J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
50(2):103-110, June 2025
DOI: 10.14710/jitaa.50.2. 103-110

Genetic diversity of coding sequence (CDS) region of HSP70 gene in Bali cattle

Y. G. M. Bonawati[1], I. Suhendro[2], Jakaria[3] and R. R. Noor [3]
[1]*Postgraduate student of Animal Production and Technology, IPB University;*
[2]*National Research and Innovation Agency, Cibinong, Bogor 16911, Indonesia;*
[3]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia.*
Corresponding e-mail: ronny_noor@yahoo.com

Received December 09, 2024; Accepted April 10, 2025

ABSTRACT

This study aimed to investigate the genetic diversity of the Heat Shock Protein 70 (HSP70) gene in the coding sequence (CDS) region and its association with physiological responses in Bali cattle. The samples used in this study consisted of 62 Bali cattle from two different locations in Bali and Serading. Physiological data, including respiratory rate, heart rate, rectal temperature, and heat tolerance coefficient (HTC), were collected. The HSP70 gene was amplified using two pairs of primers to target the coding region. SNPs of the HSP70 gene were identified through sequencing. The diversity of SNPs in the coding sequence of the HSP70 gene was determined using the FinchTV 1.4.0 application and version X of the Molecular Evolutionary Genetic Analysis (MEGA) program. In contrast, the association of HSP70 gene SNPs with physiological responses in Bali cattle was evaluated using the GLM method in IBM SPSS Statistics 26. The research discovered nine SNPs within the CDS region of the HSP70 gene, comprising eight synonymous SNPs (c.24C>T, c.31C>T, c.117C>A, c.126G>A, c.324G>A, c.333C>T, c.573G>C, c.1074C>T) and one nonsynonymous SNP (c.1265C>T), which caused an amino acid substitution from threonine (T) to methionine (M). The SNP diversity in the coding sequence of the HSP70 gene showed no significant association ($P > 0.05$) with physiological responses, including respiratory rate, heart rate, rectal temperature, and heat tolerance coefficient (HTC). The SNPs discovered in the coding region of the HSP70 gene exhibited polymorphism in Bali cattle.

Keywords: *Bali cattle, coding sequence, HSP70 gene, SNP*

INTRODUCTION

Climate change adversely affects multiple industries, including livestock (Kim *et al.,* 2022). The effects of climate change on cattle illustrate how heat stress affects livestock productivity. While livestock can adapt to environmental stress, their survival strategies often reduce overall performance (Sejian *et al.,* 2018). Increased ambient temperatures impair livestock adaptability and survival due to heat stress. Heat stress in livestock increases respiratory and heart rates, as well as body temperature, functioning as a physiological response to regulate heat production and release it to the environment (Suhendro *et al.,* 2024). Heat stress reduces feed consumption, milk production, growth, and reproductive efficiency (Hu *et al.,* 2019; Liu *et al.,* 2019).

Indonesia has a diverse range of livestock genetic resources, comprising a various of types and breeds. This diversity is crucial in adapting to the increasingly hot tropical climate. One of

Indonesia's native livestock genetic resources is Bali cattle (Hariyono, 2022). Bali cattle are native to Indonesia and represent 27% of the national population (Purwantara *et al.,* 2012). They are prevalent throughout almost all regions of Indonesia and are essential in meeting national beef demand, as well as that of other large livestock and ruminants. Bali cattle display high adaptability in marginal habitats with limited feed resources and high reproductive capability (Martojo, 2012).

Strategies to investigate the genetic potential of Bali cattle, especially genes related to heat tolerance, through Marker-Assisted Selection (MAS) techniques. Marker-assisted selection (MAS) is a vital source of information for identifying marker-based candidate genes. It serves as a guideline for establishing breeding strategies, particularly in Bali cattle (Aliyya *et al.,* 2020). Several environmental stressors, genetic potential, and management practices induce the HSP70 gene. Under stress, HSP70 interacts with several cellular proteins to preserve homeostasis and protect cells from damage caused by high temperatures (Kaushik *et al.,* 2022). The HSP70 gene consists of a 5' untranslated region (5'UTR), a coding sequence (CDS) that encodes the heat-shock 70-kilodalton protein (HSPA1B) spanning 1926 bp, a promoter, and a termination region (Sugimoto *et al.,* 2003).

Genetic polymorphisms in the HSP70 gene have been reported in other livestock species, mainly because they protect cells against heat stress-induced damage in Romosinuano cattle (Taborda-Charris *et al.,* 2023). The variability of the 5' untranslated region of the HSP70 gene has been discovered in Indonesian Indigenous cattle (Prihandini *et al.,* 2022). Particular SNPs, including g.1117G>A (Haddar *et al.,* 2022), g.-69T>G (Suhendro *et al.,* 2022), and g.136G>T (Suhendro *et al.,* 2024), within the HSP70 gene, have been recognized as prospective biomarkers for thermotolerance characteristics in Bali cattle.

Based on the above report, the genetic diversity of the HSP70 gene in response to heat stress has been extensively studied in various livestock worldwide. However, investigations on the HSP70 gene throughout the coding sequence (CDS) region of Bali cattle are still needed. The study aimed to identify SNPs in the coding sequence of the HSP70 gene and their association with physiological responses in Bali cattle. Furthermore, it is expected to provide insights for Marker-Assisted Selection (MAS) and support future research and development aimed at improving the heat tolerance of Bali cattle.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Animal Ethics Committee of Udayana University, Denpasar, Indonesia (Code ID: B/184/un14.2.9/pt.01.04/2021).

Animals and Sample Selection

The study aimed to identify SNPs in the coding sequence of the HSP70 gene in Bali cattle and was conducted from March to September 2024. This research utilized DNA samples from 62 Bali cattle, including 15 samples obtained from the Breeding Center (BPTU-HPT) in Denpasar, Bali (8°25’3” S, 114°51’49” E, altitude 46 m), and 47 samples from the Livestock and Forage Breeding Center (BPTHMT) in Serading, West Nusa Tenggara (8°34'04" S, 117° 29'48" E, altitude 50 m). Measurements were taken under controlled conditions in a livestock squeeze chute. Physiological responses, including respiratory rate (RR), heart rate (HR), and rectal temperature (RT), were recorded during the sample month of October 2021. The Heat Tolerance Coefficient (HTC) was utilized to evaluate heat adaptation in Bali cattle. Livestock with an HTC value of 2 was designated heat-tolerant, while animals with a value larger than 2 were classified as possessing reduced heat tolerance (Singh *et al.,* 2016).

Amplification and Sequencing

DNA extraction was conducted using the Promega DNA Extraction Kits. The amplification of the HSP70 gene in the coding region was performed using two primer sets developed with the Primer3 program (https://primer3.ut.ee/) according to the GenBank reference sequence AY149618.1. The first primer set produced a 963 bp product, the forward primer 5'-CGCAGATCCTCTTCACCGAT-3' and the reverse primer 5'-CCTGGTGATGGACGTGTAGA-3'. The second primer set produced a 1060 bp product, the

forward primer 5'-TCTACACGTCCATCACCAGG-3' and the reverse primer 5'-AAGTAAGGCCCCTAGTCCAC-3'.

The PCR reaction was formulated with a total volume of 25 µL, comprising 1 µL of DNA template, 0.3 µL of forward primer (at a concentration of 25 pmol), 0.3 µL of reverse primer (at a concentration of 25 pmol), 10.9 µL of nuclease -free water, and 12.5 µL of red master mix. The PCR protocol consisted of pre-denaturation at 95 °C for 1 minute, followed by denaturation at 95 ° C for 15 seconds, annealing at 58 °C for 15 seconds, and extension at 72 °C for 10 seconds, repeated for 35 cycles, concluding with a final extension at 72 °C for 5 minutes.

The amplification results were visualized on a 1.5% agarose gel stained with FloroSafe DNA and viewed using a UV transilluminator (Bio-Rad, Hercules, CA, USA). Finally, the successfully amplified PCR products were sequenced using the ABI PRISM BigDye Kit v3.1 by First BASE Laboratory (Selangor, Malaysia).

Data Analysis

The SNPs in the chromatogram were identified using FinchTV 1.4.0 (http://www.geospiza.com), and double-peak bands were considered heterozygous positions. The target gene sequence was aligned with the reference sequence (GenBank®: AY149618) using the Molecular Evolutionary Genetic Analysis (MEGA) software version X (Kumar *et al*., 2020). Allele and genotype frequencies were determined using the methodology of Nei and Kumar (2000), and heterozygosity was assessed according to the methodology of Allendorf *et al* (2010). The Hardy-Weinberg equilibrium was evaluated by Chi-square analysis (Hartl and Clark, 1997).

The association between single nucleotide polymorphisms (SNPs) in the HSP70 gene coding sequence and the physiological responses of Bali cattle from various regions was examined using the Generalized Linear Model (GLM) algorithm in IBM SPSS Statistics version 26. The employed model was

$$Y_{ijk} = \mu + L_i + G_j + e_{ijk}$$

Where Y_{ijk} signified observations of traits/ characteristics, µ represented the overall mean, L_i denoted the effect of the i-th location, G_j indicated the fixed effect of the j-th genotype, e_{ijk} and represented the error (Bhat *et al.,* 2016).

RESULTS AND DISCUSSION

Amplification of Coding Sequence Region of HSP70 Gene in Bali Cattle

The amplification of the coding region of the HSP70 gene in 62 Bali cattle identified DNA fragments of 963 bp and 1060 bp (Figure 1). Amplification was observed at annealing conditions at 58°C for 15 seconds. The PCR reaction using the designed primers was optimized by evaluating different annealing temperatures, primer concentrations, and extension durations (Li *et al.,* 2016). The annealing temperature affected the primer annealing process, an essential phase in PCR, and determined the specificity of primer binding (Erjavec, 2019).

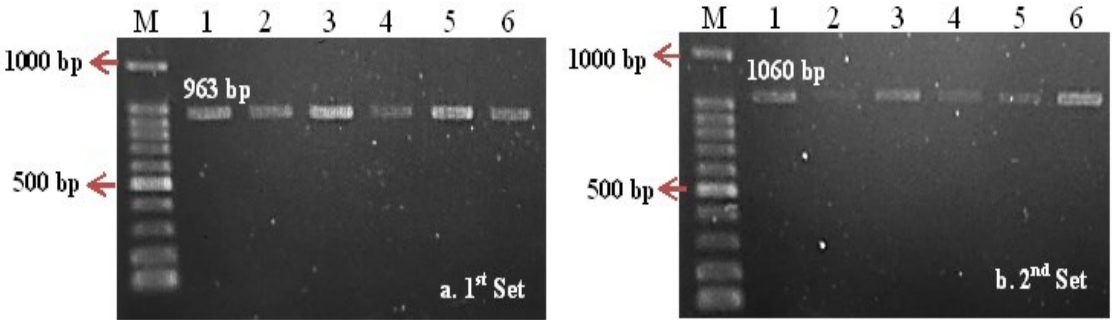

Figure 1. Visualization of the PCR product of the CDS of the HSP70 gene in Bali cattle. The first set measures 963 bp, and the second set measures 1060 bp. M represents the 100 bp marker, and numbers 1-6 indicate the samples.

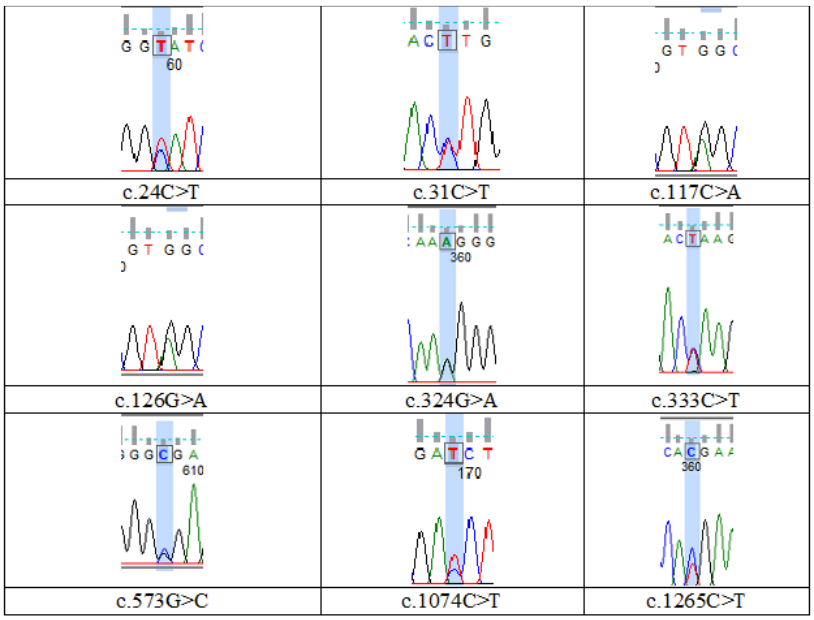

Figure 2. Chromatogram of CDS showing HSP70 mutations in Bali cattle

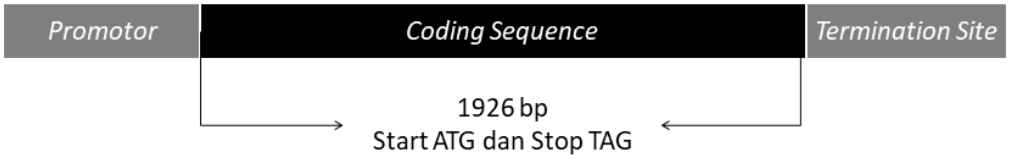

Figure 3. Illustration of the amplification target for the coding sequence region of the HSP70 gene in Bali cattle (AY149618). The CDS of the HSP70 gene has a total exon length of 1926 bp.

SNP Diversity of Coding Sequence Region of HSP70

DNA sequencing of the target gene was performed using the Sanger DNA sequencing technique on the ABI PRISM 3730 DNA Analyzer. This technique produced chromatogram data representing the DNA samples (Hagemann and Kwan, 1999). The chromatogram analysis of all samples, aligned with the reference nucleotide sequence of the HSP70 gene from NCBI (accession code AY149618), identified nine mutation loci in the coding sequence region (Figure 2).

The alignment of nitrogenous bases in the coding sequence region of the HSP70 gene was conducted from the start codon (ATG) to the stop codon (TAG). The HSP70 coding sequence has 1926 base pairs and encodes 641 amino acids (Figure 3). Seven transition substitution mutations (at positions 24, 31, 126, 324, 333, 1074, and 1265) and two transversion substitutions (at positions 117 and 573) were identified. Loci revealed dominant homozygous, heterozygous, and recessive homozygous genotypes, except for the exceptions at mutation sites 31, 333, and 1265. A nonsynonymous amino acid substitution was identified at mutation position c.1265C>T, where threonine (T) was replaced by methionine (M). The coding sequence diversity of the HSP70 gene was determined through SNP analysis, revealing nine SNPs, as shown in Table 1.

Allele frequency was determined as the proportion of a specific allele relative to the total number of alleles at an SNP locus within the population (Table 1). All SNPs were considered polymorphic if the frequency of one of their al-

Table 1. The diversity of CDS in the HSP70 Gene of Bali Cattle

SNP	Allele Frequency		Genotype Frequency			H_o	H_e	χ^2
	A	B	A/A	A/B	B/B			
c.24C>T	0.976	0.024	0.968	0.016	0.016	0.016	0.048	26.877*
c.31C>T	0.968	0.032	0.935	0.065	NA	0.065	0.063	0.067^{ns}
c.117C>A	0.726	0.274	0.532	0.387	0.081	0.387	0.401	0.047^{ns}
c.126G>A	0.363	0.637	0.419	0.435	0.145	0.435	0.466	0.210^{ns}
c.324G>A	0.919	0.081	0.855	0.129	0.016	0.129	0.149	1.045^{ns}
c.333C>T	0.960	0.04	0.919	0.081	NA	0.081	0.078	0.109^{ns}
c.573G>C	0.355	0.645	0.145	0.419	0.435	0.419	0.462	0.332^{ns}
c.1074C>T	0.871	0.129	0.774	0.194	0.032	0.194	0.227	1.196^{ns}
c.1265C>T	0.887	0.113	NA	0.226	0.774	0.226	0.202	1.004^{ns}

NA= not available; *= significantly different at the 5% level (χ^2out > χ^2table 0,05 = 3,84); ns: not significant (P>0.05); H_o = Observed Heterozygosity; H_e= Expected Heterozygosity.

leles was below 0.99 in small populations (Hartl and Clark, 1997). The equilibrium of the HSP70 coding sequence gene in the population was assessed using heterozygosity values and chi-square tests. This analysis aimed to determine if the observed data diverged from the expected values. Heterozygosity, the ratio of heterozygous individuals, was quantified on a scale ranging from 0 to 1. Observed heterozygosity (H_o) represents the proportion of heterozygotes that are identified. In contrast, expected heterozygosity (He) indicates the proportion of heterozygotes that would be predicted in a randomly mating population at Hardy-Weinberg equilibrium. The observed heterozygosity for all SNPs had values primarily consistent with the expected heterozygote genotype frequencies in the population. Heterozygosity values (H_e and H_o) under 0.5 signify comparatively low heterozygosity across all populations (Ismaeel *et al.,* 2024). The imbalance results from variable allele and genotype frequencies between generations.

Based on the chi-square test, eight SNPs were in the Hardy-Weinberg equilibrium, whereas only the SNP c.24C>T was not in equilibrium. A population is considered in equilibrium when allele and genotype frequencies are stable throughout generations. Hardy-Weinberg disequilibrium at SNP c.24C>T can arise from unbalanced genetic mutations, leading to an increase in the CC genotype and a corresponding decrease in the CT and TT genotypes within the population. This disequilibrium indicates that the population has altered allele frequencies in response to specific evolutionary pressures, such as mutation, natural selection, or genetic drift, resulting in deviations from the expected Hardy-Weinberg equilibrium. Population disequilibrium may result from insufficient random mating, frequently caused by a limited number of males. Population disequilibrium may also happen due to genetic selection and mutations (Allendorf *et al.,* 2010). Population equilibrium indicates that the allele frequencies observed remain constant throughout generations, dependent upon the absence of mutations, a substantial population size, the lack of natural selection, no migration, and random mating (Noor, 2008). A previous investigation of the SNP g.-69T>G in the promoter region of the HSP70 gene exhibited Hardy-Weinberg equilibrium (Suhendro *et al.,* 2022). The significant genetic variability of HSP70 in local cattle offers the potential for selecting cattle with greater heat stress tolerance (Haddar *et al.,* 2022).

Association of SNP on Physiological Response of Bali Cattle

HSP70 is an essential protein because of its primary function in maintaining cellular environmental stability during conditions of increased heat stress in mammals. The HSP70 protein acts as a chaperone and helps with DNA repair, cell death, signaling, and keeping protein balance inside cells (Hassan *et al.,* 2019). Table 2 presents the findings of the association analysis between SNPs in the CDS of the HSP70 gene and physiological responses in Bali cattle.

Table 2. Association of SNPs in the CDS of the HSP70 gene with the physiological response of Bali cattle

No	SNPs (n)	RR (beats/min) Mean±SE	HR (beats/min) Mean±SE	TR (ºC) Mean±SE	HTC Mean±SE	Sig
1	c.31C>T					
	CC (58)	32.24±1.12	72.14±2.87	38.88±0.05	3.64±0.07	ns
	CT (4)	31.50±4.79	58.25±3.25	38.40±0.27	3.41±0.29	
2	c.117C>A					
	CC (33)	32.18±1.42	69.00±4.08	38.88±0.09	3.61±0.09	
	CA (24)	32.08±1.95	70.38±4.23	38.77±0.07	3.62±0.12	ns
	AA (5)	32.80±2.87	79.20±15.82	39.02±0.07	3.78±0.34	
3	c.126G>A					
	GG (26)	31.69±1.65	74.27±4.17	38.92±0.10	3.63±0.10	
	GA (27)	33.26±1.74	68.78±3.82	38.80±0.08	3.65±0.11	ns
	AA (9)	30.44±2.47	69.89±9.21	38.79±0.12	3.53±0.23	
4	c.324G>A					
	GG (53)	32.42±1.09	71.26±2.96	38.84±0.06	3.65±0.08	
	GA (8)	31.75±4.35	73.13±8.01	38.91±0.19	3.55±0.19	ns
	AA (1)	24.00±0.00	55.00±0.00	38.85±0.43	2.99±0.00	
5	c.333C>T					
	CC (57)	31.96±1.16	69.12±2.90	38.85±0.06	3.60±0.07	ns
	CT (5)	34.80±2.15	84.40±15.85	38.80±0.06	3.95±0.28	
6	c.573G>C					
	GG (9)	30.67±2.08	67.67±9.53	38.79±0.15	3.51±0.21	
	GC (26)	33.54±1.85	69.96±3.98	38.83±0.08	3.63±0.11	ns
	CC (27)	31.41±1.59	71.63±4.76	38.88±0.09	3.61±0.10	
7	c.1074C>T					
	CC (48)	31.67±1.06	71.02±2.94	38.86±0.07	3.61±0.08	
	CT (12)	35.67±3.45	68.83±9.93	38.83±0.11	3.78±0.17	ns
	TT (2)	24.00±2.00	63.50±8.50	38.70±0.40	3.11±0.23	
8	c.1265C>T					
	CT (13)	33.23±2.17	74.62±7.90	38.90±0.11	3.74±0.17	ns
	TT (49)	31.92±1.25	69.22±3.24	38.84±0.06	3.59±0.08	

n = Sample; RR = Respiratory Rate; HR = Heart Rate; TR = Rectal Temperature; HTC = Heat Tolerance Coefficient; Sig = Significance; ns = not significant (P > 0.05).

The total number of SNPs discovered in the HSP70 gene within the coding region was not associated with the physiological responses of Bali cattle from two distinct localities. These findings contrast with several studies that indicate SNPs in the promoter region of the HSP70 gene are linked to physiological responses in Bali cattle (Prihandini *et al*., 2022; Suhendro *et al*., 2022; Suhendro *et al*., 2024). Additional studies have also recognized correlations, such as g.149G>T (NM_174550.1), with thermotolerance characteristics in Tharparkar cattle (Bhat *et al.,* 2016) and Zebu cattle (Onasanya *et al.,* 2021). The heat tolerance coefficient (HTC) measures an animal's ability to adapt to hot and unfavorable environments (Nurhidayat *et al.,* 2024). Respiratory rate and rectal temperature serve as indicators for assessing heat stress based on the HTC value. Typically, the HTC value should be below 2 for heat-resistant cattle. A higher HTC value signifies lower heat resistance (Singh *et al.,* 2014). Genetic variation in cattle has been linked to heat stress, which can be leveraged to enhance heat tolerance traits. However, increased adaptation to heat stress is not primarily determined by genetic variation; epigenetic alterations are also considered a significant mechanism affecting production and health parameters in cattle (Cheruiyot *et al.,* 2022). Heat resistance is a complex trait influenced by various genes and environmental factors. Both genetic and epigenetic elements contribute to determining livestock's ability to tolerate heat (Asea and Kaur, 2017).

CONCLUSION

In Bali cattle, the coding sequence (CDS)

of the HSP70 gene exhibited genetic diversity through nine single nucleotide polymorphisms (SNPs). Among these, there were eight synonymous SNPs and one nonsynonymous SNP, c.1265C>T, which alters the amino acid threonine (T) to methionine (M). The SNPs in the CDS of HSP70 demonstrated polymorphism. No association was found between the SNPs in the CDS of HSP70 and the physiological responses of Bali cattle. Further studies should utilize the Restriction Fragment Length Polymorphism (RFLP) technique, employing cutting enzymes, to determine the representation of each HSP70 CDS gene genotype.

ACKNOWLEDGMENTS

The author would like to thank the Ministry of Education, Culture, Research, and Technology for funding this research through the Master's Thesis Research (PTM) scheme with contract number 15852/IT3.D10/PT.01.02/P/T/2024.

REFERENCES

Aliyya, W.L.N, R.R. Noor and Jakaria. 2020. Exploring SNPS (single nucleotide polymorphisms) of myostatin gene in coding region in Bali cattle. IOP Conference Series: Earth and Environmental Science. 492. P. 012064.

Allendorf, F.W., P. A. Hohenlohe and G. Luikart. 2010. Genomics and the future of conservation genetics. Nat Rev Genet. 11:697–709.

Asea, A.A.A. and P. Kaur. 2017. Heat Shock Proteins in Veterinary Medicine and Sciences. Springer International Publishing. Cham.

Bhat, S., P. Kumar, N. Kashyap, B. Deshmukh, M.S. Dige, B. Bhushan, A. Chauhan, A. Kumar and G. Singh. 2016. Effect of heat shock protein 70 polymorphism on thermotolerance in Tharparkar cattle. Vet World. 9:113–117.

Cheruiyot, E.K., M. Haile-Mariam, B.G. Cocks, I.M. MacLeod, R. Mrode and J.E. Pryce. 2022. Functionally prioritised whole-genome sequence variants improve the accuracy of genomic prediction for heat tolerance. Genet Sel Evol. 54:17.

Erjavec, M.S. 2019. Annealing Temperature of 55°C and Specificity of Primer Binding in PCR Reactions. Biotechnical Faculty, University of Ljubljana. Slovenia

Haddar, M., Jakaria and R. Noor. 2022. SNP g.1117G>A HSP70 gene polymorphism in domestic beef cattle using PCR-RFLP method. IOP Conference Series: Earth and Environmental Science. 1020. P. 012002.

Hagemann, T.L. and S-P. Kwan. 1999. ABI sequencing analysis: manipulation of sequence data from the ABI DNA sequencer. MB. 13:137–152.

Hariyono, D.N.H. 2022. Application of microsatellite markers for genetic diversity analysis of Indonesian Local cattle. WARTAZOA. 32:105–118.

Hartl, D.L. and G.C. Clark. 1997. Principles of Population Genetics. Sinauer Associates. Sunderland.

Hassan, F., A. Nawaz, M.S. Rehman, M.A. Ali, S.M.R. Dilshad and C. Yang. 2019. Prospects of HSP70 as a genetic marker for thermo-tolerance and immuno-modulation in animals under climate change scenario. Anim. Nutr. 5:340–350.

Hu, L., Y. Ma, L. Liu, L. Kang, L.F. Brito, D. Wang, H. Wu, A. Liu, Y. Wang and Q. Xu. 2019. Detection of functional polymorphisms in the HSP70 gene and association with cold stress response in Inner-Mongolia Sanhe cattle. Cell Stress and Chaperones. 24:409–418.

Ismaeel, F., M. Moussa, H.A. Eltahir and H.M. Shakam HM. 2024. Identification of single nucleotide polymorphisms (SNPs) in 5′ and 3′ untranslated regions (5′ UTR and 3′ UTR) of HSP70 gene in some Western Sudan indigenous cattle. J. Adv. Vet. Res. 14:742 -748.

Kaushik, R., A. Goel and P.K. Rout. 2022. Differential expression and regulation of HSP70 gene during growth phase in ruminants in response to heat stress. Sci Rep. 12:18310.

Kim, W.S., J. G. Nejad, D.Q. Peng, Y.H. Jo, J. Kim and H.G. Lee. 2022. Effects of different protein levels on growth performance and stress parameters in beef calves under heat stress. Sci Rep. 12:8113.

Kumar, B., A.K. Sahoo, S. Dayal, A.K. Das, S.

Taraphder, S. Batabyal, P.K. Ray and R. Kumari. 2020. Investigating genetic variability in HSP70 gene-5′-fragment and its association with thermotolerance in Murrah buffalo (*Bubalus bubalis*) under subtropical climate of India. Cell Stress and Chaperones. 25(2):317–326

Li, R., J. Chiou, E.W-C. Chan and S. Chen. 2016. A novel PCR-based approach for accurate identification of vibrio parahaemolyticus. Front. Microbiol. 7:1–5.

Liu, J., L. Li, X. Chen, Y. Lu and W. Wang. 2019. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: a novel idea for monitoring and evaluation of heat stress — A review. Asian -Australas J Anim Sci. 32:1332–1339.

Martojo, H. 2012. Indigenous Bali cattle is most suitable for sustainable small farming in Indonesia. Reprod Domest. Anim. 47:10–14.

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press. New York.

Noor, R.R. 2008. Genetika Ternak. Penebar Swadaya. Jakarta

Nurhidayat. I., A. Suprayogi, A.S. Sismin, E.D. Rita, H. Maheshwari, I. Bustamam, K. Santoso, P. Achmadi, W. Manalu and R. Tarigan. 2024. Heat tolerance of several beef cattle breeds in Astomulyo village, Lampung Tengah district. ARSHI vet lett. 8:17–18.

Onasanya, G.O., G.M. Msalya, A.K. Thiruvenkadan, C. Sreekumar, G.K. Tirumurugaan, A.O. Fafiolu, M.A. Adeleke, A. Yakubu, C.O.N. Ikeobi and M. Okpeku. 2021. Heterozygous single-nucleotide polymorphism genotypes at heat shock protein 70 gene potentially influence thermo-tolerance among four Zebu breeds of Nigeria. Front. Genet. 12:642213.

Prihandini, P.W., A. Primasari, A. Aryogi, M. Luthfi and D.N.H. Hariyono. 2022. Genetic polymorphisms of the 5' untranslated regions of the HSP70 gene in Indonesian cattle populations. Vet World. 15:168–172.

Purwantara, B., R. Noor, G. Andersson and H. Rodriguez-Martinez. 2012. Banteng and Bali cattle in Indonesia: status and forecasts. Reprod Domest, Anim. 47:2–6.

Sejian, V., R. Bhatta, J.B. Gaughan, F.R. Dunshea and N. Lacetera. 2018. Review: Adaptation of animals to heat stress. Animal. 12:431–444.

Singh, A.K., R.C. Upadhyay, D. Malakar, S. Kumar and S.V. Singh. 2014. Effect of thermal stress on HSP70 expression in dermal fibroblast of Zebu (Tharparkar) and crossbred (Karan-Fries) cattle. J. Thermal Biol. 43:46–53.

Singh, K.M., S. Singh, I. Ganguly, A. Ganguly, R.K. Nachiappan, A. Chopra and H.K. Narula. 2016. Evaluation of Indian sheep breeds of arid zone under heat stress condition. Small Rum. Res. 141:113–117.

Sugimoto, M., H. Furuoka and Y. Sugimoto. 2003. Deleting one of the duplicated HSP70 genes causes hereditary myopathy of diaphragmatic muscles in Holstein-Friesian cattle. Anim. Gen. 34:191–197.

Suhendro, I., J. Jakaria, R. Priyanto, W. Manalu and R.R. Noor. 2022. The association of single nucleotide polymorphism -69t>g hspa1a gene with Bali cattle heat tolerance. Trop. Anim. Sci. J. 45:429–435.

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

Taborda-Charris, J.C., R. Rodríguez-Hernández, M.P. Herrera-Sánchez, H.F. Uribe-García, R.J. Otero-Arroyo, J.S. Naranjo-Gomez, K.J. Lozano-Villegas and I.S. Rondón-Barragín. 2023. Expression profiling of heat shock protein genes in whole blood of Romosinuano cattle breed. Vet World. 16:601–606.