Association of HSP70 gene with milk yield and milk quality of Friesian Holstein in Indonesia

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Abstract. The aim of this study was to identify the polymorphism of the ScrFi locus of HSP70 and the association of genetic variant of HSP70 with milk yield and milk quality of Holstein-Friesian (HF) cows in Indonesia. A total of 115 heads of HF lactating cows which come from different locations of lowland, medium, and highland. Genotyping of HSP70 genes was performed by nested PCR-RFLP method. The variant of HSP70 was polymorphism: 47 and 49 bp for homozygous wild type (CC) genotype and 47, 49 and 96 for heterozygous cytosine deletion mutant (C-) genotype. Dairy cows with CC genotypes were higher 4% FCM, protein, fat, milk total solid and lactose (P <0.05) compare to C- genotype. It could be concluded that dairy cows with CC genotypes produce higher 4% FCM and better milk quality than dairy cows with C-.

1. Introduction
Heat Shock Proteins (HSPs) are proteins which are expressed under stress conditions. HSPs that play a role in the thermoregulation process and adaptation to environments with extreme heat stress is HSP70 [1-2]. The expression of the HSP70 gene was influenced by changes in environmental temperature [3-4]. The HSP70 gene has the role and general molecular mechanism that is displayed by almost all different animal cells in managing stress. In human mononuclear peripheral cells, the HSP70 gene polymorphism was associated with increased ability to respond to heat stress [5], whereas in Holstein Friesian cows in China, polymorphisms of HSP70 gene coding area were associated with an increase adaptation ability in mammary gland cell culture exposed to 40°C stress [6-7]. The mutation on the promoter region of HSP70 gene was predicted affecting to the level of expression and stability HSP70 mRNA and contribute to heat stress tolerance in pig [1]. HSP70 gene transcription that plays a role in the ability of thermoregulation is determined by HSFs and HSE bonds located in the gene promoter of
HSP70 region. A number of studies show polymorphism in the UTR 5 HSP70 gene region caused by a single point, deletion, insertion or SNP mutations in the HSP70 promoters are closely related to the milk yield and milk quality [5-8]. Mutation in guanine to cytosine in 2033 bases cause glycine to change to alanine [8] and cytosine deletion in bases 859 of HSP70 AP2 promoter boxes [9] reported to be related to changes in milk yield and quality.

Milk yield is a quantitative trait controlled by polygenes, each of which has little effect [10]. Mammary gland efficiency in the transcription, translation, and milk secretory mechanisms during the lactation phase is controlled by polygenes and is influenced by many factors. The environment which consists of air temperature and relative humidity is a factor that greatly influences milk yield and quality [11]. Although many previous studies reported the association of HSP70 gene polymorphism with thermoregulation ability and milk yield. However, the availability of information about the association between HSP70 gene polymorphism and the milk yield and milk quality of dairy cattle in Indonesia is scanty. Therefore, studies to elaborate the association between HSP70 gene polymorphism with the milk yield and milk quality of Holstein Friesian dairy cows in Indonesia are necessary.

2. Materials and Methods
The research was conducted from March to December 2016. The sampling period was based on the dry season condition with the lowest rainfall and highest ambient temperature. Three locations of dairy farms research institute with different altitude were used in this study, namely Pondok Ranggon West Jakarta (06° 15’ S and 106° 54’ E; 97 meter above sea level), Ciawi Bogor (6°40’ S and 106°51’ T; 574 meter above sea level), and Lembang Bandung (06 °.50’ S and 107°.37’ E; 1241 meter above sea level).

A total of 115 heads of HF lactating cows with 1 to 4 months of lactation ranges and 1 to 3 of lactation periods were used in this study. All cattle were housing in shaded pens and fed on forage, commercial pellet and additional feed derived from agricultural waste product to meet daily energy and protein requirement [12]. Milk production data are average production 305 days and milk yield standardized by 4% FCM. Milk quality observed included protein-, fat-, lactose-, dry matter and solid non-fat contents. Blood samples for genomic analysis were taken from the caudal vein section and stored with EDTA preservation for DNA extraction.

Genomic DNA was extracted from blood using the phenol-chloroform extraction method [13]. PCR reactions were performed on thermocycler machines with taq polymerase enzymes and buffers to isolate the target gene segment of the HSP70 gene promoter region by a two step protocol for PCR amplification [1]. Firstly, generating a bovine HSP70 gene specific DNA fragment using gene diagnostic primer (Forward and reserve, respectively): 5'GTCGCCAGGAAACCAGAGAC and 5'GGAACACCCCTACG CAGG (positions 181-200 and 712-731, respectively, GenBank accession number M98823). The generated PCR products were used in a second PCR for diagnostic amplification of the polymorphic site using modified primers (modified nucleotides are underlined) to eliminate three native ScrFI restriction sites remaining the diagnostic ScrFI restriction site only: 5'-GTTCTGGGAGGAGAGGCA and 5'CTGCCATGTCGGGAATATTCAAGG (positions 284-306 and 356-379, respectively GenBank accession number M98823). Frequencies of polymorphisms in the populations were analyzed by restriction digestion of PCR fragments using ScrFI and the PCR product was visualized using 1.5% agarose gel.

HSP70 allele frequency was calculate according to [14] formula and the genotype frequency were calculated based on the comparison of the number of genotypes in the population. The effect of different altitude and variant genotype of HSP70 on the milk yield and milk quality were analyzed using the General Linear Model (GLM) of SAS®. Experimental data were analyzed with Completely Randomized Design (CRD) One Way ANOVA and if responses showed the difference (P<0.05) followed by Tukey test.
3. Results and Discussion

Genotyping of HSP70 gene by nested PCR-RFLP with ScrFI restriction enzyme was produced two genotypes, namely homozygous wild type genotype (CC: 47 and 49 bp) and heterozygous cytosine deletion mutant genotype (C-: 47, 49, and 96 bp) (Figure 1). Genotyping the HSP70 by the nested PCR-RFLP method with the ScrFI restriction enzyme that recognizes the NG/NGG cutting site. The diversity of the HSP70 genes is attributed to deletions in cytosine to base 895 [15-4]. The deletion in cytosine causes the ScrFI restriction enzyme does not recognize the cutting point to produce a 96 bp fragment. The CC genotype has a C base, which is cut off by the restriction enzyme ScRFI and produces fragments of 47 and 49 bp, whereas genotype C- is a combination of the two resulting in fragments of 96, 47 and 49 bp fragments.

![Figure 1](image)

HSP70 genotype frequency was 32.17% and 67.83% for CC and C- respectively (Table 1). However, homozygous cytosine deletion mutant /- - genotype was complete absence in the targeted population. [15] reported the polymorphism of locus ScrFI of HSP70 gene in pure Holstein cow having three genotypes. However, Indian HF crossbreed cow only has two variant genotypes that are CC and C- [9]. The C- genotype frequency was higher than CC in both lowlands, medium, and highland. This result shows that consistently dairy cattle populations with C- genotypes are higher than C- genotypes in both low-, medium- and highland. Similar results were reported by [16], [15] and [17] who conducted research on dairy cows in a four-season country, and [9] and [4] who conduct research on crossbreed dairy cows in India.

| Location   | N  | Genotype frequency | Allele frequency |
|------------|----|--------------------|------------------|
|            |    | CC (%)             | C- (%)           | C (%)  | -      |
| Lowland    | 64 | 31.25              | 68.75            | 0      | 0.66   | 0.34   |
| Medium     | 27 | 40.74              | 59.26            | 0      | 0.70   | 0.30   |
| Highland   | 24 | 25.00              | 75.00            | 0      | 0.63   | 0.38   |
| All population | 115 | 32.17              | 67.83            | 0      | 0.66   | 0.34   |

Association between HSP70 polymorphism with milk yield and milk quality are presented in Table 2. The variant genotype of HSP70 had a significant(P<0.05) effect on milk yield standardized by 4%FCM and the parameters of fat, TS, SNF and lactose levels, both in the lowland, medium, highland and combined of the three locations (P<0.05). Cow with CC genotypes is consistently associated with higher levels of fat, TS, SNF, and lactose when compared with C-genotype. The average protein
content of CC genotype was higher than C-genotype for highland locations and combined three locations, but not for dairy cows in the medium and lowland.

Table 2. Association between HSP70 polymorphism with milk yield and milk quality.

| Location     | Parameter                  | Genotype | CC   | C-   |
|--------------|----------------------------|----------|------|------|
| Lowland      | N                          | 20       | 44   |
|              | Milk Production (kg/day)   | 6.7±3.36 | 7.1±3.36 |
|              | Milk Production 4%FCM (kg)| 6.5±3.54 | 6.5±3.80 |
|              | Protein (%)                | 3.3±0.41 | 3.2±0.52 |
|              | Fat (%)                    | 4.5±1.62a | 3.8±1.88b |
|              | TS (%)                     | 13.2±1.72a | 12.1±2.13b |
|              | SNF (%)                    | 8.5±0.43a | 8.2±0.66b |
|              | Laktosa (%)                | 4.1±0.30a | 3.8±0.64b |
| Medium       | N                          | 11       | 16   |
|              | Milk Production (kg/day)   | 12.0±5.21 | 10.3±3.71 |
|              | Milk Production 4%FCM (kg)| 12.5±6.76a | 9.7±4.59b |
|              | Protein (%)                | 3.4±0.31 | 3.4±0.31 |
|              | Fat (%)                    | 4.0±0.55a | 3.6±0.71b |
|              | TS (%)                     | 12.4±1.02a | 11.6±0.99b |
|              | SNF (%)                    | 7.9±0.63 | 7.8±0.70 |
|              | Laktosa (%)                | 4.6±0.30 | 4.5±0.29 |
| Highland     | N                          | 6        | 18   |
|              | Milk Production (kg/day)   | 12.8±4.46 | 13.2±3.1 |
|              | Milk Production 4%FCM (kg)| 11.7±5.96 | 11.6±5.04 |
|              | Protein (%)                | 2.8±0.30a | 2.5±0.45b |
|              | Fat (%)                    | 2.2±0.79 | 2.2±0.85 |
|              | TS (%)                     | 10.2±1.35 | 10.2±0.95 |
|              | SNF (%)                    | 7.9±1.03 | 7.9±0.65 |
|              | Laktosa (%)                | 4.0±0.90b | 4.4±0.40a |
| Combine      | N                          | 37       | 78   |
| location     | Milk Production (kg/day)   | 10.4±5.19 | 9.6±4.33 |
|              | Milk Production 4%FCM (kg)| 10.3±6.26a | 8.7±4.93b |
|              | Protein (%)                | 3.2±0.43a | 3.0±0.59b |
|              | Fat (%)                    | 3.7±1.38a | 3.2±1.65b |
|              | TS (%)                     | 12.1±1.78a | 11.4±1.90b |
|              | SNF (%)                    | 8.1±0.75 | 8.0±0.68 |
|              | Laktosa (%)                | 4.3±0.57a | 4.1±0.60b |

Description: different superscripts on the same line show significant differences (P <0.05)

Dairy cows with CC genotypes produce the same milk yield as C- genotypes. However, the dairy cow with CC genotypes in the medium and the combined location has higher 4% FCM (P <0.05) than the C- genotype, whereas in the lowlands and highlands there was no difference in the effect of HSP70 genotypes with 4% FCM. These results indicate no consistency of the HSP70 genotype in controlling milk yield standardized by 4% FCM. Milk production trait is a quantitative trait controlled by polygenes so that one gene only gives little effect on milk production.
The results of this study are different from the results of [9] research which carried out the ScrFI locus of HSP70 genotype control in Frieswal cattle on the milk yield and milk quality. [9] stated that the CC genotype of the ScrFI locus HSP70 genotype produced higher milk production, fat and protein content when compared to C-genotype. Furthermore, Brown et al. [18] reported that there was a relationship between HSP70 gene genotype and milk production in Angus, Brahman cattle and their crossing.

In general, HSP70 genotype polymorphism may have different effects on different populations and environments or so-called phenotypic plasticity. [11] showed that environmental conditions greatly affect the genetic relationship with phenotypic properties so that dairy cows with the same genetic potential will give a different production appearance when in different environments. There are several possibilities that cause the absence of a permanent effect of ScrFI locus of HSP70 genotype on milk yield standardized 4% FCM and some milk quality parameters for dairy cows maintained in different altitude, namely (1) Research carried out in the prolonged dry season so that physiologically, animals exposed to stress over a long period of time have been able to adapt to environmental conditions outside the comfort zone; (2) The breeding system which is not random or the number of males is very limited causes the gene composition to be unbalanced; (3) The presence of a sustainable selection process to produce dairy cows with the superior trait of milk production. Further, [19] explained differences in the effect of genotype polymorphism on milk quality can occur due to differences in population size, breed cattle, frequency of genotype, parameters examined (such as daily tests, lactation, milk quality characteristics) and also statistical methods used to eliminate various factors such as the influence of parentage age, parity, season, lactation stage, health status and genetic variation itself.

4. Conclusion
Polymorphism of HSP70 genotype was related to the milk yield standardized 5%FCM and milk quality in Indonesia FH dairy cows. The CC genotype is consistently associated with milk yield standardized by 4% FCM, fat content, total solid, Solid Non-Fat and lactose contents which are higher compared to C-genotype.

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