Impact of Seasonal Variation on the Expression of Heat Shock Protein in Goats

Mihir Bhatta*, Probal Ranjan Ghosh, Nabi Kanta. Jha, Debasish Das, Anshuman Mandal, Manisha Tambuly

Abstract— Goats are predominantly found as small ruminants in the Indian sub-continent and are generally showing a noted seasonal variation in their concurrent with the thermo-tolerance. Present work has been initiated to attain a perception of the effect of heat-related stress on the expression profile of Heat shock protein (HSP) in goats kept in two different agro-climatic regions during pre-monsoon and post-monsoon seasons. Specificity of the expected DNA products has been established using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting techniques. The expression of heat shock protein (here HSP 70) in the goats, is found significantly higher ($P<0.05$) during pre-monsoon as related to post-monsoon season in both the regions under study. From the present findings, it could be concluded that the expression of heat shock protein in the Black Bengal goat lymphocyte is increased with the degree of thermal stress as well as with the magnitude of thermal fluctuations experienced by the animals.

Index Terms—Goat, ambient temperature, Lymphocytes, Heat shock protein, HSP70, Purulia, Nadia.

1 INTRODUCTION

HEAT shock proteins (HSPs) are extremely conserved proteins present in more or less all the cells of a living organism. HSPs belong to multi-gene families which range in molecular size from 10 to 150 kDa (kilo Dalton) and could be observed in all-important cellular sections. HSPs are very much essential for cellular viability as these have a major function in protein homeostasis1. This family of proteins are particularly concerned with the regulation of the folding of the other proteins to the secondary structures 2. These proteins are named heat shock proteins because they were first recognized on the basis of their increased synthesis during exposure to the elevation of ambient temperature [2]. The normal synthesis of most proteins is hindered when any living organisms are exposed to the thermal stresses, but the HSPs, a group of highly conserved proteins is rapidly translated during this period of time. HSP has been proven to contribute a major role in protecting stressed cells and organs and thus preventing disorders caused by stress [3]. It is true that the enhanced expression of HSPs has been accredited to the accumulation of abnormal proteins in the cells. There are lots of proteins present in the HSP family, among them the heat shock proteins, HSP 70 has been the most heat susceptible and is positively concurrent with the thermo-tolerance [4].

The different levels of expression of the diverse numbers of HSPs can be taken as an indication of animal adaptation to environmental stress [4-5]. The 70 kDa heat shock protein family performs a role during the protein folding event leading to the translation in the cytosol of both prokaryotic and eukaryotic cells as reported by different genetic and biochemical analyses [6]. HSP can perform as molecular chaperones by involving in the assemblage of proteins devoid of being a part of the ultimate protein configuration [7]. HSP 70 has been studied for a long time and the purification of HSP from major livestock species (such as cattle, buffalo and sheep) and for many breeds of cattle, has been achieved [8], but the work on caprine HSPs are still in the stage of its beginning. It has been a commonly recognized factor that the portion of the energy which can be metabolized is usually used for the animal's physical growth has been diverted to attain thermal stability beyond the thermo-neutral zone for that specific animal. Therefore, the productivity of the farm has been reduced under severe heat stress [9]. The thermal stress severely affects the productivity of goats similar to other livestock species [10]. However, there is little work present that can completely depict how goats respond to seasonal heat stress exposure at the molecular level in different agro-climatic regions in West Bengal. Therefore, the present study has been designed to gain insight into the impact of heat stress on physiological responses and expression profile of HSPs in Black Bengal goats. Also, the objective of the present study was to determine how the lymphocytes from the Black Bengal goat breed respond to increased ambient temperature in two different seasons with the expression of HSP 70.

- *Dr. Mihir Bhatta, Scientist-B, ICMR-National Institute of Cholera and Enteric Disease; Prof. (Dr.) Probal Ranjan Ghosh, West Bengal University of Animal and Fishery Sciences; Dr. Nabi K. Jha, Asst. Divisional Forest Officer, Buxa Tiger Reserve; Prof. (Dr.) Debasish Das, University of Kalyani; Mr. Anshuman Mandal, Lab.Tech., NRL, ICMR-NICED; Ms. Manisha Tambuly, Asst. Teacher, Stratford Day School.*
- **Corresponding Author,* ***mihiebhatta@gmail.com***

2 MATERIALS AND METHODS

2.1 Animals

The small ruminant animal has been chosen for this study were clinically healthy, non-pregnant Black Bengal does of 2 – 3 years of age and has an average body weight of more or less 15 Kg showing no parasitic infestation. The animals were taken from the different local rearers of Purulia and Nadia districts, both from the state of West Bengal but in two different agro-climatic regions, there are no feed restrictions on the goats. Animals were maintained in their ambient condition for four weeks prior to the study between 2019 - 2021.

2.2 Study areas

Among the fifteen agro-climatic zones, present studies have

been carried out in two different agro-climatic zones of India. The first one is Purulia, which belongs to Eastern Plateau and Hills region, [10-11] and the other one is Nadia belongs to the Lower Gangetic Plains region [10- 11]. The total goat population of the Purulia and Nadia are just more than eight thousand and nine thousand respectively [12]. Details of temperature and humidity have been collected from the state meteorological department during the study period are described in Table1.

2.3 Climatological measurement

The mean of the three years temperature with standard deviation has been calculated using MS-Excel 2019 (Table 1).

Table 1.

Mean maximum and minimum temperature of last three years

Temperature					
		March	**April**	**May**	**June**
Pre-Monsoon					
Purulia	Max (℃)	40.4 ± 2.3	42.6 ± 1.5	42.6 ± 1.5	38.6 ± 5.0
	Min (℃)	21.6 ± 10.9	21 ± 0.7	22.4 ± 1.1	23.2 ± 0.8
Nadia	Max (℃)	37 ± 2.45	38 ± 1	39.2 ± 1.5	36 ± 4.7
	Min (℃)	16 ± 3.9	19.4 ± 3.3	23.4 ± 1.5	23.6 ± 1.3
		November	**December**	**January**	**February**
Post-Monsoon					
Purulia	Max (℃)	32.0 ± 1.0	30.8 ± 2.2	30.0 ± 2.5	34.6 ± 2.5
	Min (℃)	13.6 ± 1.1	9.4 ± 1.7	8.6 ± 0.9	11 ± 2.5
Nadia	Max (℃)	31.6 ± 1.2	28.75 ± 0.5	28.6 ± 1.5	32.2 ± 3.6
	Min (℃)	14.4 ± 2.8	11.5 ± 1.3	10.2 ± 1.6	12.6 ± 3

Foot Note: standard deviation (SD) is denoted by ± and degree of centigrade is denoted by ℃).

2.4 Blood collection

About 4 mL of blood has been collected from apparently healthy goats using the purposive sampling technique [13], by jugular venipuncture in between 12 o'clock to 2 pm under the intense sun using disposable vacutainer needles and tubes [14]. The collected blood has been dispensed into di-potassium ethylene di-amine tetra acetic acid (K2EDTA) vials and labeled.

2.5 Lymphocytes isolations

Lymphocytes have been isolated according to the standard procedure [15]. Cell viability has been found through trypan blue exclusion assay [16]. Lymphocytes have been collected through centrifugation (4,000 g) and frozen at -20˚C for further use. Prior to the work, lymphocytes have been suspended in 1 mL of 10 mM Tris-HCL buffer, pH 7.4, containing 0.9% NaCl and 1% SDS (MERCK) and then boiled for 5 min.

2.6 SDS PAGE

The constitutive HSP 70 in Black Bengal goat lymphocytes has been separated using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) [17] with a 10% Tris-glycine acrylamide gel using the vertical mini gel apparatus. The gels have been subjected to electrophoresis (100V) till the tracking dye reached the base (for about 90 min) in 1X running buffer (1× Tris/glycine/ SDS buffer). The fractionated proteins have been visualized by Coomassie brilliant blue (Thermo Scientific™ 20278) staining.

2.7 Western Blot analysis

The gel slab was then transferred onto a nitrocellulose membrane (Sigma-Aldrich). The non-specific binding sites have been blocked after using 10 mL of cold blocking buffer (1X TBST with 5% w/v non-fat dry milk) for thirty minutes. The membranes have been incubated overnight at 45˚C with 5mL of blocking buffer containing antiserum (HSP 70 mouse anti-chicken monoclonal antibody, BioSource) against HSP 70 in a 1000X dilution. After the overnight incubation, the blots have been washed four to five times for five minutes each with 10 mL of cold (not chilled) blocking buffer. The blots then reacted with goat anti-mouse secondary antibody conjugated to horse-radish peroxidase (abcam®ab6789) for another sixty minutes

at room temperature with little agitation (dilution 1:1000 in 5% skim milk) and after that three 15-minute wash in 0.1% TBST. The beta-actin expression has been found with the same procedure as described above. Anti-mouse secondary antibody conjugated to horseradish peroxidase (abcam® ab97051) has been added [17].

2.8 Densitometric analysis

Relative density has been determined using a gel documentation system provided by UVP (UVP Inc.).

2.9 Statistical analysis

All analyses were performed using the general linear models' procedure of SPSS 27.02 (IBM SPSS). A one-way analysis of variance (ANOVA) has been performed to analyze the data and the means were separated by MS-Excel 2019. Duncan's multiple range tests (DMRT) have been performed [18].

3 RESULTS

Different HSP-genes have the capability to upregulation of their transcription immediately after the cell is exposed to diverse stressors by characterizing a typical endogenous mechanism in the defense of different essential proteins and are capable to resist any environmental harshness [19-20]. During the period of the present study, the highest HSP 70 expression has been observed in May in both of the studied regions. Even though, an increased HSP 70 expression has observed in May, which was coincide with the highest average ambient temperature for the month of the year, the highest ambient temperature during the month has been 42.6 ± 1,5˚C, while the lowest ambient temperature has been observed during the month of January again in Purulia (8.6 ± 0.9 ˚C) (Table: 1).

A more or less high HSP 70 expression has been observed during the season of pre-monsoon (Figure: 1) from March to June, which has been parallel with the higher temperatures during the pre-monsoon season (Figure: 1 a). The mean HSP 70 expression through the month of March in Purulia has been found significantly higher (P<0.01) than the HSP 70 expression observed in Nadia, also for this season, the lowest mean HSP 70 expression has been observed during this month in Nadia. During the month of April, there are no significant differences (P>0.05) in the mean HSP 70 expression has been found between the two zones. However, the highest mean HSP 70 expressions have been observed during May in both of the zones. The HSP 70 expression in Purulia May has been significantly higher (P<0.01) than the expression observed in Nadia. The mean HSP 70 expression in Nadia during June has been found significantly lower (P<0.05) than the expression observed during Purulia (Figure: 1 b).

More or less low HSP 70 expressions have been observed during the season of post-monsoon (Figure: 2) from November to February has been found to be concomitant with the lower temperatures during that season of post-monsoon. During November and December, there are no significant differences (P>0.05) in the mean HSP 70 expression that has been found between the two zones i.e. Purulia and Nadia (Figure: 2 a) However, low mean HSP 70 expressions were observed during November in both of the regions studied here also lowest mean HSP 70 expressions were observed in Nadia. The mean HSP 70 expression in Nadia during January has been found significantly lower (P<0.05) than the expression observed during Purulia. The mean HSP 70 expression through the month of February in Purulia has been found significantly higher (P<0.01) than the HSP 70 expression observed in Nadia (Figure: 2 b).

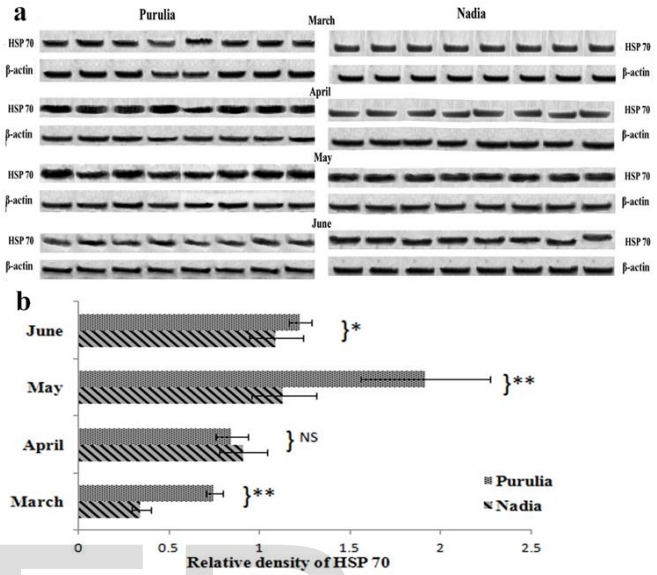

Figure 1: Relative protein expression (mean ± SD, band densitometry) of HSP 70 in goat lymphocytes; during pre-monsoon.

a. Western blot bands of β-actin (42 KD, used as reference protein) and HSP70 in goat lymphocytes during pre-monsoon in Purulia and Nadia. HSP70 and β-actin have been separated on SDS-PAGE and analysed by immuno-detection using HSP 70 and β-actin specific antibodies.

b. Relative densities of HSP 70 have been put into the horizontal axis whereas subsequent months have been added to the vertical axis of the bar diagram. Here a one-way ANOVA has been performed, where ** denotes P<0.01, * denotes P<0.05 and NS denotes no significant difference between means.

The expression of HSP 70 has been found in the May in Purulia is the highest level of HSP 70 expression has been recorded during this study (Figure. 3). The month of June also showed higher HSP 70 expression, which has been lower than the expression level of May and higher than the rest of the months. March and April sho a significantly (P<0.05) higher rate of expression than the months of post-monsoon seasons. During in post-monsoon season in Purulia, the months of December, January, and February show a similar level of HSP 70 expressions. The significantly (P<0.05) lowest level of HSP 70 expression has been found during the month of November in Purulia (Figure. 3 a).

Moreover, the relatively highest HSP 70 expression in Black Bengal goat lymphocytes has been observed during May and

June in Nadia. However, the expression level of HSP 70 during May has been higher than that in June but the difference has been not significant. The expression level of HSP 70 during these months i.e. May and June are significantly higher than the month of April, which on the other hand is significantly higher than the rest of the months of the year.

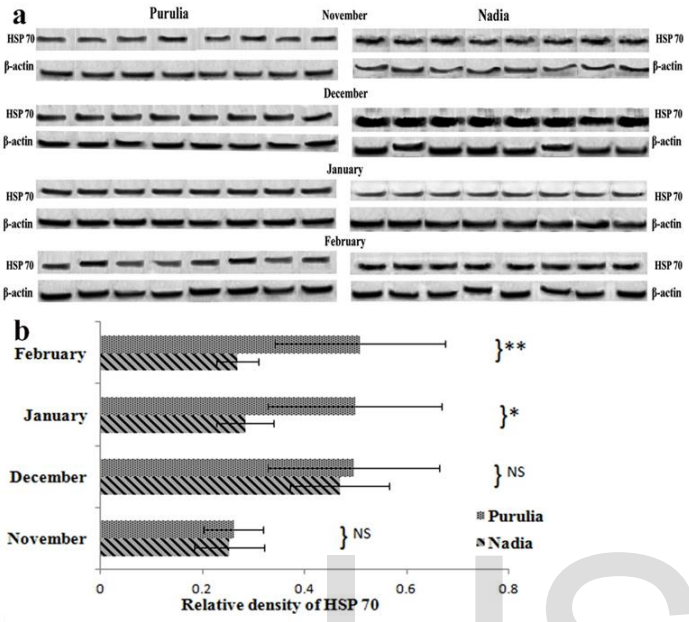

Figure 2: Relative protein expression (mean ± SD, band densitometry) of HSP 70 in goat lymphocytes; during post-monsoon.

a. Western blot bands of β-actin (42 KD, used as reference protein) and HSP70 in goat lymphocytes during post-monsoon in Purulia and Nadia. HSP70 and β-actin have been separated on SDS-PAGE and analysed by immuno-detection using HSP 70 and β-actin specific antibody.

b. Relative densities of HSP 70 have been put into the horizontal axis whereas subsequent months have been added to the vertical axis of the bar diagram. Here a one-way ANOVA has been performed, where ** denotes P<0.01, * denotes P<0.05 and NS denotes no significant difference between means.

December, it has been found that the expression level of HSP 70 has been significantly higher than the month of March in Nadia. There is a similar level of HSP 70 expression has been found significantly lowest during the months of November, January, and February (Figure. 3 b).

Stress is the consequence of environmental forces continuously acting upon living organisms which interrupt homeostasis resulting in new adaptations that can be unfavourable or favourable to that particular living organism [21]. There are different types of stressors but thermal stress has been considered the major worry in the context of declining livestock productivity in arid, tropical as well as subtropical areas [10, 22]. During the month of March of the season of pre-monsoon the ambient temperature has been found lower in Nadia than Purulia (Table 1), this has been the cause behind the lower expression of HSP 70 in Nadia. After the month of March, a severe heat stress condition arises in April, so, there may be a chance of a similar kind of HSP70 expression during this month in both of the regions studied here. In the month of May, the highest ambient temperature of the two different regions has been observed, which may be one of the key factors that elevated the HSP70 expression in the Black Bengal goat lymphocytes in both of the agro-climatic regions studied here.

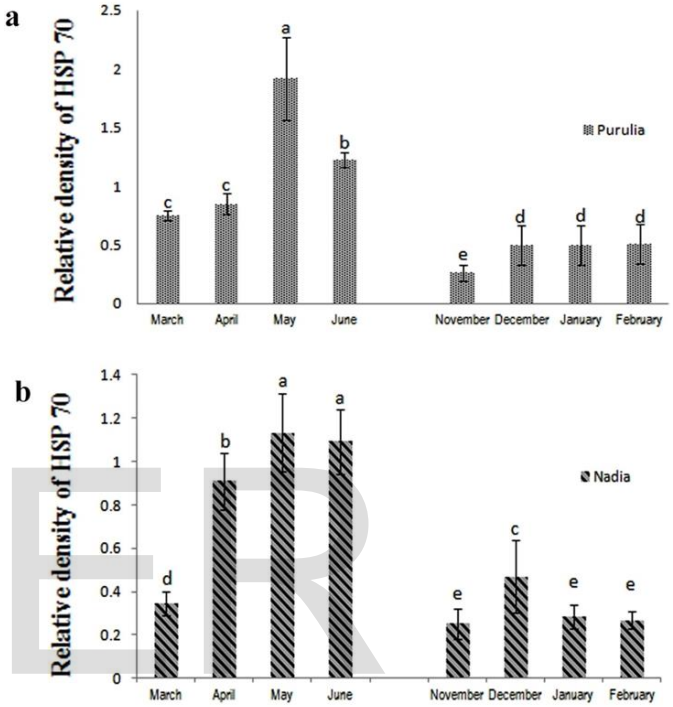

Figure 3: Relative protein expressions (mean ± SD, band densitometry) of HSP 70 in goat lymphocytes; during pre and post-monsoon in Purulia and Nadia.

a. Relative protein expressions of HSP 70 in goat lymphocytes; during pre-monsoon and post-monsoon seasons in Purulia. Means bearing different superscripts (a–e) differ significantly (P <0.05) at different months where the study animals are exposed to different ambient temperatures. Here the month May shows the highest level of the HSP 70 expressions, whereas the month November, has the lowest level of the expressions.

b. Relative protein expressions of HSP 70 in goat lymphocytes; during pre-monsoon and post-monsoon seasons in Nadia. Means bearing different superscripts (a–e) differ significantly (P <0.05) at different months where the study animals are exposed to different ambient temperatures.

4 Discussions

The elevation of the ambient temperature secondarily upregulated the HSP70 expression in the body of the animal and this situation has formed due to the combined effects of the thermal stress. Animals from both regions have been suffering from similar kinds of thermal stress in the month of May,

which leads to an elevated HSP 70 expression during this month. The present result suggested that the goats from Purulia have been suffering more from heat stress. Similarly, during in the month of June local environmental temperature has been found higher in Purulia and because of this the difference between the mean HSP 70 expressions in the two regions has been found significant (Figure. 1). Heat shock Protein is known as a chaperon, which has a crucial role to stabilize the overall structure of proteins and endurance of the ability of survival of cells at the period of high heat stress [23]. An elevated expression of HSP 70 can be a response to the environmental or any kind of stress and thus secondarily increase cell survival through a protective mechanism that shield the proteins from degradation and providing the refolding of the protein [24-25]. Different previous studies an induction of HSP 70 was reported due to heat stress in buffalo lymphocytes [26], in the kidneys of goats 27, in the PBMC of goats [28] and also in goat lymphocytes [29].

During the post-monsoon, no significant difference was observed between the means expressions of the HSP 70 have been found during the months of November and December. This may be because; November and December are the initial months of post-monsoon season after the long season of monsoon. The changes in the ambient temperature can be found similarities between the two regions studied here. Moreover, the month of November has been showing the least HSP 70 expression in both of the regions. However, Black Bengal goats of Nadia have been found the lower (not significantly) expressions of the HSP70 in the lymphocytes between them also it has been the lowest expressions of the HSP70 in Black Bengal goat lymphocytes of the present study. On the other hand, throughout the month of January, the HSP70 expression has been found significantly higher in Purulia. The temperature rapidly falls down during this period as well as there are huge thermal fluctuations can be observed between day and night during Purulia. The higher HSP70 values found during this period may not be due to the effect of the higher ambient temperature. However, it could be due to the combined effects of lowering the ambient temperature as well as temperature fluctuations between the periods of day and night, which may lead to a stage where an animal could be suffered from cold stress. A similar type of result has been found during the month of February.

In Purulia, the month of May shows the highest level of HSP70 expressions in Black Bengal goat lymphocytes, whereas the expression level in June is just less than that of May. It may be the cause of the amelioration of the animal to the surrounding environment. This may be due to the exposure of the animals to the prolonged heat for several days of pre-monsoon. On the other hand, there are no dissimilarities between March and April regarding to the HSP70 expressions in Black Bengal goat lymphocytes. November shows least level of HSP expression due to most suitable climate for goat's normal physiological conditions. However, in Purulia, the months of December, January and February have been showing (Figure: 3) the similar levels of HSP70 expressions in Black Bengal goat lymphocytes due to similar kind of ambient temperature and other possible environmental factors. In Nadia, months of May and June both show a higher level of HSP70 expressions, this may be due to the higher ambient temperature continues during these months. Some other lower levels of HSP70 expressions in goat lymphocytes have been observed during in April, December, March and November respectively [30-31]. However, in Nadia the months of January and February have been showing same level of HSP70 expressions as similar as the situation describe earlier in Purulia (Figure. 3).

5 Conclusions

However, it can be suggested that not only the highest ambient temperature but also the thermal fluctuations may refer as an environmental stressor to the Black Bengal goats. Enhanced HSPs expression signifies the assurance of security from toxic effects of the thermal stress. It has been known for quite some time that goats have been performing better than other ruminants of economic importance, during harsh ambient temperature. The adaptableness of the goats to harsh atmosphere have been gained primarily due to their capacities for water preservation, moderately high sweating rate, comparatively low basal metabolism rate or BMR as well as a high rate of respiration and skin temperature with stable heart rate. On the basis of relative HSP 70 density in the Black Bengal goat lymphocytes, present study reveals that the HSP 70 expressions increase with the degree of thermal stress as well as with the magnitude of thermal fluctuations experienced by the animals.

6 Significance Statements

Present work has been initiated to attain a perception of the effect of heat-related stress on the expression profile of Heat shock protein (HSP) in goats kept in two different agro-climatic regions during pre-monsoon and post-monsoon seasons. DNA products have been subjected to SDS-PAGE followed by western blot. The expression of HSP 70 in the goats, is found significantly higher ($P<0.05$) during pre-monsoon as related to post-monsoon season in both of the region. It is found that the expression of heat shock protein in the Black Bengal goat lymphocyte is increased with the degree of thermal stress as well as with the magnitude of thermal fluctuations.

7 Conflict of Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgment

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