

Heat shock protein 90 alpha family class B member 1 (HSP90AB1) gene polymorphism and its effect on milk production traits in friesian holstein cattle

Muhammad Mumtaz Adzdzakiy¹, Septiana Widayanti², Rebecca Vanessa², Galih Pambuko³, Ari Susilowati², Sigit Prastowo^{3}, and Sutarno²*

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia

²Graduate School of Bioscience, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia

³Faculty of Animal Science, Universitas Sebelas Maret, Surakarta, Indonesia

Abstract. Increasing temperature caused by climate change is main contributor of heat stress in dairy cattle. Heat stress has a major impact on the milk production efficiency in dairy cattle. The HSP90AB1 gene is involved in overcoming heat stress response in cattle. The purpose of this study is to find single-nucleotide polymorphism (SNP) of HSP90AB1 gene and its relationship to milk production traits in Friesian Holstein (FH) cattle. In this study, 50 FH blood samples were used. Allele Specific Polymerase Chain Reaction (AS-PCR) method was used to successfully identify three genotypes: CC, CT, and TT. The CT genotype is the most common in the sample population. When compared to the C-allele, the T-allele is more common. Hardy-Weinberg (HW) analysis using Chi-Square method revealed that the population was not equilibrium ($P < 0.05$). Association study between genotypes and milk production trait was not significant ($P > 0.05$), but the CC genotype had a trend of higher mean milk yield in the first and second lactation. It was determined that the HSP90AB1 gene could be used as molecular marker for FH cattle heat stress response, in order to increase milk production capacity.

1. Introduction

The climate change causes the rising global temperatures. The rising of earth temperature is directly and indirectly affecting the production of livestock, as indicated by heat stress. Heat stress can cause major problems with the health, reproductive and milk production performance of dairy cows [1]. That problems lead to profitability losses. In the normal range of temperature, it is possible for bovines to keep their body temperatures down with a balance between metabolic warmth production and heat's flow from its surface into the environment by means of thermoneutral conditions. Further, when the temperature is above the comfort

* Corresponding author: prastowo@staff.uns.ac.id

zone the cattle become hyperthermia. hyperthermia is caused by high ambient temperature above a thermal comfort zone, as well as extremely humidities and strong solar radiation that can affect the effectiveness of animal heat exchange only or in combination [2].

The mammals have the mechanism to control the heat exposure from the environment. In the cellular system, the adaptation response to high temperature is the activation and aggregation of the protein set, namely Heat Shock Protein (HSP). Some families classify HSP based on the weight and molecule mass, such as HSP60, HSP70, and HSP90. Further, the 90 kilodaltons heat shock proteins (HSP90) have prominent function in Molecular chaperones that are constitutively expressed as a result of heat or stress induction [3]. two isoforms called HSP90 α or HSP90AA1 (inducible) and HSP90 β or HSP90AB1(constitutive) [4]. The Heat Shock Protein 90 Alpha Family Class B Member 1 (HSP90AB1) gene plays a role in the physiological response of the body to heat shock. HSP90AB1 located in *Bos taurus* autosome (BTA) 23 and consist of 12 exons. The polymorphism in this gene has association in heat tolerant. One of the HSP90AB1 SNP that associate with the tolerant is SNP g.4338T>C with reference ID rs109251249. The g.4338T>C has significant effect on heat tolerant and milk production [5–7].

Increasing global temperature especially in Indonesia lately can affect dairy cattle production. The annual temperatures ranged from 26.8°C to 26.9°C between 2000 and 2024, with a projected increase to 27°C in 2024 [8]. The ideal temperature for dairy cattle is 13°C to 25°C [9]. Furthermore, the ideal Temperature Humidity Index (THI) for the dairy cattle is 68 to 74, above that the cattle can experience stress and decreasing the milk productivity [10]. Therefore, this study aimed to determine the genotype variant and milk production association at g.4338T>C in the HSP90AB1 gene in the Friesian Holstein population. The findings from this research can potentially serve as a molecular marker for temperature-tolerant dairy cattle selection programs.

2. Material and methods

2. 1. Blood collection and DNA extraction

Fifty blood samples of FH cows were obtained from the Dairy Cattle Breeding Center in Central Java Province, Indonesia. The blood was collected by venipuncture from the coccygeal vein and stored with EDTA for blood preservation. All the blood collection procedure was supervised by a veterinary and also comply with the Indonesian Law number 19, 2009 regarding animal husbandry and health. Furthermore, the DNA Extraction was performed by Promega Wizard® Genomic DNA Purification Kit, Promega. Blood Collection and DNA extraction were used following the previous study, according to [11].

2. 2. HSP90AB1 gene genotyping

HSP90AB1 genotyping was done by the Allele Specific-Polymerase Chain Reaction (AS-PCR) method. Each allele is amplified with specific primer sets (**Table 1**). The T allele was amplified with the F-T and R primer set, while the F-C and R primer set was used to amplify the C allele. Then, the amplification products were electrophorized and visualized by agarose 2%. The HSP90AB1 gene genotyping were used following the previous research, according to [11].

Table 1. Primer list for amplification HSP90AB1 gene fragment and genotyping.

Primers (5'-3')		Tm (°C)	PCR Cycle	Product Size (bp)	Reference
F-T	CTGGAGTCACACTGAGGAAT	58.9	35	561	[7]
F-C	CTGGAGTCACACTGAGGAAC	57.9			
R	TGTTGGAGATCGTCACCTG				

2. 3. Data Analysis

2. 3. 1. Milk production data

The milk production data were acquired from the first lactation record. The 305 2X Mature Equivalent (ME) factor was used for milk production data standardization. The Kg/lactation was used for unit measurement.

2. 3. 2. Genotype-Allele frequency and Hardy-Weinberg Equilibrium (HWE) analysis

The genotype-allele frequency and HWE analysis by Chi-Square (χ^2) was calculated by R with Genetics Package [12,13].

2. 3. 3. Association milk production trait with HSP90AB1 genotype

The association of milk production with the HSP90AB1 genotype were analyzed with ANOVA. Duncan’s Multiple Range Test (DMRT) was used for further testing. P<0.05 was set for statistical differences. The Analysis was carried out by R with Agricolae Package [13,14].

3. Result and discussion

The AS-PCR amplified HSP90AB1 specifically with a 561 bp amplification product size. The genotyping discovered three variation of genotypes (TT, CC, and CT) and two alleles (C and T) in the FH population (Fig 1). The TT genotype just amplified T-allele primer set and the amplification product presented in well 1^T and no amplification in well 1^C. Otherwise, only the C-allele primer set amplified 2^C successfully, and 2^T had no band present in the CC genotype. Further, C and T primer sets were amplified successfully in 3^C and 3^T wells in the CT genotype. The homozygous genotype only amplifies one of the allele-specific primer sets, while in the heterozygote genotype, all the allele-specific primer sets are amplified well. The previous study on the local Indonesian Friesian Holstein and Sahiwal cattle confirmed this result, but the study on Thai Crossbreds Frisian Holstein TT genotype was not found, while in White Lumphun (Thai indigenous cattle) only found the TT genotype [5,7,11].

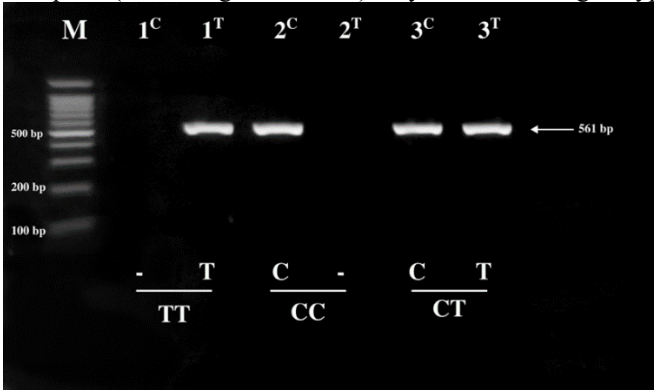


Fig 1. HSP90AB1 Genotyping by AS-PCR Result. Note: (M): Marker 100 bp, (1^C-3^C): C-allele samples, (1^T-3^T): T-allele samples, (TT, CC, CT): the HSP90AB1 genotype.

The CC genotype had the lowest genotype frequency (0.06), while the CT genotype had the highest (0.66). Further, the T allele (0.61) was higher than the C allele (0.39). The HWE analysis revealed the FH population was not in equilibrium (P-value = 0.0072) with a chi-square value of 7.49 (**Table 2**). The highest in CT genotype and the lowest TT genotype result was found in local Indonesian Friesian Holstein and Sahiwal cattle; in another study on the Frieswal cattle the TT genotype was the lowest genotype [7,11]. The inequilibrium state in this study caused by the selection activity and non-random mating with elite bulls in FH population [15].

Table 2. HSP90AB1 HWE analysis in FH population.

n	Genotype Frequency			Allele Frequency		HWE Analysis	
	CC	CT	TT	C	T	χ^2	P-Value
50	0.06	0.66	0.28	0.39	0.61	7.49	0.0072*

*Inequilibrium = (P<0.05)

The association analysis of milk production with the HSP90AB1 genotype in the FH population revealed the CC genotype had the highest milk production with 4126.26±1600.23 kg. The TT genotype had the lowest milk production with 2950.50±1143.37 kg. There was no association between milk production and HSP90AB1 genotype (P-value > 0.05). Although there was no association, the CC genotype performs better in milk production than the CT and TT genotypes.

Table 3. Association of milk production with HSP90AB1 genotypes in FH population.

Trait	Genotype			P-Value
	CC	CT	TT	
Milk Production (Kg)	4126.26±1600.23	3687.49±1459.16	2950.50±1143.37	0.19

Significant = (P<0.05)

The previous studies, the genotype variation in HSP90AB1 g.4338T>C had better heat tolerance in the TT genotype, following CT genotype and CC genotype as the lowest heat tolerant among others genotype in Frieswal and Sahiwal cattle [7]. Here in after, T allele in g.4338T>C intensified the heat tolerant in Thai crossbred Friesian Holstein, White Lumphun and White Mountain cattle [5]. The TT genotype in HSP90AB1 g.4338T>C was associated with better milk production performance and the CC genotype was the lowest milk production in Sahiwal and Frieswal cattle [7]. On other study had different result, the CT genotype had the higher milk production than CC genotype in Sahiwal cattle [6]. In this association study, there was an inverse trend in milk production. The CC genotype had the highest milk production, followed by the CT genotype, and the TT genotype had the lowest milk production. The various breeds of cattle cause the differences in genotype associated with milk production in HSP90AB1.

The milk production belongs to quantitative trait that influence of the many gene interaction. Furthermore, environmental condition and climate have major contribution to the milk production performance of the cattle. The high temperature climate will perish the milk production in dairy cattle[1]. In this study, the population of Friesian Holsteins came from a subtropical climate country, then the cattle were transported to Indonesia during pregnancy and give birth in Indonesia. In this situation, cattle are being affected the stress by changes in the environment from that of a subtropical to an equatorial climate. As a consequence, the

milk production which is not as genetically potent has been disrupted and inconsistent, this has been explained in previous study [16, 17].

4. Conclusion

The rising environmental temperatures due to climate change can disrupt dairy cattle milk production. Implementing a selection program for temperature tolerance adaptation in dairy cattle is essential to maintain milk production. The HSP90AB1 variation in g.4338T>C in Friesian Holstein population had three genotypes and two alleles: CC, CT, TT genotype and C, T allele. The CT genotype had the highest genotype frequency, and the T allele had the highest allele frequency. The Friesian Holstein population was not equilibrium. The milk production was no association with the HSP90AB1 genotypes. Nonetheless, the CC genotype had better milk production among the other genotypes. The HSP90AB1 gene is the potential candidate genetic marker for heat tolerance dairy cattle. The further study is needed in different dairy cattle breeds and more of quantitative traits.

References

1. J. Liu, L. Li, X. Chen, Y. Lu, and D. Wang, *Asian-Australas J Anim Sci* **32**, 1332 (2019)
2. S. Foroushani and T. Amon, *Int J Biometeorol* **66**, 1811 (2022)
3. S. Maiti and D. Picard, *Biomolecules* **12**, (2022)
4. L. Sailo, I. D. Gupta, A. Verma, R. Das, M. V. Chaudhari, and S. Singh, *Indian J Anim Res* **50**, 856 (2016)
5. R. Charoensook, K. Gatphayak, A. R. Sharifi, C. Chaisongkram, B. Brenig, and C. Knorr, *Trop Anim Health Prod* **44**, 921 (2012)
6. S. Lalrengpuui, D. G. I, V. Archana, D. Ramendra, and V. C. M, *African Journal of Biochemistry Research* **9**, 99 (2015)
7. B. Sajjanar, R. Deb, U. Singh, S. Kumar, M. Brahmane, A. Nirmale, S. K. Bal, and P. S. Minhas, *Anim Biotechnol* **26**, 45 (2015)
8. (n.d.)
9. D. H. Lim, T. Il Kim, S. M. Park, K. S. Ki, and Y. Kim, *J Anim Sci Technol* **63**, 872 (2021)
10. P. Herbut, S. Angrecka, and J. Walczak, *Int J Biometeorol* **62**, 2089 (2018)
11. S. Prastowo, M. Mumtaz Adzdzakiy, R. Vanessa, G. Pambuko, Purwadi, A. Susilowati, and Sutarno, in *E3S Web of Conferences* (2021)
12. G. Warnes, G. Gorjanc, F. Leisch, and M. Man, (2021)
13. R Core Team, (2023)
14. F. de Mendiburu and M. Yaseen, (2020)
15. R. S. Waples and F. Allendorf, *Journal of Heredity* **106**, 1 (2015)
16. S. Prastowo, T. Nugroho, N. Mahfudhoh, F. Y. Putra, Subiakti, A. Ratriyanto, A. Susilowati, Sutarno, and N. Widyas, *IOP Conf Ser Mater Sci Eng* **633**, (2019)
17. N. Widyas, N. Mahfudhoh, Subiakti, and S. Prastowo, *AIP Conf Proc* **2202**, 1 (2019)