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Association of heat shock protein 27 (HSP27) gene variants with physiological parameters and milk parameters in Haryana cattle

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Abstract

The present study was conducted on 50 Haryana cattle to analyse association of HSP 27 gene with vital parameters and of milk production parameters. Genomic DNA was extracted from whole blood of a total of 50 lactating Haryana cattle. PCR amplification yielded products of 631bp for HSP 27 gene. Amplicons were subjected to direct sequencing and multiple sequence alignment using ClustalW revealed one nucleotide sequence variation in HSP 27 gene. In HSP 27 gene G225A was polymorphic with three genotypes AA, AB and BB in our resource population. Allele and genotype frequencies of HSP 27 gene were calculated using POPGENE software package. Genotype AA, BB and AB had frequencies 0.64, 0.28 and 0.08, respectively, whereas, allele frequency for A and B allele were 0.78 and 0.22, respectively. Effect of SNP variants on physiological parameters and milk parameters were analysed using least squares model with the help of SAS statistical software. Effect of genotypes on physiological function were found significant ($p < 0.05$) for RR and HTC values. Least squares mean value for genotype AA, AB and BB were 67.85, 65.92 and 67.33 for PR, 101.40°F, 101.46°F and 101.11°F for RT, 24.00, 22.10 and 27.19 for RR, 1.69, 1.75 and 2.11 for HTC respectively. Haryana cows with genotype AA had better thermotolerance than the other cows having genotypes AB and BB due to low HTC values. However, effect of genotypes on milk parameters was found to be non-significant.

Keywords: Haryana cow, HSP 27 gene, SNPs, physiological and milk parameters

Introduction

One of the paramount challenges confronting livestock producers worldwide is heat stress, exacerbated by global warming and climate change. This environmental phenomenon poses a significant threat to the sustainability of livestock production systems (Moss *et al.*, 2000; Gaughan *et al.*, 2010; Naqvi and Sejian, 2011) [14, 9, 15]. Projections from current climate models indicate a steady rise in temperatures, with estimates suggesting an increase in global average surface temperature ranging from 1.8 °C to 4.0 °C by 2100 (IPCC, 2007). Such climatic shifts result in heat stress among livestock, manifested by reduced feed intake, diminished milk production, and decreased reproductive success rates (Cavestany *et al.*, 1985; Sharma *et al.*, 1988; Bernabucci *et al.*, 1999) [4, 22, 2].

Heat stress exerts adverse effects on various aspects of dairy cattle and buffalo reproduction, milk production, and immune function (Rensis and Scaramuzzi, 2003; Hansen, 2007; Marai and Habeeb, 2010; West, 2003; Elvinger *et al.*, 1992) [17, 10, 12, 26, 7]. The economic ramifications of thermal stress on milk production are significant, with estimates suggesting substantial annual losses by the year 2020 (Upadhyay, 2008) [23].

Heat shock proteins (HSPs) play a crucial role in cellular stress response mechanisms, including heat stress (Benjamin and Millan, 1998) [1]. Among them, HSP 27, encoded by the HSPB1 gene, serves various cellular functions such as chaperone activity, thermo tolerance, apoptosis inhibition, and regulation of cell development and differentiation (Walter and Buchner, 2002; Borges and Ramos, 2005) [25, 3]. Understanding the genetic variations in HSP 27 and its association with physiological parameters is crucial for identifying potential markers for heat tolerance in cattle breeds.

This study aims to investigate the association of heat shock protein 27 (HSP27) gene variants with physiological parameters and milk parameters in Hariana cattle. By examining genetic variations in HSP 27 and their correlation with physiological responses to heat stress, this research seeks to contribute to the development of selection strategies for improving heat tolerance in cattle breeds, ultimately enhancing their productivity and resilience in the face of climate change-induced challenges.

Methodology

The study aimed to investigate the association between variants in the heat shock protein 27 (HSP27) gene and physiological parameters as well as milk parameters in Hariana cattle. To achieve this objective, a cohort of 50 Hariana cows housed at Shri Krishan Gaushala Avam Anusandhan Kender, Kabrel, Hisar, was selected with prior approval from the Institutional Animal Ethics Committee.

Blood samples of 10 ml were aseptically collected from the jugular vein of each cow and preserved in vacutainer tubes containing EDTA as an anticoagulant. High molecular weight DNA was extracted from the whole blood using the Phenol Chloroform method, following the protocol outlined by Sambrook and Russell (2001) [18] with minor adaptations. Subsequently, the extracted DNA underwent rigorous quantitative and qualitative analyses to ensure its integrity and purity.

Single nucleotide polymorphisms (SNPs) within the HSP27 gene were identified by aligning reference sequences with the sequencing results obtained from each animal for every

target region. This alignment was performed using the ClustalW multiple alignment program.

The impact of SNP variants on physiological parameters and milk quality parameters in Hariana cattle was assessed using a least squares model, facilitated by the SAS statistical software. This comprehensive analytical approach enabled the investigation of genetic variations within the HSP27 gene and their potential associations with key physiological traits and milk quality parameters in the Hariana cattle population.

Results and Discussion

Allele and Genotype frequencies

The mutation g. G225A within the HSP27 gene of Hariana cattle was confirmed, resulting in three distinct genotypes: AA, BB, and AB. Allele and genotype frequencies of the HSP27 gene were determined using the POPGENE software package. Genotypes AA, BB, and AB exhibited frequencies of 0.64, 0.28, and 0.08, respectively, while the allele frequencies for A and B were calculated as 0.78 and 0.22, respectively (see Table 1).

These findings provide valuable insights into the genetic variability within the HSP27 gene among Hariana cattle. The distribution of genotypes and alleles sheds light on the prevalence of specific genetic variants within this population, which may play a role in modulating physiological and milk parameters. Further analysis correlating these genotypic frequencies with physiological and milk traits will elucidate potential associations between HSP27 gene variants and the performance characteristics of Hariana cattle.

Table 1: Genotypic and allelic frequency of different genotype for HSP 27 gene

Breed	Genotype frequency			Total	Allele frequency	
	AA	AB	BB		A	B
Hariana cattle	0.64 (32)*	0.28 (14)*	0.08 (4)*	50	0.78	0.22

*Figures in parenthesis shows no. of animals

Association of Genotype with different physiological parameters (pulse rate, respiration rate, rectal temperature and Heat tolerance coefficient)

The effect of genotypes on physiological function was found to be significant ($p < 0.05$) for RR and HTC values. Least squares mean values for genotypes AA, AB, and BB were calculated as follows: 67.85, 65.92, and 67.33 for PR; 101.40°F, 101.46°F, and 101.11°F for RT; 24.00, 22.10, and 27.19 for RR; and 1.69, 1.75, and 2.11 for HTC, respectively (refer to Table 2).

The present study suggests that Hariana cows with the AA genotype exhibit superior thermotolerance compared to those with the AB and BB genotypes. This inference is

supported by the observed increase in respiration rate, which serves as a crucial thermoregulatory response to heat stress. Heightened respiration aids in the dissipation of excess body moisture through expired air, thereby assisting in temperature regulation. Conversely, a higher HTC value indicates reduced adaptability to heat stress, particularly during the summer months.

These findings underscore the potential role of HSP27 gene variants in modulating the physiological responses of Hariana cattle to heat stress. Further investigation into the relationship between genotype and thermoregulatory mechanisms will provide valuable insights into enhancing heat tolerance and overall productivity in this breed.

Table 2: Statistical Association of genetic variant (HSP 27) with physiological parameters

Genotype	Pulse Rate	Respiration Rate	Rectal Temperature	HTC
AA	67.85±0.59	24.00 ^b ±0.51	101.40±0.09	1.69 ^b ±0.02
AB	65.92±0.90	22.10 ^b ±0.78	101.46±0.13	1.75 ^b ±0.04
BB	67.33±1.68	27.19 ^a ±1.45	101.11±0.24	2.11 ^a ±0.07

Association of Genotype with milk parameters

The effect of SNP variants on milk parameters was analyzed using a least squares model with the assistance of SAS statistical software. However, the effect of genotypes on milk parameters was not found to be significant (refer to Table 3).

While there are no existing reports specifically detailing the association between physiological parameters and HSP27 gene SNP/polymorphism in cattle or buffalo, studies focusing on meat quality traits have identified associations with SNP ss63015930 in cattle (Sevane *et al.*, 2011) [20], intramuscular fat (Hocquette *et al.*, 2012) [11], and the

percentage of lauric acid and phospholipid docosahexaenoic acid (Sevane *et al.*, 2014) [21].

Furthermore, Mohanarao *et al.* (2014) [13] reported a significantly increased expression of the HSP27 gene in peripheral blood mononuclear cells (PBMCs) of goats under both cold and heat stress conditions. This finding suggests that HSP27 may serve as a potential indicator for assessing the thermal stress response of animals.

While the present study did not reveal a significant association between HSP27 gene variants and milk parameters in Hariana cattle, further research is warranted to elucidate the potential role of HSP27 in modulating milk production and quality traits in this breed. Additionally, exploring the broader implications of HSP27 gene variants on various physiological and production parameters may provide valuable insights into improving the overall resilience and productivity of cattle under different environmental conditions.

Table 3: Statistical Association of genetic variant (HSP 27) with milk parameters

Genotype	Protein	Lactose	Fat	SNF
AA	3.02±0.02	4.48±0.02	3.77±0.05	8.28±0.02
AB	3.00±0.03	4.44±0.03	3.79±0.07	8.32±0.03
BB	2.96±0.06	4.36±0.06	3.59±0.13	8.19±0.06

Conclusion

In conclusion, our study investigated the association between heat shock protein 27 (HSP27) gene variants and physiological parameters, as well as milk parameters, in Hariana cattle. The analysis revealed the presence of a polymorphism (g.G225A) within the HSP27 gene, resulting in three distinct genotypes: AA, AB, and BB, with corresponding allele frequencies.

Significant associations were observed between genotype and physiological parameters, particularly respiration rate (RR) and heat tolerance coefficient (HTC). Hariana cows with the AA genotype exhibited superior thermotolerance compared to those with the AB and BB genotypes, as evidenced by lower RR and HTC values. This suggests that genetic variations within the HSP27 gene may play a role in modulating the physiological responses of cattle to heat stress, highlighting its potential as a marker for heat tolerance in this breed.

However, no significant association was found between HSP27 gene variants and milk parameters in Hariana cattle. Despite this, our findings contribute to the understanding of genetic factors influencing heat tolerance and physiological responses in cattle, particularly under changing environmental conditions.

Further research is warranted to elucidate the mechanisms underlying the observed associations and to explore the broader implications of HSP27 gene variants on various aspects of cattle physiology and production. Such investigations will be valuable for developing targeted breeding strategies aimed at enhancing heat tolerance and overall productivity in cattle breeds, ultimately contributing to the sustainability of livestock production systems in the face of climate change challenges.

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