



Impact of Heat Stress on Physiological Characteristics, Blood Constituents and HSP Genes Expression in Mithun during Summer Season

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ABSTRACT

Six healthy Mithun animals being reared in the shade and were not exposed to sunlight in the morning hours were termed as control group (T_1). The same animals were exposed to sunlight from 6.00 am to 2.00 pm to induce heat stress and were termed as the heat stressed group (T_2). The samples for observations on physiological, blood constituents and gene expression were recorded at 6.00 am (T_1) and 2.00 pm (T_2). The values for physiological characteristics, viz., rectal temp (100.04 ± 0.26 vs 103.34 ± 0.20), respiration rate per minute (26.67 ± 0.52 vs 33.00 ± 0.74) and pulse rate per minute (42.44 ± 1.02 vs 59.83 ± 0.84) were significantly ($P < 0.05$) higher in the heat stressed group than non stress group. The values for blood constituents viz., glucose (50.82 ± 1.27 vs 60.09 ± 1.46), SGOT (44.91 ± 0.89 vs 51.18 ± 1.08), SGPT (44.17 ± 0.87 vs 49.92 ± 0.87), cortisol (712.48 ± 0.03 vs 712.49 ± 0.02) and THI (77.56 ± 0.39 vs 81.42 ± 3.40) were also significantly ($p < 0.05$) higher in the heat stress group than non stress group. The values for expression of HSP 70 gene (28.24 ± 0.15 vs 28.88 ± 0.59) and HSP 90 gene (36.13 ± 1.24 vs 35.87 ± 1.90) were also significantly ($p < 0.05$) higher in the heat stressed group of Mithun than non stress group. The results of the study revealed that summer heat stress had a significant effect in Mithun.

KEYWORDS: Temperature, glucose, SGOT, SGPT, HSP70, HSP90, cortisol

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1. INTRODUCTION

Mithun is a domesticated animal, reared in the hilly tracts of North Eastern region of India. They undergo various kinds of stress like physical, chemical, nutritional and thermal stress and increase their susceptibility to 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 led to in arise of different environmental stress affecting productivity, reproductive efficiency and health disorders leading to severe economic losses (St-Pierre et al., 2003, Sejian et al., 2012). Virtually, stress was the cumulative detrimental condition caused due to various factors on health and performance, disturbed homeostasis or unbalanced the physiological equilibrium, animals failed to achieve their genetic potential and an animal faces a sudden change in its environment due to various kinds of stress viz. chemical, physical, nutritional and thermal stress (Dobson and Smith, 2000, Kumar et al., 2011, Sejian et al., 2012). Heat stress negatively affects the productivity of the animals (Niyas et al., 2015). Response to heat stress varies in physiological, behavioural and hormonal ways in an animal. 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. 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). De Rensis et al. (2015) defined THI <68 as thermal comfort zone, 68 to 74 as mild and ≥ 75 as severe signs of heat stress. 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). Heat shock proteins (HSPs) are specific proteins that are generated by the cells when exposed to stressful conditions and ranges from 10-150 kDa in molecular size. They are synthesized due to the outcome of heat stress and protect the cells from the severe ill-effects of heat stress and other environment stress. They heat shock proteins (HSP70 and HSP90) function as molecular chaperone and exhibit thermoregulatory protective mechanisms when affected by heat stress (Collier et al., 2008, Kumar et al., 2015), highly conserved proteins and enables cell survival caused by injury and oxidative stress (Yang et al., 2006, Belhadj et al., 2016), get easily activated by heat and other environmental stressors and counterbalance between survival and effective immunity system by creating stress tolerance and thermal adaptation (Sorensen et al., 2003, Beckham et al., 2004). These HSPs

permit the cells to resist damage resulted due to stressful conditions (Byoung Hwa Roh et al., 2008). 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 and Bukau, 2006). The level of stress leads to the expression of HSPs. In view of the above fact, the present study was postulated with the objective to know the physiological and biochemical status and analyze the expression of Heat Shock Protein genes in Mithun as the animals are reared under free grazing conditions without any kind of housing.

2. MATERIALS AND METHODS

The experiment was carried out at 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 levels during the month of June to August, 2019. Six healthy animals with same age were housed in shed and were fed, watered and maintained under hygienic conditions. The animals were not exposed to sunlight up to 6.00 am and treated as control (T_1). The same animals were later exposed to sunlight up to 2.00 pm and treated as heat stress (T_2). All the observations for physiological, blood constituents and gene expression were recorded at 6.00 am for control group and 2.00 pm for heat stress group (T_2). The animals were comforted before recording the observations and care was taken to avoid any other kind of stress. Rectal temperature ($^{\circ}$ F) was recorded by inserting digital thermometer in the rectum for two minutes. Respiration rate (breaths min^{-1}) was determined by counting the frequency of flank movement per minute. Pulse rate (pulse min^{-1}) was obtained by palpation underside of the base of the tail. Blood samples (7 ml) were collected by the jugular vein puncture under aseptic conditions. Serum samples were preserved for the analysis of glucose, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and cortisol. RNA samples were isolated for the analysis of HSP70 and HSP90 genes. Serum samples were procured after centrifuging the samples at 7500 rpm for 10 minutes at room temperature. The serum samples were labelled and stored at -80°C until further analysis of Glucose, SGOT, SGPT and cortisol. Glucose was determined by using commercial kits (Diatek, GOD-PAP: enzymatic photometric test). SGOT and SGPT were also determined by using commercial kits (Diatek, UV Kinetic method). Ambient temperature and relative humidity values were obtained from the meteorological department of ICAR Research complex, Nagaland which is located at close proximity for Temperature Humidity Index (THI) calculation. THI was calculated using the formula as $\text{THI} = 0.72(\text{W} + \text{D}) + 40.6$



where, W=Wet bulb temperature ($^{\circ}\text{C}$) and D=Dry bulb temperature ($^{\circ}\text{C}$) (Kadzere et al., 2002). The data were analysed statistically and expressed as the mean \pm SEM. The means were analysed using paired t-test as per Snedecor and Cochran (1994) to the study the effect of heat stress in Mithun during summer season.

3. RESULTS AND DISCUSSION

3.1. Pulse rate

The mean values of pulse rate (pulse/min) were 42.44 ± 1.02 and 59.83 ± 0.84 in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for pulse rate were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The findings in the present study might be due to thermoregulation which resulted to exert higher pulse rate in the heat stressed as compared to the non-heat stressed mithun during summer season. The observations of the present study were well corroborated with findings of Gupta et al. (2013), Indu et al. (2014) and Popoola et al. (2014) who reported that there was significant increase in the pulse rate of mithun under heat stress condition as compared to the non stressed group which might be primarily due to reflection in the homeostasis of circulation along with increase in metabolism and muscle activity during the stress condition.

3.2. Rectal temperature

The mean values for rectal temperature ($^{\circ}\text{F}$) were 100.04 ± 0.26 and 103.34 ± 0.20 in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for rectal temperature were significantly ($p < 0.05$) higher in heat stressed group (T_2) as compared to non heat stressed group (T_1). Increase in rectal temperature of the heat stressed mithun might be due to increased thermo-regulatory effect of mithun as compared to the non heat stressed mithun. The observations of the present study were well corroborated with findings of Gudev et al. (2007), Sarkar et al. (2010) and Rodrigo et al. (2017) who stated that rectal temperature was a sensitive indicator of body temperature in heat stressed animals and increase in rectal temperature influenced the thermo - regulatory mechanisms of the animals due to continuous exposure of intense heat stress.

3.3. Respiration rate

The average values for respiration rate (breaths/min) were 26.67 ± 0.52 and 33.00 ± 0.74 in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for respiration rate were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The findings of the present study were well corroborated with observations of Gudev et al. (2007), Sarkar et al. (2010), Renaudeau et al. (2012), Gupta et al. (2013), Ribeiro et al. (2014) and Rodrigo (2017) who found that respiration rate

increased due to continuous exposure to intense heat stress influencing thermo - regulatory mechanisms. They used their respiratory mechanism to avoid increase in rectal temperature and thus maintained homeotherms during hot periods and the first and foremost mechanism of an animal subjected to heat stress was increase in the respiration rate causing loss of heat through evaporation. They further explained that increase in respiration rate at THI of 77.83 showed that the animals were heat stressed and unable to maintain their thermoneutral zone.

Table 1: Physiological parameters of Mithun under different conditions

| S1. No. | Parameters | Non-Heat Stressed (T_1) | Heat Stressed (T_2) |
|---------|---|-----------------------------|-------------------------|
| 1. | Pulse Rate (pulse min^{-1}) | 42.44 ± 1.02 | 59.83 ± 0.84 |
| 2. | Rectal Temperature ($^{\circ}\text{F}$) | $100.04^a \pm 0.26$ | $103.34^b \pm 0.20$ |
| 3. | Respiration Rate (breaths min^{-1}) | $26.67^a \pm 0.52$ | $33.00^b \pm 0.74$ |

a, bMeans bearing various superscripts in a row differ significantly ($p < 0.05$)

3.4. Glucose

The average values of glucose (mg dl^{-1}) of mithun during summer season were 50.82 ± 3.10 and 60.09 ± 3.58 in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for glucose were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group of mithun. The results of the present study were well corroborated with observations of Bagha et al. (2009), Calamari et al. (2011), Cincovic et al. (2011) and Sejian et al. (2013) who reported that serum glucose was significantly ($p < 0.05$) higher in hot than cold climate. They further explained that concentration of serum glucose was significantly higher in control compared to the animals that were exposed to cooling systems during hot and dry ambient conditions. In heat stressed animals, the mean serum glucose values were significantly higher from the values of thermoneutral zone cows. However, supplementation of selenium during heat stress showed significant negative correlation between THI and plasma concentration of glucose.

3.5. Serum glutamic oxaloacetic transaminase (SGOT)

The average values of SGOT (U l^{-1}) during summer season were 44.91 ± 2.19 and 51.18 ± 2.64 in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for SGOT were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The present results were in close agreement with the findings of Singh et al. (2012) who also reported that the plasma concentration of SGOT



increased significantly ($p < 0.05$) due to thermal exposure observed which might be due to increase in the stimulation of gluconeogenesis by corticoids.

3.6. Serum glutamic pyruvic transaminase (SGPT)

The average values of SGPT (U l^{-1}) were 44.12 ± 2.56 and 49.69 ± 1.97 in T_1 and T_2 group, respectively during the summer season. From the data, it was revealed that the values for SGPT were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The above results were in collaboration with Singh et al. (2012) who reported that the plasma concentration of SGPT increased significantly ($p < 0.05$) due to thermal exposure.

3.7. Cortisol

The average cortisol values of during summer season were 712.48 ± 0.06 and 712.49 ± 0.05 nmol l^{-1} in T_1 and T_2 group, respectively. The average values of T_2 group were significantly ($p < 0.05$) higher as compared to T_1 group. The above results were in collaboration with Chaurasia et al. (2011), Sejjian et al. (2013) and Sharma et al. (2013) who had also found that plasma cortisol levels were significantly ($p < 0.05$) higher in the season of March-June compared to the season of November-February. Virtually, acute environmental heat exposure caused a transient increase in circulating glucocorticoids that might subsequently decreased even though body temperature remained elevated during chronic heat exposure.

Table 2: Haematological parameters of mithun under different conditions

| Sl. No. | Parameters | Non-heat stressed (T_1) | Heat stressed (T_2) |
|---------|-----------------------------------|-----------------------------|-------------------------|
| 1. | Glucose (mg dl^{-1}) | $50.82^a \pm 1.27$ | $60.09^b \pm 1.46$ |
| 2. | SGOT (U l^{-1}) | $44.91^a \pm 0.89$ | $51.18^b \pm 1.08$ |
| 3. | SGPT (U l^{-1}) | $44.17^a \pm 0.87$ | $49.92^b \pm 0.87$ |
| 4. | Cortisol (nmol l^{-1}) | $712.48^a \pm 0.03$ | $712.49^b \pm 0.02$ |
| 5. | THI | $77.56^a \pm 0.39$ | $81.42^b \pm 3.40$ |

3.8. HSP 70

The HSP 70 expression during summer season was recorded as 77.56 ± 0.39 and 81.42 ± 3.40 kDa in T_1 and T_2 group, respectively. From the data, it was revealed that the values for HSP 70 were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The results of the present study were in line with observations of Lacetera et al. (2006) and Parmar et al. (2015) who had also found increased HSP 70 concentration due to stressful condition in cattle and the expression of gene HSP mRNA was virtually higher during summer than winter season which might be due to the prevailing higher THI (> 80) conditions during the summer season. Actually, HSP 70 expression was temperature

sensitive and the increase in the expression of HSP 70 during summer than winter season might be induced by heat and hypothermic stress and a unique feature of the physiological function of these molecules with the changing environment (Beckham et al., 2004). Further, expression of HSP 70 increased due to the increase in the THI during the seasons (Rajoriya et al., 2014) and due to exposure to heat stress, thermoregulatory mechanisms were triggered to up regulation of HSP 70.

3.3.2 HSP 90

The expression of HSP 90 during the summer season was 37.08 ± 1.24 and 37.20 ± 0.64 kDa in T_1 and T_2 groups, respectively. From the data, it was revealed that the values for HSP 90 were significantly ($p < 0.05$) higher in T_2 group as compared to T_1 group. The present result coincided with the results reported by Sharma et al. (2013), Deb et al. (2014) and Kumar et al. (2015) who had also revealed significantly ($p < 0.001$) higher expression pattern of heat shock protein genes (HSPA 1A, HSPA 1B, HSP 10, HSP 60 and HSP 90) during both winter and summer seasons, whereas the magnitude of expression was higher during summer as compared to winter. Further, expression of HSP 70 increased due to the increase in the THI during the seasons (Rajoriya et al., 2014) and due to exposure to heat stress, thermoregulatory mechanisms were triggered to up regulation of HSP 90 and the higher expression of HSPs during thermal stress was an indication to maintain cellular integrity and homeostasis.

Table 3: HSP gene expression in mithun under different conditions

| Sl. No. | Genes | Non-heat stressed (T_1), N=6 | Heat stressed (T_2), N=6 |
|---------|--------------|----------------------------------|------------------------------|
| 1. | HSP 70 (kDa) | $28.24^a \pm 0.15$ | $28.88^b \pm 0.59$ |
| 2. | HSP 90 (kDa) | $36.13^a \pm 1.24$ | $35.87^b \pm 1.90$ |

4. CONCLUSION

The increase in expression of HSP 90 would be due to the increase in the ambient temperature and THI values during the seasons which triggered the up regulation of HSP 90 to maintain homeostasis.

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