

ASSOCIATIONS BETWEEN POLYMORPHISM OF GROWTH HORMONE GENE WITH MILK PRODUCTION, FAT AND PROTEIN CONTENT IN FRIESIAN HOLSTEIN CATTLE

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Received July 15, 2015; Accepted August 26, 2015

ABSTRAK

Penelitian ini bertujuan untuk menganalisis hubungan polimorfisme gen hormon pertumbuhan (leu/val) dan produksi susu pada sapi Friesian Holstein. Sampel penelitian berasal dari 62 ekor sapi yang diambil dari dua kelompok sapi Friesian Holstein (dari New Zealand=19 ekor and Australia=43 ekor). Polymerase Chain Reaction (PCR) yang dilanjutkan dengan Restriction Fragment Length Polymorphism (RFLP) menggunakan enzim restriksi *AluI* untuk menentukan genotip sapi. Genotip ditentukan berdasarkan target gen GH 211 bp yang terletak pada intron 4 dan exon 5 dari gen hormon pertumbuhan. Genotypes Leu/Leu (LL) pada kedua grup menunjukkan frekuensi yang lebih tinggi dibandingkan dengan genotip Leu/Val (LV). Sapi Friesian Holstein dari New Zealand memperlihatkan genotip LL dan LV sebesar 84% dan 16%. Sapi Friesian Holstein dari Australia memiliki genotip LL dan LV sebesar 79% and 21%. Hubungan antara polimorfisme Leu/Val pada gen hormon pertumbuhan terhadap produksi susu, kadar lemak dan protein tidak menunjukkan pengaruh yang nyata pada kedua grup. Berdasarkan pengelompokan asal sapi, hasil penelitian menunjukkan perbedaan yang nyata pada kadar lemak dan protein susu. Kadar lemak dan protein susu pada sapi FH yang diimpor dari Australia lebih tinggi dibandingkan dengan kadar lemak dan protein susu pada sapi FH yang diimpor dari New Zealand.

Kata kunci: Friesian Holstein import, gen hormon pertumbuhan, produksi susu, kadar lemak dan protein

ABSTRACT

The aim of the research was to determine the associations between polymorphism of the bovine growth hormone (GH) gene (Leu/Val) and milk production of Friesian Holstein Cattle. A total of 62 cows which consist of two Friesian Holstein cattle groups (from New Zealand=19 heads and Australia=43 heads) were used for the research. We performed the Polymerase Chain Reaction and followed by Restriction Fragment Length Polymorphism using *AluI* restriction enzyme. The genotype was observed base on the target gene GH 211 bp in the part of intron 4 and exon 5 of growth hormone gene. The frequencies of genotypes LL were found higher than genotype LV in both groups. Friesian Holstein cattle from New Zealand showed the genotype LL and LV as 84% and 16%, respectively. Friesian Holstein cattle from Australia show the genotype LL and LV as 79% and 21%, respectively. The association between Leu/Val polymorphism on milk production, fat and protein content in both groups did not show the significant effect. Base on two groups of the origin of cattle, the result showed the significant different on fat and protein content of milk. Fat and protein contents of milk were higher in breed of FH imported from Australia compared to those in breed of FH imported from New Zealand.

Keywords : imported FH, growth hormone gene, milk production, fat and protein content

INTRODUCTION

The selection of dairy cattle can be focused on the yield and composition of milk. Recently, the potential candidate genes related to milk production have been widely recognized. The candidate genes are selected on the basis of known relationships between physiological or biochemical processes. The variation of allelic genes in the regulatory and structural regions of those genes may influence the diversification of quantity and composition of milk. One of the candidate genes is growth hormone (GH) gene. Bovine growth hormone gene consists of five exons separated by interval introns. This polymorphism was localized in the exons V of the gene in position 2141 (Lucy *et al.*, 1993). The previous study detected a polymorphic site located in the exon V of the GH gene (transversion C → G in position 2141 of the gene) and consequently the substitution of *Leu* by *Val* in a protein product (Lucy *et al.*, 1993). The other finding also reported the polymorphism in the exon V of the GH gene, in position 2241 showed the transversion from A → C (Yao *et al.*, 1996). The additional polymorphic site in the somatotropin gene (point mutation C → T in the codon 172) was found in the Japanese cattle and consequently threonine was changed into methionine in a protein chain (Chikuni, 1994). The studies on the influence of the *Leu/Val* polymorphism on production traits were quite advanced but the results obtained by various researchers were not always corresponding. Friesian Holstein cows homozygous for *Leu*-127 of bGH produced more milk compared with *Leu/Val* animals (Lucy *et al.*, 1993). The aim of this study was to investigate the allelic frequencies at the *Leu/Val* locus at the bovine GH gene Friesian Holstein cattle and the relationship of polymorphism growth hormone gene with milk production, fat and protein content.

MATERIALS AND METHODS

Sample and Data Collection

The total numbers of samples were 62 heads of Friesian Holstein (FH) cattle at Balai Pembibitan Ternak Unggul Hijauan Pakan Ternak (BPTU-HPT) Baturraden-Purwokerto. The samples consisted of 19 heads FH imported from New Zealand and 43 heads FH imported from Australia. The secondary data of milk yield, fat and protein content were also collected.

DNA Extraction and Amplification of 211 bp GH Gene

About 3 mL of blood sample were collected from 62 animals. The genomic DNA was extracted from blood samples using extraction method described by Sambrook *et al.* (1989). A 211 bp fragment of GH gene was amplified with the primer of GH-forward: 5'GCTGCTCCTGAGGGC-CCTTC-3', and the primer of GH-reverse: 5'CATGACCCTCA-GGTACGTCTCCG-3' (Reis *et al.*, 2001). Amplification of 211 bp of GH gene were conducted by using thermal cycler machine (Peqlab, Advanced Primus 25, Germany). The PCR condition was programmed for initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 65 °C for 30 seconds, extension at 72 °C for 30 seconds, then followed by final extension at 72 °C for 5 minutes (Mu'in, 2008).

Restriction Fragment Length Polymorphism

The 211 bp product size of GH gene was digested with *AluI* enzyme at 37°C for three hours to identify the polymorphism of GH gene. The digestion products were separated by electrophoresis using 2.5% agarose gels in 1XTBE buffer and running with 50 V for an hour for separation of the DNA fragments. The agarose gels were stained with ethidium bromide in order to visualize the DNA fragments by UV light. The size of the DNA fragments were compared with DNA marker ϕ X174 DNA/BsuRI (*HaeIII*) (Fermentas).

Genotype, Allel Frequency and Data Analysis

Genotyping base on *Leu/Val* polymorphism of GH gene was obtained from the results of digestion of each sampel with *AluI* restriction enzyme. Three patterns (genotypes) were produced as the result of *AluI* restriction site (AGCT) such as VV (211 bp), LV (211, 159 and 52 bp) and LL (159 and 52 bp) band pattern could be distinguished on the gel (Hartatik *et al.*, 2013; Mu'in, 2008).

RESULTS AND DISCUSSION

Growth hormone, encoded by the GH gene, modulates growth and lactation in domestic animals, and then genetic variants at the GH locus might affect its level stage or tissue of expression, as well as its functions, having an impact on production traits. The restriction fragment length

polymorphism of growth hormone product was digested using AluI restriction enzyme showed the DNA band as 159 and 52 bp (Leu/Leu genotype) and the DNA band as 211, 159 and 52 bp (Leu/Val genotype) (Figure 1). It was found 79% to 81% Leu/Leu genotype in this study. The frequency of the Leu allele (CTG) was higher than Val allele (GTG). Friesian Holstein cattle from New Zealand have 92% Leu allele and 8% Val allele. Friesian Holstein cattle from Australia have 90% Leu allele and 10% Val allele (Table 1).

The frequencies of Leu/Val alleles in this study were similar to the frequencies of the same allele in earlier study for Friesian Holstein cattle. Higher frequency of the Leu allele was mostly observed in the Holstein breed (Lucy *et al.*, 1993; Reis *et al.*, 2001; Mu'in, 2008; Lee *et al.*, 1996; Zhang *et al.*, 2007; Sabour *et al.*, 1997; Vukasinovic *et al.*, 1999; Dario *et al.*, 2005; Pawar *et al.*, 2007; Mu'in and Zurahmah, 2007; Mohammadabadi *et al.*, 2010). Leu allele is a common allele in growth hormone gene. Based on the observed vs. expected genotype frequencies

the whole pool was in Hardy-Weinberg genetic equilibrium ($X^2=1.04$). Total samples of 50 animals from 62 animals had homozygote Leu/leu alleles. The allele frequencies of total sample showed the same result for that of Australia sample (0.90 Leu and 0.10 Val). This similarity was due to the proportion of sample and the frequency of each genotype. Two digits of calculation in decimal were also influencing the result (0.903 vs 0.895).

Table 2 represents the differences of fat content and protein content between Friesian Holstein cattle imported from New Zealand and Australia. However there is no significant effect of genotype with milk production, fat and protein content in both Friesian Holstein cattle. This result may be influenced by the condition of management in BPTU-HPT Baturaden had the standard of feeding with minimum requirements for cattle. So, the function of potential genetic in cattle cannot be optimized. On the other hand, the results of statistical analysis showed statistically differences ($P<0.05$) between the mean values of

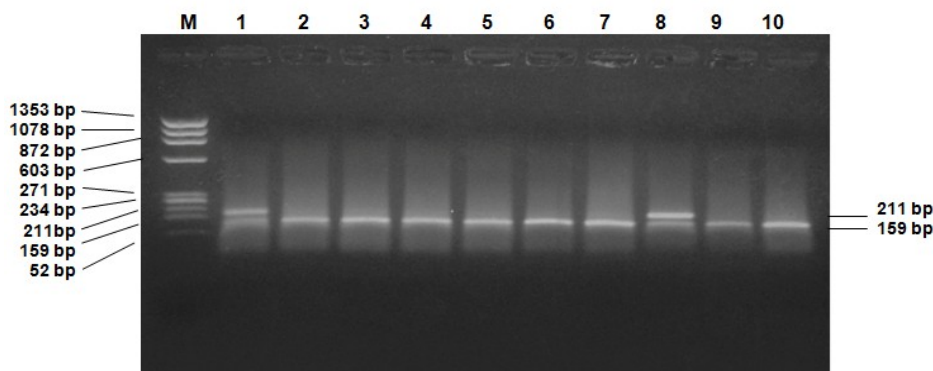


Figure 1. Representative Result of GH-Alu I Analysis Detected by Agarose Gel Electrophoresis. M=DNA Marker ϕ X174/BsuRI(HaeIII); 1-10: PCR-RFLP GH-Alu I

Table 1. Frequencies of Genotypes and Alleles of the *GH* Gene

| FH imported | N | Genotypes | | | Alleles | |
|--------------|----|-----------|------|------|---------|------|
| | | LL | LV | VV | L | V |
| New Zealand | 19 | 0.84 | 0.16 | 0.00 | 0.92 | 0.08 |
| Australia | 43 | 0.79 | 0.21 | 0.00 | 0.90 | 0.10 |
| Total sampel | 62 | 0.81 | 0.19 | 0.00 | 0.90 | 0.10 |

N: The number of samples; L; Leu; V: Val

Table 2. Association of the Leu/Val Genotype with Milk Production, Fat and Protein Content in the FH Cows

| Parameter | Type | Genotype | N | Average Value of Each Genotype | Average Value of Each Breed |
|----------------------------|------|----------|----|--------------------------------|-----------------------------|
| Milk Production (x1000, L) | NZ | LL | 16 | 4.00 ± 1.56 | 4.18±1.49 |
| | | LV | 3 | 5.19 ± 0.23 | |
| | AU | LL | 34 | 5.66 ± 1.83 | 5.68±1.79 |
| | | LV | 9 | 5.79 ± 1.49 | |
| Fat content (%) | NZ | LL | 16 | 3.98 ± 0.64 | 3.99 ^a ±0.59 |
| | | LV | 3 | 4.04 ± 0.26 | |
| | AU | LL | 34 | 4.74 ± 0.76 | 4.78 ^b ±0.72 |
| | | LV | 9 | 4.93 ± 0.59 | |
| Protein content (%) | NZ | LL | 16 | 2.94 ± 0.06 | 2.94 ^c ± 0.66 |
| | | LV | 3 | 2.95 ± 0.07 | |
| | AU | LL | 34 | 3.23 ± 0.39 | 3.23 ^d ± 0.40 |
| | | LV | 9 | 3.23 ± 0.47 | |

^{a, b}: significant different (P<0,05) in fat content of different breed; ^{c, d}: significant different (P<0,05) in protein content of different breed; NZ: New Zealand ; AU: Australia; LL: Leu/Leu ; LV: Leu/Val

fat and protein content in studied population of Friesian Holstein cattle imported from New Zealand and Australia. Lucy *et al.* (1993) reported that L allele was closely related with higher milk production traits for Holstein cattle, whereas in Jersey the V allele was associated with higher milk yield.

CONCLUSION

Polymorphism of Growth Hormone in *AluI* site restriction showed non-significant effect of milk yield, fat and protein content. However, different breed of Frisian Holstein cattle imported from New Zealand and Australia significantly influenced the yield of fat and protein content, but not for milk yield. Fat and protein contents were higher in breed of FH imported from Australia compared with those in breed of FH imported from New Zealand.

ACKNOWLEDGMENT

The authors wish to express thanks to Head of BPTU-HPT Baturraden for permitting the research. This study was partly supported by Research Grant 2013 from Graduate School

Program, Faculty of Animal Science, Gadjah Mada University.

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