

Original Research Article

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The Effect of Exogenous HSP70 on Expression Kinetics of HSP70, HSP90, HSP110 and IL2 and IL6 in Peripheral Blood Mononuclear Cells and Possible Crosstalk between Them in Black Bengal Goat

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ABSTRACT

The present study was aimed to investigate the modulatory effect of eHSP70 on major heat stressed genes in *in-vitro* cell culture system. HSP70 (eHSP70) on mRNA expression profile of Heat shock protein 70 (HSP70), Heat shock protein 90 (HSP90), Heat shock protein 110 (HSP110), Interleukin-2 (IL-2), Interleukin-6 (IL-6) in peripheral blood mononuclear cells (PBMC) in cell culture model of black Bengal Goat. The cultured PBMCs were treated with a specific dose of eHSP70 (50 ng/ml) at 37°C for 0, 1, 2, 4, 8, 12, 16, 20, 24 hours. The mRNA expression of the above mentioned genes were determined by quantitative real-time PCR (qRT-PCR). The proteins were immunochemically localized by the cultured PBMCs. The study showed that on administration of eHSP70 to the PBMCs cell culture system, the relative mRNA expression of iHSP70, HSP90 and HSP110 increased significantly ($p < 0.05$) at 4, 20 and 24 h compared with control and Interleukins (IL-2), (IL-6), showed significantly higher expression ($p < 0.05$) at 4 and 24 h compared with control. Immunoreactivity revealed that all genes under study were localized in cytoplasm as well as in nucleus. The differential expression pattern of above mentioned genes at specific dose of eHSP70 might indicate their role in maintaining the cellular homeostasis during heat stress.

Keywords

Goat, Kinetics, Heat stress, HSP, IL, PBMC

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Introduction

Heat is a naturally occurring factor of environment and show deleterious effect on animals and causes serious consequences. Higher temperature above 42°C is generally considered to be life threatening (Bettaieb and Averill-Bates, 2008). Heat shock proteins

(HSP) are molecular chaperones. They protect cells from extreme physiological, pathological, and environmental conditions (Kiang, 2004). In an unstressed cell the constitutively expressed HSP regulate protein folding, protein translocation across membranes, assembly, and disassembly of protein oligomers, degradation of aged

proteins, etc. Inducible HSP can correct protein misfolding and preserve immature polypeptides from aggregation under stresses. Nevertheless 210 genes were up regulated and 250 genes were down regulated by the heat stress (Kolli *et al.*, 2014). In the presence of HSP70 in extracellular compartment, it has been shown to facilitate innate immunity (Aneja *et al.*, 2006). eHSP70 has vital role in pro-inflammatory immune response (Pockley, 2003); therefore, changes in eHSP70 may be an indication of cellular damage within the intestines (Dokland *et al.*, 2006).

Black Bengal Goat is the most common indigenous breed of Goat, mostly found in the Eastern and North-Eastern region of India and famous worldwide mainly for its high quality chevon and skin. In India, Goats are kept free for grazing most of the daytime. So, there is always a great chance to encounter with heat stress. To obtain optimum productivity from Black Bengal Goats, an insight into the heat stress induced genes expression would provide a key to other basic researches on stress amelioration.

Cytokines are soluble signals of paramount importance mediating cell-to-cell communication during inflammatory and immune responses. Among the cytokines, IL-6 was found to be produced by cells at local tissue sites and the circulating IL-6 plays an important role in the induction of acute phase reactions by acting upon the liver and the hypothalamic-pituitary-adrenal axis (Ruzek *et al.*, 1997).

IL-6 can even modulate thermal stress in some animal tissues (Parikh *et al.*, 1998; Yildirim and Yurekli, 2010). The lymphokine IL-2, released by stimulated T-cells, is required for lymphocyte proliferation and it has been demonstrated that stressors may cause decreased IL-2 production in calves (Blecha and Baker, 1986). The higher

expression of IL-2 and IL-6 during summer is also in accordance with the previous study of (Hershko *et al.*, 2003) that induction of the heat shock response increased IL-6 production in gut mucosa *in vivo* and in cultured Caco-2 cells *in vitro*. Recent studies showed that acute heat stress promoted the production of IL-2 by lymphocytes due to increased lymphocyte proliferation in broiler (Han *et al.*, 2010). The HSP act cognitively in cellular and tissue homeostasis (DeJong *et al.*, 2009) and are released intra-cellularly and extra-cellularly in an inducible form in response to stress (Hecker and McGarvey, 2011). The higher expression of interleukin genes may have some cell protective role in thermal stress. To the best of our knowledge, no study has so far given an in-depth insight into the analysis of exogenous effect of HSP70 variation on the relative expression of major heat stress genes in PBMCs of Black Bengal breed of Goats. Hence, the present *in vitro* study was planned to assess the exogenous effect of HSP70 on expression kinetics of HSP70, HSP90, HSP110, IL2, IL6, in PBMCs of the black Bengal Goat.

Materials and Methods

Animals and blood collection

Six female Black Bengal Goat (2-3 years old) apparently healthy with average body weight of 20–24 kg were selected from the experimental herd of the Division of Physiology and Climatology, Indian Veterinary Research Institute (IVRI), Izatnagar, India. Animals were maintained under semi intensive housing conditions with access to *ad lib* water and feeds. Blood samples (10 ml) were collected from animals by jugular vein puncture in heparinized vacutainer under aseptic conditions and were transported to the laboratory in cold chain. The blood samples were collected at mean temperature humidity index of 66.97 ± 1.01 .

Separation of PBMCs

PBMCs were isolated by density gradient centrifugation method using Histopaque 1077 (Sigma, USA) as per manufacturer's instructions. The cells were washed thrice in Dulbecco's phosphate buffer saline (DPBS; pH: 7.4) by centrifugation at 425× g for 5 min at 25°C. Cells were counted using hemocytometer and viability was determined by the trypan blue exclusion method (Tolnai, 1975). Cells viability was typically greater than 90%.

Heat challenge treatment of PBMCs

The PBMCs were then re suspended at a concentration of 2×10^6 live cells/ml in RPMI-1640 (Cat no.- 1260454, Lot no.- MR29830, MP biological, France) medium containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin, 100 µg/ml streptomycin. The cells were then plated out at 2×10^6 viable cells per well in 4x6 12-well plate and incubated in a humidified CO₂ (5%) incubator at 37°C over a period of 24 hours at different time interval i.e (0, 1, 2, 4, 8, 12, 16, 20, 24 hr). After the incubation of specific time interval total RNA was extracted from PBMCs and the incubated culture plate at 37°C was used for immunocytochemistry.

Primers

The primers of HSP70, HSP90, HSP105/110, Ribosomal protein S15a (RPS15A) and beta actin (Dangi *et al.*, 2014), IL2, IL6, were taken from published sequence. The sequences, efficiency, and expected PCR Product length are presented in Table 1.

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 by using spectrophotometer 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.

Quantitative RT-PCR analysis

Quantitative real-time PCR was performed using Ssofast Eva Green @ qPCR kit, Biorad, USA. Each sample was run in triplicate in 20 µl reaction mixture which was consisted of 10 µl Eva green mix, 0.5 µl each of forward (0.25 µM) and reverse primer (0.25 µM), 1 µl of cDNA and 8 µl nuclease-free water. The real-time PCR (MxPro3005P Stratagene, Agilent Technologies, USA) was run with initial denaturation at 95°C for 30 sec followed by 40 cycles of denaturation at 95°C for 5 sec, annealing for 10-12 sec and extension at 72°C for 10 sec. The optimum annealing temperatures for different genes are presented in Table 1.

Real time PCR efficiencies were determined by amplification of a standardized dilution series, and slopes were obtained. To assess the specificity of amplified product, dissociation curve for each gene was generated at temperature of 65-95°C. Results were expressed as changes in threshold cycle values (CT) which reflects the cycle number when the fluorescence of reporter dye is higher than background. The threshold, automatically adjusted by instrument, was used for generating CT values. 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 confirm that transcripts were of exact molecular size. The mRNA expression of samples with 0 dose treatment was used as calibrator of the corresponding temperature for obtaining relative mRNA expression. The geometric mean of C_t values of the beta actin and RPS15A was used as C_t of reference gene. Efficiency corrected relative quantification of mRNA was obtained by Pfaffl (2001) for which efficiencies of primers were determined by serial dilution of template cDNA sample and running in triplicate.

Immunocytochemistry

The PBMCs cultured at 37°C were centrifuged at 400xg for 5 min and the cell pellet was washed and re-suspended with 100 µl 1xPBS. Approximately 1×10^5 cells were spotted onto glass slides pre-treated with polyethylenimine p70 @ 0.3%. After 5 min when the cells settle down, the fluid was carefully discarded and 50 µl of 3% PFA in PBS was added, incubated for 15 min at room temperature. The PFA solution was discarded and the slides were immersed in 100% methanol in a glass jar at -20 °C for overnight for fixing. On next day the slides were washed in PBS for 5 min, followed by 0.5% TritonX-PBS for 15 min and another wash with PBS for 5 min with a gentle shaking. Just after washing PBMCs were incubated in 50 µl blocking solution (3% bovine serum albumin, BSA in PBS) for 30 min at 37°C to block non-specific binding. Thereafter the BSA-PBS was removed and appropriate primary antibodies *viz.*, anti-HSP90 (MA1051, Lot # 08A12) at a 1:200 dilution, anti-HSP70 (MA1050, Lot # 08A12) at a 1:500 dilution, anti-IL2 (Catalogue no. OAS08776, Lot #120315) at 1:200 dilution, anti-IL6 (Catalogue no. GTX82951, Lot #201204) at 1:200 dilution and 3% BSA as control were added. After incubation with primary antibody the slides were extensively rinsed 3 times with 0.2% BSA-PBS for 5-10

min each. After rinsing PBMCs were incubated in a dark humidified chamber at 37°C with Cruz Fluor TM (CFL) 488 conjugated secondary antibodies (Goat anti-rabbit or anti-mouse IgG-CFL 488 and alexa flour 596, 1:1500 dilutions) for 40 min. The primary and secondary antibodies were diluted in 1% BSA in PBS. After immune staining, the PBMCs were stained with DAPI (0.4 µg/ml in PBS) to stain the nuclei of the cells over slides. The control was processed under similar conditions except for the omission of the primary antibody. Fluorescently stained cell cultures on glass slides were mounted with anti-fade mounting media (MP Biomedical, France) and images were captured using Axio Observer.Z1 (Carl Zeiss Micro Imaging GmbH, Germany) microscope.

Statistical analyses

In accordance with the Pfaffl (2001), without treatment sample was used as the calibrator. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests, General Linear Model (GLM) using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Mean values were expressed with \pm SEM.

Results and Discussion

In this study, PBMCs of Indian Black Bengal Goat were utilized as cellular *in vitro* model to describe and chemical chaperone on expression of HSP's during heat stress. The transcriptional induction of HSP70, HSP90, HSP110, IL2, IL6 and mRNA was used as indicator to evaluate the comparative cellular tolerance ability of PBMCs. Good quality RNA as reflected A_{260}/A_{280} ratio of 1.9 ± 0.22 was obtained in all the samples types. The melt curve analysis showed single melting peak for each of the analyzed genes indicating quality of qPCR data. Furthermore, high PCR efficiency that ranged from 99.9 to 109.5 was

observed for different studied genes. In order to normalize the target gene expression data, geometric mean of beta actin and RPS15a was considered as internal control genes. In our study, all the PBMCs that were exposed to the 37°C temperature were found to be responsive to *in vitro* condition. mRNA expression was calculated for HSP70, HSP90 and HSP110, IL2, IL6 taking 0h sample as control (Table 2–6).

Expression Kinetics of Heat shock proteins

The Expression Kinetics of HSP70, HSP90, and HSP110 have been depicted in Figure 1 (A), 1 (B) and 1 (C). eHSP70 at 50ng/ml in cell culture media up-regulated the relative mRNA expression of HSP70, 90 in treatment group at 24hrs of incubation reaching peak at 4 hrs ($p<0.05$), again up-regulated at 20 hours reaching second peak at 24 hrs ($p<0.05$) compared to control whereas HSP110 reaching peak at 4hrs ($p<0.05$), and second peak occurred at 20 hrs, the level maintained till 24 hrs as compared to control. Immunocytolocalization of HSP70, 90, 110 showed their expression in PBMCs in Figure 2a, b, c respectively.

Expression kinetics of interleukins

eHSP70 at 50ng/ml in cell culture media up-regulated the relative mRNA expression of IL2 (Fig. 1-D) and IL6 (Fig. 1-E) in treatment group reaching peak at 4 hrs ($p<0.05$), maintaining the level at 8 hrs and decreases at 12, 16 and 20 hrs and again upregulated at 24 hrs reaching second peak ($p<0.05$), compared to control. Immunocytolocalization of IL2, 6 showed their expression in PBMCs (Fig. 2d, e).

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). Thermal stress triggers a complex cellular response including altering gene expression (Sonna *et al.*, 2002; Kültz, 2005). It is widely accepted that changes in gene expression are an integral part of the cellular response to thermal stress. Cytokines play a key role in bidirectional communication between the neuro-endocrine and immune system.

HSP70 family of proteins is most temperature sensitive and highly conserved among heat shock proteins. In the present investigation the mRNA expression of HSP70 was found to be significantly higher at higher temperature.

Increased expression of HSP70 mRNA upon exposure to heat stress has been reported in caprine PBMCs (Sharma and Kiran, 2013; Dangi *et al.*, 2014) and bovine lymphocytes (Mishra, 2010; Patirand Upadhyay, 2010; Bharati *et al.*, 2017a). HSP70 play the most dominant role among all the HSP in protecting cells from damage caused by acute thermal stress (Dangi *et al.*, 2014). Our findings are attuned with the findings of Dangi *et al.*, (2012) who reported similar findings of mRNA expression in PBMCs of tropical region Goats.

We obtained higher expression for HSP90 at higher temperature, which is in confirmation with, earlier investigations in Tharparkar cattle PBMCs reporting up regulation of HSP90 expression (Bharati *et al.*, 2017b) during heat stress.

In the present study, the mRNA expressions of HSP70, HSP90, HSP110, IL2 and IL6 were found to be significantly upregulated upon exposure of exogenous HSP70. The transcriptional and translational pattern of HSP70 and HSP90 was upregulated upon exposure to heat stress has been reported in caprine PBMCs and bovine lymphocytes (Mishra, 2010).

Fig.1 Relative mRNA Expression of heat shock protein and interleukins in PBMC at 0, 1, 2, 4, 8, 12, 16, 20, 24 h time interval after in vitro cultured with exogenous HSP70 at 37°C (A) Relative mRNA expression of HSP70 (B) Relative mRNA expression of HSP90 (C) Relative mRNA expression of HSP110 (D) Relative mRNA expression IL2 (E) Relative mRNA expression IL6. The data are presented for each individual and as mean \pm SD. The genes were calculated taking zero dose at zero hour (without treatment) as control. * Significant difference from control at 0h ($p < 0.05$)

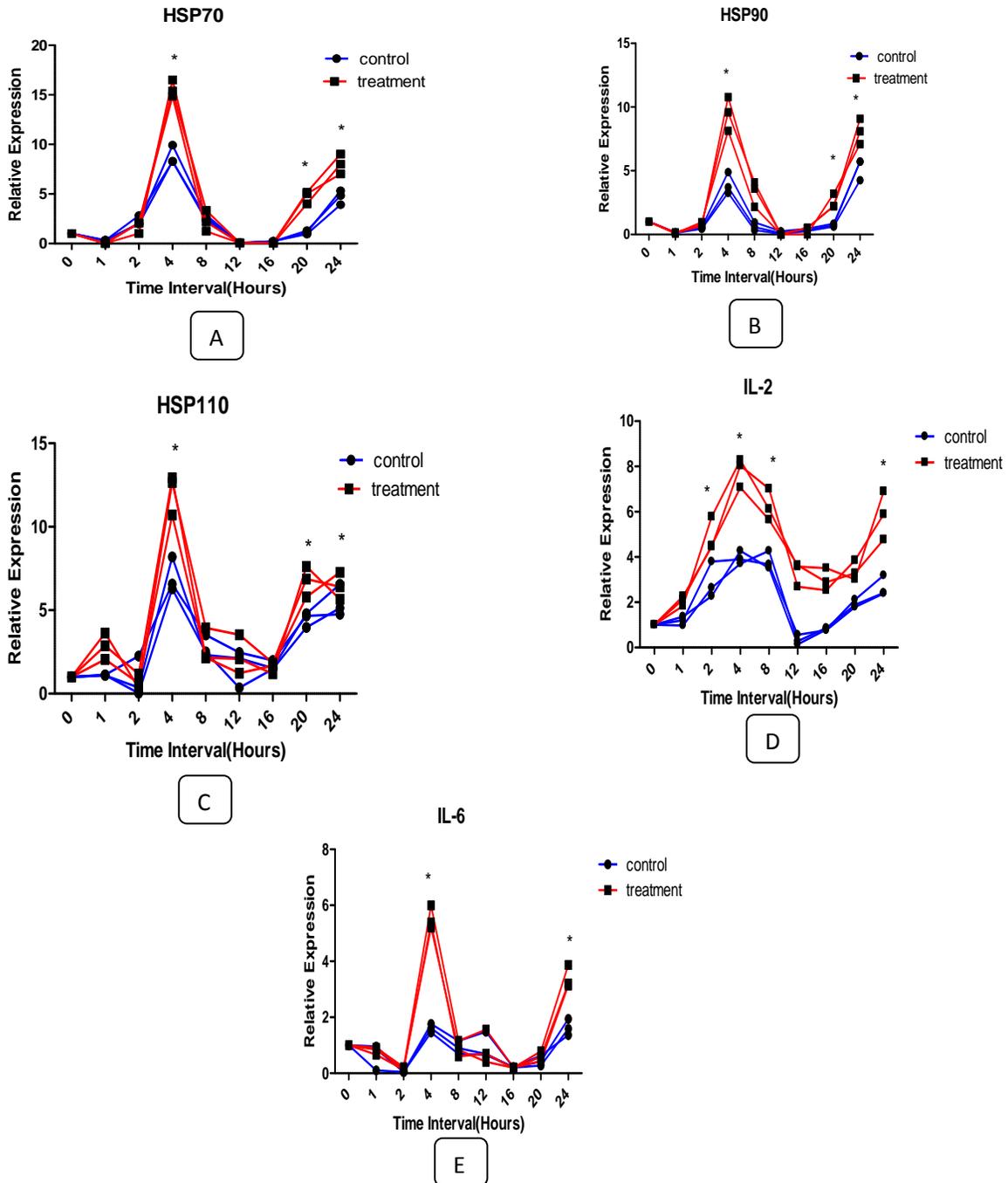


Fig.2 Immunocytochemical localization of (a) HSP70 (b) HSP90 (c) HSP110 (d) IL2 (e) IL6 in PBMCs of Black Bengal Goat. The cultured PBMCs were stained with ALEXA FLUOR 594 and CFL 488 and merged with DAPI counter-stain (blue), indicating the nuclei of all cells in the sections. Negative control sections were presented without primary antibody labelling.

Scale bar = 20µm

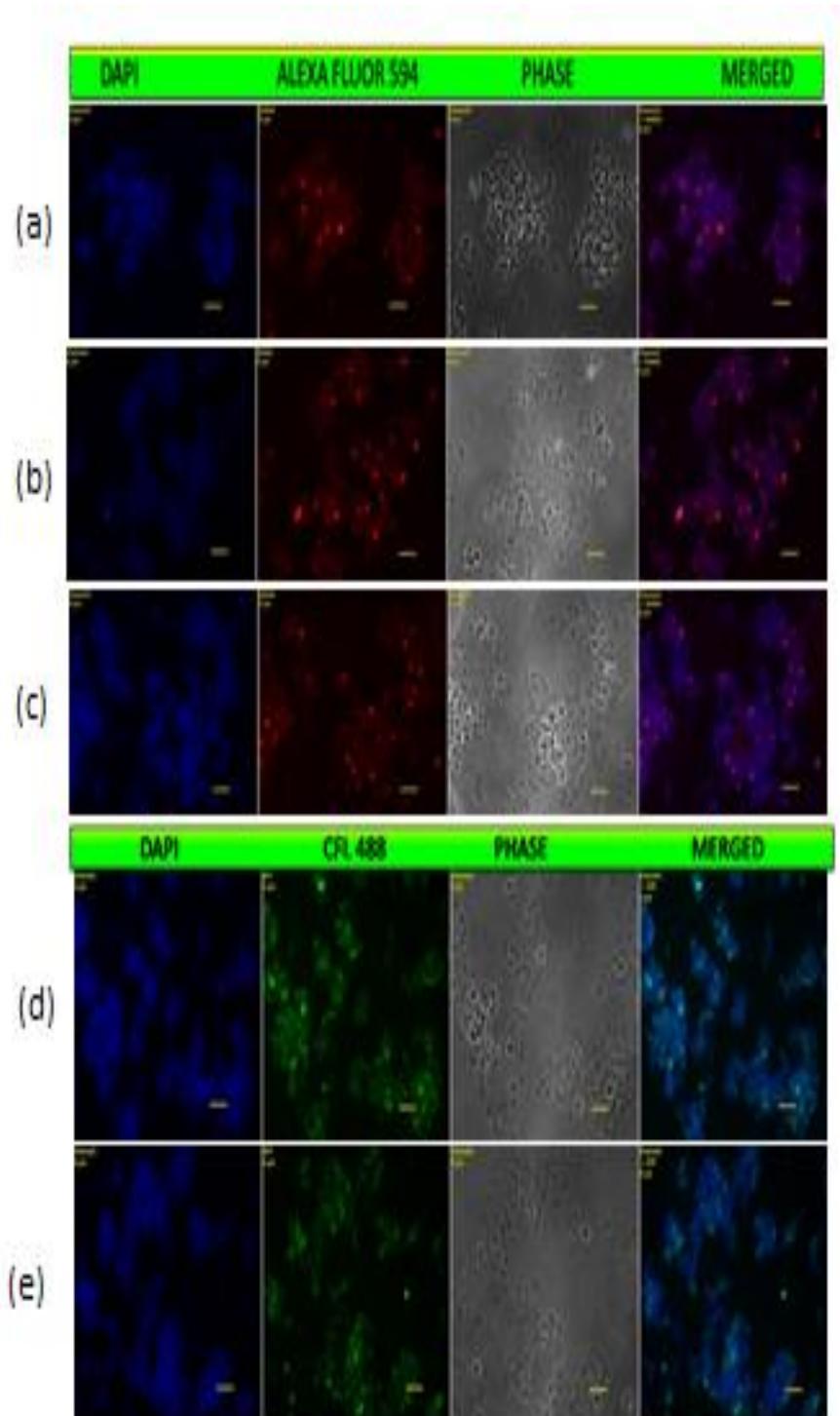


Table.1 Gene transcripts, primer sequence and resulting fragment size

Gene	Sequence of nucleotide	Fragment size (bp)	Annealing temperature	EMBL/reference
HSP 70	For: 5'- GACGACGGCATCTTCGAG -3' Rev: 5'- GTTCTGGCTGATGTCCTTC -3'	132	58° C	Dangi <i>et al.</i> , (2012)
HSP 90	For: 5'-GCATTCTCAGTTCATTGGCTATCC- 3' Rev: 5'-GTCCTTCTTCTTCTCCTCCTCTTC- 3'	190	58° C	Dangi <i>et al.</i> , (2012)
HSP105/ 110	For: 5'-CACAGCCCCAGGTACAAACT - 3' Rev:: 5'-TCCCTAACTGCCAGACCAAG- 3'	204	60° C	NM_001075302.1
IL 2	For: 5'-ACGGGGAACACAATGAAAGAAG- 3' Rev: 5'-TGCATCCTGGAGAGCTTGAG- 3'	101	58° C	AF535145.1
IL6	For: 5'-GCTGCTCCTGGTGATGAC- 3' Rev: 5'- CGACGATGTGCTTAATGAGAG- 3'	134	60° C	D86569.1
RPS 15a	For: 5'-AATGGTGCATGAATGTC- 3' Rev: 5'- GACTTTGGAGCACGGCCTAA- 3'	100	60° C	XM_005679050.1
Beta actin	For: 5'-AGTTCGCCATGGATGATGA-3' Rev: 5'-TGCCGGAGCCGTTGT-3'	54	60° C	Dangi <i>et al.</i> , (2012)

Table.2 Mean±SEM of relative mRNA expression of HSP70 during different time interval on treatment of PBMCs with eHSP70 at 37 °C in *in vitro* cell culture system

Genes	Time interval								
	0hr	1hr	2hr	4hr	8hr	12hr	16hr	20hr	24hr
HSP70 Control	1±00	0.32±0.02	2.88±0.26	8.83±0.54	2.60±0.15	0.07±0.00	0.23±0.00	1.13±0.09	4.62±0.40
HSP70 Treatment	1±00	0.01±0.00	1.72±0.34	15.58±0.60	2.25±0.60	0.06±0.00	0.04±0.01	4.73±0.36	8.01±0.57

Table.3 Mean±SEM of relative mRNA expression of HSP90 during different time interval on treatment of PBMCs with eHSP70 at 37 °C in *in vitro* cell culture system

Genes	Time interval								
	0hr	1hr	2hr	4hr	8hr	12hr	16hr	20hr	24hr
HSP90 Control	1±0.00	0.144±0.01	0.508±0.03	3.95±0.48	0.62±0.17	0.140±0.05	0.35±0.05	0.736±0.06	5.22±0.48
HSP90 Treatment	1±0.00	0.119±0.2	0.84±0.08	9.49±0.76	3.28±0.57	0.013±0.00	0.34±0.16	2.52±0.32	8.08±0.57

Table.4 Mean±SEM of relative mRNA expression of HSP110 during different time interval on treatment of PBMCs with eHSP70 at 37 °C in *in vitro* cell culture system

Genes	Time interval								
	0hr	1hr	2hr	4hr	8hr	12hr	16hr	20hr	24hr
HSP110 Control	1±0.00	3.44±0.33	0.88±0.69	7.01±0.59	2.77±0.37	1.64±0.65	2.00±0.30	4.46±0.25	5.48±0.54
HSP110 Treatment	1±0.00	9.18±0.76	0.714±0.24	12.10±0.70	2.75±0.59	2.28±0.67	1.57±0.19	6.75±0.92	6.44±0.46

Table.5 Mean±SEM of relative mRNA expression of IL2 during different time interval on treatment of PBMCs with eHSP70 at 37 °C in *in vitro* cell culture system

Genes	Time interval								
	0hr	1hr	2hr	4hr	8hr	12hr	16hr	20hr	24hr
IL-2 Control	1±0.00	1.180±0.11	2.89±0.45	3.95±0.16	3.81±0.22	0.33±0.12	0.801±0.02	1.93±0.09	2.66±0.26
IL-2 Treatment	1±0.00	2.07±0.12	4.91±0.4	7.79±0.37	6.25±0.40	3.30±0.30	2.97±0.28	3.38±0.24	5.85±0.61

Table.6 Mean±SEM of relative mRNA expression of IL-6 during different time interval on treatment of PBMCs with eHSP70 at 37 °C in *in vitro* cell culture system

Genes	Time interval								
	0hr	1hr	2hr	4hr	8hr	12hr	16hr	20hr	24hr
IL-6 Control	1±0.00	0.64±0.26	0.04±0.00	1.61±0.08	0.91±0.13	0.94±0.26	0.26±0.0	0.44±0.10	1.63±0.16
IL-6 Treatment	1±0.00	0.80±0.07	0.16±0.02	5.53±0.24	0.86±0.16	0.88±0.34	0.19±0.0	0.59±0.10	3.40±0.23

Thermal stress induces characteristic patterns of circulating cytokines in man and animals that are dominated by acute elevations in various cytokines like IL-6, IL-10 and TNF- α (Bouchama *et al.*, 2005). HSP70 families of proteins are most temperature sensitive and highly conserved among heat shock proteins. TLRs are again the pattern recognition receptor which can recognize microbial markers and or endogenous ligands such as HSP 70 to initiate a complex signalling cascade that activate a wide variety of transcription factors and inflammatory cytokines (Akira and Takeda, 2004). Previous study conducted in baboon shows that heatstroke activates complex, systemic, inflammatory, and regulatory responses characterized by an early and simultaneous release of anti-inflammatory cytokine and soluble cytokine receptors (Bouchama *et al.*, 2005). Through binding to TLR2 and TLR4, recombinant HSP70 can stimulate sentinel cells to release cytokines and increase their antigen-presenting capacity (Vabulas *et al.*, 2002; Gobert *et al.*, 2004). Thus, there may be many possible signalling pathways that could account for stimulation of cytokine production in conditions of heat stress. However, two possible reasons for up-regulation of interleukins in regards to HSP may exist. The first arises from the possibilities of HSP70 induced activation of NF-kB and subsequent transcription of cytokines (like IL6) via acting through its membrane receptor. As it has been reported that exogenous HSP70 can activate the production of cytokines via a pathway dependent on CD14 and intracellular calcium

influx (Asea *et al.*, 2000). eHSP70 has been shown to activate monocytes, macrophages, and dendritic cells, and up-regulate the expression of pro-inflammatory cytokines (Mortaz *et al.*, 2006). HSP70, in its ability as a chaperone, was involved in antigen acceptance and presentation by macrophages and other followers of the APC group (Suzue and Young, 1996). Furthermore, antigens associated with HSP70 were found to elicit a more pronounced immune response than they did on their own (Barrios *et al.*, 1994). It has been shown that eHSP70 can interact with APCs via a receptor and elicit the release of pro-inflammatory cytokines (Asea *et al.*, 2000; Binder *et al.*, 2000).

Thus, the pattern of cytokine expression in our study may function to suppress subsequent proinflammatory signaling locally or systemically, as the anti-inflammatory effects of IL-6 include suppression of both TNF- α and IL-1 β (Starkie *et al.*, 2003). Together, these responses could play critical roles in dampening the proinflammatory pathways, in initiating survival programs and maintaining cell homeostasis during heat stress exposure in Black Bengal Goats.

Persisting stress alters physiological state referred to as acclimation via gene expression in response to endocrine signals or external signals. HSP expression pattern is at least biphasic or two-peak phenomenon. It requires a long term study to further study HSP peak phenomenon consistency. Some genes play important role during initial phase whereas others at later phase to smoothen the

acclimation process to nullify deleterious effect of thermal stress by maintaining cellular homeostasis. In the acclimated state, metabolism is minimized to counteract the detrimental effects of increased thermal heat load.

Competing Interests

The authors declare that they have no competing interests.

References

- Akira, S. and Takeda, K. (2004). Toll-like receptor signalling. *Nat. Rev. Immunol.* 4 (7): 499-511.
- Aneja, R., Odoms, K., Dunsmore, K., Shanly, T.P. and Wong, H.R. (2006). Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. *J. Immunol.* 177:7184–7192.
- Asea, A., Kraeft, S.K., Kurt-Jones, E.A., Stevenson, M.A., Chen, L.B., Finberg, R.W., Koo, G.C. and Calderwood, S.K. (2000). HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat. Med.* 6: 435–442.
- Barrios, C., Georgopoulos, C. and Lambert, P.H. (1994). Heat shock proteins as carrier molecules: *In vivo* helper effect mediated by *Escherichia coli* GroEL and DnaK proteins requires cross-linking with antigen. *Clin. Exp. Immunol.* 98, 229–233.
- Bettaieb, A. and Averill-Bates, D.A. (2008). Thermotolerance induced at a fever temperature of 40C protects cells against hyperthermia-induced apoptosis mediated by death receptor signalling. *Biochem. Cell Biol.* 86 (6): 521-538.
- Bharati, J., Dangi, S.S., Bag, S., Maurya, V.P., Singh, G., Kumar, P. and Sarkar, M. (2017b). Expression dynamics of HSP90 and nitric oxide synthase (NOS) isoforms during heat stress acclimation in Tharparkar cattle. *Int. J. Biometeorol.* 7: 1-9.
- Bharati, J., Dangi, S.S., Chouhan, V.S., Mishra, S.R., Bharti, M.K., Verma, V., Shankar, O., Yadav, V.P., Das, K., Paul, A. and Bag, S. (2017a). Expression dynamics of HSP70 during chronic heat stress in Tharparkar cattle. *Int. J. Biometeorol.* 61 (6): 1017-1027.
- Binder, R.J., Han, D.K. and Srivastava, P.K. (2000). CD91: A receptor for heat shock protein gp 96. *Nature Immunol.* 1: 151–155.
- Blecha, F. and Baker, P.E. (1986). Effect of cortisol *in vitro* and *in vivo* on production of bovine interleukin 2. *Am. J. Vet. Res.* 47 (4): 841-845.
- Bouchama, A., Al Mohanna, F., El-Sayed, R., Eldali, A., Saussereau, E., Chollet-Martin, S. and Roberts, G. (2005). Experimental heatstroke in baboon: analysis of the systemic inflammatory response. *Shock*, 24 (4): 332-335.
- Dangi, S.S., Gupta, M., Maurya, D., Yadav, V.P., Panda, R.P., Singh, G., Mohan, N.H., Bhure, S.K., Das, B.C., Bag, S. and Mahapatra, R. (2012). Expression profile of HSP genes during different seasons in Goats (*Capra hircus*). *Trop. Anim. Health Prod.* 44 (8): 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. *Int. J. Biometeorol.* 58 (10): 2085-2093.
- DeJong, P.R., Schadenberg, A.W.L., Jansen, N.J.G. and Prakken, B.J. (2009). Hsp70 and cardiac surgery: Molecular chaperone and inflammatory regulator with compartmentalized effects. *Cell Stress Chaperones.* 14: 117–131.

- Doklandy, K., Moseley, P.L. and Ma, T.Y. (2006). Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *Am. J. Physiol. Gastrointest. Liver Physiol.* 290: G204–G212.
- Gobert, A.P., Bambou, J.C., Werts, C., Balloy, V., Chignard, M., Moran, A.P. and Ferrero, R.L. (2004). *Helicobacter pylori* heat shock protein 60 mediates interleukin-6 production by macrophages via a toll-like receptor (TLR)-2-, TLR-4-, and myeloid differentiation factor 88-independent mechanism. *J. Biol. Chem.* 279 (1): 245-250.
- Han, A.Y., Zhang, M.H., Zuo, X.L., Zheng, S.S., Zhao, C.F., Feng, J.H. and Cheng, C. (2010). Effect of acute heat stress on calcium concentration, proliferation, cell cycle, and interleukin-2 production in splenic lymphocytes from broiler chickens. *Poult. Sci.* 89 (10): 2063-2070.
- Hecker, J.G. and McGarvey, M. (2011). Heat shock proteins as biomarkers for the rapid detection of brain and spinal cord ischemia: A review and comparison to other methods of detection in thoracic aneurysm repair. *Cell Stress Chaperones.* 16: 119–131.
- Hershko, D.D., Robb, B.W., Luo, G.J., Paxton, J.H. and Hasselgren, P.O. (2003). Interleukin-6 induces thermotolerance in cultured Caco-2 cells independent of the heat shock response. *Cytokines*, 21 (1): 1-9.
- Kiang, J.G. (2004). Inducible heat shock protein 70 kD and inducible nitric oxide synthase in hemorrhage/resuscitation-induced injury. *Cell Res.* 14 (6): 450-459.
- Kolli, V., Upadhyay, R.C. and Singh, D. (2014). Peripheral blood leukocytes transcriptomic signature highlights the altered metabolic pathways by heat stress in zebu cattle. *Res. Vet. Sci.* 96 (1): 102-110.
- Kültz, D. (2005). Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol.* 67: 225-257.
- Mishra, R.P. (2010). Role of housing and management in improving productivity efficiency of Goats. Goat production-processing of milk and meat. First edition, page 45. *Missouri Agric. Exp. Stat. Res. Bull.* No 475.
- Mortaz, E., Redegeld, F.A., Kijkamp, F.P., Wong, H.R. and Engels, F. (2006). Acetylsalicylic acid-induced release of HSP70 from mast cells results in cell activation through the TLR pathway. *Exp. Hematol.* 34: 8–18.
- Parikh, A.A., Moon, M.R., Kane, C.D., Salzman, A.L., Fischer, J.E. and Hasselgren, P.O. (1998). Interleukin-6 production in human intestinal epithelial cells increases in association with the heat shock response. *J. Surg. Res.* 77 (1): 40-44.
- Patir, H. and Upadhyay, R.C. (2010). Purification, characterization and expression kinetics of heat shock protein 70 from *Bubalus bubalis*. *Res. Vet. Sci.* 88 (2): 258-262.
- Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29: 2002–2007.
- Pockley, A.G. (2003). Heat shock proteins as regulators of the immune response. *The lancet.* 362 (9382): 469-476.
- Ruzek, M.C, Miller, A.H., Opal, S.M., Pearce, B.D. and Biron, C.A. (1997). Characterization of early cytokine responses and an interleukin (IL)-6–dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. *J. Exp. Med.* 185 (7): 1185-1192.

- Sharma, A. and Kiran, R. (2013). Corporate social responsibility: driving forces and challenges. *Int. J. Bus. Res. Dev.* 2 (1).
- Sonna, L.A., Fujita, J., Gaffin, S.L., Lilly, C.M. (2002). Invited review: effects of heat and cold stress on mammalian gene expression. *J. Appl. Physiol.* 92 (4): 1725-1742.
- Starkie, R., Ostrowski, S.R., Jauffred, S., Febbraio, M. and Pedersen, B.K. (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF- α production in humans. *FASEB J.* 17 (8): 884-886.
- Stott, G.H. (1981). What is animal stress and how is it measured? *J. Anim. Sci.* 52 (1): 150-153.
- Suzue, K. and Young, R.A. (1996). Heat shock proteins as immunological carriers and vaccines. *EXS.* 77: 451-465.
- Tolnai, S. (1975). A method for viable cell count. *Methods Cell Sci.* 1 (1): 37-38.
- Vabulas, R.M., Wagner, H. and Schild, H. (2002). Heat shock proteins as ligands of toll-like receptors. *Curr. Top. Microbiol. zImmunol.*, 270: 169-184.
- Yildirim, N.C. and Yurekli, M. (2010). The effect of adrenomedullin and cold stress on interleukin- 6 levels in some rat tissues. *Clin. Exp. Immunol.* 161 (1): 171-175.

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