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Genetic Polymorphism in 220 bp Fragment of HSP 70 Gene in Kankrej Cattle by PCR-SSCP

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ABSTRACT

The experiment was conducted during January, 2022 to January, 2023 at the Department of Animal Genetics & Breeding, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India to identify polymorphisms in HSP 70 gene. Heat Shock Proteins (HSPs) known as molecular chaperones are essential for cells' ability to recover from stress and serve as the primary defence mechanism within cells. They are extremely conserved and essential to the response to heat stress and cellular thermotolerance. Even though there are numerous HSP genes, in livestock species, heat tolerance is primarily associated with HSP 70. This gene polymorphisms have been linked to heat tolerance, milk production, fertility and cattle susceptibility to disease. They can be utilised as genetic markers to help choose animals that are more resilient to climate change, have stronger immune systems and perform better overall. A 220 bp fragment of bovine HSP 70 gene was subjected to Polymerase Chain Reaction- Single-Strand Conformation Polymorphism (PCR-SSCP) technique to identify the polymorphism. PCR-SSCP pattern was associated with the thermotolerance traits in Kankrej cattle using the univariate GLM model of SPSS 26. HSP 70 gene (220 bp fragment) was found to be monomorphic documented on SSCP gel which revealed only one genotype (AA) in all Kankrej cattle. It is concluded that genotype and its association with thermotolerance traits were found to be non-significant. However, The HSP 70 polymorphism is expected to strongly predict cattle heat tolerance, aiding in selection for thermotolerance.

KEYWORDS: Kankrej cattle, PCR-SSCP, Polymorphism, thermotolerance traits, HSP 70

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

The burden that an ever-growing human population is placing on natural resources, especially livestock is immense (Herrero and Thornton, 2013) another significant danger to the sustainability of animal production systems is the changing global environment (Polsky and Keyserlingk, 2017). Livestock immune competence, metabolic health and performance in reproduction and production are all impacted by global warming.

Selection and breeding methods are the only tools available for animal breeders to meet these challenges. Though crossbreeding was proved to be the fastest way of increasing milk production, adaptability of crossbreds was poor as reflected by lower breeding efficiency. Further, crossbreds are found to be highly susceptible to diseases and less tolerant to heat stress (Singh, 2016). The only implementation accessible to animal breeders to handle these issues are selection and breeding techniques. The current approaches, which concentrate on short-term management adjustments to lessen the consequences of heat stress are only partially effective (Berman, 2005). A viable long-term plan to mitigate the consequences of heat stress might involve feeding and housing improvements with a focus on genetic modifications (Boonkum et al., 2011; Scholtz et al., 2013).

Heat shock proteins (HSPs) are molecular chaperones that aid in the healing process of stressed cells and offer cytoprotection, shielding them from further assaults. By identifying nascent polypeptides, unstructured protein regions and exposed hydrophobic amino acid segments, they protect stressed cells. Although there are other HSP genes, the HSP 70 and HSP 90 genes are mostly associated with heat tolerance in farm animals. HSP 70 is the most prevalent and temperature-sensitive and it is thought to play a key role in environmental stress and thermal adaptation. Higher levels of expression of proteins of the HSP 70 and HSP 90 families have been observed in various livestock species in summer season (Archana et al., 2017). Animal traits such as heat tolerance, milk production, fertility and susceptibility to disease have been associated with variations in the HSP 70 and HSP 90 genes (Shergojry et al., 2014; Kumar et al., 2015, Bhat et al., 2016). They might be helpful candidate gene markers to find animals with better immune responses, climate resilience and overall performance (Hassan et al., 2019).

While there have been observed variations in heat tolerance between Bos indicus and Bos taurus cattle at the physiological and cellular level (Collier et al., 2006; Chaiyabutr et al., 2008; Wilson and Crandall, 2010 and Dalcin et al., 2016). Additionally, heat stress can also alter follicular growth (Roth et al., 2000), steroid secretion (Wolfenson et al., 2000; Ozawa et al., 2005) and gene expression (Argov et al., 2005). Although, zebu cattle (Bos indicus) have gained genes that provide thermotolerance at the physiological and cellular levels throughout their independent evolution from Bos taurus. The zebu genotype can be further exploited for cattle production systems through breeding techniques like marker assisted selection, once the specific genes causing thermotolerance in zebu have been found or mapped (Hansen, 2004). However, there is limited data available on HSP gene polymorphism in crossbred Holstein Friesian cattle and Sahiwal cattle. However, few reports from India about the relationship of HSP 70 and HSP 90 gene polymorphism with heat tolerance in Tharparkar cattle (Bhat et al., 2016), Deoni cattle (Kerekoppa et al., 2015), Jersey crossbred cows (Sailo et al., 2015), Sahiwal cattle (Prasanna et al., 2022) and from abroad in Holstein cow (Li et al., 2011). Therefore, the present study assessed the polymorphisms of the 220 bp fragment of HSP 70 gene and its association with several physiological parameters viz. Rectal temperature, respiration rate and pulse rate in Kankrej cattle.

2. MATERIALS AND METHODS

2.1. Experimental animals

The present study was carried out during January, 2022 to January, 2023 which included a total of 50 Kankrej cows maintained at the Livestock Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardar Krushi Nagar, Dantiwada and 50 Kankrej cows from nearby areas of Banaskantha District North Gujarat (India).

2.2. Genomic DNA isolation

Blood samples (10 ml) were collected by jugular vein puncture from all experimental animals ascetically in vacutainers containing K_3EDTA as an anti-coagulant and stored at -20°C till further use. Genomic DNA was isolated from blood as per standard phenol: chloroform extraction protocol described by John et al. (1991) with required modification. The purity of the genomic DNA samples was determined by measuring the optical densities (OD) at 260 nm and 280 nm against a blank using Nanodrop (Thermo Fisher Scientific) and storing them at -20°C until further use.

Table 1: Primer sequence for amplification of HSP 70 gene

Primer Set	Sequence	Amplicon size	Reference
HSP 70 fragment	F 5'CTAAGGTGCAGGTGAGCTACAAAG3'	220 bp	Bhat et al., 2016
	R 5'TTGATGATCCTCAGCACGTTCAGC3'		

2.3. Physiological parameters

The physiological parameters, rectal temperature (RT), respiration rate (RR) and pulse rate (PR) of each animal in the current study were recorded in each of the three seasons, i.e winter, spring and summer.

2.4. PCR primers and amplifications

Published forward and reverse primers used for amplification of HSP 70 gene length of 220 bp for identification of various allele variants present in Kankrej cattle.

PCR was carried out in a final reaction volume of 20 µl using 2X PCR Master mix (Emerald, TaKara) containing 0.05 U μl^{-1} Taq DNA polymerase in reaction buffer (Mg Cl_2 4 mM, dNTPS 0.4 mM). A master mix was prepared and aliquoted 17 µl in each of PCR tube. Three µl sample DNA was added in each to make the final volume of 20 µl. All the reactions were carried in thermal cycler (Ependorf) and subjected to PCR with 5 min of initial denaturation at 95°C followed by 30 cycles of 60 s denaturation at 95°C, 45 s annealing at 60°C for HSP 70 Fragment, 60 s extension at 72°C and 10 min of final extension at 72°C. PCR products were detected by electrophoresis on 2% agarose gel stained with ethidium bromide.

2.5. Single strand conformation polymorphism (SSCP)

Polymorphism in 220 bp fragment of HSP 70 gene was screened using the single-strand conformation polymorphism (SSCP) technique using the amplified PCR products. About 10 µl of PCR product was taken with 10 µl formamide solution and mixed properly followed by denaturation at 95°C for 10 min then snap chilling on ice for 15 min. The mixture was loaded on 8% polyacrylamide gel (acrylamide/bis-acrylamide 29:1, w/w) and electrophoresis was carried out using×0.5 tris-borate EDTA (45 mM tris-borate 1^{-1} mM EDTA) at 150 V for 4 h. Distinct band patterns were detected by silver staining of the SSCP product (Bassam et al., 1991). Each animal showing different conformation banding pattern was assigned a specific genotype. The frequency of HSP 70 genotypes and their allelic frequencies were estimated by standard procedure.

2.6. Association analysis

Association study between thermotolerance traits with the HSP 70 genotypes was analyzed by the univariate general linear model of SPSS v 26 according to the following statistical model:

$Yijkl=\mu+Gi+BFk+\varepsilon ik$

Where, Yijkl=Observation for rectal temperature, respiration rate and pulse rate, µ=Overall mean for each trait, Gi=Fixed effect of Genotype, BFk=Fixed effects of breed, and εik=Random environment effect.

Significant differences between the means of different thermotolerance traits and genotype were tested by Duncan's multiplerange test (DMRT). Values were considered significant at $p<0.05$ and are presented as means±SE.

3. RESULTS AND DISCUSSION

The PCR reactions were set for all the animals with species-specific primers available in the literature for the amplification in 220 bp fragment of HSP 70 gene. Amplification of desired size was noticed in all the tested samples. The representative figure showing the PCR amplified products of HSP 70 gene, showing the size of 220 bp are presented in Figure 1. While following SSCP technique, only one genotypes was observed for this fragment of HSP 70 gene and arbitrarily assigned as AA (Figure 2), which revealed monomorphic nature of selected loci. Thus, genotypes and gene frequencies are eventually one.

Bhat et al. (2016) demonstrated monomorphic SSCP pattern in Tharparkar cattle which is found similar to the present study. Similarly, Prasanna et al. (2022) also revealed the 220 bp fragment was found to be monomorphic in both Sahiwal and crossbred cows. No further reports were available to compare or contrast the present findings.

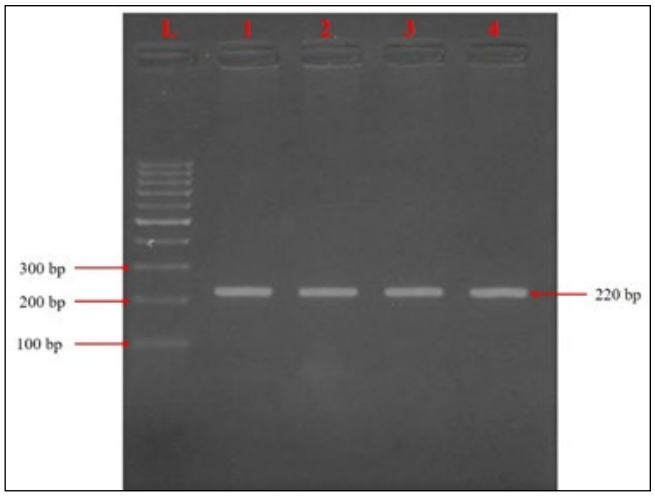

Figure 1: Amplified HSP 70 fragment (220 bp) (L: 100 bp ladder, 1-4 sample)

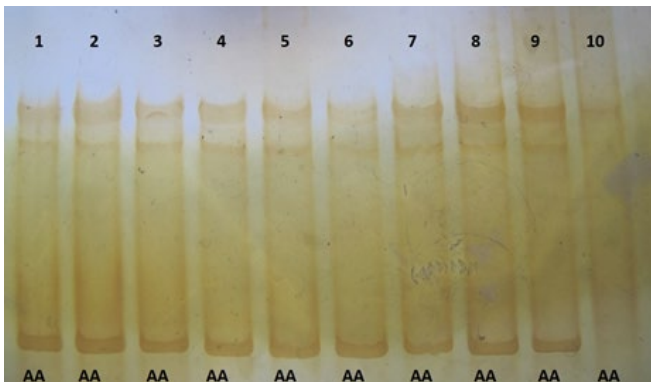

Figure 2: SSCP genotype of 220 bp fragment – HSP 70 gene in Kankrej cattle (1-10 sample)

Effect of genotypes on physiological parameters like rectal temperature, respiration rate and pulse rate of Kankrej cattle in winter, spring and summer season as well as overall effect on morning and evening has been presented in Table 2. Genotype (AA) of Kankrej cattle under study were found to have non-significant effect on rectal temperature,

Table 2: Effect of genotype (AA) on thermotolerance traits in all season and overall effect

Physiological parameter		Seasons			Overall
		Winter	Spring	Summer	
Rectal temperature (°F)	Morning	99.48±0.05	100.74±0.02	101.48±0.02	100.57±0.05
	Evening	100.69±0.07	101.55±0.01	102.63±0.02	101.63±0.05
Respiration rate (breaths/min)	Morning	23.75±0.07	24.60±0.05	28.90±0.08	25.75±0.13
	Evening	28.48±0.07	29.35±0.07	34.69±0.07	30.84±0.16
Pulse rate (beats/min)	Morning	52.89±0.07	55.19±0.07	61.60±0.05	56.56±0.21
	Evening	57.52±0.05	60.57±0.05	67.52±0.05	61.87±0.24

respiration rate and pulse rate both in the morning and evening (p<0.05).

Rectal temperatures, respiration rate and pulse were higher during evening than morning and during summer season as compared to spring and winter season but were not significantly different. Normally these parameters are associated with thermotolerance and they tend to be higher in bovines during heat stress. Non-significant rise in these parameters is associated with adaptive capabilities for thermal stress. In the present study non-significant differences between these parameters indicate that the genotype (AA) is thermotolerant and adapted for heat stress.

4. CONCLUSION

A 220 bp fragment of HSP 70 gene was successfully amplified with PCR-SSCP technique and found to be monomorphic with only one genotype AA that was associated non-significantly with its thermotolerance.

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