



Heat shock protein and gene regulation in goats during heat stress

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ABSTRACT

Heat shock proteins (HSPs), also known as molecular chaperons are prominent stress markers. Heat shock proteins consist of highly conserved protein expressed at the time of stress, and play an important role in adaptation to the environmental stress. Although, the expression pattern of HSP70 gene is species and breed specific, variations in adaptation and thermal tolerance is due to the nature of environment and adaptive capacity of a species. The present study was conducted to evaluate the adaptive capability of different goat (*Capra hircus*) breeds, i.e. Jamunapari, Barbari, Jakhrana and Sirohi under peak dry summer. The targeted gene HSP70 (HSPA6) was evaluated for this purpose using specific primers. The expression of HSP70 gene and protein was estimated by RT PCR and ELISA kits respectively. The expression of HSP70 gene was found lowest in sirohi breeds implying that this breed was more adapted followed by Jakhrana, Barbari and Jamunapari during peak summer season. Whereas, the level of HSP70 protein in blood was significantly higher in Jamunapari, followed by Barbari, Jakhrana and lowest in Sirohi. These results indicated that, during adverse climatic stress the quantum of expression (HSP70 gene and protein) was more in Jamunapari. It is concluded that Sirohi breed is better adapted to heat stress than Jamunapari, Jakhrana and Barbari and HSP70 may be a potential molecular biomarker in the future for selection of climate resilient animals.

Keywords: Adaptation, Expression, Heat stress, Heat tolerance, HSP70

Goats are distributed in different ecology and supposed to be more tolerant to extreme weather conditions because of their metabolic size and water conservation capacity (Silanikove 2000). Exposure of goats to ambient temperatures above the upper critical limit results in heat stress (Yousef 1985). Goats are more resilient and adjust to different higher environment by expressing different adaptive strategies (Silanikove *et al.* 2015) and generally utilize its thermoregulatory mechanism to relieve from stress. Cellular tolerance to heat stress is regulated by heat shock proteins (HSPs) and these proteins are responsible for maintaining the balance in organism and to acclimatize the stress (Morange 2006). HSPs are released intra cellularly and extra cellularly in response to various environmental stresses (Sonna *et al.* 2002, Hecker *et al.* 2011) in inducible form and can be an indicator of stress in cells (Sonna *et al.* 2002). The regulation of HSP production is critical to cell survival and among the HSPs, Hsp70 has a significant role in cell thermo-tolerance and animal survival (King *et al.* 2002). Understanding the regulation of heat stress at cellular level and expression pattern of Hsp70 gene will throw light on the mechanism of heat stress adaptation in goats. Hsp70

gene expression has been positively correlated with variations in thermo tolerance in different organisms since this protein plays a multifarious role at the cellular and tissue levels. The present study was therefore carried out to analyse heat shock protein 70 gene expression in different goat breeds in semi-arid climate.

MATERIALS AND METHODS

Animals, management and experimental protocol: The present study was carried out on 40 male, 10 each of Jamunapari, Barbari, Jakhrana and Sirohi goat breeds belonging to arid and semi-arid region of India. All the experimental goats were carrying an average body weight of 17.21 ± 0.71 kg and 7 months of age. The experiment was carried out during summer season (May–June) for 45 days. All experimental goats were housed under well ventilated animal shed ($27^{\circ}10' \text{ N}$, $70^{\circ}02' \text{ E}$) and were apparently healthy and free from any anatomical and physical abnormalities. All experimental goats were maintained under semi intensive system of feeding management, allowed to graze for 6 h (08:00–14:00 h) and were supplemented with gram straw (*ad lib.*) and concentrate mixture (1.5% of body weight) and green fodder daily. All the animals were closely monitored and were provided similar managerial inputs during whole experimental period. The observations on meteorological

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variables (relative humidity, temperature, Dry bulb temperature, Wet bulb temperature) were collected and temperature humidity index (THI) was calculated.

Blood sample collection, RNA extraction and cDNA preparation: Blood samples were collected aseptically from all experimental goats through jugular vein around 1400 h during grazing hours and stored under refrigeration condition for further use. Total RNA was isolated using TRI Reagent (T3809-Sigma) method and impurities of genomic DNA was finally digested by RNase-free DNase I (NEB, US). Purity of RNA was checked by Nanodrop spectrophotometer (QIAxpert, Qiagen, Germany) and was preserved at -20°C . Further, preserved RNA (1 μg) was used for cDNA preparation using high script tool one step kit (Biotools B and M Labs, Spain) in 50 μl reaction. One reaction mixture includes Master mix 25 μl , MgSO_4 (100 mM) 8 μl , primer 1 μl , PCR astringent 7 μl , RT enzyme 0.5 μl , nuclease free water 6.5 μl and RNA template 2 μl . PCR was carried out at following conditions—Initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 58°C to 60°C and extension at 72°C for 30 sec followed by final extension step for 7 min. The cDNA was stored at -20°C for further use. Control was also set during this process without reverse transcriptase. Primer of HSPA 6 gene (forward CTCAGACACCGAGCGGCTGG and reverse CCCC GCGGTAGGACACGCG) was used with an annealing temperature 59°C and produce 197 bp amplified product. Similarly, the primer of β -actin (forward CCAACCGTGAGAAGATGACC and reverse CGCTCCGTGAGAATCTTCAT) was used with annealing temperature 60°C and produce 247 bp amplified product.

Gene expression analysis and quantification of HSP70 protein: Analogous expression level of mRNA transcripts of HSPA6 genes was measured by real-time PCR in Roche Light Cycler 480, Version 1.5 (Roche Diagnostics, Switzerland) using the Quantimix easy SYG kit (Biotools B and M Labs, Germany). Real time PCR was used for expression of HSPA6; housekeeping gene and nuclease free water was used as negative control. A master reaction was prepared following the protocol of manufacturer (easy SYG kit). The master reaction contained, master mix 10 μl , 50 mM MgCl_2 22.5 μl , primer (forward and reverse) 1 μl each and 2 μl of cDNA to make final volume of 20 μl . The PCR conditions were set to optimal amplification, initial denaturation at 95°C for 15 min, followed by denaturation at 94°C for 10 sec, annealing at 59°C — β actin and HSPA6 for 30 sec, extension at 72°C for 30 sec for 35 cycles and last cycle at 95°C for 30 sec and final melting step at 60°C to 95°C gradual increment @ 0.5/sec and final cool down. The relative expression of HSPA6 was calculated by $\Delta\Delta\text{ct}$ method (Livak *et al.* 2001). Plasma samples stored earlier at -20°C were used in duplicates for ELISA (Blue gene, China), specific for goats and optical density (OD) was determined at 450 nm (Spectra Max plus 384, USA). The final concentration of HSP70 in ng/ml was calculated by regression standard curve.

Statistical analysis: All the analysis was performed in

duplicate. Results were expressed as the means $\pm$ SEM. A difference with value $P < 0.05$ was considered statistically significant. The different group mean were compared by student's *t* test by the SPSS Version 16.0.1 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Gene expression analysis in different goat breeds under heat stress: THI varied from 73.72 to 93.16, indicating that the animals were under stress during the experiment period (Table 1). Relative expression of HSPA6 genes varied amongst the four goat breeds and its expression pattern indicated that Jamunapari goats had highest expression of HSPA6 gene (1.61 folds) during heat stress period followed by Barbari (1.36 folds), Jhakrana (1.26 folds) and lowest in Sirohi (1.16 folds). The Sirohi goat had lowest expression pattern indicating better adaptive capability during heat stress period. The units of HSP70 protein concentration was found highest in Jamunapari (76.29 ng/mL) followed by Barbari (73.29 ng/mL), Jhakrana (72.34 ng/mL) and lowest in Sirohi (67.54 ng/mL). These results depicts that Jamunapari goat breed is more sensitive and Sirohi is most adaptive to heat stress condition as compared to remaining two breeds.

Table 1. Environmental condition during dry summer

Season	Environmental parameters Dry hot Summer
Duration (days)	45
Temp range ($^{\circ}\text{C}$)	33.1 $^{\circ}\text{C}$ –46.5 $^{\circ}\text{C}$
Humidity range (%)	13.2–61.8%
THI range	73.72–93.16

The mRNA level of HSP70 was observed higher in all the goat breeds and highest expression was observed in Jamunapari goats. These findings are corroborated with the view of Patir *et al.* (2007) and (2010) who reported that heat stress is responsible for HSP70 expression in bovine lymphocytes. The higher expression of HSPA6 has also been reported in human blood in response to heat stress (Sonna *et al.* 2002, 2004) and similar observation has been reported in camel indicating that the temperature change affects the level of expressed HSP70 (Garbuz *et al.* 2011, Ulmasov *et al.* 1993). Stress tolerance is a critical characteristic and its methodology is not fully understood (Mizzen *et al.* 1988). The findings of the present study showed that gene expression of HSP70 is breed specific and confirms the view of Dangi *et al.* (2012), who reported that in Indian conditions, tropical goats have showed significantly higher HSP70 mRNA expression in PBMCs during peak summer season compared to the winter season. On contrary, the goats of temperate region did not show significant levels of HSP70 transcriptional response between peak summer and winter season (Dangi *et al.* 2012). In yet another study involving heat stress of goat

PBMCs *in vitro* showed significantly higher up-regulation of HSP70 mRNA compared to unstressed cells (Mohanarao *et al.* 2014).

Stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be detrimental or advantageous to the animal (Stott 1981). Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, subtropical, and arid areas (Silanikove *et al.* 1997). The ability of animal to acclimatize and produce under the specific climate condition signifies the adaptation to a particular environmental niche. *In vitro* studies on heat stress exposure and recovery effect on HSP expression are undertaken by different researchers (Kishore *et al.* 2016, Deb *et al.* 2014). Hsp70 concentration in blood is also a reliable indicator of chronic stress in feedlot cattle (Gaughan 2013). There is considerable evidence that the synthesis of Hsp70 is temperature-dependent (Zulkifli *et al.* 2003) and thus Hsp70 responses could be considered as a cellular thermometer. The acute phase includes the heat shock response at the cellular level and the chronic phase results in acclimation to the stressor and involves the reprogramming of gene expression and metabolism (Horowitz 2002). In ruminants, there is a loss in productivity as animals pass through the acute phase and return to productivity as they undergo acclimation to the stress (Collier *et al.* 2006). The species specific difference in HSP70 is due to variation in thermal tolerance (Silanikove 2000, Hightower *et al.* 1999).

Although identical pattern of expression in Sirohi and Barbari goat breed was reported (Banerjee *et al.* 2014) but the expression level in Sirohi goats was observed to be relatively lower. Sirohi is a breed of arid regions and is highly adapted to heat stress whereas Barbari is a breed of semi-arid regions and is comparatively less well adapted to heat stress conditions. Similarly, under the present study, Sirohi breed exhibited a higher mRNA level of Hsp70 gene indicating that it was better at regulating heat stress compared to other three breeds. Especially, HSPA1A mRNA showed higher expression during winter in heat-adapted breeds (Sirohi and Barbari) compared to cold-adapted breeds (Gaddi and Chegu) again re-iterating the fact that heat-adapted breeds are also equal prone to cold stress as cold-adapted breeds to heat stress (Banerjee *et al.* 2014). There are also considerable points to leverage our findings that portrayed differences between breeds with regard to heat stress in ruminants which has the ability to subdue the metabolism thereby negatively influencing the body heat production and augmenting its effective dissipation (Silanikove 2000, Kadzere *et al.* 2002). It has been suggested that the expression of Hsp70 was significantly higher during the summer season as compared to the winter in tropical region goats, which might play an important role in thermal stress tolerance against harsh environmental conditions (Dangi *et al.* 2012).

Under the present study, the values of THI was observed under the mild and severe stage during whole experimental

period causing heat stress to the goats and the elevated values of heat stress affects the thermoregulatory mechanisms (Kadzere *et al.* 2002, Brown *et al.* 2009). Amelioration of heat stress begins at the cellular level, where there is an interplay of various molecules including activation of heat shock transcription factor 1 that positively correlates to an increased expression of heat shock proteins by binding to promoter region of heat shock elements (HSE) of the HSP genes (Ruell *et al.* 2009). The present finding is in agreement of studies carried out in bovine lymphocytes (Ptir *et al.* 2010, Mishra *et al.* 2010), in bovine PBMCs (Kishore *et al.* 2013), in caprine PBMCs (Dangi *et al.* 2012), and in kidneys of goats (Zulkifli *et al.* 2010). The above results indicated that the expression level of HSP70 was positively correlated to the level of heat stress based on THI parameters. The fluctuations among the breeds studied with respect to the levels of HSP70 both by qRT-PCR (genotypic levels) and ELISA (phenotypic levels) suggests that the breeds that best suit to a particular micro-environment or micro-climatic conditions will help tap the production potential.

Based on the present results, it is concluded that Sirohi breed is better adapted to heat stress than Jamunapari, Jakhrana and Barbari and HSP70 may be a potential molecular biomarker in the future for selection of climate resilient animals

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