B-5574
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research

Heat Shock Protein 70 (HSP70) m-RNA Expression Pattern during Different Seasons in Red Kandhari Cattle of India

Maharudra J. Sanap[1], Pranesh V. Yeotikar[2], Prabhakar B. Ghorpade[2], S. Sajid Ali[2], Vilas B. Dongre[1], Suhas A. Amrutkar[3], M.F.M.F. Siddiqui[2]

10.18805/IJAR.B-5574

ABSTRACT

Background: Heat shock proteins (HSPs), particularly HSP70, are highly conserved and ubiquitously expressed proteins that are synthesized in response to a variety of stressors. Among these, HSP70 plays a crucial role in cellular protection during heat stress by being rapidly up-regulated, thereby exerting a cytoprotective effect. Acting as molecular chaperones, HSPs help maintain the proper three-dimensional structure of proteins under adverse environmental conditions. As a result, they enhance cellular adaptability and contribute significantly to stress tolerance and thermal regulation. The present study aimed to examine the expression pattern of the HSP70 gene in Red Kandhari cattle across different seasons, with a particular focus on assessing relative thermal stress during summer in comparison to the winter and rainy seasons.

Methods: In the present experiment, apparently healthy, male (n=12) and females (n=12) experimental animals of Red Kandhari cattle with the average age ranging from 1-4 yrs were selected from Livestock Farm Complex, College of Veterinary and Animal Sciences, Parbhani. Blood samples (2 mL) were collected once for the three different seasons *viz.* summer: April-May (May: THI 83.36), rainy: June-September (September: THI 78.24) and winter: January-February (January: THI 67.84) during 2023. A quantitative Real-Time PCR (qPCR) expression study was undertaken to evaluate the relative m-RNA expression pattern of the HSP70 gene during different seasons.

Result: The study revealed that the relative mRNA expression of the HSP70 gene was significantly higher ($p<0.01$) during the summer season compared to the winter and rainy seasons in Red Kandhari cattle. Additionally, expression levels were significantly higher ($p<0.01$) in the rainy season than in winter. These findings suggest that fluctuations in ambient temperature and humidity lead to increased HSP70 gene expression. Notably, during the summer, the elevated expression of heat shock proteins in Red Kandhari cattle appears to support cellular adaptability, enhance stress tolerance and maintain thermal homeostasis.

Key words: HSP70, Red kandhari cattle, Summer season, Thermal stress, THI.

INTRODUCTION

The Red Kandhari cattle categorized as an indigenous breed believed to be originated from Kandhar Tehsil of the Nanded district of Maharashtra state. Red Kandhari animal are well adapted to agro-climatic conditions of various breeding tracts *e.g.* Nanded, Parbhani, Hingoli and Beed districts of Maharashtra state and the Bidar district of Karnataka. Red Kandhari breed is used as draft animal for agricultural work. Now a day, Red Kandhari breed is reared as a milch animal for the purpose of milk in Maharashtra state.

Among stressors, one of the major environmental stressor is heat stress that affects the animal production (Behera *et al.,* 2022). It can cause disturbances in homeostasis such as physiological, thermoregulatory responses (West, 1994). Cattle are homoeothermic animals which live in thermo-neutral zone ranging from 4°C to 25°C. Animals can maintain their core body temperature by making evaporative and non-evaporative heat losses. It was revealed that, the adverse effects of high ambient temperature are aggravated when heat stress is accompanied by high ambient humidity (Marai *et al.,* 2007).

Temperature humidity index (THI), is most extensively used parameter for the determination heat load on the

[1]College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Udgir-431 402, Maharashtra, India.

[2]College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Parbhani-413 517, Maharashtra, India.

[3]Institute of Veterinary and Animal Sciences, Akola-444 001, Maharashtra, India.

Corresponding Author: Maharudra J. Sanap, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Udgir-431 402, Maharashtra, India.
Email: maharudrasanap@mafsu.ac.in
ORCIDs: https://orcid.org/0009-0002-1079-3262; https://orcid.org/0000-0003-0316-1486; https://orcid.org/0000-0002-8793-3553; https://orcid.org/0000-0003-1226-0910; https://orcid.org/0000-0002-5681-4789; https://orcid.org/0009-0006-6487-2037; https://orcid.org/0009-0002-0943-6233.

How to cite this article: Sanap, M.J., Yeotikar, P.V., Ghorpade, P.B., Ali, S.S., Dongre, V.B., Amrutkar, S.A. and Siddiqui, M.F.M.F. (2025). Heat Shock Protein 70 (HSP70) m-RNA Expression Pattern during Different Seasons in Red Kandhari Cattle of India. *Indian Journal of Animal Research.* 1-8. doi: 10.18805/IJAR.B-5574.

Submitted: 22-02-2025 **Accepted:** 08-09-2025 **Online:** 07-10-2025

animals which is good indicator of thermal stress of climatic conditions (McDowell *et al.,* 1976; Behera *et al.,* 2018). The thermoregulation mechanism is vital along with thermo neutrality and comfort zone for maintaining body conditions in response to stress (Kadzere *et al.,* 2002). It is governed by stable heat proteins known as Heat Shock Proteins (HSP) in ruminants (Collier *et al.,* 2008).

Heat shock proteins comprise a large group of molecular chaperones, classified based on molecular size and similarities in amino acid sequences (Kumar *et al.,* 2015). Amongst members of the HSP family, HSP70 is the most abundant and temperature sensitive essential protein (Beckham *et al.,* 2004) well known to be conserved and ubiquitous in nature (Liu and Steinacker, 2001; Kregel, 2002) produced by cells in response to the stress conditions (Lanneau *et al.,* 2007).

Heat shock protein 70 (HSP70) are dynamic, conferring cryoprotection against different stressors. Amongst the stressors, thermal stress triggers a complex mechanism of gene expression as well as adaptive biochemical responses (Lindquist, 1986 and Fujita, 1999). Heat shock proteins can prevent the cells from oxidative stress through its antioxidant and thermo tolerance effect (AL-Jaryan *et al.,* 2023). It is also functioning as molecular chaperon which is attributed to fold, unfold and refold stress-denatured proteins.

Numerous research activities have been conducted based on HSP70 gene expression patterns during different seasons in cross-bred cattle throughout the globe. However, very meagre studies were found in the Indigenous cattle breeds of India. Therefore, the present study was undertaken to determine the relative mRNA expression pattern of HSP70 gene with special reference to the summer season in response to thermal stress in Red Kandhari cattle.

MATERIALS AND METHODS

The present search was conducted in the department of Veterinary Biochemistry, College of Veterinary and Animal Sciences (MAFSU), Parbhani, total twenty-four (24) apparently healthy animals of either sex with average age ranging from 1-4 yrs. were selected from Livestock Farm Complex, COVAS, Parbhani. They were kept under similar managemental and nutritional regimen throughout the duration (January 2023 to September 2023) of the experimental period as shown in Table 1.

Temperature humidity index

Temperature and relative humidity was recorded inside the shed with the help of dry bulb/wet bulb thermometer and thermo hygrometer (Mader *et al.,* 2006). Temperature humidity index (THI) of the animal shed was calculated using the formula as follows:

$$THI = (0.81 \times Tdb) + \{(RH \div 100) \times (Tdb - 14.4)\} + 46.6$$

Where,
Tdb = Average temperature in °C of the dry bulb thermometer
RH = Average relative humidity.

Collection of samples

The blood samples (2 mL) were collected aseptically by jugular venipuncture with EDTA vacutainer from Red Kandhari cattle during peak summer month (May, THI: 83.36); peak rainy month (September, THI: 78.24); and peak winter (January, THI: 67.84); seasons. During the collection of samples, cold chain was maintained by use of ice cubes and were immediately processed further for the isolation of total RNA.

Reagents, media and kits

The glassware and plastic ware procured from various firms such as Borosil, Himedia *etc.* The reagents or kits obtained namely *e.g.* Agarose, Ethidium bromide, DEPC, Hi Temp PCR master mix, SYBR green master mix, DNase-I, Amplification grade, Invitrogen life Technologies, California, USA.

Isolation of total RNA from whole blood

The pure linkTM total RNA blood purification kit (Invitrogen Life Technologies, California, USA) was used for Isolation of total RNA from whole blood by following the recommended manufacturer's protocol. Due to its Instability, immediately RNA was processed further for the synthesis of cDNA.

Synthesis of cDNA

The protocol was optimized for cDNA. The quality and quantity of cDNA was recorded (Nanodrop Spectrophotometer, Himedia). ThecDNA samples showing OD: 260/280 more than 1.6 were processed further for RT- qPCR analysis.

Primers

The cattle specific published primer sequences complementary to HSP70 and GAPDH genes were purchased from Barcode Bioscience, Bangalore (Karnataka). The primer sequences, annealing temperature and predicted fragment size shown (Table 2).

Optimization of PCR conditions and primers specificity

The primers were diluted with double distilled water to make 100 µM stock solutions and were stored at -20°C in Deep freezer until use. PCR conditions were optimized by

Table 1: Experimental animals for study.

Red kandhari cattle	Seasons		
Male (n=12) Female (n=12)	Winter: January-February (*Peak month: January of the winter season)	Summer: April-May (*Peak month: May of the summer season)	Rainy: June-September (*Peak month: September of the rainy season)

employing various annealing temperature corresponding to the Tm of the primes in Thermo cycler.

The confirmation of cDNA

The PCR product revealed distinct bands for HSP70 and GAPDH genes on *GelDoc* (Bio-rad) by Agarose gel electrophoresis with 1.5% Agarose gel as shown (Fig 1).

Amplification plot for HSP70 and GAPDH gene expression

The amplification plot for expression of HSP70 and GAPDH genes was obtained. This was used for examining the unspecific binding, primer dimer or secondary structure formation as shown (Fig 2).

Confirmation of qPCR product

The real time PCR product was confirmed on 1.5% Agarose gel through Gel Doc(Bio-Rad) system as shown (Fig 3).

Validation of qPCR product by melt curve analysis

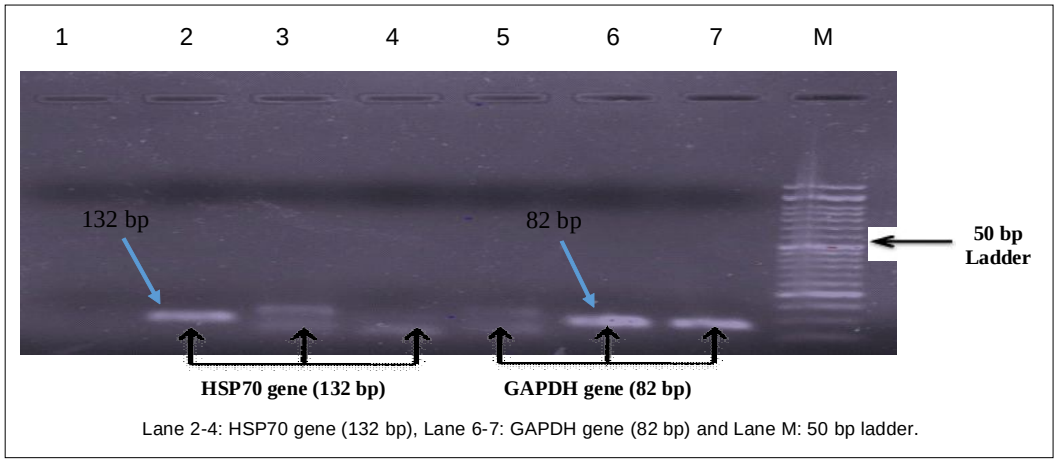
The dissociation characteristics of double stranded DNA were assessed during thermal cycles of qPCR as shown (Fig 4).

Statistical analysis

Statistical analysis of data was done by using SPSS statistical software for windows (version 24). One way ANOVA was applied to analyse the variance about mean Ct

Table 2: Primer sequence of genes.

Target gene	Prime sequence	References	Predicted size of gene product
HSP70	For: GACGACGGCATCTTCAAG	Dangi *et al.*, 2012	132 bp
(Gene of interest)	Rev: GTTCTGGCTGATGTCCTTC		
GAPDH	For: GCGATACTCACTCTTCTACTTTCGA	U85042.1	82 bp
(Housekeeping gene)	Rev: TCGTACCAGGAAATGAGCTTGAC		



Lane 2-4: HSP70 gene (132 bp), Lane 6-7: GAPDH gene (82 bp) and Lane M: 50 bp ladder.

Fig 1: Confirmation of cDNA and specificity of primers on 1.5 Agarose gel electrophoresis.

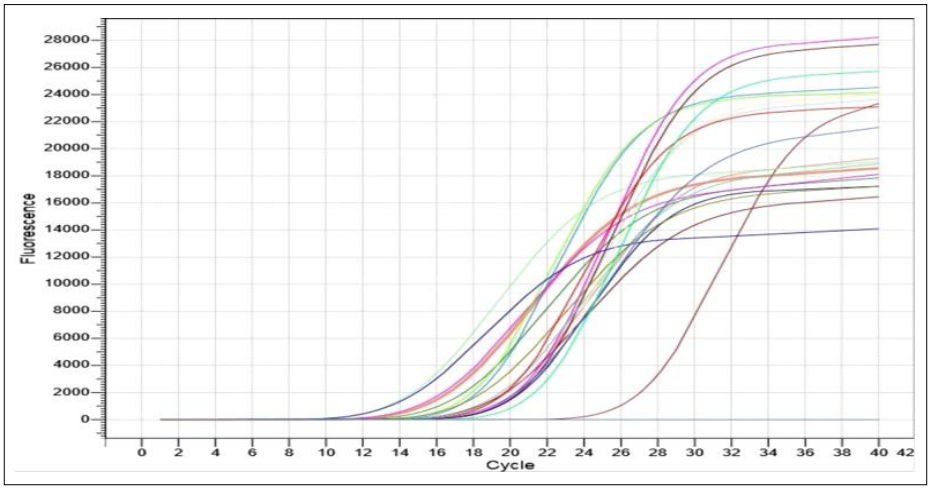


Fig 2: Real time pCR (qPCR) amplification plot of HSP70 and GAPDH gene expression.

value for relative mRNA expression of HSP70 gene between seasons.

RESULTS AND DISCUSSION

Temperature humidity index

The mean values of THI recorded during the peak months of different (summer, winter and rainy) seasons are presented (Table 3).

In the present study, the highest THI (83.36) was recorded in the month of May and the lowest (67.84) in January. However, the intermediate value (78.24) was recorded in the September.

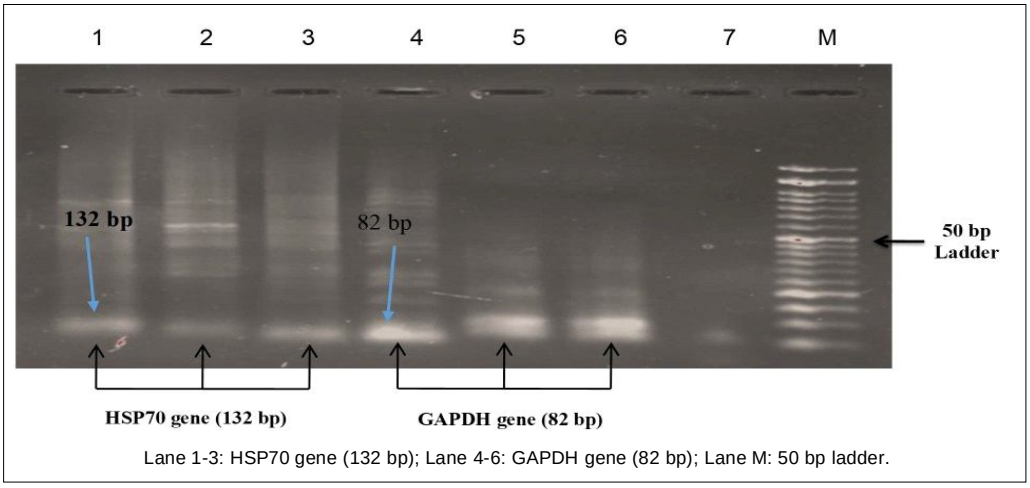
The values of THI 74 or less indicating comfort ability**,** 75-78 showing an alert status, greater than 79 to 83 could be vulnerable, THI equal to or above 84 is an emergency situation (McDowell, 1976 and Kohli, 2014).

We observed that temperature and humidity were the factors responsible for increase in m-RNA expression during peak summer (May: THI 83.36) as compared to peak rainy (September: THI 78.24) and peak winter (January: THI 67.84) seasons. Temperature humidity index (THI) is used to estimate the degree of stress on the animals (Armstrong, 1994 and Vaidya *et al.,* 2010).

It is worldwide accepted that THI equal to 72 is the threshold for environmental heat stress. However, Thatcher *et al.* (2010) reported that, if THI above 72, dairy cows could be exposed to heat stress and when it exceeds 88, then animals might be living under severe heat stress conditions. Our findings indicate that the animal may be living under moderate heat stress conditions, particularly during the summer season, where THI is 83.36. Moreover, during the rainy season, animals might be living under heat stress condition, where, THI is 78.24, during these conditions, animal was unable to maintain thermoregulatory responses.

HSP70 gene expression

The HSP70 gene expression was significantly differed amongst all the three seasons. It was found significantly ($P<0.01$) higher in the experimental animals during peak summer season followed by rainy season, whereas the HSP70 gene expression was significantly ($P<0.01$) lower during winter season (Table 4 and Fig 5).



Lane 1-3: HSP70 gene (132 bp); Lane 4-6: GAPDH gene (82 bp); Lane M: 50 bp ladder.

Fig 3: Confirmation of real time PCR products on 1.5 Agarose gel elctrophoresis.

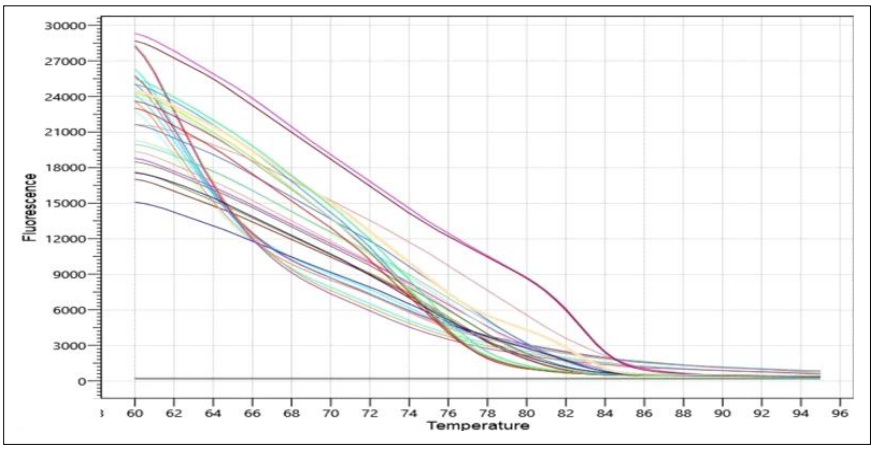


Fig 4: Melt curve analysis of real time PCR (qPCR) reaction products.

The HSP70 gene expression corresponds to the low Ct values indicating the higher gene expression during the summer as compared to the other two seasons *i.e.* rainy and winter.

The results of present study revealed that, the significantly (P<0.01) elevated levels of mRNA expression of HSP70 gene during the peak summer season as compared to rainy and winter seasons against hot dry and hot humid conditions in Red Kandhari cattle. These findings are in accordance with the outcomes of Parmar *et al.* (2015), who found that the relative mRNA expression of HSP70 gene was increased in peripheral blood mononuclear cells (PBMCs) of Sahiwal cows during the summer season as compared to winter. Further, the findings of the present study corroborated by De Maio (1999), who revealed that the cells can increase the expression of several classes of proteins when exposed to environmental stresses. Yadav *et al.* (2015) and Kumar *et al.* (2017) have also observed significantly(P<0.01) increase in mRNA and protein expression of HSP70 and HSP90 during summer (high THI) as compared to thermo-neutral season in all the goat breeds.

Red Kandhari cattle are acclimatized to hot and dry breeding tract of Maharashtra region. But during peak summer, ambient temperature was higher reaches to peak 44°C which may exert heat stress on animals. The upregulation of gene expression was recorded which is influenced by rise in THI during the summer and rainy seasons, these observation were collinear with the Bharati *et al.* (2017), who investigated the mRNA expression of HSP70 gene in cultured PBMCs of Tharparkar cattle on exposure to thermal stress. They reported that the HSP70 expression was increased in temperature and time dependent manner in cultured PBMCs as compared to control. These responses potentiate the protective effect of antioxidant enzymes through its chaperon functions (Uttarani, 2017). The upregulation of HSP70 mRNA reduces the oxidative stress at cellular level (Kalmar and Greensmith, 2009), improving antioxidant capacity and inhibits lipid peroxidation in order to protect the animals from harmful effect of heat stress.

Droge (2002) who reported that the body releases the HSPs proteins along with the antioxidants on exposure to heat stress to combat the cellular effects of ROS. However, Han *et al.* (2016), who observe that the transcription of the genes (HSP27, HSP70 and HSP90) of heat shock protein (HSP) was significantly enhanced under heat stress (HS) wherein, the peak transcription of HSP70 was 14 times higher than the control at 1 h.

Banerjee *et al.* (2014), who explored the effect of temperature sensitivity and seasonal variation on the expression patterns of HSP70 genes in Indian goats. They observed a significant (P<0.01) variation between different seasons for all HSP70 gene expressions, the expression pattern was higher during summer season. They concluded that, the animals exposed to ambient temperature

Table 3: Temperature humidity index (Mean ± SE) of three different seasons.

THI, Summer	THI, Winter	THI, Rainy
83.36±2.74	67.84±2.57	78.24±1.44

Table 4: The relative quantitative m-RNA expression of HSP70 and GAPDH gene in terms of Ct value (Mean ± SE) amongst different seasons in Red Kandhari cattle.

Genes	Winter (n=24)	Summer (n=24)	Rainy (n=24)	P- value
Target gene (HSP 70)	28.64^{a}±0.09	17.86^{c}±0.26	26.72^{b}±0.57	0.00
Housekeeping gene (GAPDH)	25.87^{a}±0.14	21.49^{c}±0.48	23.86^{b}±0.60	0.00

Means with no common superscripts are significantly different (P<0.01) within each row between different seasons.

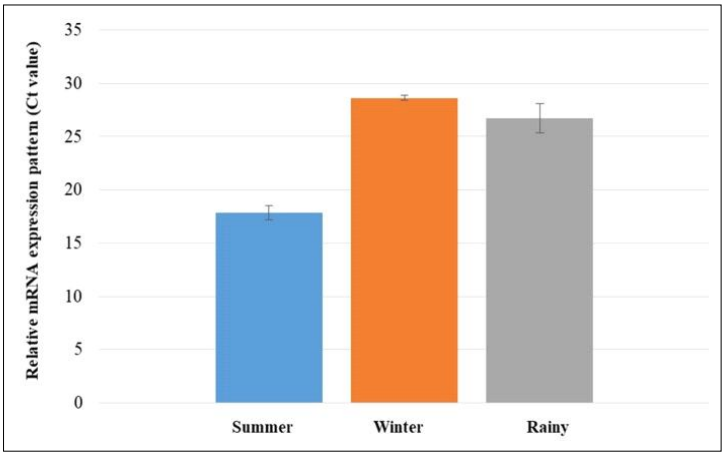


Fig 5: The relative m-RNA expression pattern of HSP70 gene in Red Kandhari cattle.

beyond the comfort zones were living under thermal stress during summer than that of winter. Also, the similar results were observed by Lacetera, 2006; Patir and Upadhaya, 2007, 2010, who found that the heat induced stress causes upregulation of HSP70 gene expression.

The comparable results were reported by Ambade, 2023, who found that the expression of HSP70 gene was significantly ($P<0.05$) higher during summer as compared to winter season in Pandharpuri buffaloes. They recorded the upregulation of HSP70 gene during both summer and rainy seasons whereas, during winter down regulation of gene was observed. The higher expression of HSP70 genes during the thermal stress suggested that there was involvement of HSP70 to rearrange the harmful effect of heat stress for maintaining the cellular integrity and homeostasis (Dangi, 2012).

Our study was corroborated by Kumar *et al.* (2019) who were undertaken the profiling of HSP70 gene in Murrah buffalo (*Bubalus bubalis*) under sub-tropical climate of India. They reported that there was relatively higher mRNA expression in summer, when THI was more than 84 as compare to the spring and winter seasons. Moreover, they also reported that there was fold change increase by 4.5 times in summer than the spring seasons.

The present study was supported by Pangaonkar *et al.* (2023) who estimated seasonal effect of THI on HSP70 gene expression in Deccani sheep, they predicted that HSP70 could be used as a potential bio marker for selecting climate-resilient animals that would show superior thermo-tolerance to enhance livestock productivity.

Suhendro *et al.* (2024) who investigated the association of heat shock protein 70.1 gene with physiological and physical performance in Bali cattle. They reported the higher expression of HSP70.1 that could mitigate the deleterious effect of heat stress.

Kumar *et al.* (2024) carried out the expression and SNP profiling of HSP70 gene associated with thermo tolerance traits in Munjal Sheep. Sheep were categorized into two groups-heat stress susceptible (HSS) and heat stress tolerant (HST)-based on their heat tolerance coefficient. Significant differences in HSP70 gene expression were observed between the HSS and HST groups.

Biradar *et al.* (2024) who studied the single nucleotide polymorphism in promoter region of HSP70 gene in Deoni cattle. They revealed that HSP70 gene polymorphism in genotype AA could be associated with physiological parameter *e.g.* respiration rate and rectal temperature.

In line with the present results, Kim *et al.* (2025), who reported that the HSP70 gene expression in hair follicle was increased with increase in temperature humidity index which implies that increase in HSP expression indicating thermo tolerance in diary calves. They also reported that there was genetic variation in stress tolerance that could be attributed to HSP70 gene polymorphism which is affecting the cellular responses and gene expression in animals exposed to heat stress.

CONCLUSION

The present study demonstrated that elevated temperature-humidity index (THI) during the summer season significantly influences HSP70 gene expression, suggesting its vital role in the cellular defence mechanism against thermal stress. In Red Kandhari cattle, the upregulation of HSP70 during summer supports cellular adaptability, stress tolerance and thermal regulation. However, the increased expression also indicates potential cellular vulnerability to heat stress during peak summer. HSP70 may serve as a reliable molecular marker for thermotolerance and could be utilized to identify heat-resilient animals in organized breeding programs. Further studies on genetic polymorphisms, particularly SNPs in the HSP70 gene, may enable the selection of heat-tolerant animals for future breeding strategies.

ACKNOWLEDGEMENT

The author wish to thank Associate Dean, College of Veterinary and Animal Sciences, Parbhani for providing the animals and data for this research. The Author also, thankful to The Director of Research, MAFSU, Nagpur for providing valuable guidance.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Study was approved by Institutional Animal Ethical Committee (IAEC) of the College of Veterinary and Animal Sciences, Parbhani (Maharashtra Animal and Fishery Sciences University, Nagpur) with vide No. IAEC / 100/2022.

Conflict of interest

The authors declare that there are no conflicts of interest in relation to this publication.

REFERENCES

AL-Jaryan, I.L., AL-Thuwaini, T.M. and AL-Jebory, H.H. (2023). Heat shock protein 70 and Its role in alleviating heat stress and improving livestock performance. *Reviews in Agricultural Science*. **11:** 234-242.

Ambade, R.B., Dalvi, S.H., Dighe, V.D., Doiphode, A.Y., Ramteke, B.N. and Jadhav, S.N. (2023). *Hsp70* gene expression pattern in peripheral blood mononuclear cells during different seasons in Pandharpuri buffaloes. *Indian Journal of Animal Research*. doi: 10.18805/IJAR.B-4888.

Armstrong, D. (1994). Heat stress interaction with shade and cooling. *Journal of Dairy Science*. **77(7):** 2044-2050.

Behera, R., Chakravarty, A.K., Sahu, A., Kashyap, Rai, S. and Mandal, A. (2018). Identification of best temperature humidity index model for assessing impact of heat stress on milk constituent traits in Murrah buffaloes under subtropical climatic condition of Northern India. *Indian Journal of Animal Research*. **52(1):** 13-19. doi: 10.18805/ijar.B-3359.

Behera, R., Mandal, A., Rai, S., Karunakaran, M., Mondal, M. and Ghosh, M.K. (2022). Genotype environment interaction on milk production traits of crossbred dairy cows under tropical climatic condition of India. *Indian Journal of Animal Research*. **56(11):** 1422-1427. doi: 10.18805/IJAR.B-4121.

Banerjee, D., Ramesh, C., Upadhyay, U., Chaudhary, B., Kumar R., Singh. S., Jagan, A., Mohanarao, G., Polley, S., Mukherjee, A., Das T.K. and Sachinandan, D. (2014). Seasonal variation in expression pattern of genes under HSP70 family in heat and cold-adapted goats (*Capra hircus*). *Cell Stress and Chaperones*. **19:** 401-408.

Beckham, J.T., Mackanos, M.A., Crooke, C., Takahashi, T., O'Connell-Rodwell, C., Contag, C.H., Jansen, E.D. (2004). Assessment of cellular response to thermal laser injury through bio luminescence imaging of heat shock protein 70. *Photochemistry and Photobiology*. **79:** 76-85.

Bharati, J., Dangi, S., Chouhan, V., Mishra, S. R., Bharti, M.K., Verma, V., Shankar, O., Yadav, V.P., Das, K., Paul, A., Bag, S., Maurya, V.P., Singh, G., Kumar, P. and Sarkar, M. (2017). Expression dynamics of HSP70 during chronic heat stress in Tharparkar cattle. *International Journal of Biometeorology*. **61:** 1017-1027.

Biradar, S.R., Komatwar, S.J., Dongre, V.B., Jadhav, P.V., Bhosale, A.V. and Vaidya M.M. (2024). Molecular characterization of heat shock protein 70 gene in Deoni cattle (*Bos indicus*). *Journal of Livestock Science*. **15:** 203-207.

Collier, R.J., Collier, J.L., Rhoads, R.P. and Baumgard, L. H. (2008). Invited review: Genes involved in the bovine heat stress response. *Journal of Dairy Science*. **91:** 445-454.

Dangi, S.S., Gupta, M., Maurya, D., Yadav, V.P., Panda, R.P., Singh, G. and Sarkar, M. (2012). Expression profile of HSP genes during different seasons in goats (*Capra hircus*). *Tropical Animal Health and Production*. **44:** 1905-1912.

De Maio, A. (1999). Heat shock proteins: facts, thoughts and dreams. *Shock*. **11(1):** 1-12.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Review*. **82:** 47-95.

Fujita, J. (1999). Cold shock response in mammalian cells. *Journal of Molecular Biology*. **1:** 243 - 255.

Han, H.U., Zhang, Y., Zheng, N., Cheng, J. and Wang, J. (2016). The effect of heat stress on gene expression and synthesis of heat-shock and milk proteins in bovine mammary epithelial cells. *Animal Science Journal*. **87:** 84-91.

Kadzere, C.T., Murphy, M.R., Silanikove, N. and Maltz, E. (2002). Heat stress in lactating dairy cows: A review. *Livestock Production Science*. **77:** 59-91.

Kalmar, B. and Greensmith, L. (2009). Induction of heat shock proteins for protection against oxidative stress. *Advanced Drug Delivery Reviews*. **61:** 310-318.

Kim, W.S., Jo, Y.H., Nejad, J.G., Lee, H.G. (2025). Effects of heat shock protein 70 gene polymorphism on heat resistance in beef and dairy calves based on proliferation and heat shock protein 70 gene expression in peripheral blood mononuclear cells and hair follicles. *Animals*. **15:** 475. https://doi.org/10.3390/ani15040475.

Kohli, S., Atheya, U.K. and Thapliyal, A. (2014). Assessment of optimum thermal humidity index for crossbred dairy cows in Dehradun district, Uttarakhand, India. *Veterinary World*. **7(11):** 916-921.

Kregel, K.C. (2002). Molecular biology of thermoregulation Invited review: Heat shock proteins modifying factors in physiological stress responses and acquired thermo tolerence. *Journal of Applied Physiology*. **92:** 2177-2186.

Kumar, A., Ashraf, S., Goud, T.S., Grewal, A., Singh, S.V., Yadav, B.R. and Upadhyay, R.C. (2015). Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. *Journal of Thermal Biology*. http://dx.doi.org/10.1016/j.jtherbio.2015.03.006.

Kumar, B., Sahoo, A.K., Dayal, S., Das, A.K. Taraphder, S., Batabyal, S. Ray, P.K. and Kumari, R. (2019). Geneticprofiling of Hsp70 gene in Murrah buffalo (*Bubalus bubalis*) under sub-tropical climate of India. *Cell Stress and Chaperones*. **24:** 1187-1195. https://doi.org/10.1007/s12192-019-01042-7 (2019.

Kumar, S., Magotra, A., Kumar, N. Bangar, Y.C. (2024). Expression and SNP profiling of HSP70 gene associated with thermotolerance traits in Munjal Sheep. DOI: https://doi.org/10.21203/rs.3.rs-4423115/v1.

Kumar, D., Yadav, B., Choudhury, S., Priyambada, K., Madan, A.K., Singh, S.P., Rout, P.K., Ramchandran, N.and Yadav, S. (2017). Evaluation of adaptability to different seasons in goat breeds of SEi-arid region in India through differential expression pattern of heat shock protein genes. *Biological Rhythm Research*. DOI: 10.1080/09291016.2017.1377984.

Lacetera, N., Bernabucci, U., Scalia, D., Basirico, L., Morera, P., Nardone, A. (2006). Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein Cows. *Journal of Dairy Science*. **89:** 4606-4612.

Lanneau, D., Brunet, M., Frisan, E., Solary, E., Fontenay, M. and Garrido C. (2007). Heat shock proteins: Essential proteins for apoptosis regulation. *Journal of Cellular and Molecular Medicine*. **12(3):** 743-761.

Lindquist, S. (1986). The heat shock response. *Annual Review of Biochemistry*. **55:** 1151-1191.

Liu, Y. and Steinacker, J. (2001). Changes in skeletal muscle heat shock proteins: Pathological significance. *Frontiers in Bioscience*. **6:** 12-25.

Mader, T.L., Davis, M.S., Brown-brandl, T. (2006). Environmental factor influencing heat stress in feedlot cattle. *Journal of Animal Science*. **84:** 712-719.

Marai, I.F.M., Darawany, A.A., Fadiel A. and Abdel Hafez M.A.M. (2007). Physiological traits as affected by heat stress in sheep a review. *Small Ruminant Research*. 71(1-12).

McDowell, R.E., Hooven, N.W. and J.K. Camoens (1976). Effects of climate on performance of Holsteins in first lactation. *Journal of Dairy Science*. **59:** 965-973.

Parmar, M.S., Madan, A.K., Huozha, R., Rastogi, S.K. and Milli, B. (2015). Protein70 (HSP70) gene expression pattern in peripheral blood mononuclear cells (PBMCs) during different seasons in sahiwal cows (*Bos Indicus*). *Journal of Animal Research*. **5(1):** 109-113.

Pangaonkar, L.A., Patodkar, V.R., Mehere, P.V., Amle, M.B., Khanvilkar, A.V., Lambate, S.B. and Yeotikar, P.V. (2023). Seasonal effect of THI on HSP70 gene expression pattern in deccani sheep. *Indian Journal of Animal Research*. **57(6):** 811-814. doi: 10.18805/IJAR.B-5041.

Patir, H. and Upadhyay, R.C. (2007). Interrelationship between heat shock protein 70 (HSP70) and lymphocyte proliferation in thermal exposed buffalo heifers. *Italian Journal of Animal Science.* **6:** 1344-1346.

Patir, H. and Upadhyay, R.C. (2010). Purification, characterization and expression kinetics of heat shock protein 70 from *Bubalus bubalis*. *Research in Veterinary Science*. **88:** 258-262.

Suhendro, I., Noor, R.R., Jakaria, J., Priyanto, R., Manalu, W. and Andersson, G. (2024). Association of heat-shock protein 70.1 gene with physiological and physical performance of Bali cattle. *Veterinary World*. **17(1):** 17-25.

Thatcher, W.W., Flamenbaum, I., Block, J. and Bilby, T.R. (2010). Interrelationships of Heat Stress and Reproduction in Lactating Dairy Cows. High Plains Dairy Conference. Amarillo, Texas. pp: 45-60. 2010.

Uttarani, M., Hooda O.K., Sharma, P.S., Singh, S.V. and Upadhyay R.C. (2017). Seasonal Change in oxidative stress markers in blood plasma of Tharparkar (*Bos indicus*) and Karan Fries (*Bos indicus*x *Bostaurus*) cattle under tropical climate. *International Journal of Current Microbiology and Applied Sciences*. **6(2):** 172.

Vaidya, M., Kumar, P. and Singh, S.V. (2010). Effect of temperature humidity index and heat load on physiological parameters of Murrah buffaloes and Karan Fries cattle during different seasons. *Wayamba Journal of Animal Science.* **578:** 57-58.

West, J.W. (1994). Interactions of energy and bovine somatotropin with heat stress. *Journal of Dairy Science.* **77:** 2091-2102.

Yadav, V.P., Dangi, S.S., Chouhan, V.K., Gupta, M., Dangi, S.K., Singh, G., Maurya, V.P., Kumar, P. and Sarkar, M. (2015). Expression analysis of NOS family and HSP genes during thermal stress in goat (*Capra hircus*). *International Journal of Biometeorology*. (DOI: 10.1007/s00484-015-1035-5).