95 IU/ ml) in Sahiwal cows during hot dry. Singh *et al.* (2012) reported that the plasma concentration of SGOT and SGPT increased significantly ($P < 0.05$), whereas the concentration of plasma alkaline phosphatase and acid phosphatase decreased due to thermal exposure. Chandra Bhan *et al.* (2012) recorded a significantly higher AST activity during summer compared to the spring season in young and adult Sahiwal cattle. Significant increase was reported in the plasma AST activity (U/l) during summer (70.9 ± 17.07) against winter (61.9 ± 16.6) in local cattle. It was concluded that the increase in the plasma AST activity in cattle suggests the cellular damage in the liver (Al-Saeed *et al.* 2009). Chowlu *et al.* (2020) reported that the heat stressed group had significantly ($P<0.05$) higher level of SGOT compared to the control group of mithuns. Thompson (1973); Habeeb (1987); Marrai *et al.* (1995) observed that SGOT increased in the heat stressed animals due to increase in the stimulation of gluconeogenesis by corticoids.

In contrary of the above results, Marai *et al.* (1992) reported that SGOT levels were insignificantly affected by summer, winter and autumn seasons in Ossimi X Suffolk. Similar reported were made by Srikandakumar *et al.* (2003) who found significant decrease in the plasma AST during heat stress and the decrease was within the normal range. Non significant increase in SGOT was observed during spring season (38.68 ± 4.83 Units/ml) compared to during summer (38.44 ± 4.95 Units/ml) in crossbred calves (Bagha *et al.* 2009).

4.2.3. SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

The observations of SGPT (U/L) in mithun in different treatment groups under various seasons is presented in the Table 4.2.3 and graphically represented in Fig. 4.2.3. The statistical analysis of average values of glucose is given in the Appendix 1.

Table 4.2.3: SGPT (U/L) in mithun under different treatment during various seasons.

TREATMENT	REPLICATION						AVERAGE
	1st	2nd	3rd	4th	5th	6th	
AUTUMN							
T_1	38.13	37.39	37.33	36.66	36.61	39.08	$37.54^{b}\pm0.94$
T_2	43.57	42.94	43.43	41.68	42.68	45.59	$43.31^{a}\pm1.30$
WINTER							
T_1	42.04	41.20	42.39	40.87	40.98	40.40	$41.31^{b}\pm0.76$
T_2	44.70	44.89	45.59	44.27	44.31	43.24	$44.50^{a}\pm0.78$
SPRING							
T_1	43.87	43.49	45.21	43.25	44.33	44.93	$44.18^{b}\pm0.78$
T_2	46.41	48.25	48.37	46.22	47.54	48.55	$47.56^{a}\pm1.02$
SUMMER							
T_1	41.03	44.97	43.53	42.95	47.16	45.38	$44.17^{b}\pm2.13$
T_2	47.89	51.66	49.06	47.26	52.29	51.33	$49.92^{a}\pm2.12$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

The average values of SGPT during autumn season were recorded as 41.12 ± 2.08 and 41.48 ± 3.09 (U/L), respectively in T_1 and T_2 groups. The

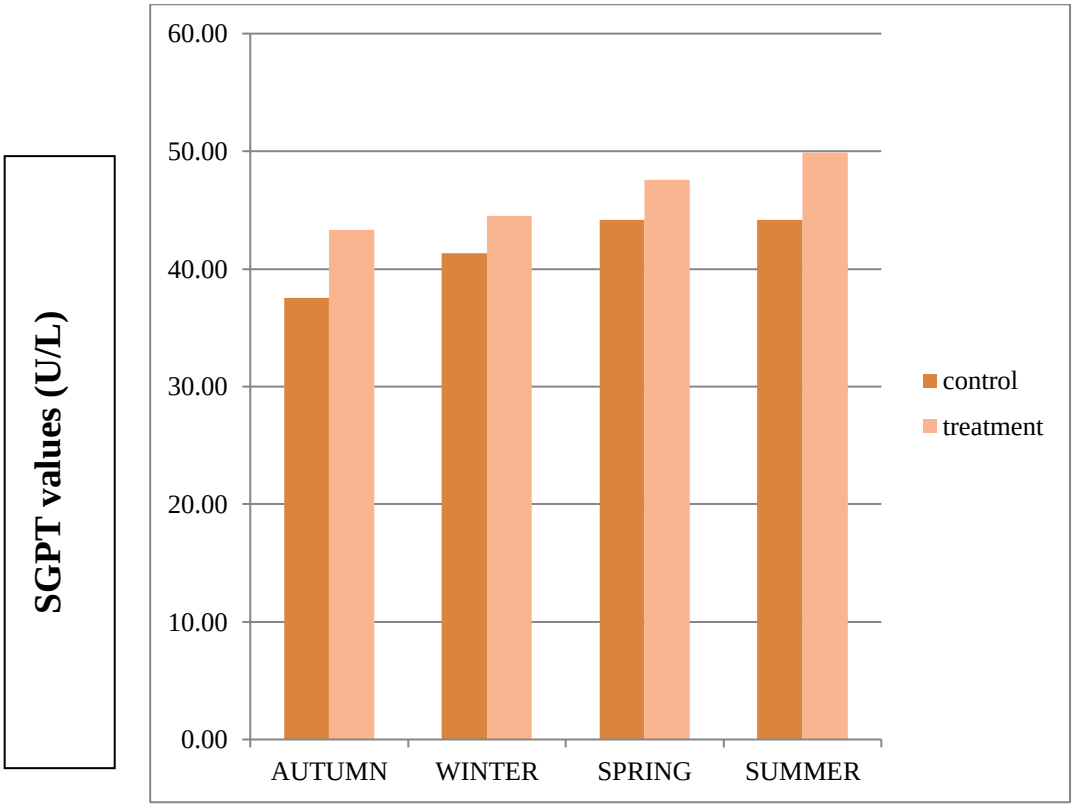

Fig. 4.2.3: SGPT values (U/L) of mithun under different treatment during various seasons.

average values of T_2 group were found to be significantly ($P<0.05$) higher than T_1 group.

The average values of SGPT during winter season were 50.06 ± 3.91 and 50.87 ± 3.91 (U/L) in T_1 and T_2 group, respectively. There was significant ($P<0.05$) difference between T_1 and T_2 group.

The average values of SGPT during spring season were recorded as 46.79 ± 2.33 and 53.06 ± 3.17 (U/L) in T_1 and T_2 group respectively. The T_2 group values were found to be significantly ($P<0.05$) higher compared to T_1 group.

The average values of SGPT were found to be 44.12 ± 2.56 and 49.69 ± 1.97 (U/L) in T_1 and T_2 group, respectively during the summer season. The T_2 group values were found to be significantly ($P<0.05$) different compared to T_1 group.

The above results were in collaboration with Pandey *et al.* (2013) revealed a significant increase in the serum ALT activity from morning (27.33 IU/ ml) to evening (30.60 IU/ ml) indicating an increased enzyme activity in the liver when the temperature was more in the evening hours. Similarly, Singh *et al.* (2012) reported that the plasma concentration of SGOT and SGPT increased significantly ($P < 0.05$) due to thermal exposure. Chandra Bhan *et al.* (2012) recorded a significantly higher activity of ALT activity during summer compared to the spring season in young and adult Sahiwal cattle. Significant increase in ALT activity was recorded during summer compared to the spring season in young and adult Sahiwal cattle (Chandra Bhan *et al.* 2012). Chowlu *et al.* (2020) reported that the mithuns under heat stressed recorded significantly ($P<0.05$) higher level of SGPT levels during autumn season compared to the control group of mithuns.

In contrary to the above reports, Marai *et al.* (1992) reported that SGOT and SGPT levels were insignificantly affected by season of the year (summer,

autumn and winter) in Ossimi x Suffolk, under Egyptian conditions. It was concluded by Bagha *et al.* (2009) that summer stress reduced the growth rate and lowers SGPT activity. They found non-significant (P<0.05) difference in SGPT levels during summer and spring season.

4.2.4. CORTISOL

The observations of cortisol (nmol/L) in mithun in different treatment groups under various seasons is presented in the Table 4.2.4 and graphically represented in Fig. 4.2.4. The statistical analysis of average values of glucose is given in the Appendix 1.

Table 4.2.4: Cortisol values (nmol/L) in mithun under different treatment under various season.

TREATMENT	REPLICATION						AVERAGE
	1st	2nd	3rd	4th	5th	6th	
AUTUMN							
T_1	712.45	712.52	712.42	712.41	712.50	712.38	712.45^{b}±0.05
T_2	712.51	712.53	712.40	712.47	712.51	712.36	712.46^{a}±0.07
WINTER							
T_1	712.53	712.57	712.47	712.51	712.56	712.44	712.51^{b}±0.05
T_2	712.55	712.60	712.56	712.52	712.59	712.54	712.56^{a}±0.03
SPRING							
T_1	712.54	712.62	712.44	712.52	712.62	712.41	712.53 ±0.09
T_2	712.54	712.60	712.44	712.51	712.59	712.41	712.52 ±0.08
SUMMER							
T_1	712.51	712.55	712.43	712.48	712.53	712.39	712.48^{b}±0.06
T_2	712.53	712.54	712.45	712.51	712.51	712.41	712.49^{a}±0.05

[a, b] Means with different superscript differ significantly (P<0.05) during the season among the treatment groups.

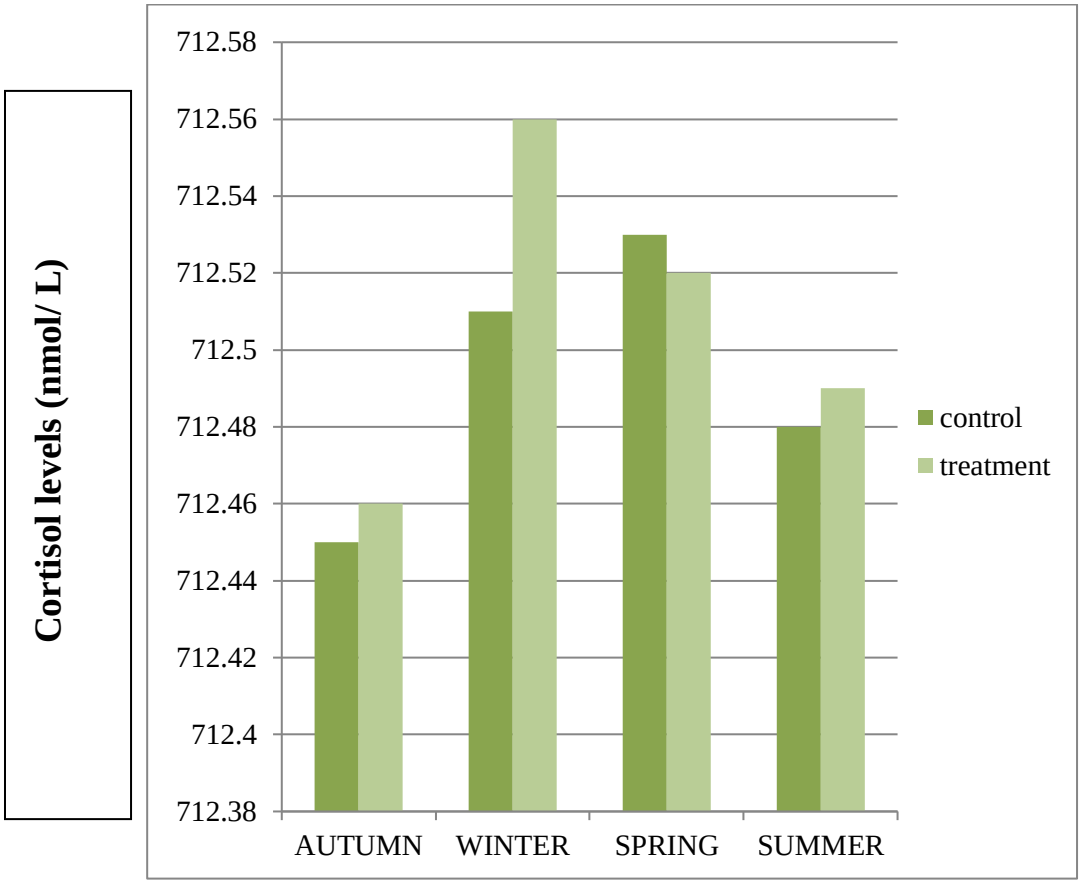

Fig. 4.2.4: Cortisol level (nmol/ L) in mithun under different treatment during various seasons.

The average values of cortisol during autumn season were recorded as 712.45± 0.05 and 712.46± 0.07 (nmol/L) in T_1 and T_2 group, respectively. The average values of T_2 group were significantly ($P<0.05$) different compared to T_1 group.

The average values of cortisol level in mithun during winter season were recorded as 712.51± 0.05 and 712.56± 0.03 (nmol/L) in T_1 and T_2 group, respectively. The average values of T_2 group were significantly ($P<0.05$) different compared to the T_1 group.

The average values of cortisol level in mithun during spring season were recorded as 712.53± 0.09 and 712.52± 0.08 (nmol/L) in T_1 and T_2 group, respectively. The cortisol values of the groups during the season did not vary significantly ($P>0.05$) between the T_1 and T_2 treatment groups.

The average cortisol values of during summer season were recorded as 712.48± 0.06 and 712.49± 0.05 (nmol/L) in T_1 and T_2 group, respectively. The average values of T_2 group were significantly ($P<0.05$) different compared to T_1 group.

The average cortisol values were found to be significantly different among T_1 and T_2 group of mithun during autumn, winter and summer season.

The above results were in collaboration with Chaurasia *et al.* (2011) also found that plasma cortisol (ng/ml) level (4.08± 0.14; 2.89± 0.10 and 3.21± 0.12; 2.70±0.13) were significantly higher ($P<0.05$) at an altitude of 300m MSL and 2100m MSL in different seasons. They reported that the concentrations of plasma cortisol levels were higher in the season of March-June (S_1) compared to the season of November-February (S_3). Christison et al. (1972) also reported that acutely exposed to a moderate heat stress (35°C) cows accustomed to the experimental procedure increased plasma cortisol significantly ($P<0.05$) in the first 20 minutes of exposure from 30 to 37 µg/litter. The earlier reports indicated that acute environmental heat exposure

can cause a transient increase in circulating glucocorticoids that may subsequently deceases even though body temperature remained elevated during chronic heat exposure (Alvarez and Johnson, 1973). Seijian *et al.* (2013) plasma cortisol concentration was found to be highest in the group-3 (34.73 nmol/L) and lowest in group-2 (nmol/L) when Malpura ewes were exposed to 23°C, 40°C and 42°C temperatures for group-1, group-2 and group-3 respectively. Sharma *et al.* (2013) found significant ($P<0.05$) increase in cortisol level in the control group as the exposure temperature increased compared to the melatonin treated group.

In contrary reports were also made by Abdel (1991) who found that the cortisol hormone level was not correlated with either ambient temperature or temperature humidity index (THI) in Hampshire x Suffolk withers. Scott and Wiersma (1971) who found that decrease in plasma cortisol during acclimatization which helps in reducing heat production in animals. Kamal *et al.* (1989) found that when polygastric species are exposed to high temperature, there was a decrease in cortisol levels.

4.3. HEAT SHOCK PROTEIN GENES

4.3.1. HSP70 gene

The expression of HSP70 in mithun under different treatment groups among various seasons is presented in the Table 4.3.1 and graphically represented in Fig. 4.3.1. The statistical analysis of average values of glucose is given in the Appendix 1.

The HSP70 expression of mithun was recorded as 28.24±0.15 and 28.88±0.59 in T_1 and T_2 group during autumn season. In the autumn season, the expression of HSP70 was found to be significantly ($P<0.05$) higher in T_2 than T_1 group. It was observed that with the increase in the ambient temperature, the animal exposed to heat stress expressed higher HSP70 expression.

Table 4.3.1: Expression of HSP70 in mithun under different treatment during various seasons.

Seasons	Groups	HSP70 expression	THI
Autumn	Control	$28.24^{b} \pm 0.15$	$69.59^{b} \pm 7.12$
	Treatment	$28.88^{a} \pm 0.59$	$78.71^{a} \pm 4.01$
Winter	Control	$25.87^{b} \pm 0.55$	$55.86^{b} \pm 2.25$
	Treatment	$26.26^{a} \pm 1.10$	$70.41^{a} \pm 0.88$
Spring	Control	$29.62^{b} \pm 1.19$	$68.32^{b} \pm 5.54$
	Treatment	$29.90^{a} \pm 0.48$	$77.89^{a} \pm 2.92$
Summer	Control	$31.00^{b} \pm 0.62$	$77.56^{b} \pm 0.39$
	Treatment	$31.14^{a} \pm 1.17$	$81.42^{a} \pm 3.40$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

The HSP70 expression during winter season was recorded as 25.87± 0.55 and 26.26 ±1.10 in T_1 and T_2 group, respectively. The HSP70 expression of T_2 group differed significantly ($P<0.05$) compared to T_1 group during winter stress.

The HSP70 expression during the spring season was recorded as 68.32± 5.54 and 77.89 ±2.92 in T_1 and T_2 group respectively. The T_2 group recorded a significant ($P<0.05$) difference due to the heat stress caused during the spring season.

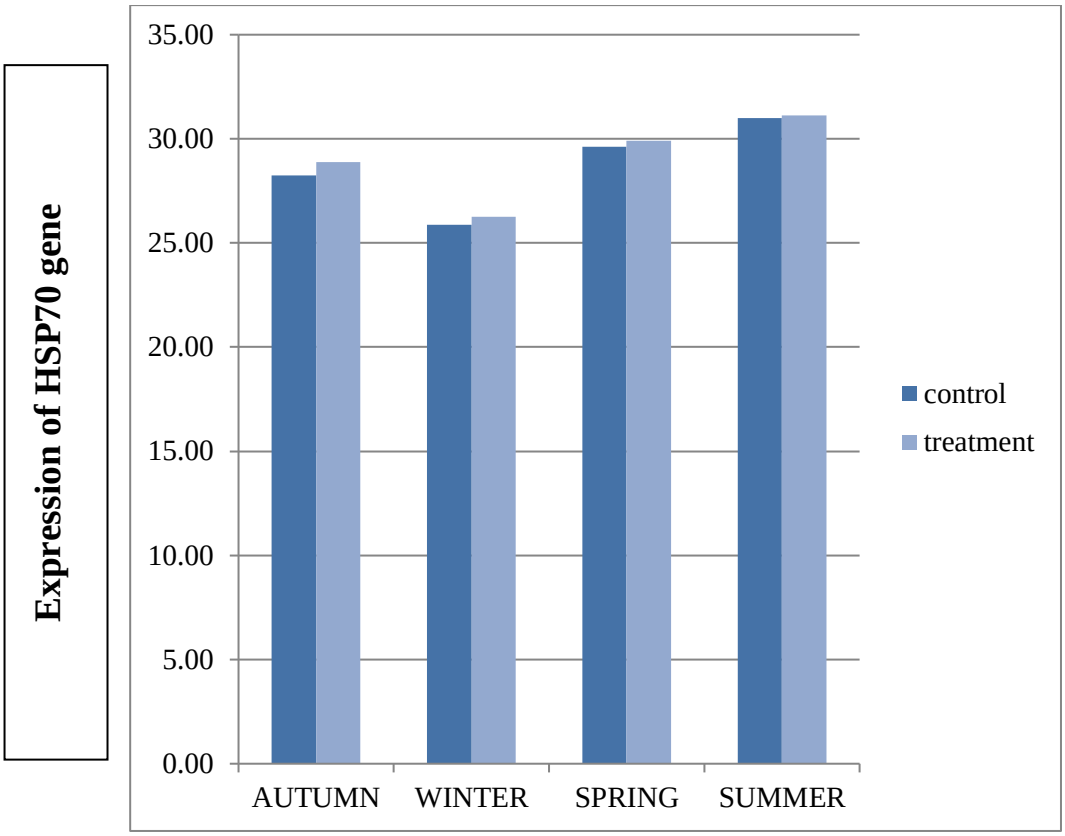

Fig. 4.3.1: mRNA expression of HSP70 gene of mithun under different treatment during various seasons.

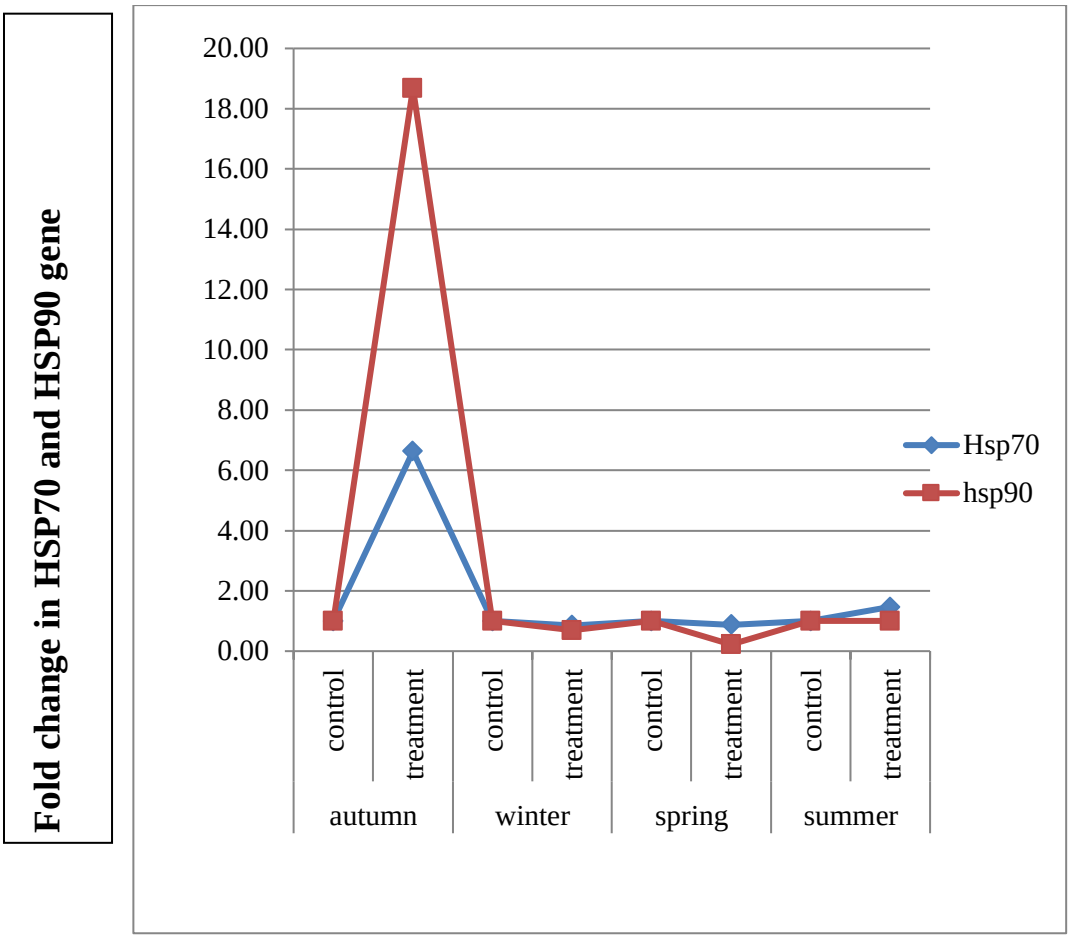

Fig. 4.3.2: Fold change in HSP70 and HSP90 gene expression of mithun under different treatment during various seasons.

The HSP70 expression during summer season was recorded as 77.56 ±0.39 and 81.42 ±3.40 in T_1 and T_2 group respectively. There was a significant ($P<0.05$) difference between T_1 and T_2 group. The T_2 group showed increase in expression profile during the heat stressed during summer season in mithun.

The above results were in line with De Maio 1999 and Lacetera *et al.* (2006) who found that the HSP70 concentration increased due to stressful condition in cattle. Similar results were also reported by Hahn *et al.* (1992) who observed that HSP mRNA expression in skin of Tharparkar and Karan Fries cattle was found to be higher during summer than winter season. They stated that the increase in HSP concentration might be due to the prevailing higher THI (>80) conditions during the summer season, which is suppose to cause heat stress to the animals. It was reported by Beckham *et al.* (2004) that HSP70 expression is temperature sensitive and the increase in the expression of HSP70 during summer and winter season might be induced by heat and hypothermic stress. They stated that the increase in HSP70 expression is a unique feature of the physiological function of these molecules with the changing environment. Parmar *et al.* (2015) reported that the relative expression of HSP70 in Sahiwal during summer season was found significantly higher ($P<0.05$) than the corresponding values during winter season.

In contrary to these results, Rajoriya *et al.* (2014) reported no significant differences in the mRNA expression of HSP70 during winter and summer seasons. It could be concluded that the expression of HSP70 increased due to the increase in the THI during the seasons. It can be stated that due to exposure to heat stress, thermoregulatory mechanisms were triggered to upregulation of HSP70 in mithun. The higher expression of HSPs during thermal stress is an indication to maintain cellular integrity and homeostasis.

4.3.2. HSP90 gene

The expression of HSP90 in mithun under different treatment groups among various seasons is presented in the Table 4.3.2 and graphically represented in Fig. 4.3.2. The statistical analysis of average values of glucose is given in the Appendix 1.

Table 4.3.2: Expression of HSP90 in mithun under different treatment during various season.

Seasons	Groups	HSP90 expression	THI
Autumn	Control	$36.13^{b} \pm 1.69$	$69.59^{b} \pm 7.12$
	Treatment	$35.87^{a} \pm 1.90$	$78.71^{a} \pm 4.01$
Winter	Control	$27.40^{b} \pm 1.26$	$55.86^{b} \pm 2.25$
	Treatment	$27.72^{a} \pm 1.10$	$70.41^{a} \pm 0.88$
Spring	Control	$34.68^{b} \pm 2.04$	$68.32^{b} \pm 5.54$
	Treatment	$35.88^{a} \pm 1.36$	$77.89^{a} \pm 2.92$
Summer	Control	$37.08^{b} \pm 1.24$	$77.56^{b} \pm 0.39$
	Treatment	$37.20^{a} \pm 0.64$	$81.42^{a} \pm 3.40$

^{a,b} Means bearing different superscripts in a column within a season differs significantly ($P<0.05$).

The expression of HSP90 during the autumn season was 36.13 ± 1.69 and 35.87 ± 1.90 in T_1 and T_2 group, respectively. The expression of HSP90 were found to be significantly ($P<0.05$) different among the groups. With the increase in THI values, the expression profile of HSP90 increased in the groups.

The expression of HSP90 during the winter season was 27.40 ± 1.26 and 27.72 ± 1.10 in T_1 and T_2 group, respectively. The expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

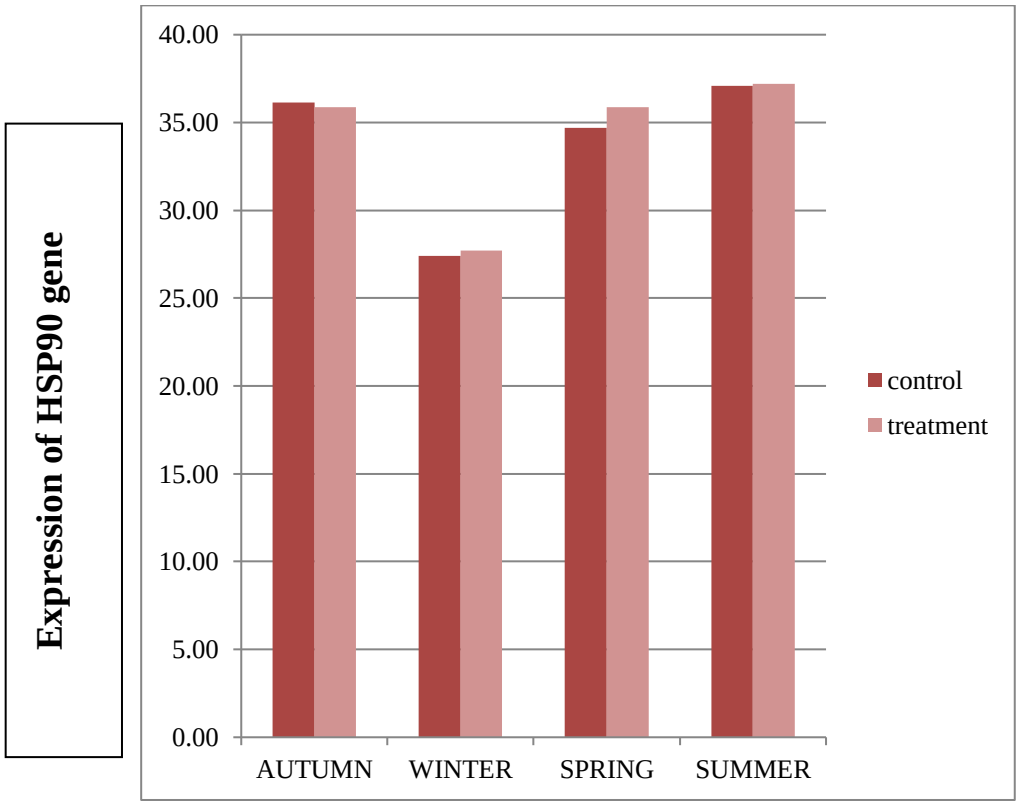

Fig. 4.3.3: Expression of HSP90 gene of mithun under different treatment during various seasons.

The expression of HSP90 during the spring season was 34.68±2.04 and 35.88±1.36 in T_1 and T_2 group respectively. The expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

The expression of HSP90 during the summer season was 37.08±1.24 and 37.20±0.64 in T_1 and T_2 group respectively. The expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

The present result coincides with the results reported by Kumar *et al.* (2015) revealed significantly higher ($P < 0.001$) expression pattern of heat shock protein genes (HSPA1A, HSPA1B, HSP10, HSP60 and HSP90) in winter and summer seasons, whereas summer season recorded higher magnitude of expression as compared to winter. Similarly, Deb *et al.* (2014) also revealed with increase in temperature from 37°C to 45°C, there was increase in the mRNA expression of HSP90 during summer stress. They found that the mRNA expression was significantly ($P<0.01$) higher among Sahiwal (0.953 ± 3.41 at 37°C, 1.83 ± 2.93 at 39°C, 2.86 ± 2.39 at 41°C and 3.67 ± 2.99 at 45°C) than Frieswal (0.874 ± 3.85 at 37°C, 1.52 ± 2.21 at 39°C, 1.98 ± 3.61 at 41°C and 2.98 ± 2.52 at 45°C) samples. Sharma *et al.* (2013) also reported that at heat stress the relative expression of Ubiquitin, HSP60 , HSP70 and HSP90 increased significantly ($P<0.05$) with the increase in the exposure temperature (25°C, 35°C and 40°C) in the control and treatment group in goats.

In contrary to the above results, Rajoriya *et al.* (2014) stated that there was no significant difference in the mRNA expression in HSP70 and 90 during summer and winter season.

It can be concluded that the expression of HSP90 varied significantly among the treatment group at different season. The increase in expression of HSP90 would be due to the increase in the ambient temperature and Increase in THI values during the seasons which triggered the upregulation of HSP90 to maintain homeostasis.

4.4. TEMPERATURE HUMIDITY INDEX (THI)

The average THI values recorded under different treatment groups under various seasons are presented in the Table 4.4 and graphically plotted in Fig. 4.4.1.

Table 4.4.1: THI values during various seasons under different treatment.

GROUPS	THI VALUES			AVERAGE
AUTUMN				
T_1	76.312	70.336	62.128	69.59^b ±7.12
T_2	82.864	78.400	74.872	78.71^a ±4.01
WINTER				
T_1	57.664	53.344	56.584	55.86^b ±2.25
T_2	70.048	69.760	71.416	70.41^a ±0.89
SPRING				
T_1	62.488	68.968	73.504	68.32^b ±5.54
T_2	74.872	78.112	80.704	77.89^a ±2.93
SUMMER				
T_1	77.176	77.536	77.968	77.56 ±0.39
T_2	82.864	77.536	83.872	81.42 ±3.41

a, b Means bearing different superscripts in a column within the season differs significantly ($P<0.05$).

The THI values obtained during autumn season were 69.59 ±7.12 and 78.71 ±4.01 respectively, for T_1 and T_2 groups. The THI values was found to be non-significant ($P>0.05$) between T_1 and T_2 groups.

The THI values recorded during winter season were 55.86 ±2.25 and 70.41 ±0.89 respectively, for T_1 and T_2 groups. The THI values were significantly ($P<0.05$) higher in T_2 compared to T_1 group during the season.

The THI values recorded during spring season were 68.32 ±5.54 and 77.89 ±2.93 respectively, for T_1 and T_2 groups. The THI values did not vary significantly ($P>0.05$) between the group T_1 and T_2 during the season.

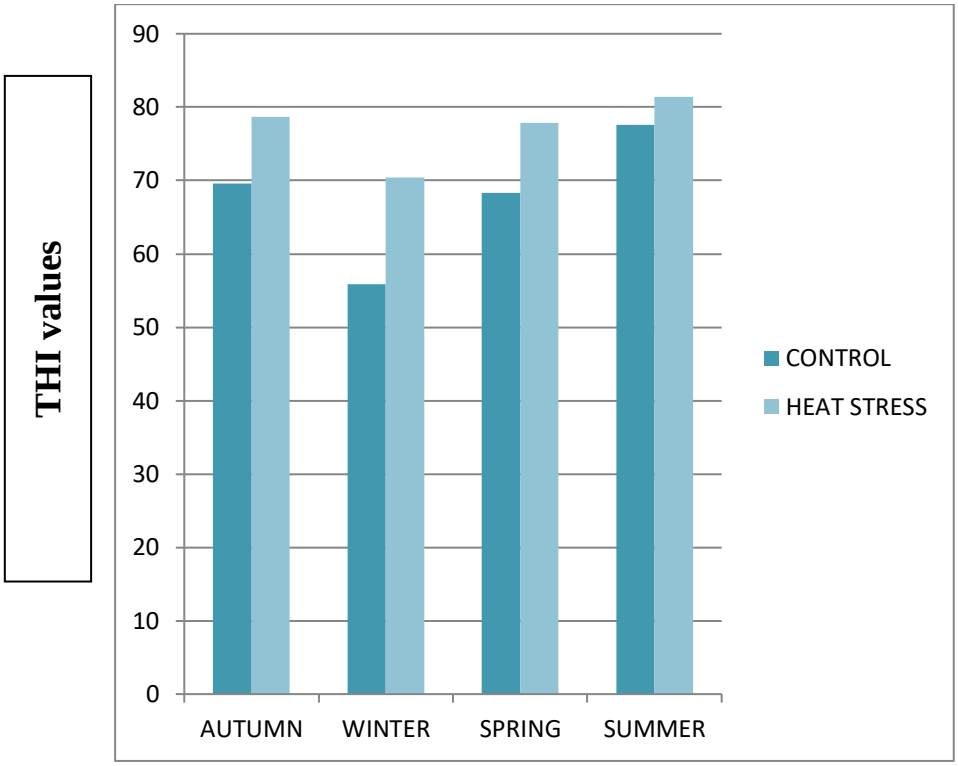

Fig. 4.4.1: THI values under different treatment during various seasons.

The THI value recorded during summer season were 77.56 ±0.39 and 81.42 ±3.41 respectively, for T_1 and T_2 groups. The THI values did not vary significantly ($P>0.05$) between T_1 and T_2 group during the season.

The THI value were found to be non-significant ($P>0.05$) during autumn, spring and summer season except for winter season where, the THI values varied significantly ($P<0.05$) among the group T_1 and T_2. There was no significant ($P>0.05$) difference among the seasons between the groups.

During the study period, the THI value increased in the T_1 and T_2 group in the seasons. It was observed that with the increase in THI values, rectal temperature, respiration rate, pulse rate, glucose level, SGOT, SGPT, cortisol and the HSP gene expression increased significantly in all the seasons. The effect of heat stress (THI) can be observed in the physiological, haematological parameters and gene expression profile.

As per many researchers THI has been categorized in different categories. Armstrong (1994) used THI <71 as a thermal comfort zone (assuming that THI does not drop below the thermoneutral conditions of dairy cows, which induces cold stress), 72 to 79 as mild heat stress, 80 to 90 as moderate heat stress, and >90 as severe heat stress. Comparatively, De Rensis *et al.* (2015) defined THI <68 to be outside the thermal danger zone for cows. Mild signs of heat stress are observed at THI of 68 to 74, and a THI ≥75 will cause drastic decreases in production performance (De Rensis *et al.* 2015). The THI value is usually the main determinant for management decisions related to heat stress as most meteorological stations close to farms provide this data. Thom (1959) categorized THI as normal when THI=<74, alert when THI is 75-78, danger status when THI is 79-83 and THI=> 84 as emergency level. McDowell *et al.* (1976) categorized THI as comfortable when THI =>70, stressful when THI is 75-78 and if THI greater than 78 as extreme distress condition above which the animal cannot maintain thermoregulatory

mechanisms. Perez (2000) categorized THI as, normal if THI =<70 that is no heat stress; alert when THI= 70-80, where the animals need to take extra precautions; danger when THI=79-83 where the animal needs additional precautions for protection like use of sprinklers or fans; emergency when THI=>84, where there is need to minimise handling of animals to avoid any kind of stress on animals.

Yousef *et al.* (1985) stated that temperature-humidity index (THI) is a single value representing the combined effect of air temperature and humidity associated with the level of thermal stress. Collier *et al.* (2011) found that the correlation between THI values and increase in rectal temperature, respiration rate and decrease in milk yield in heat stressed cattle. They found the as the THI values increased, the rectal temperature of cows increased ($P<0.0001$, $r^2=0.2691$); $y=0.0587x + 34.888$, x=THI, y=rectal temperature, respiration rate of cows increased ($P<0.0001$, $r^2=0.5658$); $y=0.028x + 37.438$, x=respiration rate, y=rectal temperature. El-Tarabany *et al.* (2017) found that there was a significant decrease in lactose and solids-not-fat percentages in dairy Baladi goats at high THI in comparison with low and moderate. They also found a negative correlation of THI values with serum glucose ($r= 0.370$, $p= 0.013$). Du Preez *et al.* (1990) reported that dairy cows in the Southern African countries were affected by heat stress when THI values were higher than 72. Gantner *et al.* (2011) found that heat stress conditions indicated with mean daily values of THI>72 were determined during spring and summer season and absence of heat stress conditions during autumn and winter season. Bouraoui et al. (2002) reported the negative correlation between daily THI to milk yield ($r= -0.24$) and feed intake ($r= -0.24$) when the THI value increased from 68 to 78 during spring (68± 3.75) and summer (78± 3.23) period. Habeeb *et al.* (2018) concluded that elevated temperature and humidity as presented in THI negatively affects feed intake and altered hormone concentration leading to negatively affecting the productive and reproductive performance of farm animals.

4.5 EFFECT OF SEASON AND TREATMENT

4.5.1 Effect of season, treatment and interaction effect of season and treatment on the physiological parameters

The effect of season, treatment and their interaction effect on the physiological parameters are presented in Table 4.5.1, Table 4.5.2 and Table 4.5.3, respectively.

Table 4.5.1: Effect of season on the physiological parameters

Season	Rectal temperature	Pulse rate	Respiration rate	THI
Autumn (S_1)	602.050	259.500 [b]	172.000	222.456
Winter (S_2)	601.245	312.495 [a]	177.165	189.408 [b]
Spring (S_3)	602.510	305.665	189.500	219.324
Summer (S_4)	610.165	306.835	178.005	238.479 [a]
CD (P=0.05)	15.379	9.206	39.174	29.469

[a, b] Means bearing different superscript within the column differ significantly (P<0.05)

From the Table 4.5.1, the average rectal temperatures of mithun during autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 602.050, 601.245, 602.510 and 610.165 beat per minute, respectively. The average pulse rate of mithun during autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 259.5, 312.495, 305.665 and 306.835 beat per minute, respectively. The average respiration rate of mithun recorded for autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 172, 177.165, 189.5, 178.005 breaths per minute, respectively.

Statistical analysis revealed a non-significant (P<0.05) difference of the effect of season on the rectal temperature and respiration rate of mithun, whereas the pulse rate was statistically (P<0.05) significant due to the effect of season in mithun. The rectal temperature was recorded to be highest in summer season compared to spring, autumn and winter season. The pulse rate was

observed to be significantly (P<0.05) different among the seasons. Pulse rate recorded highest in the winter season followed by summer, spring and autumn season. Significant (P<0.05) difference between winter and autumn season was found. Respiration rate was recorded to be highest during spring season compared to summer, winter and autumn season.

Similar findings were reported by Chaurasia *et al.* (2010) found that altitude and season had a significant influence on the rectal temperature, respiration rate and pulse rate in mithun. Sarkar *et al.* (2010) reported that seasonal effect (P<0.01) on rectal temperature was observed in yaks with the higher mean in warm season and the rectal temperature and respiration rates were (P<0.01) negatively correlated (-0.42) and (-0.36) with higher plasma corticoid concentration. Gudev *et al.* (2007) also reported increase in respiration rate at THI of 77.83, showing that the animals are heat stressed and unable to maintain their thermoneutral zone. Indu *et al.* (2014) found that there was an increase in respiration rate during afternoon compared to morning in Malpura ewes. Phulia *et al.* (2010) reported increased respiration rate during afternoon (77.33) compared to morning (43.66) in goats. Chowlu *et al.* (2020) also reported that the values of rectal temperature, pulse rate and respiration rate were significantly (P<0.05) higher in heat stressed group compared to non heat stressed group of mithun during autumn season.

Table 4.5.2: Effect of treatment on the physiological parameters

Treatment	**Rectal Temperature**	**Pulse Rate**	**Respiration Rate**	**THI**
T_1	595.880 [b]	242.670 [b]	157.505 [b]	203.502 [b]
T_2	612.105 [a]	349.575 [a]	200.835 [a]	231.330 [a]
CD (P=0.05)	6.174	6.517	11.363	20.834

[a, b] Means bearing different superscript within the column varied significantly (P<0.05)

From the Table 4.5.2 it can be observed that the statistical analysis revealed that the rectal temperature under different treatment groups varied

significantly (P<0.05). The statistical analysis revealed that rectal temperature, pulse rate and respiration rate was significantly (P<0.05) higher in the heat stress group (T_2) compared to the control group (T_1). It can be observed that pulse rate and respiration rate and rectal temperature increased in the heat stressed (T_2) group. It might be due to the effect of increase in ambient temperature and relative humidity which led to higher values of Temperature

Table 4.5.3: Interaction effect of season x treatment on the physiological parameters

S X T	Rectal Temperature	Pulse Rate	Respiration Rate	THI
S_1T_1	99.43^{e}	34.33^{d}	24.83 ^{d}	69.592 ^{c}
S_1T_2	101.25^{c}	52.17^{b}	32.50 ^{b}	78.712 ^{a}
S_2T_1	98.88^{f}	43.44^{c}	26.33 ^{cd}	55.864 ^{d}
S_2T_2	101.54^{bc}	60.72^{a}	32.72 ^{b}	70.408 ^{c}
S_3T_1	98.90^{f}	41.56^{c}	27.17 ^{c}	68.320 ^{c}
S_3T_2	101.94^{b}	60.33^{a}	36.00 ^{a}	77.890 ^{a}
S_4T_1	100.04^{d}	42.44^{c}	26.67 ^{c}	77.560 ^{ab}
S_4T_2	103.34^{a}	59.83^{a}	33.00 ^{b}	81.424 ^{a}
CD	0.490	2.044	1.555	7.253

$^{a, b, c, d, e, f}$ Means bearing different superscript within the column differ significantly (P>0.05)

From Table 4.5.3, it can be observed that the highest and lowest rectal temperature due to interaction of season and treatment was in S_4T_2 (103.34) and S_2T_1 (98.88), respectively. The highest and lowest pulse rate due to interaction of season and treatment was in S_2T_2 (60.72) and S_1T_1 (34.33), respectively. The highest and lowest respiration rate observed due to interaction effect of season and treatment was in S_3T_2 (36.00) and S_1T_1 (24.93), respectively.

Statistical analysis revealed that interaction effect of season and treatment had significant effect on the physiological parameters of mithun within the seasons. The increase in rectal temperature might be due to the higher THI values and the decrease in rectal temperature might be due to the lower THI values. The increase in pulse rate and respiration rate might be due to addition stress condition occurred during sample collection along with the high THI values.

It can be concluded that the significant difference in the interaction effect of season and treatment on the physiological parameters was due to increase in ambient temperature and relative humidity resulting to increase in THI values causing heat stress conditions during the various seasons in mithun. Gudev *et al.* (2007) reported significant increase in the rectal temperature ($P<0.05$) and respiration rate ($P<0.01$) at 3 p.m. when exposure directly to solar radiation, indicating that the animals could not maintain rectal temperature within thermoneutral zone in spite of the increased respiration rate and air velocity at the time. He also stated that the same level of THI did not induce significant changes in the rectal temperature of buffaloes when kept in the barn, whereas, the enhanced levels of respiration rate indicated heat stress due to direct exposure to solar radiations (THI- 77.83).

4.5.2 Effect of season, treatment and interaction effect of season and treatment on the haematological parameters

The effect of season, treatment and their interaction effect on the haematological/blood biochemical parameters are presented in Table 4.5.4, Table 4.5.5 and Table 4.5.6, respectively.

Table 4.5.4: Effect of season on Haematological parameters

Season	Glucose	SGOT	SGPT	Cortisol	THI
Autumn (S_1)	242.440	250.405^{b}	242.545 ^{c}	4274.73^{b}	222.456
Winter (S_2)	345.580	249.800 ^{b}	257.440 ^{bc}	4275.22^{a}	189.408 ^{b}
Spring (S_3)	442.030	276.210 ^{a}	275.210 ^{ab}	4275.12^{a}	219.324
Summer (S_4)	332.730	288.260 ^{a}	282.255 ^{a}	4274.92^{ab}	238.479 ^{a}
CD	115.865	18.368	19.346	0.319	29.469

$^{a,\ b,\ c}$ Means bearing different superscript within the column differ significantly($P<0.05$)

From the Table 4.5.4, it can be observed that the highest glucose levels were recorded in spring (442.030) followed by winter (345.580), summer (332.730) and autumn (242.440) season, respectively. Statistical analysis revealed non-significant ($P>0.05$) difference in the level of glucose between the seasons.

The level of SGOT was recorded highest in summer (288.266) followed by spring (276.210), autumn (250.405) and winter (249.800), respectively. Statistical analysis revealed significant ($P<0.05$) difference in the level of SGOT during various seasons. Significant difference was observed between summer and winter season. The increase in the level of SGOT might be due to the increase in THI values resulting in thermal stress in mithun.

Statistical analysis revealed significant ($P<0.05$) difference in the level of SGPT during autumn and summer seasons. The highest level of SGPT was recorded in summer (282.255) followed by spring (275.210), winter (257.440) and autumn (242.545), respectively. The increase in THI values might

contribute for thermal stress resulting to upregulation of SGPT values during the season.

Statistical analysis revealed significant ($P<0.05$) difference in the level of Cortisol during autumn and winter seasons. The highest level of cortisol was recorded in winter (4275.220) followed by spring (4275.120), summer (4274.920) and autumn (4274.730), respectively. Low THI value during winter season might have contributed for the stress resulting in increased cortisol level.

Rasooli *et al.* (2004) observed that there was a significant decrease in the serum glucose and cholesterol concentrations of heifers in summer compared to winter season. They also observed that the serum AST and CK activities in the heifers were extremely high during hot season compared to cold season. Eldon *et al.* (1988) also found higher serum glucose concentrations of cows during winter season.

In contrast reports were made by Shaffer *et al.* (1981) who stated that there was no effect of season on the serum CK activity in cattle. They also stated that the increased activity of some enzymes with rising temperature might be due to the accelerated reactions of the enzymes at higher temperatures. Soveri *et al.* (1992) reported that there was no significant effect of season on serum AST activity in camel. Bagha *et al.* (2009) stated that serum glucose was significantly ($P<0.05$) higher in summer (51.69 ± 4.40 mg %) than spring season (38.62 ± 4.81 mg %). Significant increase in serum glucose concentrations (mg/dL) were reported in heat stressed rams compared to winter season (95.07 ± 2.16 vs. 71.29 ± 2.80) (Al-Haidary *et al.* 2012).

It can be concluded that the variation in the levels of haematological parameters due to the effect of season might be due to the effect of high ambient temperature and relative humidity resulting to increased THI values leading the animals to maintain homeostasis.

Table 4.5.5: Effect of treatment on haematological parameters

Treatment	Glucose	SGOT	SGPT	Cortisol	THI
T_1	306.375	252.195[b]	250.795[b]	4274.950	203.502 [b]
T_2	375.015	280.145[a]	277.93[a]	4275.045	231.330 [a]
CD	115.888	12.983	13.688	0.279	20.834

[a, b] Means bearing different superscript within the column differ significantly ($P<0.05$)

From the Table 4.5.5, it can be observed that the levels of glucose is higher in T_2 (375.015) than T_1 (306.375) group. Similarly, SGOT levels were higher in T_2 (280.145) than T_1 (252.195), respectively. The SGPT levels were also higher in T_2 (277.93) than T_1 (250.795), respectively. The cortisol levels were found to be higher in T_2 (4275.045) than T_1 (4274.95), respectively. The statistical analysis revealed that there was no significance ($P>0.05$) difference in the levels of glucose and cortisol levels among the T_1 and T_2 groups. Whereas, a significant ($P<0.05$) difference in the levels of SGOT and SGPT was observed in between the T_1 and T_2 groups.

Naqvi *et al.* (1991) reported higher levels of AST and CK activity might be due to the influence of thermal stress in Avikalin sheep compared to Malpura sheep. Chaurasia *et al.* (2010) found significant ($P<0.05$) increase in cortisol values at altitude A_1 and A_2 during S_1 compared to S_3 season at altitude A_1 and A_2. They stated that high cortisol level at low altitudes during hot season might be due to the higher values of THI and low cortisol values at high altitudes might be due to lower THI values.

It can be concluded that the increase in enzyme activity with increase in temperature might be due to accelerated reaction at higher temperatures. It reveals the effect of heat stress on the haematological parameters due to treatment.

Table 4.5.6: Effect of season x treatment on Haematological parameters

S X T	Glucose	SGOT	SGPT	Cortisol	THI
S_1T_1	39.46^{f}	40.04^{d}	37.53^{e}	712.45^{e}	69.592^{c}
S_1T_2	41.35^{ef}	43.42^{c}	43.31^{c}	712.46^{de}	78.712^{a}
S_2T_1	43.32^{e}	39.78^{d}	41.31^{d}	712.51^{bc}	55.864^{d}
S_2T_2	71.80^{b}	43.48^{c}	44.50^{c}	712.56^{a}	70.408^{c}
S_3T_1	70.65^{b}	43.40^{c}	44.18^{c}	712.53^{b}	68.320^{c}
S_3T_2	76.70^{a}	48.67^{b}	47.56^{b}	712.52^{bc}	77.890^{a}
S_4T_1	50.82^{d}	44.91^{c}	44.17^{c}	712.48^{d}	77.560^{ab}
S_4T_2	60.09^{c}	51.18^{a}	49.92^{a}	712.49^{cd}	81.424^{a}
CD	3.384	1.833	1.418	0.030	7.253

$^{a, b, c, d, e}$ Means bearing different superscript within the column differ significantly (P>0.05)

From the Table 4.5.6, it can be observed that the highest and lowest level of glucose was recorded in S_3T_2 (76.70) and S_1T_1 (39.46), respectively. While the highest and lowest values of SGOT was recorded in S_4T_2 (51.18) and S_2T_1 (39.78), respectively. The highest and lowest values of SGPT was recorded in S_4T_2 (49.92) and S_1T_1 (37.53), respectively. The highest and lowest values recorded for cortisol was in S_2T_2 (712.56) and S_1T_1 (712.45), respectively. Statistical analysis revealed that there was significant effect of heat stress in mithun during different seasons. The lowest values were observed in the T_1 group and the highest values from the T_2 group in all the haematological parameters. The increase in the activity of the enzymes might be due to the rise in the temperature accelerating the reaction in high ambient temperature and relative humidity resulting to high THI values. It can be concluded that there was a significant (P<0.05) difference in the haematological parameters under different treatment groups due to stress during various seasons.

4.5.3 Effect of season, treatment and interaction effect of season and treatment on the HSP gene expression

The effect of season, treatment and their interaction effect on the expression of HSP genes are presented in Table 4.5.7, Table 4.5.8 and Table 4.5.9, respectively.

Table 4.5.7: Effect of season on HSP gene expression

Season	HSP70	HSP90	THI
Autumn (S_1)	171.335 ^{c}	21.005 ^{ab}	222.456
Winter (S_2)	156.380 ^{d}	165.360 ^{c}	189.408 ^{b}
Spring (S_3)	178.550 ^{b}	211.573 ^{b}	219.324
Summer (S_4)	186.395 ^{a}	222.865 ^{a}	238.479 ^{a}
CD	2.878	8.363	29.469

$^{a, b, c}$ Means bearing different superscript within the column differ significantly ($P<0.05$)

From the Table 4.5.7, it can be observed that the expression of HSP70 gene was highest in summer (186.395) and lowest in winter (156.380) season. Statistically, it was found that there was significant ($P<0.05$) difference in the expression of HSP70 gene between winter, spring and summer seasons.

The expression of HSP90 was highest in summer (222.865) and lowest in winter (165.360) season. From the above table, it can be interpreted that the expression of HSP90 gene was significantly ($P<0.05$) different between summer, spring and winter season.

Mishra *et al.* (2011) found high concentration of HSP70 in serum lymphocytes of Murrah buffaloes during dry heat exposure compared to controlled conditions. Manjari *et al.* (2015) reported a significant increase in the expression of HSP70 gene in buffalo during summer season (2.37± 0.12) as compared to winter season (0.29± 0.04). Banerjee *et al.* (2014) observed higher

expression of HSP70 genes during summer season compared to winter season in heat and cold adapted goat breeds. Dangi *et al.* (2012) also observed significantly (P<0.05) higher HSP70 mRNA expression during summer season, which might be the reason for thermal stress tolerance against the stressful environmental conditions in tropical goats.

It can be concluded that the increase in the expression of HSP70 and HSP90 gene during summer season might be due to the rise in THI value in summer causing heat stress in mithun. The possible reason for increased expression of HSPs could be a natural protective and homeostatic response of the cells to the deleterious effects of the hypothermic stress.

Table 5.3.2: Effect of treatment on HSP gene expression

Treatment	**HSP70**	**HSP90**	**THI**
T_1	172.075 [b]	202.945	203.502 [b]
T_2	174.265 [a]	205.000	231.330 [a]
CD	2.033	38.679	20.834

[a, b] Means bearing different superscript within the column differ significantly (P<0.05)

From the Table 4.5.8, it can be noted that T_2 (174.265) recorded higher HSP70 expression value than T_1 (172.075) group. Similarly, HSP90 expression was recorded to be high in T_2 (205.000) than T_1 (202.945) group.

Statistical analysis revealed significant (P<0.05) difference between the heat stressed (T_2) group and control (T_1) group in the expression of HSP70 genes, whereas, no significant variation was observed between the treatment groups in HSP90 gene expression.

Singh *et al.* (2014) found the expression of HSP70 genes was moderate to high during heat stress (40 and 44°C) whereas, the expression was low during cold stress (25°C). Bhanuprakash *et al.* (2016) observed a significant

increase in the expression of HSP70 level during thermal stress compared to thermo-neutral condition.

The increased expression of HSPs might be due to natural protective and homeostatic response of the cells to the deleterious effects of the hypothermic stress. It can be concluded that heat stress had significant effect on the increased expression of HSP70 genes in the heat stressed (T_2) group.

Table 4.5.9: Effect of season x treatment on HSP gene expression

S X T	HSP 70	HSP90	THI
S_1T_1	28.24^{d}	36.13^{ab}	69.592^{c}
S_1T_2	28.88^{cd}	35.87^{ab}	78.712^{a}
S_2T_1	25.87^{e}	27.40^{c}	55.864^{d}
S_2T_2	26.26^{e}	27.72^{c}	70.408^{c}
S_3T_1	29.62^{bc}	34.68^{b}	68.320^{c}
S_3T_2	29.90^{b}	35.88^{ab}	77.890^{a}
S_4T_1	31.00^{a}	37.08^{a}	77.560^{ab}
S_4T_2	31.14^{a}	37.20^{a}	81.424^{a}
CD	2.07	1.733	7.253

$^{a, b, c, d}$ Means bearing different superscript within the column differ significantly ($P>0.05$)

From the Table 4.5.9, it can be observed that the highest and lowest HSP70 expression was in S_4T_2 (31.14) and S_2T_1 (25.87), respectively. Similarly the highest and lowest HSP90 expression was recorded in S_4T_2 (37.20) and S_2T_1 (27.40), respectively. Statistical analysis had revealed significant ($P<0.05$) difference due to various season and treatment interaction. Significant difference was found between autumn and spring seasons in the expression of HSP70 and 90 genes.

Similar, reports were made by King *et al.* (2002) that an induction of HSP70 was observed at 41°C when the mice were exposed for 30 min and they concluded that HSP70 played a significant role for the survival of mice during

heat stress. Patir and Upadhyay (2010) observed increase in HSp70 concentration after exposure for 2 hour for 45°C in Murrah buffaloes. Haque *et al.* (2012) observed significant ($P<0.05$) increase in the concentration of HSP70 at 40, 42 and 45°C in young as well as adult buffaloes. Rajoriya *et al.* (2014) observed non-significant difference in the mRNA expression of HSP70 and 90 genes between winter and summer season in the Tharparkar bull semen. They stated that there might be presence of similar type of stress resistant spermatozoa in the Tharparkar bull semen. Kumar *et al.* (2015) found that HSP90 mRNA expression was significantly higher ($P<0.05$) in Tharparkar, Sahiwal and Murrah buffalo during summer season compared to winter season. The increase in HSP90 expression could be due to the fact that the ambient heat stress stimulated and rapidly initiated the transcription of HSP90 mRNA and translated HSP90 protein to protect cells from heat stress.

The increase in the expression of HSP70 and 90 genes might be due to the increase in the THI values during the season and treatment and the decrease in the HSP gene expression might be due to decrease in the THI value in the season and treatment. The increase in expression of HSPs might be due to the fact that it helps the animal to cope up with the heat stress to maintain thermotolerance.

It can be concluded that heat stress had significant effect on the expression of HSP70 and 90 genes in mithun kept under various seasons causing rise in the expression of the HSPs.

SUMMARY AND CONCLUSION

The livestock industry in India is threatened severely by effects of climate change as the environmental temperature differs from 10 to 44°C throughout the year. Combination of high environmental temperature with other extreme weather conditions like high relative humidity with intense solar radiations exerts great influence on the productivity of an animal. Heat stress is a condition in which the body temperature is increased (hyperthermia) which cause failure in the physiological systems to maintain body temperature within the thermoneutral zone. Heat stress causes stress in livestock leading to negative consequences on animal health, quality of product and also on productivity of the animals. Heat shock proteins (HSP) are a group of proteins that are synthesized in the cells in order to protect the cell from adverse effects due to exposure to stressful conditions. HSPs help to thermal adaption and deal with environmental stress tolerance. HSP 70 and 90 are believed to be the most temperature sensitive and are easily induced by physiological and environmental stressors. Mithun are reared under free-range grazing conditions without any proper housing facilities under cool hilly ecosystem. These animals are exposed to different kind of environmental conditions during a complete year. The present experiment was conducted to investigate the "**Impact of Stress on Account of Seasonal Variations on the Expression Profile of Heat Shock Protein Genes in Mithun (*Bos Frontalis*)"** was carried out at Nagaland University, SASRD, Medziphema in collaboration with National Research Centre on Mithun at Jharnapani, with the objective to study the physiological and haematological responses to heat stress in different seasons in mithun.

In the present study, six healthy number of mithun were kept under four different season viz. autumn (August- October), winter (November- January),

spring (February- April) and summer (May- July) of a year. The experimental animals were grouped as control group (T_1) or non heat stress group in which the observations and blood samples were recorded and collected in the morning hours at 0600 hours before the animals were exposed to sunlight. After the observations were recorded the same animals were exposed to sunlight to induce heat stress. The observations were again recorded and collected in the afternoon at 1400 hours for the treatment group (T_2) or the heat stress group. The data on physiological, biochemical parameters and expression of Heat shock protein gene 70 and 90 in mithun (*Bos frontalis*) were recorded and analyzed using Paired T-Test design for analyzing the difference between treatment and control group and split plot design for determining the difference among seasons and interaction between season, treatment and control group.

AUTUMN

PHYSIOLOGICAL PARAMETERS

RECTAL TEMPERATURE

In the autumn season, the average rectal temperature recorded was 99.43± 0.53 vs. 101.25± 0.58 (°F/min) in T_1 and T_2 group respectively. Statistical analysis revealed that there were significant ($P<0.05$) difference among the T_2 group and T_1 group. It can be interpreted that the rectal temperature increased due to heat stress in mithun.

PULSE RATE

The average pulse rate recorded during autumn season was 34.33± 0.68 vs. 52.17± 0.68 in T_1 and T_2 groups, respectively. Statistical analysis revealed

there were significant ($P<0.05$) difference between the T_2 and T_1 group. It can be interpreted that the pulse rate increased due to heat stress in mithun.

RESPIRATION RATE

The average values of respiration rate recorded during autumn season are 24.83± 0.93 vs. 32.50± 1.34 in T_1 and T_2 group respectively. Statistical analysis revealed significant ($P<0.05$) difference between T_2 compared to T_1 group. It can be interpreted that respiration rate increased due to heat stress in mithun.

HAEMOTOLOGICAL PARAMETERS

GLUCOSE

The average values of glucose of mithun during autumn season in T_1 and T_2 group were 39.46 ± 2.11 and 41.35 ± 1.07, respectively. Statistical analysis revealed that the T_2 group were significantly ($P<0.05$) higher compared to T_1 group. It can be interpreted that glucose level increased due to heat stress in mithun.

SERUM GLUMATIC OXALOACETIC TRANSAMINASE (SGOT)

The average values of SGOT of mithun during autumn season were 40.04± 1.59 and 43.42± 2.28 in T_1 and T_2 group, respectively. Statistical analysis revealed that the SGOT values differed significantly ($P<0.05$) in T_2 group compared to T_1 group of mithuns. It can be interpreted that SGOT levels increased due to heat stress.

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

The average values of SGPT during autumn season were recorded as 41.12 ± 2.08 and 41.48 ± 3.09, respectively in T_1 and T_2 groups. Statistical analysis revealed significant ($P<0.05$) difference between T_2 and T_1 group. It can be interpreted that SGPT levels increased due to heat stress.

CORTISOL

The average values of cortisol during autumn season were recorded as 712.45± 0.05 and 712.46± 0.07 in T_1 and T_2 group, respectively. Statistical analysis revealed significant ($P<0.05$) difference between T_2 and T_1 group. It could be interpreted that the increase in cortisol level could be due to heat stress.

HEAT SHOCK PROTEIN GENES EXPRESSION

HSP70

The HSP70 expression of mithun was recorded as 28.24 ±0.15 and 28.88 ±0.59 in T_1 and T_2 group during autumn season. Statistical analysis revealed that the expression of HSP70 was found to be significantly ($P<0.05$) higher in T_2 than T_1 group. It was observed that with the increase in the ambient temperature, the animal exposed to heat stress expressed higher HSP70 expression.

HSP90

The expression of HSP90 during the autumn season was 36.13 ± 1.69 and 35.87 ± 1.90 in T_1 and T_2 group, respectively. Statistical analysis revealed that the expression of HSP90 were found to be significantly ($P<0.05$) different among the groups. With the increase in THI values, the expression profile of HSP90 increased in the groups.

TEMPERATURE HUMIDITY INDEX (THI)

The THI values obtained during autumn season were 69.59 ±7.12 and 78.71 ±4.01 respectively, for T_1 and T_2 groups. The THI values were found to be non-significant (P>0.05) between T_1 and T_2 groups.

WINTER

PHYSIOLOGICAL PARAMETERS

RECTAL TEMPERATURE

In winter season, the average rectal temperature recorded was 98.88± 0.35 vs. 101.54± 0.16 (°F/min) in T_1 and T_2 group respectively. Statistical analysis revealed that the values recorded were significantly (P<0.05) higher in T_2 group than T_1 group.

PULSE RATE

In winter season, the average pulse rate recorded was 43.44 ± 2.18 vs. 60.72 ± 1.93 in T_1 and T_2 group, respectively. Statistical analysis revealed significantly (P<0.05) higher pulse rate in T_2 group as of T_1 group.

RESPIRATION RATE

In the winter season, the average respiration rate recorded for T_1 and T_2 group are 26.33 ± 0.70 and 32.72 ± 1.42, respectively. Statistical analysis revealed that the values recorded for T_2 group were significantly (P<0.05) higher in T_2 compared to the T_1 group during the season.

HAEMOTOLOGICAL PARAMETERS

GLUCOSE

The average values of glucose during winter seasons in mithun were recorded as 43.32 ± 1.73 and 71.87 ± 1.57 in T_1 and T_2 group, respectively. Statistical analysis revealed that the T_2 group had significantly ($P<0.05$) higher values compared to T_1 group. The mithuns were affected by winter heat resulting in increase in glucose level in the heat stressed group (T_2) of mithun compared to the control (T_1) group of mithuns.

SERUM GLUMATIC OXALOACETIC TRANSAMINASE (SGOT)

The average values of SGOT during winter season were 39.78± 1.20 and 43.48 ± 1.08 respectively in T_1 and T_2 groups. Statistical analysis revealed that the average values of T_2 group were significantly ($P<0.05$) higher compared to T_1 group.

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

The average values of SGPT during winter season were 50.06 ± 3.91 and 50.87 ± 3.91 in T_1 and T_2 group. Statistical analysis revealed significant ($P<0.05$) difference among the T_1 and T_2 group.

CORTISOL

The average values of cortisol level in mithun during winter season were recorded as 712.51± 0.05 and 712.56± 0.03 in T_1 and T_2 group, respectively. Statistical analysis revealed that the average values of T_2 group were significantly ($P<0.05$) different compared to the T_1 group.

HEAT SHOCK PROTEIN GENES

HSP70

The HSP70 expression during winter season was recorded as 25.87± 0.55 and 26.26 ±1.10 in T_1 and T_2 group, respectively. Statistical analysis revealed that the HSP70 expression of T_2 group differed significantly ($P<0.05$) compared to T_1 group during winter stress.

HSP90

The expression of HSP90 during the winter season was 27.40 ± 1.26 and 27.72 ± 1.10 in T_1 and T_2 group, respectively. Statistical analysis revealed that the expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

TEMPERATURE HUMIDITY INDEX (THI)

The THI values recorded during winter season were 55.86 ±2.25 and 70.41 ±0.89 respectively, for T_1 and T_2 groups, respectively. Significantly ($P<0.05$) higher THI values were recorded in T_2 group during the season.

SPRING

PHYSIOLOGICAL PARAMETERS

RECTAL TEMPERATURE

In spring season, the average rectal temperature recorded was 98.90± 0.50 vs. 101.94± 0.32 (°F/min) in T_1 and T_2 group respectively. Statistical analysis revealed that the values recorded were significantly ($P<0.05$) higher in T_2 group than T_1 group. Increase in rectal temperature could be due to heat stress.

PULSE RATE

In spring season, the average pulse rate recorded was 41.56 ± 1.34 vs. 60.33 ± 2.17 in T_1 and T_2 group. Significantly ($P<0.05$) high values in pulse rate was recorded in statistical analysis in T_2 group as compared to T_1 group. Increase in pulse rate could be due to heat stress.

RESPIRATION RATE

The average values of respiration rate recorded for spring season were 27.17±39 and 36.00± 1.33 for T_1 and T_2 group, respectively. Statistical analysis revealed that the T_2 group varied significantly ($P<0.05$) compared to T_1 group during the season. Increase in respiration rate could be due to heat stress.

HAEMOTOLOGICAL PARAMETERS

GLUCOSE

The average values of glucose during spring season were recorded as 70.65 ± 2.35 and 76.70± 4.80 in T_1 and T_2 group. Statistical analysis recorded significantly ($P<0.05$) high level of glucose values in T_2 group compared to T_1 group of mithun. Heat stress can be the reason for the increase in the glucose level.

SERUM GLUMATIC OXALOACETIC TRANSAMINASE (SGOT)

The average values of SGOT during spring season were recorded as 43.40 ± 1.40 and 48.67 ± 2.74 respectively in T_1 and T_2 groups. Statistical analysis showed significantly ($P<0.05$) higher levels in the average values of SGOT in T_2 group compared to T_1 group.

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

The average values of SGPT during spring season were recorded as 46.79 ± 2.33 and 53.06 ± 3.17 in T_1 and T_2 group respectively. Statistical analysis revealed that the T_2 group were found to be significantly ($P<0.05$) higher compared to T_1 group.

CORTISOL

The average values of cortisol level in mithun during spring season were recorded as 712.53± 0.09 and 712.52± 0.08 in T_1 and T_2 group, respectively. Statistical analysis revealed that the cortisol values did not vary significantly ($P>0.05$) between the T_1 and T_2 groups.

HEAT SHOCK PROTEIN GENES

HSP70

The HSP70 expression during the spring season was recorded as 68.32± 5.54and 77.89 ±2.92 in T_1 and T_2 group respectively. Statistical analysis revealed that the T_2 group recorded a significant ($P<0.05$) difference due to the heat stress caused during the spring season.

HSP90

The expression of HSP90 during the spring season was 34.68±2.04 and 35.88±1.36 in T_1 and T_2 group respectively. Statistical analysis revealed that the expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

TEMPERATURE HUMIDITY INDEX (THI)

The THI values recorded during spring season were 68.32 ±5.54 and 77.89 ±2.93 respectively, for T_1 and T_2 groups. The THI values did not vary significantly (P>0.05) between the group T_1 and T_2 during the season.

SUMMER

PHYSIOLOGICAL PARAMETERS

RECTAL TEMPERATURE

In summer season, the average rectal temperature recorded was 100.04± 0.64 vs. 103.34± 0.49 (°F/min) in T_1 and T_2 group respectively. Statistical analysis revealed that the values recorded were significantly (P<0.05) higher in T_2 group than T_1 group. The increase in the rectal temperature could be due to heat stress.

PULSE RATE

In summer season, the average pulse rate recorded was 42.44± 2.51 vs. 59.83± 2.05 in T_1 and T_2 group. Statistical analysis showed significantly (P<0.05) higher pulse rate recorded in T_2 group compared to T_1 group.

RESPIRATION RATE

The average respiration rate of mithun during summer was recorded as 26.67± 1.26 and 33.00 ± 1.81 in T_1 and T_2 group, respectively. Statistical analysis revealed that the values recorded for T_2 group were significantly (P<0.05) higher than T_1 group in summer season.

HAEMOTOLOGICAL PARAMETERS

GLUCOSE

The average values of glucose of mithun during summer season were found to be 50.82 ± 3.10 and 60.09 ± 3.58 in T_1 and T_2 group respectively. Statistical analysis revealed that the glucose values in T_2 group were significantly ($P<0.05$) higher compared to T_1 group of mithun. There was significant effect of summer heat stress on glucose level in mithun.

SERUM GLUMATIC OXALOACETIC TRANSAMINASE (SGOT)

The average values of SGOT during summer season were recorded as 44.91± 2.19 and 51.18 ± 2.64 in control and treatment group respectively. Statistical analysis revealed that the T_2 group recorded significantly ($P<0.05$) higher values compared to T_1 group.

SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

The average values of SGPT were found to be 44.12 ± 2.56 and 49.69 ± 1.97 in T_1 and T_2 group, respectively during the summer season. Statistical analysis revealed that the T_2 group values were found to be significantly ($P<0.05$) different compared to T_1 group.

CORTISOL

The average cortisol values of during summer season were recorded as 712.48± 0.06 and 712.49± 0.05 in T_1 and T_2 group, respectively. Statistical analysis revealed that the average values of T_2 group were significantly ($P<0.05$) different compared to T_1 group.

HEAT SHOCK PROTEIN GENES

HSP70

The HSP70 expression during summer season was recorded as 77.56 ±0.39 and 81.42 ±3.40 in T_1 and T_2 group respectively. Statistical analysis revealed that there was a significant difference ($P<0.05$) between T_1 and T_2 group. The T_2 group showed an increase in expression profile of HSP70 during the heat stressed during summer season in mithun.

HSP90

The expression of HSP90 during the summer season was 37.08±1.24 and 37.20±0.64 in T_1 and T_2 group respectively. Statistical analysis revealed that the expression of HSP90 were found to be significantly ($P<0.05$) different among the groups.

TEMPERATURE HUMIDITY INDEX (THI)

The THI value recorded during summer season were 77.56 ±0.39 and 81.42 ±3.41 respectively, for T_1 and T_2 groups. Statistical analysis revealed that the THI values did not vary significantly ($P>0.05$) between T_1 and T_2 group during the season.

INTERACTION

Effect of season on Physiological parameters

The average rectal temperatures of mithun during autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 602.050, 601.245, 602.510 and 610.165 beat per minute, respectively. The average pulse rate of mithun during autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 259.5, 312.495, 305.665 and 306.835 beat per minute, respectively. The average respiration rate of mithun recorded for autumn (S_1), winter (S_2), spring (S_3) and summer (S_4) season was 172, 177.165, 189.5 and 178.005 breathes per minute, respectively.

Statistical analysis revealed a significant ($P<0.05$) difference of the effect of season on all the physiological parameters. Significantly ($P<0.05$) high rectal temperatures were recorded during summer season as compared to spring, autumn and winter season. The pulse rate was significantly ($P<0.05$) different in winter and autumn seasons. Pulse rate recorded highest in the winter season followed by summer, spring and autumn season. Respiration rate was recorded to be highest during spring season compared to summer, winter and autumn season. Respiration rate was significantly ($P<0.05$) higher during spring season compared to summer, winter and autumn season.

Effect of treatment on the physiological parameters

The statistical analysis revealed that the rectal temperature, pulse rate and respiration rate under different treatment groups varied significantly ($P<0.05$). It can be observed that pulse rate, respiration rate and rectal temperature increased in the heat stressed (T_2) group. Rectal temperature, pulse rate and respiration rate was found to be significantly ($P<0.05$) high in the heat stressed group (T_2) compared to the control group (T_1) due to high THI values recorded in (T_2) causing heat stress in the T_2 group for maintaining homeostasis.

Interaction effect of season x treatment on the physiological parameters

The highest and lowest rectal temperature due to interaction of season and treatment was in S_4T_2 (103.34) and S_2T_1 (98.88), respectively. The highest and lowest pulse rate due to interaction of season and treatment was in S_1T_1 (60.72) and S_1T_1 (34.33), respectively. The highest and lowest respiration rate observed due to interaction effect of season and treatment was in S_3T_2 (36.00) and S_1T_1 (24.93), respectively. Statistical analysis revealed that interaction effect of season and treatment had significant effect on the physiological parameters of mithun within the seasons. The increase in rectal temperature

might be due to the higher THI values and the decrease in rectal temperature might be due to the lower THI values. The increase in pulse rate and respiration rate might be due to addition stress condition occurred during sample collection along with the high THI values.

It can be concluded that the significant difference in the interaction effect of season and treatment on the physiological parameters was due to increase in ambient temperature and relative humidity resulting to increase in THI values causing heat stress conditions during the various seasons in mithun.

Effect of season on the haematological parameters

The highest glucose levels were recorded in spring (442.030) followed by winter (345.580), summer (332.730) and autumn (242.440) season, respectively. Statistical analysis revealed non-significant ($P>0.05$) difference in the level of glucose between autumn and spring seasons.

The level of SGOT was recorded highest in summer (288.266) followed by spring (276.210), autumn (250.405) and winter (249.800), respectively. Statistical analysis revealed significant ($P<0.05$) difference in the level of SGOT during various seasons. Significant difference was observed between summer and winter season. The increase in the level of SGOT might be due to the increase in THI values resulting in thermal stress in mithun.

Statistical analysis revealed significant ($P<0.05$) difference in the level of SGPT during autumn and summer seasons. The highest level of SGPT was recorded in summer (282.255) followed by spring (275.210), winter (257.440) and autumn (242.545), respectively. The increase in THI values might contribute for thermal stress resulting to upregulation of SGPT values during the season.

Statistical analysis revealed significant ($P<0.05$) difference in the level of Cortisol during autumn and winter seasons. The highest level of cortisol was recorded in winter (4275.220) followed by spring (4275.120), summer (4274.920) and autumn (4274.730), respectively. Low THI value during winter season might have contributed for the stress resulting in increased cortisol level.

It can be concluded that the variation in the levels of haematological parameters due to the effect of season could be due to the effect of increased THI values resulting from high ambient temperature and relative humidity to leading the animals to maintain homeostasis.

Effect of treatment on haematological parameters

It was observed that the levels of glucose is higher in T_2 (375.015) than T_1 (306.375) group. Similarly, SGOT levels were higher in T_2 (280.145) than T_1 (252.195), respectively. The SGPT levels were also higher in T_2 (277.93) than T_1 (250.795), respectively. The cortisol levels were found to be higher in T_2 (4275.045) than T_1 (4274.95), respectively. The statistical analysis revealed that there was no significance ($P>0.05$) difference in the levels of glucose and cortisol levels among the T_1 and T_2 groups. Whereas, a significant ($P<0.05$) difference in the levels of SGOT and SGPT was observed in between the T_1 and T_2 groups.

It can be concluded that the increase in enzyme activity with increase in temperature might be due to accelerated reaction at higher temperatures. It reveals the effect of heat stress on the haematological parameters due to treatment.

Effect of season x treatment on Haematological parameters

It was observed that the highest and lowest level of glucose was recorded in S_3T_2 (76.70) and S_1T_1 (39.46), respectively. While the highest and lowest values of SGOT was recorded in S_4T_2 (51.18) and S_2T_1 (39.78), respectively. The highest and lowest values of SGPT was recorded in S_4T_2 (49.92) and S_1T_1 (37.53), respectively. The highest and lowest values recorded for cortisol was in S_2T_2 (712.56) and S_1T_1 (712.45), respectively. Statistical analysis revealed that there was significant effect of heat stress in mithun during different seasons. The lowest values were observed in the T_1 group and the highest values from the T_2 group in all the haematological parameters. The increase in the activity of the enzymes might be due to the rise in the temperature accelerating the reaction in high ambient temperature and relative humidity resulting to high THI values.

It can be concluded that due to the interaction effect of season x treatment, there was a significant ($P<0.05$) difference in the haematological parameters under different treatment groups due to stress during various seasons. The increase in the levels of Glucose, SGOT, SGPT and cortisol could be due to the effect of heat stress in mithun.

Effect of season on HSP gene expression

The expression of HSP70 gene was highest in summer (186.395) and lowest in winter (156.380) season. Statistically, it was found that there was significant ($P<0.05$) difference in the expression of HSP70 gene between winter, spring and summer seasons. The expression of HSP90 was highest in summer (222.865) and lowest in winter (165.360) season. Significant ($P<0.05$) difference was found between summer, spring and winter season.

It can be concluded that the increase in the expression of HSP70 and HSP90 gene during summer season might be due to the rise in THI value in

summer causing heat stress in mithun. The possible reason for increased expression of HSPs could be a response to protect and maintain homeostasis of the cells from the adverse effects of the thermal stress.

Effect of treatment on HSP gene expression

The expression of HSP70 was recorded highest in T_2 (174.265) compared to T_1 (172.075) group, respectively. HSP90 expression was recorded to be high in T_2 (205.000) than T_1 (202.945) group. Statistical analysis revealed significant ($P<0.05$) difference between the heat stressed (T_2) group and control (T_1) group in the expression of HSP70 genes, whereas, no significant variation was observed between the treatment groups in HSP90 gene expression.

Effect of season x treatment on HSP gene expression

It was observed that the highest and lowest HSP70 expression was in S_4T_2 (31.14) and S_2T_1 (25.87), respectively. Similarly the highest and lowest HSP90 expression was recorded in S_4T_2 (37.20) and S_2T_1 (27.40), respectively. Statistical analysis had revealed significant ($P<0.05$) difference due to various season and treatment interaction. Significant difference was found between autumn and spring seasons in the expression of HSP70 and 90 genes.

The interaction effect of season x treatment had significant effect on the expression of HSP70 and HSP90 genes during different seasons under different treatment groups. The increase in expression of HSPs might be due to the fact that it helps the animal to cope up with the heat stress to maintain thermotolerance.

It can be concluded that heat stress had significant effect on the expression of HSP70 and 90 genes in mithun kept under various seasons causing rise in the expression of the HSPs.

CONCLUSIONS

1. During autumn season, rectal temperature, pulse rate and respiration rate was found to be significantly different among the T_2 group and T_1 group. The haematological parameter (glucose, SGOT, SGPT and cortisol) was found to be significantly different among T_2 group and T_1 group. It can be concluded that the physiological and haematological parameters increased due to heat stress in mithun. The expression of HSP70 and 90 genes were found to be significantly ($P<0.05$) higher in the heat stressed group (T_2) compared to the control (T_1) group.
2. During winter season, rectal temperature, pulse rate and respiration rate was found to be significantly different among the T_2 group and T_1 group. The haematological parameter (glucose, SGOT, SGPT and cortisol) was found to be significantly different among T_2 group and T_1 group. The expression of HSP70 and 90 genes were found to be significantly ($P<0.05$) higher in the heat stressed group (T_2) compared to the control (T_1) group. It can be concluded that the mithuns were affected by winter stress.
3. During spring season, the physiological and haematological parameters was found to be significantly ($P<0.05$) higher in the heat stressed group (T_2) compared to the control (T_1) group except for cortisol levels. The heat stress did not affect the cortisol levels in mithun during spring season. The expression of HSP70 and 90 genes were found to be significantly ($P<0.05$) higher in the heat stressed group (T_2) compared to the control (T_1) group.
4. During summer season, it was found that summer heat stress affected the physiological parameters and haematological parameters of heat stressed mithun (T_2). The expression of HSP70 and 90 genes were also affected due to summer heat stress in the heat stressed mithun (T_2).

5. Effect of season on the physiological parameters was found to be significantly ($P<0.05$) different. Rectal temperature was found to be highest in summer season followed by spring, autumn and winter season. Pulse rate recorded highest in the winter season followed by summer, spring and autumn season. Respiration rate was higher during spring season compared to summer, winter and autumn season.
6. Effect of season on the haematological parameters was found to be significantly ($P<0.05$) different. Glucose levels were found to be highest in spring followed by winter, summer and autumn season. SGOT was found to be highest in summer followed by spring, autumn and winter season. SGPT was found to be highest in summer and lowest in autumn season. Cortisol level was found to be highest in winter and lowest in autumn season.
7. Effect of season on the expression of HSP70 and 90 genes was found to be significantly different. The expression of HSP70 and 90 genes was highest in summer and lowest in winter season.
8. THI values recorded during autumn season were 69.59 ±7.12 and 78.71 ±4.01 respectively, for T_1 and T_2 groups. For winter season, the THI values recorded were 55.86 ±2.25 and 70.41 ±0.89 respectively, for T_1 and T_2 groups. The THI values recorded during spring season were 68.32 ±5.54 and 77.89 ±2.93 respectively, for T_1 and T_2 groups. The THI value recorded during summer season were 77.56 ±0.39 and 81.42 ±3.41 respectively, for T_1 and T_2 groups. Summer season recorded the highest THI values and winter season with the lowest THI values for the study period.

From the present study, we can observe the effect of heat stress in mithun during various seasons, that mithun has to go through the whole year. The effect of heat stress on rectal temperature, pulse rate and respiration rate was found to be significantly different during various seasons. Similarly, glucose

levels, SGOT and SGPT were found to be significantly different except cortisol. Heat stress also significantly affected expression of HSP70 and HSP90 genes. Highest THI levels were seen during summer season. It can be concluded that heat stress had a significant effect on the physiological, haematological and expression of HSP genes of mithun compared to the control group.

FUTURE PLANS

1. To study different kinds of stress influencing the expression of HSP genes.
2. To study effect on physiological and haematological parameters due to the influence of different kinds of stress in mithun.

REFERENCES

Abeni, F., Calamari, L. and Stefanini, L. 2007. Metabolic condition of lactating dairy cows during the hot season in the Po Valley. 1. Blood indicators of heat stress. *International Journal of Biometeorology*. **52** (2): 87-96.

Abdel-Hafez, M.A.M. 2002. Studies on the reproductive performance in sheep. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Abdel-Samee, A.M. 1991. Detection of heat adaptability of growing lambs in subtropics. *Zagazig Veterinary Journal*, **19**: 719-731.

Avendano-Reyes, L., Hernandez, R.J.A., Alvarez-Valenzuela, F.D., Macias-Cruz, U., Molina, R.D., Correa-Calderon, A., Robinson, P.H. and Fadel, J.G. 2010. Physiological and productive responses of multiparous lactating Holstein cows exposed to short-term cooling during severe summer conditions in an arid region of Mexico. *International Journal of Biometerology*. **56** (6): 993-999.

Aggarwal, A. and Singh, M. 2008.skin and rectal temperature changes in lactating buffaloes provided with showers and wallowing during hot-dry season. *Tropical Animal Health Production*. **40**: 223-228.

Akyuz, A., Boyaci, S. and Cayli, A. 2010. Determination of critical period for dairy cows using temperature humidity index. *Journal of Animal Veterinary Advances.* **9**: 1824-1827.

Alam, M.M., Hashem, M.A., Rahman, M.M., Hossain, M.M., Haque, M.R., Sobhan, Z. and Islam, M.S. 2011. Effect of heat stress on behaviour, physiological and blood parameters of goat. *Progressive Agriculture*. **22** (1&2): 37-45.

Alameen, A.O. and Abdelatif, A.M. 2012. Influence of season and pregnay on thermoregulatory and haematological profile in crossbred dairy cows in Tropical environment. *Global Veterinaria*. **9** (3): 334-340.

Al-Haidary, A.A., Aljumaah, R.S., Alshaikh, M.A., Abdoun, K.A., Samara, E.M., Okab, A.B. and Alfuraiji, M.M. 2012. Thermoregulatory and physiological responses of Najdi sheep exposed to environmental heat load prevailing in Saudi Arabia. *Pakistan Veterinary Journal*. **32** (4): 515-519.

Al-Saeed, M.H., Haidar, K.A. and Rashad, F.G. 2009. Selective evaluation of certain blood biochemical parameters of local cattle during winter and summer seasons. *Basrah Journal of Veterinary Research*. **8** (1): 138-143.

Alvarez, M.B. and Johnson, J.D. 1973. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. *Journal of Dairy Science*. **56**: 189-194.

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. *Journal of Dairy science*. **77**: 2044-2050.

Banerjee, D., Upadhyay, R.C., Chaudhary, U.B., Kumar, R., Singh, S., Ashutosh, M., Polley, S., Mukherjee, A., Das, T.K. and De, S. 2014. Seasonal variation in expression pattern of genes under HSP70: Seasonal variation in expression pattern of genes under HSP70 family in heat- and cold-adapted goats (*Capra hircus*). *Cell Stress Chaperon*. **19** (3): 401–408.

Bhanuprakash, V., Singh, U., Sengar, G., Sajjanar, B., Bhusan, B., Raja, T.V., Alex, R., Kumar, S., Singh, R., Alyethodi, R.R., Kumar, S. and Deb, R. 2016. Differential effect of thermal stress on HSP70 expression, nitric oxide production and cell proliferation among native and cross bred dairy cattle. *Journal of thermal Biology*. **59**: 18-25.

Bahga, C.S., Sikka, S.S. and Saijpal, S. 2009. Effect of Seasonal Stress on Growth Rate and Serum Enzyme Levels In Young Crossbred Calves. *Indian Journal of Animal Research*. **43** (4): 288-290.

Barbe, M.F., Tytell, M., Gower, D.J. and Welch W.J. 1998. Hyperthermia protects against light damage in the rat retina. *Science*. **241**: 1817-1820.

Baumgartner, W. and Parnthaner, A. 1994. Influence of age, season and pregnancy upon blood parameters in Austrian Karakul sheep. *Small Ruminant Research*. **11**: 147-151.

Beckham, J.T., Mackanos, M.A., Crooke, C., Takahashi, T., O'Connell-Rodwell, C., Contag, C.H. and Jansen, E.D. 2004. Assessment of cellular response to thermal laser injury through bioluminescence imaging of heat shock protein 70. *Photochemistry and Photobiology*. **79**: 76–85.

Behl, R., Behl, J. and Joshi, B.K. 2010. Heat tolerance mechanism in cattle- status in zebu cattle: A review. *Indian Journal of Animal Sciences*. **80** (9): 891-897.

Belhadj, S.I., Najar, T., Ghram, A., and Abdrrabba, M. 2016. Heat stress effects on livestock: molecular, cellular and metabolic aspects- A review. *Journal of Animal Physiology and Animal Nutrition*. **100**: 401-412.

Berhan, T., Puchala, R., Goetsch, A.L. and Merkel, R.C. 2006. Effects of walking speed and forage consumption on energy expenditure and heart rate by Alpine does. *Small Ruminant Research*. **63** (1-2): 119-124.

Berman, A., Folman, Y., Kaim, M., Mamen, M., Herz, Z., Wolfenson, D., Arieli, A. and Graber, Y. 1985. Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. *Journal of Dairy Science*. **68**: 1488-1495.

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. and Belyea, R. 2002. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Animal Research*. **51**: 479-491.

Broucek, J., Novak, P., Vokralova, J., Soch, M., Kiaac, P. and Uhrincat, M. 2009. Effect of high temperature on milk production of cows from free-stall housing with natural ventilation. *Slovak Journal of Animal Science*. **42** (4): 167-173.

Byoung Hwa Roh, M.D., Dae Hyun Kim, M.D., Moon Kyun Cho, M.D.,Young Lip Park, M.D. and Kyu Uang Whang, M.D. 2008. Expression of heat shock protein 70 in human skin cells as a photoprotective function after UV exposure. *Annals of Dermatology* (Seoul). **20** (4): 184-189.

Calamari, L., Petrera, F., Abeni, F. and Bertin, G. 2011. Metabolic and haematological profiles in heat stressed lactating dairy cows fed diets supplemented with different selenium sources and does. *Livestock Sciences*. **142**: 128-137.

Chaurasia, R.K., Mech, A., Dhali, A., Vidyarthi, V.K., Rajkhowa, C. and Sharma, V.B. 2010. Effect of Altitude and Season on Rectal Temperature, Pulse Rate and Respiration Rate in Mithun (*Bos frontalis*). *Indian Journal of Hill Farming.* **23** (2):25-29.

Chaurasia, R.K., Vidyarthi, V.K. and Dhali, A. 2011. Variation and correlation of plasma cortisol and THI in Mithun calves during different seasons and at different altitudes. *International Journal of Research in Agricultural Sciences*. **2** (2): 2384-3997.

Chaurasia, R.K. 2012. Effect of season and climatological condition on growth growth performance and nutrient utilization in growing Mithun (*Bos frontalis*). Ph.D. Thesis, Nagaland University, School of Agricultural Sciences and Rural Development, Medziphema Campus, India.

Chandra, B., Singh, S.V., Hooda, O.K., Upadhyay, R.C., Beenam, and Vaidya, M. 2012. Influence of themperature variability on physiological, haematological and biochemical profile of growing and adult sahiwal cattle. *Journal of Environmental Research and Development*. **7** (2A): 986-994.

Chandra, G., Aggarwal, A., Singh, A.K., Kumar, M. and Upadhyay, R.C. 2013. Effect of vitamin E and zinc supplementation of erythrocyte antioxidant enzymes and plasma total antioxidant activity in Sahiwal cows. *Indian Journal of Dairy Science*. **66** : 412-417.

Chowlu, H., Vidyarthi, V.K. and Mukherjee, S. 2020. Effect of heat stress on blood constituents and physiological parameters of mithun (Bos frontalis) in autumn season. *Livestock Research International*. **8** (3): 94-98.

Christison, G.I. and Johnson, H.D. 1972. Cortisol turnover in heat-stressed cows. *Journal of Animal Sciences*. **35**: 1005-1010.

Cincovic, M.R., Branislava, B.M., Bojan, T.D., Ivan, R.V. and Bojana, V.R. 2010. The influence of THI values at different periods of lactation on milk quality and characteristics of lactation curve. *Journal of Agriculture Science.* **55**: 235-241.

Cincovic, M.R., Belic, B., Toholj, B., Potkonjak, A., Stevancevic, M., Lako, B and Ivan, R. 2011. Metabolic acclimation to heat stress in farm housed Holstein cows with different body condition scores. African Journal of Biotechnology. 10 (50): 10293-10303. http://www.academicjournals.org/AJB

Collier, R.J., Collier, J.L., Rhoads, R.P. and Baumgard, L.H. 2008. Gene involved in the bovine heat stress response. *Journal of Dairy Sciences*. **91**: 445–454.

Collier, R.J., Zimbelman, R.B., Rhoads, R.P., Rhoads, M.L. and Baumgard, L.H. 2011. A re-evaluation of the impact of temperature humidity indix (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Western Dairy Management Conference; March 9-11 Reno, N.V. 113-140.

Correa- Calderon,A., Armstrong, D., Pay, D., DeNise, S. and Enns, M. 2004. Thermoregulatory responses of Holstein and Brown Swiss heat-stressed dairy cows to two different cooling systems. *International Journal of Biometerology*. **48**: 142-148. Doi:10.1007/s00484-003-0194-y.

Cowley, F.C., Barber, D.G., Houlihan, A.V. and Poppi, D.P. 2015. Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science*. **98**: 2356-2368.

Dangi, S.S., Gupta, M., Maurya, D., Yadav, V.P. and Panda, R.P. 2012. Expression profile of HSP genes during different seasons in goats (*Capra hircus*). *Tropical Animal Health Production*. **44**: 1905-1912.

Dangi, S.S., Gupta, M., Nagar, V., Yadav, V.P., Dangi, S.K., Shankar, O., Chouhan, V.S., Kumar, P., Singh, G. and Sarkar, M. 2014. Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats. *International Journal of Biometeorology*. **58** (10): 2085–93.

David, N.K., Anthony, J.M. and Robert, E.F. 1990. Effect of stress on the immune system. *Immunology Today*. **11:** 170-175.

De Maio, A. 1999. Heat shock proteins: facts, thoughts, and dreams. Shock. **11** (1): 1–12.

De Renesis, F and Scaramuzzi, R.J. 2003. Heat stress and seasonal effects on reproduction in the dairy cows- a review. *Theriogenology*. **60**: 1139-1151.

De Rensis, F., Garcia-Ispierto, I. And Lopez-Gatius, F. 2015. Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows. *Theriogenology*. **84**: 659-666.

Deb, R., Sajjanar, B., Singh, U., Kumar, S., Singh, R., Sengar, G. and Sharma, A. 2014. Effect of heat stress on the expression profile of Hsp90 among Sahiwal

(*Bos indicus*) and Frieswal (*Bos indicus* X *Bos taurus*) breed of cattle: A comparative study. *Gene*. **536** (2): 435–440.

Devi, J., Goswami, J., Sarmah, B.C. and Sarma, K. 2014. Effect of zinc supplementation on serum enzymatic activities of Assam local kids. *Indian Journal of Small Ruminants*. **20** (1): 108-110.

Dobson, H. and Smith, R.F. 2000. What is stress and how does it affect reproduction? *Animal Reproduction Science*. **60**: 743-752.

Du Preez, J.H., Hatting, P.J., Giesecke, W.H. and Eisenberg, B.E. 1990. Heat stress in dairy cattle and other livestock under Southern African conditions. I. Temperature-Humidity index mean values during the four main seasons. *Onderstepoort Journal of Veterinary Research*. **57**: 77-86.

Eldon, J., Thorsteinsson, T.H. and Olafsson, T.H. 1988. The concentration of blood glucose, urea, calcium and magnesium in milking dairy cows. *Journal of Veterinary and Medicine,* Seri A, **35**: 44-53.

El-Tarabany, M.S., El-Tarabany, A.A. and Atta, M.A. 2017. Physiological and lactation responses of Egyptian dairy Baladi goats to natural thermal stress under subtropical environmental conditions. *International Journal of Biometeorology*. **61**: 61-68.

Feder, M.E. and Hofmann, G.E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Reverse Physiology*. **61**: 243–282.

Fuquay, J.W. 1981. Heat stress as its affects animal production. *Journal of Animal Sciences.* **52** (1): 164-174.

Ganaie, A.H., Hooda, O.K., Singh, S.V., Ashutosh and Upadhyay, R.C. 2012. Response of vitamin C supplementation on biochemical, hormonal and

physiological parameters of pregnant Murrah buffaloes during hot-humid conditions. *Indian Journal of Animal Nutrition*. **29** (3): 214-221.

Ganaie, A.H., Shanker, G., Bumla, N.A., Ghasura, R.S. and Mir, N.A. 2013. Biochemical and physiological changes during thermal stress in bovines. *Journal of Veterinary Science Technology*. **4**: 126.

Gantner, V., Mijic, P., Kuterovac, K., Soli, D. and Gantner, R. 2011. Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo*. **61** (1):56-63.

Garbuz, D.G., Astakhova, L.N., Zatsepina, O.G., Arkhipova, I.R., Nudler, E. and Michael, B.E. 2011. Functional organization of HSP70 cluster in Camel (*Camelus dromedaries*) and other animals. *Public Library of Science One*. **6**(11): 27205.

Gaughan, J.B., Holt, S.M., Hahn, G.L., Mader, T.L. and Eigenberg, R. 2000. Respiration rate: Is it a good measure of heat stress in cattle? *Asian Australian Journal for Anim*al. **13**: 329-332.

Gaughan, J.B., Mader, T.L., Holt, S.M., Sullivan, M.L. and Hahn, G.L. 2010. Assessing the heat tolerance of 17 beef cattle genotypes. *International Journal of Biometeorology*. **54**: 617-627.

Gaugharn, J.B., Boner, S.L., Loxton, I. and Mader, T.L. 2013. Effect of chronic heat stress on plasma concentration of secreted HSP70 in growing feedlot cattle. *Journal of Animal Sciences.* **91**: 120-129.

Giasuddin, M. and Islam, M. R. 2003. Physical feature, physiological character and behaviour study of Gayal (*Bos frontalis*). *Asian Australian Journal of Animal Sciences.* 16: 1599-1603.

Gudev, D., Popova-Ralcheva, S., Moneva, P., Aleksiev, Y., Peeva, T., Ilieva, Y. and Pencheu, P. 2007. Effect of heat stress on some physiological and biochemical parameters in buffaloes. Italian Journal of Animal Science. 6 (2): 1325-1328.

Gupta, M., Kumar, S., Dangi, S.S. and Jangir, B.L. 2013. Physiological, biochemical and molecular responses to thermal stress in goats. *International Journal of Livestock Research.* **3**: 27-38.

Habeeb, A.A.M. 1987. The role of insulin in improving productivity of heat stressed farm animals with different techniques. Ph.D. Thesis submitted to Faculty of Agriculture, Zagazig University. Zagazig, Egypt.

Habeeb, A.A.M., El-Marsy, K.A., Aboulnaga, A.I. and Kamal, T.H. 1996. The effect of hot summer climate under level of milk yield on blood biochemistry and circulating thyroid and progesterone hormones in Friesian cows. *Arab Journal of Nuclear Sciences and Applications.* **29**: 161-173.

Habeeb, A.A., Gad, A.E. and Atta, M.A. 2018. Temperature-Humidity Indices as indicators of heat stress of climatic conditions with relation to production and reproduction of farm animals. *International Journal of Biotechnology and Recent Advances.* 1 (1): 35-50.

Hafez, E.S.E. 1968. Principles of animal adaptation. In Hafez ESE (ed) Adaptation of domestic animals. Lea & Febiger, Philadelphia, USA.

Hafez, E.S.E. 2005. Adaptation of domestic animals. Lea & Febiger, Philadelphia, USA. 103.

Hahn, G.L., Chen,Y.R., Nienaber, J.A., Eigenberg, R.A. and Parkhurst, A.M. 1992. Characterizing animal stress through fractal analysis of thermoregulatory responses. *Journal of Thermal Biology.* **17**: 115-120.

Hansen, P.J. 2004. Physiological and cellular adaptations of zebu cattle to thermal stress. *Animal Reproduction Sciences*. (82–83): 349–360.

Haq, A. and Konwar, D. 2015. Effect of ascorbic acid and amla powder (*Embilica officinalis*) supplementation on physiological parameters in cross-bred dairy cattle during heat stress. *International Journal of Advanced Research.* **3** (4): 830-837.

Haque, N., Ludri, A., Hossain, S.A. and Ashutosh, M. 2012. Comparative studies on temperature threshold for heat shock protein 70 induction in young and adult Murrah buffaloes. *Journal of Animal Physiology and Animal Nutrition* (Berl.). **96** (5): 920-929.

Holter, J.B., West, J.W. and McGilliard, M.L. 1997. Predicting ad libitum dry matter intake and yield of Holstein cows. *Journal of Dairy Science.* **80**: 2188-2199.

Hooda, O.K. and Singh, G. 2010. Effect of thermal stress on feed intake, plasma enzymes and blood biochemicals in buffalo heifers. *Indian Journal of Animal Nutrition.* **27**: 122-127.

Indu, S., Sejian, V. and Naqvi, S.M.K. 2014. Impact of simulated heat stress on growth, physiological adaptability, blood metabolites and endocrine responses in Malpura ewes under semi-arid tropical environment. *Animal Production Sciences*. http://dx.doi.org/10.1071/AN14085.

Itoh, F., Obara, Y., Rose, M.T., Fuse, H. and Hashimoto, H. 1998. Insulin and glucagon secretion in lactating dairy cows during heat exposure. *Journal of Animal Sciences.* **76**: 2182-2189.

Johnson, H.D., Ragsdale, A.c., Berry, I.L., and Shanklin, M.D. 1963. Temperature-humidity effects including influence of acclimation in feed and water consumption of Holstein cattle. *University of Missouri Research Bulletin*. **846**.

Johnson, H.D. 1980. Depressed chemical thermogenesis and hormonal function in heat. In: Yousef, MK (Ed.), Environmental Physiology: Ageing, Heat and Altitude. Elsevier North Holland, Inc., New York, USA. 3-9.

Kadzere, C.T., Murphy, M.R., Silanikove, N. and Maltz, E. 2002. Heat stress in lactating dairy cows: a review. *Livestock Production Science.* **77**: 59-91.

Kamal, T.H., Habeeb, A.A.M., Abdel-Samee, A. and Marai, I.F.M. 1989. Milk production of heat stressed Friesian cows and its improvement in the subtropics. International Symposium on the Constraints and Possibilities of Ruminant Production in the Dry Subtropics, Cairo, Egypt, EAAP Publication No. 38: 156-158.

Kamwanja, L.A., Chase, C.C., Gutierrez, J.A., Guerriero, V., Olson, T.A., Hammond, A.C. and Hansen, P.J. 1994. Responses of bovine's lymphocytes to heat shock as modified by breed and antioxidants status. *Journal of Animal Sciences*. **72**: 438-444.

Khate, K. 2015. Effect of heat stress on physiological performance, growth, nutrient utilization and expression patterns of heat shock protein (HSP) genes in mithun (*Bos frontalis*). Ph.D. (LPM) Thesis submitted to Nagaland University, SASRD, Medziphema, Nagaland, India.

King, Y.T., Lin, C.S., Lin, J.H., Lee, W.C. 2002. Whole-body hyperthermia-induced thermotolerance is associated with the induction of Heat Shock Protein 70 in mice. *Journal of Experimental Biology*. **205**: 273–278.

Koushish, S.K., Sengupta, B.P. and Georgie, G.C. 1997. Effect of thermal stress and water restriction on cortisol level of Beetal and Black Bengal goats. *Indian Journal of Animal Science*. **67**: 1104-1105.

Kumar, S.B.V., Kumar, A. and Kataria, M. 2011. Effect of heat stress in tropical livestock and different strategies for its amelioration. *Journal Stress Physiological Biochemistry.* **7**: 45-54.

Kumar, M., Jindal, R. and Nayyar, S. 2012. Physiological and biochemical profile of summer stressed goats. *Indian Veterinary Journal*. **89** (8): 38-40.

Kumar, A., Ashraf, S., Sridhar, T.G., Grewal, A., Singh, S.V., Yadav, B.R. and Upadhya, R.C. 2015. Expression profiling of major heat shock protein genes during different seasons in cattle (Bos indicus) and buffalo (Bubalus bubalis) under tropical climatic condition. *Journal of Thermal Biology*. **51**: 55-64.

Lacetera,N., Bernabucci,U., Scalia,D., Basiricò,L., Morera,P. and Nardone,A. 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. *Journal of Dairy Sciences*. **89** (12): 4606–4612.

Lindquist, S. and Craig, D. 1988. The heat shock proteins. Annual Review of Genetics. **22**: 631-677.

Lindquist, S. 1986. The heat-shock response. *Annual Review Biochemistry*. **55**: 1151–1191.

Liu, Y.X., Li, D.Q., Cui, Q.W., Shi, H.X. and Wang, G.L. 2010. Analysis of HSP70 mRNA level and association between linked microsatellite loci and heat tolerance traits in dairy cows. *Yi Chuan.* **3**: 935-941.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and 2[-Delta Delta C (T)] method. *Methods*. **25**: 402-408.

Livestock survey of India. https://vikaspedia.in/agriculture/agridirectory/reports-and-policy-briefs/20th-Livestock-census.

Lowe, T., Gregory, N.G., Fisher, A.D.and Payne, S.R. 2002. The effects of temperature elevation and water deprivation on lamb physiology, welfare and meat quality. *Crop Pasture Science*. **53** (6): 707-714. DOI: 10.1071/AR01125

Mader, T.L., Davis, M.S. and Brown-Brandl, T. 2006. Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science.* **84:** 712-719.

Maibam, U., Hooda, O.K., Sharma, P.S., Mohanty, A.K., Singh, S.V. and Upadhyay, R.C. 2016. Expression of HSP70 genes in skin of zebu (Tharparkar) and crossbred (Karan Fries) cattle during different seasons under tropical climatic conditions. *Journal of Thermal Biology*. DOI: http://dx.doi.org/10.1016/j.jtherbio.2016.11.007.

Manjari, P., Yadav, M., Ramesh, K., Uniyal, S., Rastogi, S.K., Sejian, V. and Hyfer, I. 2015. HSP70 as a marker of heat and humidity stress in Tarai buffalo. *Tropical Animal Health and Production*. **47** (1): 111-116.

Marai, I.F.M., Daader, A.H., Makkawy, M.Y., Gabr, H.A. and Ibramin,H. 1992. Effect of housing system, season of year and age on some physiological parameters and blood constituents of Ossimi sheep. *Journal of Arid Environment.* **22**: 277-285.

Marai, I. F. M., Habeeb, A. A., Daader, A. H. and Yousef, H. M. 1995. Effects of Egyptian subtropical summer conditions and the heat stress alleviation technique of water spray and a diaphoretic on the growth and physiological functions of Friesian cows. *Journal of Arid Environment*. **30** (2): 219-225.

Marai, I.F.M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M. 2007. Physiological traits as affected by heat stress in sheep: A review. *Small Ruminants*. **71**: 1-12.

Mayer, M.P. and Bukau, B. 2006. HSP70 chaperones: Cellular functions and molecular mechanism. *Cellular and Molecular Life Sciences.* **62** (6): 670-684.

McDowell, R.E., Hooven, N.W. and Camoens, J.K. 1976. Effect of climate on performance of Holsteins in first lactation. *Journal of Dairy Science*. **59**: 965-973.

Mishra, A., Hooda, O.K., Singh, G. and Meur, S.K. 2011. Influence of induced heat stress on HSP70 in buffalo lymphocytes. *Journal of Animal Physiology and*

Animal Nutrition. (Berlin). **95**(4): 540-544.

Mondal, M., Baruah, K.K. and Rajkhowa, C. 2014. Mithun: An animal of pride. *Livestock Research for Rural Development.* **26**(1). http://www.lrrd.org/1/cont2601.htm

Mondal S.K. and Pal, D.T. 1999. Mithun: Historical perspective. *Asian Agri-History.* **3**(4): 245-260.

Morris, S.D. 2002. Heat shock proteins and the skin. *Clinical and Experimental Dermatology*. **27**: 220-224.

Mosser, D.D., Van Oostrom, J. and Bols, N.C. 1987. Induction and decay of thermotolerance in rainbow trout fibro-blasts. *Journal of Cellular Physiology*. **132**: 155–160.

Naqvi, S.M.K., Hooda, O.K. and Purmina, S. 1991. Some plasma enzymes of sheep under thermal, nutritional and exercise stresses. *Indian Veterinary Journal*. **68**: 1045-1047.

Nasr, M.A.F. and Mahmoud S. El-Tarabany. 2017. Impact of three THI levels on somatic cell count, milk yield and composition of multiparous Holstein cows in a subtropical region. *Journal of Thermal Biology*. **64**: 73-77.

Nazifi, S., Saeb, M., Rowghani, E. and Kaveh, K. 2003.The influence of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. *Comparative Clinical Pathology*, **12**:135- 139.

Niyas, P.A.A., Chaidanya, K., Shaji, S., Sejian, V., Bhatta, R., Bagath, M., Rao, G.S.L.H.V.P., Kurien, E.K. and Girish, V. 2015. Adaptation of livestock to environmental challenges. *Journal of veterinary science and medical diagnosis*. **4**:3. http://dx.doi.org/10.4172/2325-9590.1000162

NRCM Annual Report. 2013. HSP gene expression studies of heat shock protein (HSP) in mithun. *Annual Report 2012-13*, 19-21.

Pandey, A. K., Khan, J. R., Mishra, O. P. and Singh, A. 2013. Hematobiochemical responses of Sahiwal cattle during hot dry and hot humid environmental conditions. *Indian Veterinary Journal*, **90** (7): 19-21.

Pandey, N., Kataria, N., Kataria, A.K., Joshi, A., Sankhala, L.N., Asopa, S. and Pachaury, R. 2012. Extreme ambiences vis-a-vis endogenous antioxidants of Marwari goat from arid tracts in India. *Extreme Life, Biospeology & Astrobiology Bioflux.* **4** (2): 29-33.

Pangestu, M., Subagyo, Y., Yuwonol, P., and Rustomo, B. 2000. Heat tolerance and productivity of local and imported Holstein-Friesian cows in Indonesia. *Asian-Australian Journal of Animal Sciences.* **13**: 505-508.

Parmar, M.S., Madan, A.K., Ruokuobeinuo H., Rastogi, S.K. and Bhabesh Mili. 2015. Heat Shock Protein70 (HSP70) Gene Expression Pattern in Peripheral Blood Mononuclear Cells (PBMCs) during different Seasons in Sahiwal Cows (*Bos Indicus*). *Journal of Animal Research.* **5**(1): 109-113.

Patel, J.S., Jajane, K.R., Vadqaria, V.P., Kullarni, V.V and Radadia, N.S. 1991. Effect of temperature on certain blood constituents in Patanwadi and its crosses with Merino and Ramboullet. *Indian Veterinary Journal*. **68**: 1134-1138.

Patir, H. and Upadhyay, R.C. 2010. Purification, characterization and expression kinetics of heat shock protein 70 from *Bubalus bubalis*. *Research of Veterinary Sciences*. **88**: 258–262.

Pereira, A.M.F., Baccari Jr, F., Titto, E.A.L. and Almeida, J.A.A. 2008. Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in Alentejana, Mertolenga, Frisian and Limousine cattle breeds. *International Journal of Biometeorology*. **52**: 199-208.

Perez, J.H. 2000. Parameters for the determination and evaluation of heat stress in the dairy cattle in South Agriculture. *Journal of Veterinary Research*. **67** (4): 263-271.

Perumal, P., Chamuah, J.K., Nahak, A.K. and Rajkhowa, C. 2015. Effect of seasons on semen production, effect of melatonin on the liquid storage (5°C) with correlated study of birth rate in mithun (*Bos frontalis*). *Asian Pacific Journal of Reproduction.* **4** (1): 1-12.

Phulia, S.K., Upadhyay, R.C., Jindal, S.K. and Mishra, R.P. 2010. Alteration in surface body temperature and physical responses in Sirohi goats during day time in summer season. *Indian Journal of Animal Science*. **80**: 340-342.

Popoola, M.A., Bolarinwa, M.O, Yahaya, M.O., Adebisi, G.L. and Saka, A.A. 2014. Thermal comfort effects on physiological adaptations and growth performance of West African dwarf goats raised in Nigeria. *European Scientific Journal (Special Edition)*. **3**: 275-281.

Rahardja, D.P., Toleng, A.L. and Lestari, V.S. 2011. Thermoregulation and water balance in fat-tailed sheep and Kacang goat under sunlight exposure and water restriction in a hot and dry area. *Animal*. **5** (10): 1587-1593.

Rajoriya, J. S., Prasad, J. K., Ghosh, S. K., Perumal, P., Kumar, A., Kaushal, S. and Ramteke, S.S. 2014. Studies on effect of different seasons on expression of HSP70 and HSP90 gene in sperm of Tharparkar bull semen. *Asian Pacific Journal of Reproduction*. **3** (3): 192-199.

Rao, M., Yadav, M., Ramesh, K., Uniyal, S., Rastogi, S.K., Sejian, V. and Hyder, I. 2014. HSP70 as a marker of heat and humidity stress in Tarai buffalo. *Tropical Animal Health and Production*. **47**: 111-116.

Ravagnolo, O., Misztal, I. and Hoogenboom, G. 2000. Genetic component of heat stress in dairy cattle, development of heat index function. *Journal of Dairy*

Science. **83**: 2120-2125.

Renaudeau, D., Collin, A., Yahav, S., De Basilio, V. and Gourdine, J.L. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production animal. *Animal*. **6**: 707-728.

Ribeiro, N.L., Ribeiro, M.N., Bozzi, R., Givisiez, P.E.N. and Costa, R.G. 2014. Physiological and biochemical blood parameters of goats subjected to heat stress. *Journal of Biomedical Environmental Sciences*. **9**: 35.

Rodrigo, A.F., Henry, D.M.G., Vivian, H.V.A., Camilia, S.N., Cecilia, J.V., Roberto, J.S., sartori, S. and Ferreia, J.C.P. 2017. Thermoregulatory responses of Holstein cows exposed to experimentally induced heat stress. *Journal of thermal Biology*. **66**: 68-80.

Rosales, A.G. 1994. Stress syndrome in birds. *Journal of Applied Poultry Research*. **3**: 199-203.

Rasooli, A., Nouri, M., Khadjeh, G.H. and Rasekh, A. 2004. The influence of seasonal variations on thyroid activity and some biochemical parameters of cattle. *Iranian Journal of Veterinary Research*. **5** (2): 1383-1390.

Sabuncuoglu, N. 2004. Effect of barn types on physiologic traits of calves. *Indian Veterinary Journal.* **81**: 22-24.

Salem, J.A., Kobeisy, M.A., Zenhom, M. and Hayder, M. 1998. Effect of season and ascorbic acid supplementation on some blood constituents of suckling Chois lambs and its crosses with Ossimi sheep in Upper Egypt. *Assiut Journal of Agricultural Science*. **29**: 87-100.

Sano, H., Ambo, K. and Tsuda, T. 1985. Blood glucose kinetics in whole body and mammary gland of lactating goats exposed to heat. *Journal of Dairy Sciences*. **68**: 2557-2564.

Sarkar, M., Das, B.C., Mondal, D.N., Chaterjee, D.B. and Yadav, B.P.S. 1999. Studies of thermo adaptability in Yaks. *Indian Journal of Animal Sciences.* **69**: 963-964.

Sarkar, M., Krishnan, G. and Prakash, B.S. 2010. Seasonal variation in plasma glucocorticoids levels in yaks (*Poephagus grunniens*) and (*Bos grunniens). Tropical Animal Health Production.* **42**: 421-424.

Schutz, K.E., Cox, N.R. and Matthews, L.R. 2008. How important is shade to dairy cattle? Choice between shade or lying following different levels of lying deprivation. *Applied Animal Behavioural Sciences.* **114**: 307-318.

Scott, G.H. and Wiersma, F. 1971. Plasma corticosteroids as indicators of gonadotrophin secretion and infertility in stressed bovine. *Journal of Dairy Sciences.* **53**: 652.

Sejian, V., Maurya, V.P. and Naqvi, M.K. 2010. Adaptive capability as indicated by endocrine and biochemical responses of Malpura ewes subjected to combined stresses (thermal and nutritional) under semi-arid tropical environment. *International Journal of Biometeorology*. **54**: 653-661.

Sejian, V. and Srivastava, R.S. 2010. Pineal-adrenal-immune system relationship under thermal stress: effect on physiological, endocrine and non-specific immune response in goats. *Journal of Physiology and Biochemistry*. **66**: 339-349.

Sejian, V., Maurya, V.P. and Naqvi, S.M.K. 2010. Adaptive capacity as indicated by endocrine and biochemical responses of Malpura ewes subjected to combined stresses (thermal and nutritional) under semi-arid tropical environment. *International Journal of Biometeorology*. **54**: 653-661.

Sejian, V., Maurya, V.P., Sharma, K.C. and Naqvi, M. 2012. Concept of multiple stresses and its significance on livestock productivity. **In**: Environmental stress and amelioration in livestock production. Germany: Springer-Verlag GMbH Publisher. 129-152.

Sejian, V., Indu, S. and Naqvi, S.M.K. 2013. Impact of short term exposure to different environmental temperature on the blood biochemical and endocrine responses of Malpura ewes under semi-arid environment. *Indian Journal of animal Sciences*. **83** (11): 1155-1160.

Shaffer, L., Roussel, J.D. and Koonce, K.L. 1981. Effects of age, temperature, season and breed on blood characteristics of dairy cattle. *Journal of Dairy science.* **64**: 62-70.

Sharma, A.K. and Kataria, N. 2011. Effects of extreme hot climate on liver and serum enzymes in Marwari goats. *Indian Journal of Animal Sciences.* **81**: 293-295.

Sharma, S., Ramesh, K., Hyder, I., Uniyal, S. and Yadav, V.P. 2013. Effect of melatonin administration on thyroid hormones, cortisol and expression profile of heat shock proteins in goat (*Capra hircus*) exposed to heat stress. *Small Ruminant Research*. **112**: 216-223.

Sheikh, A.A., Aggarwal, A. and Aarif, O. 2014. Effect of in-vitro zinc supplementation of HSPs expression and interleukin10 production in heat treated peripheral blood mononuclear cells of transition Sahiwal and Karan Fries cows. *Journal of Thermal Biology*. **56**: 68-76.

Shrikande, G.B., Rode, A.M., Pradhan, M.S. and Satpute, A.K. 2008. Seasonal effect on the composition of blood in cattle. *Veterinary World.* **1** (11): 341-342.

Simoons, F.J. 1984. Evolution of domesticated animals. Longman press, London UK. 34-36.

Singh, S. P., Hooda, O. K., Kundu, S. S. and Singh, S. V. 2012. Biochemical changes in heat exposed buffalo heifers supplemented with yeast. *Tropical Animal Health Production,* **44**: 1383-1387.

Singh, A.K., Upadhyay, R.C., Malakar, D., Kumar, S. and Singh, S.V. 2014. Effect of thermal stress on HSP70 expression in dermal fibroblast of zebu (Tharparkar) and crossbred (Karan-Fries) cattle. *Journal of Thermal Biology*. **43**: 46–53.

Sorensen, J.G., Kristensen, T.N. and Loeschcke, V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters*. **6** (11): 1025–1037.

Soveri, T., Sankari, S. and Nieminen, M. (1992). Blood chemistry of reindeer calves (*Rangifer Tarandus*) during the winter season. *Comparative Biochemistry and Physiology*, Seri A, **102**: 191-196.

Sreedhar, S., Rao, K.S., Suresh, J., Moorthy, P.R.S. and Reddy, V.P. 2013. Changes in haematocrit and some serum biochemical profile of Sahiwal and Jersey X Sahiwal cows in tropical environments. *Veterinarski Arhiv*. **83** (2): 171-187.

Srikandakumar, A., Johnson, E.H. and Mahgoub, O. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Ruminant Research*. **49**: 193-198.

Srikandakumar, A. and Johnson, E.H. 2004. Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian Milking Zebu cows. *Tropical Animal Health Production*. **36**: 685-692.

St-Pierre, N.R., Cobanov, B. and Schnitkey, G. 2003. Economic losses from heat stress by US Livestock industries. *Journal of Dairy Sciences*. **86**: 52-77. http://dx.doi.org/10.3168/jds.S00220323(03)74040-5.

Sunil Kumar, B.V., Kumar, A. and Kataria, M. 2011. Effect of heat stress in tropical livestock and different strategies for its amelioration. *Journal of Stress Physiology & Biochemistry*. **7** 1): 45-54.

Swenson, M.J. and Reece, W.O. 2006. Dukes- Fisiologia dos Animais Domesticos, Koogan GSA, Rio de Janeiro.

Thom, E.C. 1959. The discomfort index. *Weather Index*. **12**: 57-59.

Thompson, G. 1973. Climatic physiology of cattle. *Journal of Dairy Research.* **40** (3): 441-473. DOI:10.1017/S0022029900014813

Thompson, V.A., Fadel, J.G. and Sainz, R.D. 2011. Meta-analysis to predict sweating and respiration rates for *Bos indicus*, *Bos taurus*, and their crossbreds. *Journal of Animal Sciences.* **89**: 3973-3982.

Trana, A.D., Celi, P., Salvatore, C., Fedele, V. and Rubino, R. 2006. The effect of hot season and nutrition on the oxidative status and metabolic profile in dairy goats during mid lactation. *Animal Science*. **82** (5): 717-722.

Upadhyay, R.C., Singh, S.V. and Ashutosh. 2008. Impact of climate change on livestock. *Indian Dairyman.* **60** (3): 98-102.

Verma, N.D. 1994. Physiological records on mithun. Annual Report, NRC on Mithun, Nagaland. (193-94 and 1994-95). 13-14.

Vupru, K., Sanyal, S., Ghosh, P.R., Das, P.K., Chattophadhyyay, A. and Rajkhowa, C. 2005. Studies on draught power and some physiological, haematological and biochemical changes in mithun (*Bos frontalis*) as influenced by work. M.V.Sc. Thesis West Bengal University of Animal and Fishery Sciences. 51-52.

West, J.W. 2003. Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science*. **86**: 2131–2144.

Wu, C. 1995. Heat shock transcription factors: structure and regulation. *Annual Review of Cell Developmental Biology.* **11** (1): 441-469.

Yang, M., Tan, H., Yang, Q.L., Wang, F., Yao, H., Wei, Q., Robert, M.T. and Wu, T. 2006. Association of HSP70 polymorphisms with risk of noise-induced hearing loss in Chinese automobile workers. *Cell Stress Chaperones*. **11** (3): 233-239.

Yousef, M.K. 1985. Measurements of heat production and heat loss. In:Book-series title stress physiology in livestock (ed. MK Yousef), 1: 35-46.CRC Press, Boca Raton, FL, USA.

Yousef, H.M., Habeeb, A.A.M. and El-Kousey, H. 1997. Body weight gain and some physiological changes in Friesian calves protected with wood or reinforced concrete sheds during hot summer season of Egypt. *Egyptian Journal of Animal Production.* **34**: 89-101.

Zhang, F.J., Weng, X.G., Wang, J.F., Zhou, D., Zhang, W., Zhai, C.C., Hou, Y.X. and Zhu, Y.H. 2014. Effects of temperature-humidity index and chromium supplementation on antioxidant capacity, heat shock protein 72 and cytokine responses of lactating cows. *Journal of Animal Sciences.* **92**: 3026-34.

APPENDICES 1

(RECTAL TEMPERATURE)

Source of variation	df	SS	MSS	F cal	T- Value	Logic
Replication	5	145921	291848.20		4.76	
Mp Treatment (A)	3	1459227	486409.00	3.75	3.29	Significant
MP Error (Error A)	15	1945685.52	129712.37			
Sp Treatment (B)	1	1459156	1459156.00	59.99	4.35	Significant
Interaction (AX B)	3	1459247.64	486415.88	20.00	3.10	Significant
Error (Error B)	20	486435	24321.75			

(PULSE RATE)

Source of variation	df	SS	MSS	F Cal	T- Value	Logic
Replication	5	350734.24	70146.85		4.76	
Mp Treatment (A)	3	350154.98	116718.33	3.74	3.29	Significant
MP Error (Error A)	15	468393.20	31226.21			
Sp Treatment (B)	1	346949.67	346949.67	59.04	4.35	Significant
Interaction (AX B)	3	350764.25	116921.42	19.90	3.10	Significant
Error (Error B)	20	117526.92	5876.35			

(RESPIRATION RATE)

Sov	df	SS	MSS	F Cal	T- Value	Logic
Replication	5	128754.00	25750.80		4.76	
Mp Treatment (A)	3	128707.00	42902.33	3.75	3.29	Significant
Mp Error (Error A)	15	171800.19	11453.35			
Sp Treatment (B)	1	128120.91	128120.91	59.62	4.35	Significant
Interaction (A*B)	3	128774.11	42924.70	19.98	3.10	Significant
Error (Error B)	20	42977.00	2148.85			

APPENDICES 2

(GLUCOSE)

Source of Variation	df	SS	MSS	F Cal	T-Value	Logic
Replication	5	464108.76	92821.75		4.76	
Mp Treatment (A)	3	457456.86	152485.62	3.65	3.29	Significant
Mp Error (Error A)	15	625987.72	41732.51			
Sp Treatment (B)	1	471435.00	471435.00	61.69	4.35	Significant
Interaction (AX B)	3	465508.60	155169.53	20.31	3.10	Significant
Error (Error B)	20	152831.00	7641.55			

(SGOT)

Source of Variation	df	SS	MSS	F- Cal	T- Val	Logic
Replication	5	283302.86	56660.57		4.76	
Mp Treatment (A)	3	283006.91	94335.64	3.74	3.29	Significant
Mp Error (Error A)	15	378393.84	25226.26			
Sp Treatment (B)	1	285937.65	285937.65	62.23	4.35	Significant
Interaction (AX B)	3	283175.26	94391.75	20.54	3.10	Significant
Error (Error B)	20	91893.60	4594.68			

(SGPT)

Source of Variation	DF	SS	MSS	F cal	T- Value	Logic
Replication	5	279528.09	55905.62		4.76	
Mp Treatment (A)	3	279228.63	93076.21	3.74	3.29	Significant
Mp Error (Error A)	15	373166.83	24877.79			
Sp Treatment (B)	1	280667.75	280667.75	61.11	4.35	Significant
Interaction (AX B)	3	280836.10	93612.03	20.38	3.10	Significant
Error (Error B)	20	91863.30	4593.17			

(CORTISOL)

Source of Variation	df	SS	MSS	F Cal	T-Value	Logic
Replication	5	73102452.80	14620490.56		4.76	
Mp Treatment (A)	3	73102452.90	24367484.30	3.75	3.29	Significant
Mp Error (Error A)	15	97469937.51	6497995.83			
Sp Treatment (B)	1	96793062.70	96793062.70	2860	4.35	Significant
Interaction (AX B)	3	73102452.93	24367484.31	720	3.10	Significant
Error (Error B)	20	676874.70	33843.74			

APPENDICES 3

(HSP70)

Source of Variation	df	SS	MSS	F cal	T-Value	Logic
Replication	5	119947.66	23989.53		4.76	
Mp Treatment (A)	3	119788.27	39929.42	3.74	3.29	Significant
MP Error (Errora)	15	160115.30	10674.35			
Sp Treatment (B)	1	119949.74	119949.74	59.76	4.35	Significant
Interaction (AX B)	3	119951.75	39983.92	19.92	3.10	Significant
Error (Error B)	20	40141.16	2007.06			

(HSP90)

Source of Variation	df	SS	MSS	F cal	T- Value	Logic
Replication	5	166411.86	33282.37		4.76	
Mp Treatment (A)	3	165739.06	55246.35	3.72	3.29	Significant
MP Error (Errora)	15	222706.15	14847.08			
Sp Treatment (B)	1	166421.55	166421.55	60.29	4.35	Significant
Interaction (AX B)	3	166426.42	55475.47	20.10	3.10	Significant
Error (Error B)	20	55208.11	2760.41			