

Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats

Satyaveer Singh Dangi · Mahesh Gupta · Vimla Nagar · Vijay Pratap Yadav · Saroj K. Dangi · Om Shankar · Vikrant Singh Chouhan · Puneet Kumar · Gyanendra Singh · Mihir Sarkar

Received: 6 December 2013 / Revised: 20 February 2014 / Accepted: 20 February 2014 / Published online: 9 March 2014
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Abstract Six, nonpregnant, Barbari goats aged 4–5 years were selected for the study. For the first 6 days, the animals were kept in psychrometric chamber at thermoneutral temperature for 6 h each day to make them acclimated to climatic chamber. On the 7th day, the animals were exposed to 41 °C temperature for 3 h and then to 45 °C for the next 3 h. Cardinal physiological responses were measured, and blood samples (3 ml) were collected at 1-h interval during the heat exposure period and then once after 6 h of the heat exposure. The rectal temperature (RT) and respiratory rate (RR) increased significantly ($P < 0.05$) during the heat exposure compared to pre- and postexposure. The relative messenger RNA (mRNA) expression of heat shock protein (HSP)60, HSP70, and HSP90 increased significantly ($P < 0.05$) within 1 h after exposure to heat stress at 41 and 45 °C and decreased significantly ($P < 0.05$) in next 2 h but remain significantly ($P < 0.05$) elevated from preexposure. HSP105/110 relative mRNA expression level remained unchanged during the first 4 h, and thereafter, it increased significantly ($P < 0.05$) and reached the peak at 6 h. Relative protein expression pattern of HSPs during exposure to heat stress showed similar trend as observed for the relative mRNA expression. Given the response sensitivity and intensity of HSP genes to environmental stresses, HSP70 was found to be the most sensitive to temperature fluctuation, and it could be used as an important molecular biomarker to heat stress in animals.

Keywords Goat · Heat stress · HSP · THI · Physiological response

Introduction

The energy expenditure to maintain normal body temperature is minimal and independent of environmental temperature in the homeothermic animals in their thermoneutral zone (Yousef 1987). Outside of this zone, the animal experiences stress to maintain homeothermy. Animals experience heat stress when environmental variables such as ambient temperature, humidity, solar radiation, and movement of air surpass the upper limit of the thermoneutral zone. The ability of ruminants to regulate body temperature is species- and breed-dependent. Although the lower critical temperature for goat is not specified, the limits of thermoneutrality for goats may be taken as a climatic environment having an air temperature of 13–27 °C, relative humidity of 60–70 % and wind velocity of 5–8 km/h, and a medium level of solar radiation (Misra and Puneet 2009).

Multiple cellular mechanisms are displayed to counter the stress conditions. One of the cellular responses includes altered expression of stress genes (Collier et al. 2008). Under thermal stress, transcriptional activation and accumulation of a set of proteins called “heat shock proteins” (HSPs) is well-known (De Maio 1999; Kregel 2002). HSPs constitute a large group of chaperone proteins that is classified into several families on the basis of their molecular size and amino acid sequence similarity. The heat shock response confers transient thermal tolerance, in part due to the expression of HSPs. HSPs are responsible for maintaining the balance between survival and an effective immune system in the organisms in order to acclimatize the stress (Morange 2006). The mechanism by which the HSPs confer stress tolerance is not completely understood but may relate to the important role of HSPs in

S. S. Dangi · M. Gupta · V. Nagar · V. P. Yadav · O. Shankar · V. S. Chouhan · P. Kumar · G. Singh · M. Sarkar (✉)
Division of Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243122, India
e-mail: msarkar24@gmail.com

S. K. Dangi
Division of Bacteriology & Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243122, India

the processing of stress-denatured proteins (Mizzen and Welch 1988).

HSP expression acts as a potential indicator of animal adaptation to harsh environmental stress (Valenzuela 2000; Hansen 2004). There exists a strong correlation between the induction of HSPs and the induction of thermotolerance by preventing activation of stress kinases (Gabai et al. 1997). Heat stress affects the productivity of goats, but very little information is available about how they respond to heat at a cellular level. Earlier studies report an induction of HSPs due to thermal stress in goat PBMCs (Dangi et al. 2012; Sharma et al. 2013).

To explore the role of HSPs in short-term thermal adaptation in goats, the physiological responses and expression profiles of HSP60, HSP70, HSP90, and HSP105/110 in goats exposed to short-term heat stress, simulating the natural environmental condition of peak summer months of tropical regions, were studied.

Materials and methods

Experimental site

The present study was carried out at the Division of Physiology and Climatology, IVRI, India. Location of this institute is 28° 21' N and 79° 24' E having subtropical climate. The experiment was conducted in the first week of March 2013 when the mean environmental temperature and relative humidity were 17–23 °C and 78–91 %, respectively.

Animals

Six, healthy, nonpregnant, Barbari goats aged 4–5 years and weighing 23–25 kg were selected for the study. The animals were housed in a well-ventilated room adjoining to psychrometric chamber. The animals had the access to ad lib feed and drinking water. The minimum temperature, maximum temperature, relative humidity (RH), and temperature humidity index (THI) were recorded at the experimental site at 0700, 1300, and 1800 h daily for seven consecutive days, before and on the day of short-term heat exposure to goats (Table 1). Temperature and humidity were also monitored during the experiment period in the psychrometric chamber.

The THI, a measure of thermal load on animals, was calculated from the following formula (Tucker et al. 2008):

$$\text{THI} = (1.8 \times \text{AT} + 32) + [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times \text{T} - 26)]$$

where AT=air temperature, °C, and RH=relative humidity, %.

THI values were categorized as follows: ≤ 70 =comfortable, 71–74=mild stress, 75–78=stressful, 79–85=severe stress, and >86 =lethal.

Experimental design

The experiment was conducted for the period of 7 days; during which, the animals were housed in the room adjoining to the psychrometric chamber for 6 days and on the 7th day exposed to heat stress in the psychrometric chamber. A climatic chamber (7.5×7.5×2.5 m³) insulated and thermostatically fitted with heat convector was used for exposing the animals. During first 6 days, the animals were kept in the psychrometric chamber at thermoneutral zone (25 °C) for 6 h (1000 to 1600 h) each day to make them acclimated to the climatic chamber. The thermal environmental data, rectal temperature (RT), heart rate (HR), and respiratory rate (RR) values of goats were recorded for 6 days during preexposure periods. On the 7th day, the animals were exposed to 41 °C temperature for 3 h (1000 to 1300 h) and to 45 °C for 3 h (1300 to 16:00 h). RR, HR, and RT were measured, and blood samples (3 ml) were taken in heparinized vacutainers at 1000h (preexposure); 1100, 1200, 1300, 1400, and 1500h (during exposure); 1600h (at the end of exposure); and 2200 h (6 h postexposure). Heparinized blood was used for messenger RNA (mRNA) isolation.

Primers

The primers of HSP60, HSP70, HSP90, and β -actin were taken from published sequence (Dangi et al. 2012), and the primers of HSP105/110 and ribosomal protein S15a (RPS15A) were designed by the Fast PCR (Version: 6.2.73) software. The sequences and expected PCR product length are shown in Table 2.

PBMC isolation

PBMCs were isolated by density gradient centrifugation method using Histopaque 1077 (Sigma, USA) as per manufacturer's instruction. The cells were washed thrice in phosphate buffered saline (PBS) (pH 7.4), and finally, the cell pellet was obtained.

Total RNA extraction and quality determination

Total RNA was isolated from PBMCs using ONE STEP-RNA Reagent (Bio Basic Inc., Canada) following manufacturer recommendations. To remove any contamination by genomic DNA, the samples were submitted to digestion with DNase 1 (D-5025; Sigma). The extracted RNA was quantified spectrophotometrically, and the OD260/OD280 was used for evaluation of quality. The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1 % agarose gel.

Table 1 Ambient temperature, relative humidity, and temperature humidity index data recorded for 6 days before and during short-term heat exposure of the goats ($n=6$)

	6 days before short-term heat exposure			During short-term heat exposure and recovery period (time in hours)							
	Minimum	Maximum	Mean±SEM	1000 (0)	1100 (1)	1200 (2)	1300 (3)	1400 (4)	1500 (5)	1600 (6)	2200 (R6)
Ambient temperature (°C)	13.58±0.55	27.10±0.61	19.84±0.61	21.7	41	41	41	45	45	45	23.1
Relative humidity (%)	57.38±6.54	99.00±00	86.38±1.12	80	49	47	44	39	36	32	66
Temperature humidity index (THI)	56.80	80.65	66.97±1.01	69.62	92.39	91.86	91.08	94.55	93.64	92.43	70.67

Mean±SEM of thermal environmental data

Quantitative RT-PCR analysis

Constant amounts of 1 µg of total RNA was reverse-transcribed using iScript™ Select cDNA synthesis kit (Bio-Rad Laboratories, CA) and oligo-dT18 primer at 42 °C for 90 min. The resulting complimentary DNAs (cDNAs) were used in quantitative real-time polymerase chain reaction (qRT-PCR). The qRT-PCR for HSPs and housekeeping genes (β -actin and RPS15A) was performed in duplicate using HotStart-IT SYBR Green qPCR Master Mix (2X) kit (Affymetrix, Inc., USA) in an Agilent Stratagene Mx3005P Real-Time qPCR System (Stratagene) as per manufacturers' instructions. The real-time PCR reactions were carried out in 20 µl reaction systems with 10 µl 2X HotStart-IT SYBR Green qPCR Master Mix (Affymetrix, Inc., USA), 0.5 µl forward primer (0.25 µM), 0.5 µl reverse primer (0.25 µM), 1 µl cDNA template equivalent to 100 ng total RNA, and 8 µl DNase, RNase-free water (MP Biomedicals, France/USA). Thermal cycling conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, 58 °C for 30 s, and 72 °C for

30 s. A melt curve analysis was added (65 to 95 °C, with increments of 0.58 °C/s) to demonstrate the specificity of the PCR products, as displayed by a single peak. A negative control without cDNA was run in each assay. Meanwhile, a non-reverse transcription (NRT) control reaction using the DNase 1-treated RNA as template was performed to ensure no genomic DNA contamination of RNA. After the run has ended, cycle threshold (C_t) values and amplification plot for all determined factors were acquired by using the "SYBR Green (with dissociation curve)" method of the real time machine (MxPro3005P Stratagene, Agilent Technologies, USA). Real-time PCR efficiencies were determined by amplification of a standardized dilution series, and slopes were obtained. The specificity of desired products was documented using analysis of melting temperature, which is product-specific, and a high-resolution gel electrophoresis to verify that transcripts were of exact molecular size. The mRNA expression at preexposure was used as calibrator for obtaining relative mRNA expression. The geometric mean of C_t values of the β -actin and RPS15A was used as C_t of reference gene. Efficiency-

Table 2 Gene transcripts, primer sequences, and efficiency and resulting fragment size

Target	Sequence of nucleotide	Fragment size (bp)	Efficiency (%)	EMBL/reference
HSP60	For: 5'-ACTGGCTCCTCATCTCACTC-3' Rev: 5'-TGTTCAATAATCACTGTCCTTCC-3'	148	108	Dangi et al. (2012)
HSP70	For: 5'-GACGACGGCATCTTCAAG-3' Rev: 5'-GTTCTGGCTGATGTCCTTC-3'	132	109.5	Dangi et al. (2012)
HSP90	For: 5'-GCATTCTCAGTTCATTGGCTATCC-3' Rev: 5'-GTCCTTCTTCTCTCCTCCTCTTC-3'	190	100.2	Dangi et al. (2012)
HSP105/110	For: 5'-CACAGCCCCAGGTACAAACT-3' Rev: 5'-TCCCTAACTGCCAGACCAAG-3'	204	99.9	NM_001075302.1
β -Actin	For: 5'-AGTTCGCCATGGATGATGA-3' Rev: 5'-TGCCGGAGCCGTTGT-3'	54	107.4	Dangi et al. (2012)
RPS15A	For: 5'-AATGGTGCATGAATGTC-3' Rev: 5'-GACTTTGGAGCACGGCCTAA-3'	100	103.6	XM_005679050.1

EMBL—accession number or reference of published sequence

corrected relative quantification of mRNA was obtained by Pfaffl (2001) method. For this, efficiencies of primer were determined by serial dilution of template cDNA sample and running in triplicate. The efficiency for the primers of different factors is given in Table 2.

Western blot

Total proteins were extracted from PBMCs, and different serial dilutions were used for the optimization of protein loading for quantitation. In each well, 40 μg (for HSP110, HSP90, β -actin) and 60 μg (for HSP70, HSP60) protein were loaded and resolved by SDS-PAGE on a 12.5 % (*w/v*) uniform gel (Laemmli 1970). After electrophoresis, the proteins were electrotransferred onto polyvinylidene difluoride (PVDF) membrane and blocked with 3 % bovine serum albumin (BSA) before incubation with primary antibodies viz. anti-HSP60 (MA1049, Lot # 08A12) and anti-HSP90 (MA1051, Lot # 08A12) at a 1:200 dilution, anti-HSP70 (MA1050, Lot # 08A12) at a 1:500 dilution, anti-HSP105/110 (PA1608, Lot # 0161212060831) at a 1:400 dilution, and anti- β actin (SC-81178, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at a 1:500 dilution for overnight at 4 °C. After incubation, the membrane was washed thrice with PBS-T (PBS+0.01 % Tween 20) for 5 min each then respective secondary antibody (goat anti-mouse IgG-HRP, sc-2005, Lot # C2011 and goat anti-rabbit IgG-HRP, sc-2004, Lot # B1711, Santa Cruz Biotechnology, USA.) conjugated with horse radish peroxidase was added and incubated at 37 °C for 1 h. After washing three to four times in PBS-T solution, the positive signals were detected by incubating the membrane using 0.06 % 3,3'-diaminobenzidine tetrahydrochloride (DAB, Genei) in 1X PBS (pH 7.4) containing 0.06 % H_2O_2 for 10–15 min. The bands were visualized under white light and recorded on a gel documentation system (DNR Bio-Imaging System, Minibus Pro). Densitometry of the immunospecific bands was performed using ImageJ 1.43U software (National Institute of Health, Bethesda, MD).

Statistical analyses

In accordance with the Pfaffel method (2001), the unstressed (0 h) sample was used as the calibrator. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA). Pearson's correlation was employed in the analysis of the relationships between the THI, basic physiological variables, and relative mRNA expression of HSPs. Mean values were expressed with $\pm\text{SEM}$.

Results

Thermal environmental data recorded during the study period

The mean air temperature, relative humidity, and THI recorded for 6 days were 19.84 ± 0.61 °C, 86.38 ± 1.12 %, and 66.97 ± 1.01 , respectively. On the day of exposure, the thermal microenvironmental variables recorded during preexposure, with 1-h interval on exposure to short-term heat stress, and 6 h postexposure are shown in Table 1.

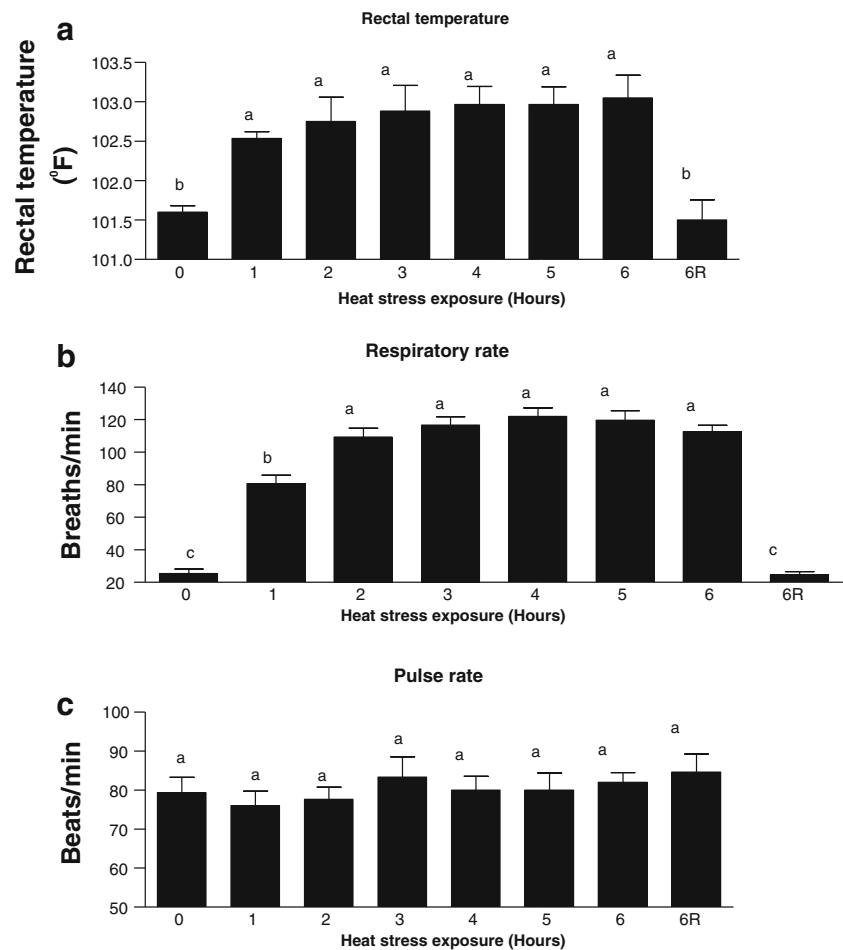
Rectal temperature and respiratory and heart rates

The effects of short-term heat exposure on RT, RR, and HR of the goats are shown in Fig. 1a–c. The RT, RR, and HR recorded for six consecutive days preexposure periods were 101.7 ± 0.09 , 24.86 ± 3.4 , and 78.6 ± 4.5 , respectively. The preexposure RT, RR, and HR values recorded in goats were not significantly ($P>0.05$) different from one another among days. The RT recorded during exposure hours was significantly ($P<0.05$) higher than pre- and postexposure hours. The RT rose progressively from the preexposure value of 101.6 ± 0.08 °F to a peak value of 103.05 ± 0.29 °F during the 6th hour of heat exposure; thereafter, the RT decreased to preexposure level. The RR of the goats rose progressively from the preexposure value of 25.33 ± 2.86 breaths/min to a peak value of 122 ± 5.22 breaths/min during the 4th hour of heat exposure; thereafter, the RR fluctuated between 112 and 119 breaths/min and became normal after 6-h recovery period at room temperature. However, no significant change was observed in heart rate.

Responses of HSP genes to short-term heat stress

The expression profile of HSP60, HSP70, HSP90, and HSP105/110 genes in PBMCs of the goat on exposure to short-term heat stress have been depicted graphically in Fig. 2a–d, respectively. Although all the four genes were induced under thermal stress, different expression patterns were observed. The relative mRNA expression of HSP60 and HSP90 increased significantly ($P<0.05$) within 1 h after exposure to heat stress at 41 and 45 °C and decreased significantly ($P<0.05$) in next 2 h, but remain significantly ($P<0.05$) elevated even at recovery for 6 h postexposure in comparison to preexposure level. HSP70 transcript level was induced immediately by heat stress, reaching the maximum (6.27- and 6.75-fold at 41 and 45 °C, respectively) at 1 h postexposure. After that, the expression level declined gradually and achieved the preexposure level at recovery. HSP105/110 mRNA expression level was almost the same at the initial 5 h of exposure (0, 1, 2, 3, and 4 h); after that, it increased significantly ($P<0.05$) with heat exposure hours and reaching the maximum (3.88-fold that of the calibrator) at 6 h. It

Fig. 1 **a** Rectal temperature at different heat stress exposure hours. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–c) differ significantly ($P<0.05$) at different heat stress exposure hours. **b** Respiratory rate at different heat stress exposure hours. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–c) differ significantly ($P<0.05$) at different heat stress exposure hours. **c** Pulse rate at different heat stress exposure hours. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–c) differ significantly ($P<0.05$) at different heat stress exposure hours



remains elevated even at 6 h recovery period. Relative protein expressions of HSP60, HSP70, HSP90, and HSP105/110 on exposure to heat stress have been depicted graphically in Fig. 3a–d, respectively, and was observed to have similar trend as observed for the relative mRNA expression.

Relationships between the short-term heat exposure hours, THI, physiological responses, and relative mRNA expression of HSPs

Table 3 shows the relationship between the short-term heat exposure hours, THI, RT, HR, RR, and relative mRNA expression of HSPs recorded during the 12-h study. It is evident from the table that THI has a significant ($P<0.01$) positive correlation with RR, RT, and relative mRNA expressions of HSP60, HSP70, and HSP90. Moreover, the RT, RR, and relative mRNA expressions of HSP60 and HSP105/110 have a significant ($P<0.01$) positive correlation with heat stress exposure hours. The relative mRNA expression of HSP60 has a significant ($P<0.01$) positive correlation with THI, RR, and relative mRNA expressions of HSP70 and HSP90.

Discussion

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 (Collier et al. 2008; Kishore et al. 2013; Deb et al. 2014). However, it is not completely understood how animals respond to short-term heat stress exposure at molecular level. Therefore, the present study was designed to gain insight into the impact of short-term heat stress on physiological responses and expression profile of HSPs in goats.

In the present study, the values of temperature, relative humidity, and THI were above the normal comfort zone for goats (Zaytsev et al. 1971; Richardson 2002; Kadzere et al. 2002; Brown-Brandl et al. 2009). This suggests that the microclimatic conditions in the psychrometric chamber in the

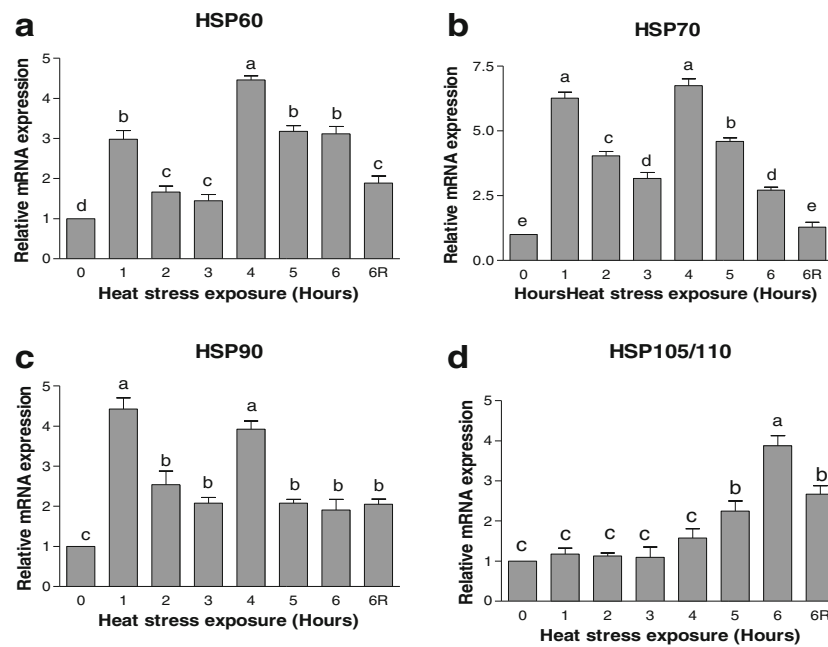


Fig. 2 **a** Expression level of HSP60 mRNA in PBMCs. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–d) differ significantly ($P<0.05$) at different heat stress exposure hours. **b** Expression level of HSP70 mRNA in PBMCs. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–e) differ significantly ($P<0.05$) at different heat stress exposure. **c** Expression level of HSP90

mRNA in PBMCs. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–c) differ significantly ($P<0.05$) at different heat stress exposure hours. **d** Expression level of HSP105/110 mRNA in PBMCs. Data represents the mean \pm SEM ($n=6$). Means bearing different superscripts (a–c) differ significantly ($P<0.05$) at different heat stress exposure hours

present investigation when the goats were exposed to THI above 90 were unfavorable to the animals as heat stress was apparent and posed a significant threat to their thermoregulatory mechanisms. Physiological parameters provide an immediate response to the climatic stress and, consequently, the level of discomfort/comfort to the animal (Bianca 1965). The RT and RR values obtained in the present study during heat stress exposure hours were above the normal range of 38.5–40.0 °C and 16–30 breaths/min (Zaytsev et al. 1971; Ayo et al. 1998) which are often used as reliable indices of detection and evaluation of acute stress (Zaytsev et al. 1971). Increased RR is the first reaction when animals are exposed to environmental temperatures above the thermoneutral zone (Maurya et al. 2007). With the increasing environmental temperature, the RR continues to rise linearly until a certain temperature thereafter slows down and depth of breathing increases (Kaushish et al. 1989). The RT is also considered as an ideal indicator for assessment of stress in animals (Lefcourt et al. 1986), and changes in RT indicates changes of a similar magnitude in deep body temperature (Rosenberg et al. 1983).

In the present investigation, it has been observed that there is positive relationship in THI, RT, RR, and HSP expression. All four genes studied showed apparent upregulations at the mRNA level, but different expression profiles were obtained. Except HSP105/110, all HSPs increased within 1 h after exposure to heat stress. All animals respond to heat stress at the cellular level by synthesizing HSPs, which help to protect

cells from heat-induced injury. In our previous study, we observed that the mRNA expression of HSP60, HSP90, and ubiquitin were higher during peak summer season as compared with peak winter season in both tropical and temperate region goats, whereas the expression of HSP70 was significantly higher during summer season as compared with winter season in tropical region goats which might play an important role in thermal stress tolerance against harsh environmental conditions (Dangi et al. 2012).

Among all the HSPs, HSP70 is the most temperature sensitive and is positively correlated with thermotolerance (Hansen 2004). HSP70 is known to be a highly inducible chaperon with a major role to stabilize the native conformation of proteins and maintenance of cell survivability during thermal stress (Beckham et al. 2004). Enhanced HSP70 expression may be a response to stressful environments and may improve cell survival by protecting proteins from degradation and facilitating their refolding (Pratt 1993; Hartl 1996). Its expression data is in accordance with studies carried out in bovine lymphocytes (Lacetera et al. 2006; Patir and Upadhyay 2010; Mishra et al. 2010), in bovine PBMCs (Kishore et al. 2013), in caprine PBMCs (Dangi et al. 2012; Sharma et al. 2013), and in kidneys of goats (Zulkifli et al. 2010). Enhanced expression of HSPs indicates that these provide protection from toxic effects of thermal stress. It has also been reported that lower heat tolerance was associated with higher expression of HSP72 (Lacetera et al. 2006). Collier et al (2008) have

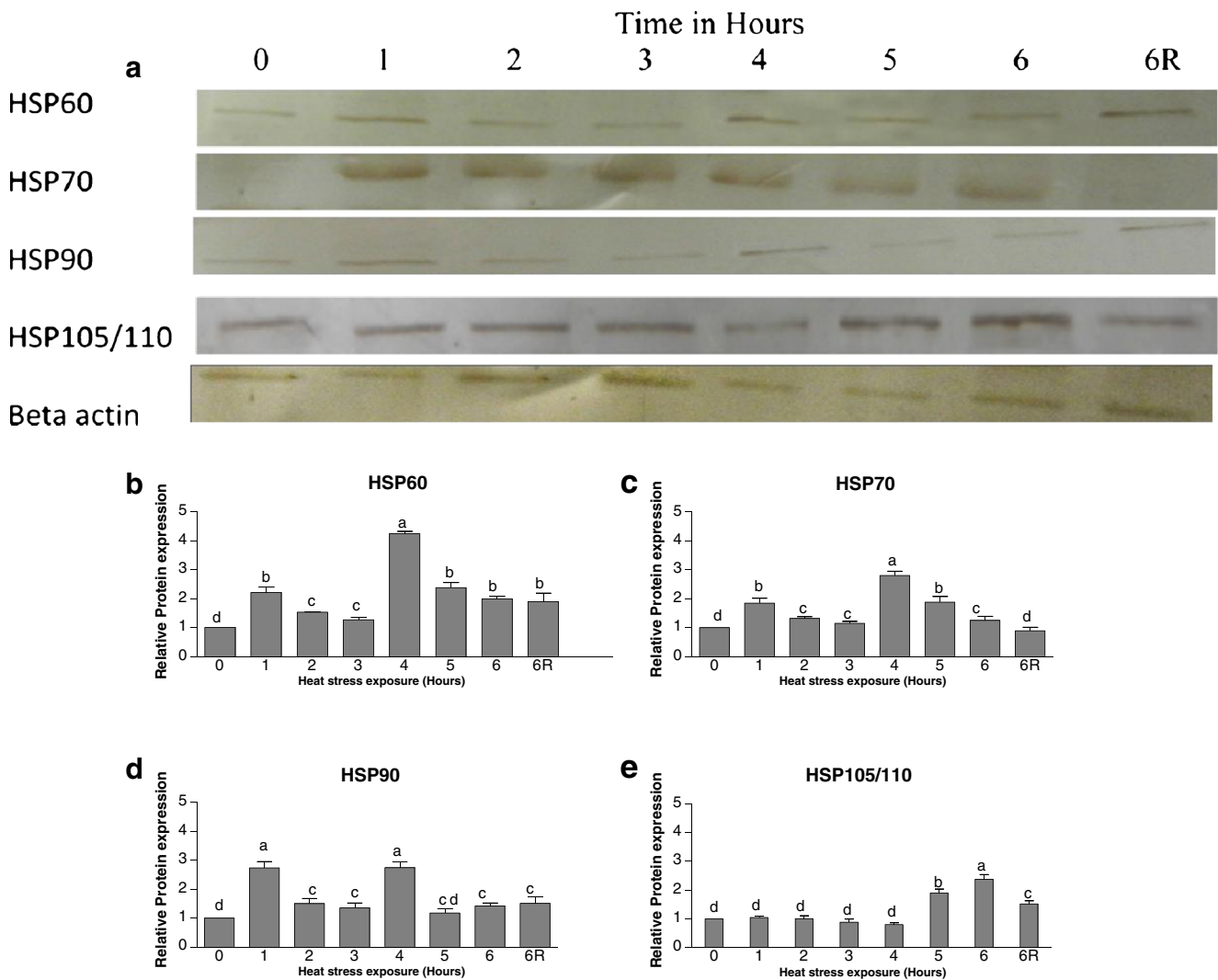


Fig. 3 Relative protein expression (mean±SEM, band densitometry) of HSPs in PBMC lysate of different heat stress exposure hours. **a** Western blot bands of HSP60, HSP70, HSP90, HSP105/110, and β-actin in PBMCs of short-term heat-stressed goats; β-actin (41 KD) used as reference protein. Relative change in expression between different heat stress exposure hours and 6-h recovery of **b** HSP60 (60 KD), **c** HSP70

(70 KD), **d** HSP90 (90 KD), and **e** HSP105 (105 KD). The proteins of different heat stress exposure hours equivalent to 40 μg (for HSP110, HSP90, β-actin) and 60 μg (for HSP70, HSP60) were separated on 12.5 % SDS-PAGE and analyzed by immunodetection using HSP family and β-actin specific antibody. Different superscripts denote statistically different values (*P*<0.05)

Table 3 Correlation coefficient among physiological parameters, THI, heat stress exposure hours, and relative mRNA expression of HSPs

	THI	RT	RR	HR	HSP60	HSP70	HSP90	HSP110	Heat stress exposure hour
THI	–	–	–	–	–	–	–	–	–
RT	0.712**	–	–	–	–	–	–	–	–
RR	0.923**	0.609**	–	–	–	–	–	–	–
HR	–0.101	–0.028	–0.034	–	–	–	–	–	–
HSP60	0.596**	0.356*	0.522**	–0.139	–	–	–	–	–
HSP70	0.774**	0.432**	0.624**	–0.173	0.718**	–	–	–	–
HSP90	0.519**	0.276	0.316*	–0.153	0.604**	0.801**	–	–	–
HSP110	0.033	0.027	0.084	0.086	0.290*	–0.187*	–0.191	–	–
Heat stress exposure hour	0.647**	0.545**	0.738**	0.147	0.577**	0.176	0.036	0.757**	–

P*<0.05; *P*<0.01

observed increased HSP70 expression in bovine mammary epithelial cells exposed to heat stress at 42 °C for the first 4 h followed by decreased expression in subsequent hours. This further pinpoints the cytoprotective role of HSP70.

In addition to HSP70, other HSP genes that were studied such as HSP60, HSP90, and HSP105/110 also contribute to thermotolerance (Duncan 2005; Kampinga et al. 2003; Yamagishi et al. 2003). HSP60 is also known to be one of the most important molecular chaperons under various stressful conditions (Oksala et al. 2006). At 2 h post heat stress, it showed higher induction in Murrah buffaloes, followed by HF and Sahiwal cows (Kishore et al. 2013). Expression of HSP90 considerably increased under heat stress (Prodromou et al. 2000). As indicated in the study by Grad and Picard (2007), HSP90 binds to glucocorticoid receptors keeping them in an innate but native state until their binding to cortisone, a steroid hormone secreted during stress conditions. Heat stress-induced expression of HSP90 was found in the heart, kidney, and liver tissues peaked after a 2-h heat exposure and reduced to basal level after increased duration of heat stress (Lei et al. 2009). Recently, *in vitro* studies also showed the increased expression of HSP90 in bovine PBMCs at 2 h post heat stress (Kishore et al. 2013; Deb et al. 2014).

HSP105 α is expressed constitutively and induced by various forms of stress, while HSP105 β is an alternatively spliced form of HSP105 α that is specifically produced following heat shock at 42 °C (Hatayama et al. 1994; Yasuda et al. 1995; Ishihara et al. 1999). HSP105/110 mRNA expression level was almost the same at the initial 5 h; thereafter, it increased with heat exposure hours and reaching the maximum (3.88-fold that of the calibrator) at 6 h. HSP105 suppress the aggregation of denatured proteins caused by heat shock *in vitro*, as does HSP70/Hsc70 (Yamagishi et al. 2000; Ishihara et al. 2003). In addition, HSP105 α and HSP105 β were suggested to function as a substitute for HSP70 family proteins to suppress the aggregation of denatured proteins in cells under severe stress, in which cellular ATP levels decrease markedly (Yamagishi et al. 2003). Increased HSP expression during short-term heat stress may be due to the fact that heat stress stimulated and quickly initiated the transcription of HSP mRNA and translation of HSP protein to protect cells from heat stress.

The above results indicated that the transcription level of HSP70 was the most sensitive to temperature fluctuations among the four genes. Elevation of temperature dramatically increased the expression of HSP70 very quickly, and a reduction in temperature inhibited the overexpression of HSP70 in a short period. The transcription variations of HSP60 and HSP90, however, were slower and at lower levels when goat experienced temperature variation and does not revert during recovery period to preexposure level. HSP105/110 expression initiation was much slower than others. Based on the present data, we speculated that, among the four HSP genes in goat,

HSP70 played the most dominant role in protecting cells from damage caused by acute thermal stress, and it may be an important molecular biomarker during the early phase, and HSP60 and HSP105/110 during late phase, of heat stress in goat farming.

Acknowledgements Funding support received from the National Initiative on Climate Resilient Agriculture (NICRA) and Indian Council of Agricultural Research (ICAR) is gratefully acknowledged.

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