Associations between polymorphisms in the growth hormone locus and reproductive efficiency in dairy cattle in Pujon

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Abstract. Selection using genetic markers (MAS) is carried out in an effort to accelerate livestock breeding programs, through molecular studies individual superior livestock can be detected earlier so that the selection program can be directed and controlled. This study aims to analyze genetic diversity at the growth hormone locus that is thought to have a relationship with reproductive and production traits in Friesian Holstein Crossed (FHC) dairy cattle through the polymerase chain reaction - restriction fragment length polymorphisms (PCR-RFLP) method. The study was conducted in Pujon subdistrict using 53 FHC cattle samples. The results showed that at the GH gene locus only one genotype was found, AA with an allele frequency of 1.00. Based on these results, it was concluded that the GH gene in FHC cattle is monomorphic. The information in this study can be used as a reference in formulating livestock breeding programs.
Keywords: Growth Hormone Polymorphism, monomorphic, Marker Assisted Selection (MAS)

1 Introduction

Dairy cattle are a livestock commodity that is widely cultivated in the tropics of Indonesia. Recorded in the data of the Director General of PKH in 2022, East Java province is one of the provinces with the largest dairy cattle population with a population of 314,385 cattle. Dairy cattle are generally raised in highland areas, with a semi-intensive maintenance system. The increase in livestock population every year indicates an increase in the level of milk consumption and processed products. This increase needs to be supported by livestock breeds that have high genetic quality through controlled breeding, to produce breeds with high fertility rates and milk production above the group average. Implementation of controlled breeding is currently done by utilizing molecular genetics-based selection or MAS (Marker Assisted Selection) gene markers which are considered quite effective in identifying phenotypic traits for improving reproductive traits such as service per conception (S/C), days open and calving interval (CI) as well as livestock production such as carcass and milk. Through MAS identification, it is possible to determine the effect of candidate genes on livestock performance based on allele detection.

Growth Hormone (GH), known as somatotropin hormone, is one of the candidate genes derived from a peptide series of about 190 amino acids synthesized by somatotroph cells in the anterior pituitary [1]. GH plays an important role in metabolism, lactation control, mammary gland development, and fertility in cattle [2]. In a previous study by [3] it was shown that there is a positive relationship between GH gene polymorphisms and growth and

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lactation performance in dairy cattle. At least four single nucleutide polymorphisms (SNPs) have been found in exon 10, which encodes the cytoplasmic domain of GHR. SNPs are located at positions 257 (A/G), 229 (T/C), 200 (G/A), 76 (T/C). Positions 200 (G/A) and 257 (A/G) induce the amino acid substance alanine to threonine, and serine to glycine. GH effects the growth, reproduction and productivity of livestock through interactions between specific receptors (GHR) on the surface of target cells. As a result, there are changes in GH activity in the target tissue due to the influence of increased signaling pathways and binding capacity. The purpose of this study was to analyze genetic diversity (genetic polymorphism) at the growth hormone locus that is thought to have a relationship with reproductive and production traits in Peranakan Friesian Holstein dairy cattle.

2 Materials and Methods

This study was conducted on 53 Friesian Holstein Crossbreed dairy cattle in lactation period II - IV with milking frequency twice per day, average body weight reached 425 ± 59.3 kg and milk production 11.13 ± 4.43 l/day kept in a smallholder farm located in Pandesari village, Pujon, Malang Regency, East Java 65391 coordinates 7°51'41.7 "S 112°28'36.6 "E with an altitude of 1200 meters above sea level with temperatures ranging from 18 oC.to 25 oC. and average humidity 74%. The research was conducted in two stages, the first stage included linear body measurements obtained from measuring body weight, body length, height, and chest circumference using digital scales, measuring rulers and measuring tapes. Daily milk production measurements were taken in the morning and evening, and reproductive data were obtained from the KOPSAE office database through the inseminator. The second stage of the research involved taking blood samples from dairy cattle using vacuntainer tubes containing EDTA anti-coagulant. Blood sampling through the jugular vein as much as 3 mL then transferred blood samples to the laboratory and stored in a refrigator at 20^{o}C.

2.1 Amplification of DNA fragments of GH gene locus

Amplification of specific DNA fragments of the GH gene of FHC dairy cattle with PCR product length determined based on the attachment of primers designed from the position of 1537 bp to 2013 bp with a product length of 476 bp, thus obtaining DNA primers consisting of, GH-forward:

5`- GTTGTTGGATGGCAGTGG -3` and GH-reverse: 5`- CTTCTCCAAGCCTGTAGG - 3`, with gene bank acese code: JQ711182. The total volume for DNA amplification was 15 µL with a composition according to Promega product requirements which included 7.0 µL Gotaqgreen, 6.4 µL FNW (Free Nucleous Water), 0.3 µL forward and reverse DNA primers respectively and 1 µL genomic DNA sample. The mixture was processed in a BioRad T100 Thermal Cycler PCR machine with the following settings: Initial denaturation of 95oC for 5 minutes, followed by denaturation process at 95oC for 45 seconds, anneling at 58oC for 45 seconds, and extension at 72oC for 45 seconds repeated for 35x cycles, continued with the final extension process at 72oC for 5 minutes and ended with cooling down process at 12oC for 2 minutes (Rahayu, Sumitro, Susilawati and Soemarno., 2006).

The DNA amplification results (Figure 1) obtained were then subjected to RFLP process using restriction enzymes Msp1 with cutting site `5-CC | GG- 3` and Alu1 with cutting site `5-AG | CT- 3`. The total volume for RFLP using Msp1 and Alu1 was 25 µL each consisting of 1 µL of PCR product DNA sample, 21 µL of FNW (Free Nucleous Water), 2.5 µL of Buffer 10-NE and 0.5 µL of restriction enzymes (Msp1 and Alu1). Then incubate the RFLP sample at 37 oC for 15 minutes. Visualization of RFLP results is then seen at the

electrophoresis stage using a 100 Volt MupidEx electrophoresis machine with agarose gel media that has been colored with bromide.

2.2 Data analysis

The visualization obtained was then continued with the calculation of genotype and allele frequencies to test the Hardy-Weinberg equilibrium (Warwick, 1990). Calculation of the magnitude of Growth Hormone gene polymorphism in PFH dairy cattle based on % PIC (Polymorphic Information Content) (Budak et al, 2003) as follows:

$$PICi = 1 - \sum p2i\ j$$

Where PICi is the polymorphic information content at the i-th locus, pij is the frequency of the j-th allele and the i-th locus.
Data in the form of reproductive efficiency traits such as S/C, DO and CI as well as data on BW, LD, PB and TB, milk production were analyzed in relation to Growth Hormone gene polymorphisms using the One Way Anova method assisted by Minitab software version 17 (2023)..

3 Results and discussion

The results of data analysis using Minitab version 17 show the results as in Table 1. Because the age distribution is quite large, age grouping is carried out, namely: Age group 1 is age 2-3.5 years, age group 2 is age 3.5 - 4.5 years, and age group 3 is age > 4.5 years. Based on the results of the study mentioned that body weight (BW) was significantly influenced ($P < 0.05$) by age group, as well as in Table 2. Presented body weight at various lactation periods.

Table 1. Body weights in FHC cattle at different age groups.

Age group	N	Mean ± standard deviation (kg)*)
1	13	367.00 ± 26.53 [a]
2	13	398.15 ± 31.85 [b]
3	7	391.6 ± 45.6 [b]

[1] Different superscripts in the same column indicate significantly different ($P < 0.05$)

Table 2. Body weight in FHC cattle in different lactation groups

Lactation period	N	Mean ± standard deviation (kg)*)
1	14	369.57 ± 27.25 [a]
2	9	393.11 ± 31.33 [b]
3	5	379.20 ± 17.25 [b]
4	3	401.00 ± 50.23 [c]
5	2	438.50 ± 74.25 [c]

[2]Different superscripts in the same column indicate significantly different ($P < 0.05$)

3.1 Reproductive Efficiency of FHC dairy cattle in different age groups and lactation periods

The results of statistical analysis show that the reproductive efficiency of female dairy cattle is not influenced by age group. This can be seen in Table 3. Meanwhile, Table 4 shows that the reproductive efficiency of female cattle (S/C, DO and CI) is not influenced by body weight group. In this case the body weight is grouped into 4, namely body weight group 1 = 360-400 kg, body weight group 2 = 400-440 kg, body weight group 3 = 440-480 and body weight group 4 = > than 480 kg. . Table 4. Presents Reproductive Efficiency grouped into 4 Body Weight groups.

Table 3. Reproductive efficiency of FHC dairy cattle in different age groups

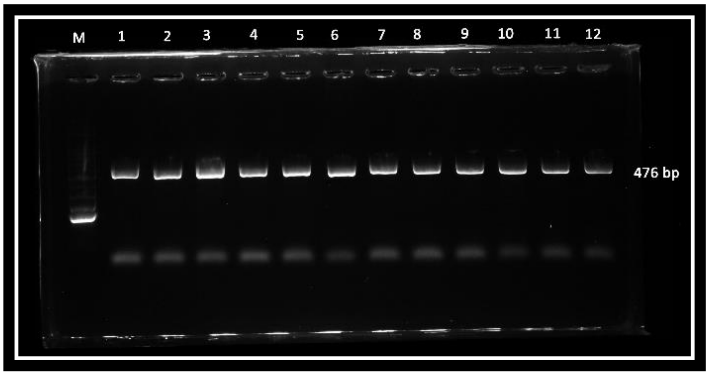
Variables	Age group	N	Mean± Standard deviation
	1	19	1.58 ± 0.51
S/C	2	21	1.38 ± 0.50
	3	13	1.23 ± 0.44
DO	1	19	101.05 ± 11.38
	2	21	96.62 ± 11.60
	3	13	95.00 ± 10.87
CI	1	19	381.05± 11.38
	2	21	376.62± 11.60
	3	13	375 ± 10.87

Table 4. Reproductive efficiency of FHC dairy cattle in 4 body weight groups

Variables	Age group	N	Mean± Standard deviation
	1	3	1.00 ± 0.00
S/C	2	25	1.28 ± 0.46
	3	2	1.5 ± 0.71
	4	3	1.33 ± 0.58
DO	1	3	102.00 ± 10.54
	2	25	99.48 ± 11.90
	3	2	91.00 ± 5.66
	4	3	105,67 ± 10.60
CI	1	3	382.00 ± 10.54
	2	25	379.48± 11.90
	3	2	371.00 ± 5.66
	4	3	385.67 ± 10.60

3.2 Allele frequencies of GH-Msp1 and GH-Alu1 loci

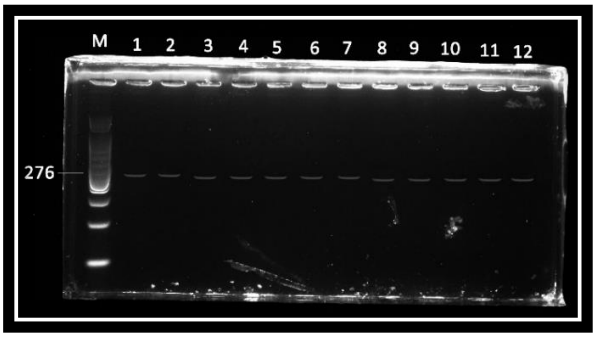
Growth Hormone gene polymorphism analysis in FHC cattle in this study has been carried out by PCR-RFLP method with visualization of DNA amplification using 1.5% and 2% agarose gel presented in Figure 1 with PCR visualization results showing a single band pattern at 476 bp.



Pic 1 Visualization of GH gene amplification on 1.5% agarose gel

3.3 GH-Msp1 gene locus

PCR-RFLP visualization of the GH-Msp1 gene locus with a base length of 476 bp produced 1 single band at position 276 bp presented in Figure 2. Based on this visualization, one A allele was obtained while the G allele or other alleles were not found. This is due to the absence of mutations in the GH-Msp1 gene locus, resulting in the same or unvaried banding pattern.In general, the GH-Msp1 gene locus in FHC dairy cattle in Pujon sub-district is monomorphic with an allele frequency (A) of 1.0 because the GH gene found 100% has the AA genotype. Similar results were also found by []stating that only AA genotype was found for the GH-Msp1 gene locus in subpopulations of Balinese and PO cattle breeds. The uniformity of the GH gene locus in livestock is due to a directed selection process or controlled breeding, so the possibility of inreedig is low



Pic 2 Visualization of PCR-RFLP with Msp1

3.4 GH-Alu1 gene locus

Presented in Figure 3 the results of cutting the GH gene using Alu1 show monomorphic results by producing 3 banding patterns at positions 130 bp, 244 bp and 476 bp. Based on this visualization, one A allele was obtained while the other alleles were not found. This is due to the absence of mutations in the GH-Alu1 gene locus, resulting in the same or unvaried banding pattern. Calculation of genotype and allele frequency at the GH-Alu1 gene locus from 53 FHC cattle obtained an allele frequency (A) of 1.0 because the GH gene found 100% has the AA genotype.

Monomorphic results at the GH gene locus were also obtained in the research of [5] which stated that in Ongole breeders only one BB genotype was found at the GH gene locus at positions 223 bp and 104 bp. Added by [6] which states that the GH-Alu1 gene locus is generally monomorphic because only the LL genotype is found in Balinese cattle at position 211 bp. It can be concluded that the frequency of genotypes and alleles in FHC cattle from generation to generation remains constant, this is possible due to several aspects such as, no mutation occurs, the number of individual cattle in the population is very large so that marriages in the population occur randomly, with this random marriage, the frequency of alleles and genotypes will be constant from generation to generation and each gene will have the same viability and fertility[7]. On the other hand, this result is different with the result of [8] in the same breed that is Local Friesian Holstein (Grati cattle) in Pasuruan, the population was polymorphic by MSp1 restriction [8].

4 Conclusion

Based on the results of the study, it was concluded that the GH (Growth Hormone) gene in Friesian Holstein Crossbreed cattle in the Pujon sub-district area was found to be monomorphic, so it could not be used in further correlation analysis between the marker gene locus and certain phenotypic traits, including reproductive traits and milk production. Further identification needs to be done using more specific gene locus markers to confirm the polymorphism status in a population.

References

1. Cattini, P.A., Bock, M.E., Jin, Y., Zanghi, J.A. and Vakili, H., *Growth Hormone & IGF Research*, **42**, 58-65 (2018)
2. Lucy, M., *Reproduction in Domestic Animals*, **43,** 31-39 (2008)
3. Sumiati, S.S., Arif, D. and Bazilah, A.T. Prosiding Seminar Nasional Industri Peternakan Bogor, 2017, **1**, 78-85 (2017)
4. Amiri, S., B. Jemmali, M.A. Ferchichi, H. Jeljeli, R. Boulbaba and A.B. Gara. Arch. Anim. Breed. **61**, 481 – 489 (2018)
5. Anggraeni, A., Talib, C., Asmarasari, S. A., Herawati, T., & Andreas, E. JITV. 22 (4): 165-172. (2017)
6. Renaville, R; Hammadi, M and Portetelle, D. Domest Anim Endocrinol. **23**, 351-36. (2002)
7. Ciptadi, G., Budiarto, A. and Oktanella. Brawijaya University Press (2019)
8. S. Maylinda. Int. J. Biotechnol. Mol. Biol. Res. **2**, 117 – 120 (2011)