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Identification of Gh|Alu-I Gene Polymorphisms in Indonesian Simeulue Buffalo

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ABSTRACT: The purpose of this study was to identify the GH/AluI gene polymorphism in Indonesian Simeulue buffalo. To the best of our knowledge this is the first published data on the polymorphism of growth hormone (GH) gene in Simeulue buffalo. The 178 DNA samples buffalo were collected from three districts in Simeulue Island, Teupah Selatan (71), Teupah Barat (59), and Salang (48). Result shows that a gene fragment of the GH|AluI gene at 432 bp located on exon 3 were successfully amplified by using the techniques of PCR (polymerase chain reaction) and genotyped by PCR-RFLP (restriction fragment length polymorphism). Based on that results showed no polymorphisms were detected in these genes. All buffaloes tested had LL genotype for locus GH|AluI.

Keywords: Simeulue Buffalo, Growth Hormone Gene, Polymorphism

INTRODUCTION

Buffalo is one of the importance domestic animals in Indonesia. They are also regarded as the excellent meat producer. To increasing demand for its products, attention has been focused on the genetic improvement of these species. The local buffalo is a source of germplasm that can be used in order to increase food availability, to improve public welfare, to create employment and to generate foreign exchange. Animals that are genetically adapted to specific environmental conditions would be more productive because it can be developed using low cost, supporting the diversity of food, agriculture and culture, as well as effective in achieving the objectives of food security (FAO, 2000).

Growth hormone (GH) is an anabolic hormone synthesized and secreted by cells of lobe somatotropin in anterior pituitary (Ayuk and Sheppard, 2006). GH has an important role in the growth and postnatal development, growth tissue, lactation, reproduction, and proteins, lipids and carbohydrates metabolism (Akers, 2006; ThidarMyint *et al*., 2008). The study of GH gene MspI and AluI loci have been reported in Hereford and Composite cattle (Sutarno *et al*., 1996; Sutarno 1998), Ongole Grade (PO) cattle (Sutarno *et al*., 2005), Pesisir cattle (Jakaria *et al*., 2007), Aceh cattle (Sari *et al*., 2013), Indonesia local buffalo (Andreas *et al*., 2010; Sumantri *et al*., 2013). but the use of GH gene as molecular marker in Simeulue buffalo has never done. The aim of this research was conducted in order to identify the polymorphism of growth hormone (GH) gene of AluI loci in Indonesian Simeulue buffalo.

MATERIAL AND METHODS

DNA Sample

DNA samples obtained from blood buffalo. The blood samples were used as a source of as much as 178 DNA samples originating from three different regions, namely 59 samples from Teupah Barat, 71 samples from Teupah Selatan, and 48 samples from Salang.

Primer

Primers to amplify gene segments of GH followed Balogh *et al.* (2009), with forward primer 5'-CGGACCGTGTCTATGAGAAGCTGAAG-3' and reverse primer 5'-GTTCTTGAGCAGCGCGTCGTCA-3'. The amplified product length was 432 bp.

DNA Extraction

DNA was extracted from blood buffalo. Extraction procedure followed the phenol chloroform method (Sambrook and Russell, 2001) was modified with the following procedure:

Sample preparation

The blood in the alcohol were as much as 200 µl. Sample was inserted to a 1.5 ml tube. Alcohol was eliminated from the sample by adding distilled water until 1000 µl, and left in room temperature for 20 minutes. Then it was precipitated by centrifugation at a speed of 8,000 rpm for 5 minutes.

Protein degradation

The samples were cleared from alcohol and added by 200 µL 1x STE (sodium tris EDTA), 40 µL sodium dosesil sulfate 10%, and 20 µl proteinase K (5 mg/ml). The mixture were incubated overnight at 55 °C temperature while shaken gently.

Organic material degradation

After incubated, samples were added by 400 µl phenol solution, 400 µL chloroform/isoamyl alcohol (24:1), and 40 µL 5M NaCl. Then, the mixture was shaken at room temperature for one hour.

DNA precipitation

Samples were centrifuged at a speed of 5,000 rpm for 10 minutes to separate the water phase with phenol phase. Water phase was transferred in a new tube with the volume measured. DNA molecules deposited by adding a 2x volume of alcohol absolute and 0.1 x volume of 5M NaCl. Then the mixture was incubated at a temperature of -20 °C over night. Subsequent DNA was precipitated by centrifugation at a speed of 12,000 rpm for 10 minutes. Obtained DNA precipitate was washed by 70% alcohol, and then precipitated again. Precipitated DNA clean from alcohol restored by adding 100 µl TE (Tris EDTA). DNA samples were stored at -20 °C and ready for use.

Amplification of GH Gene

Amplification of GH fragment was done by using PCR (polymerase chain reaction) methods. Reagents used for amplification of both target fragment were a 2 µL sample DNA, each primer 25 pmol, 200 µM dNTPs mixture, 1 mM $MgCl_2$, and 0.5 units of DreamTaq™ DNA Polymerase and 1x buffer (Fermentas) in total solution 25 µL. Amplification in vitro within Gene Amp® PCR System 9,700 (Applied Bio systems™) done with the condition of pra-denaturation at 94ºC for 5 minutes, 35 cycles consisting of denaturation at 94ºC for 45 seconds, annealing primers at 62ºC for 45 seconds and extension of new DNA at 72ºC for 1 minute, and the final extension at 72ºC for 5 minutes.

Genotyping by using RFLP Method

Determination of genotypes of each individual was done by using restriction fragment length polymorphism (RFLP), follow by visualized on 2% agarose gel with 0.5 x TBE buffer (tris borate

EDTA) at 100 V for 40 minutes. Gel was stained by ethidium bromide, and visualized on UV transiluminator. Cutting enzyme that is used for both sides of the target gene was AluI.

Genotype and Allele Frequency

Genotype frequency represents the ratio of a genotype to total population. Allele frequency is a ratio of an allele to the overall allele at a locus in the population. Mathematical model genotype and allele frequency (Nei and Kumar, 2000) is represented as follows:

$$X_{ii} = \frac{n_{ii}}{N} \times 100\%$$

$$X_i = \frac{(2\,n_{ii} + \sum_{i \neq j} n_{ij})}{2\,N}$$

where :
x_{ii} = ii^{th} genotype frequency
n_{ii} = number sample of ii genotype
n_{ij} = number sample of ij genotype
N = total sample
x_i = i^{th} allele frequency

RESULTS AND DISCUSSION

Amplification of Buffalo GH Gene

Amplification of GH gene fragment was carried on Gene Amp® PCR System 9700 (Applied Biosystems™) with temperature of 62°C. The amplified gene fragments were visualized on 1.5% agarose gel. The amplified product length GH gene fragment was 432 bp, including 55 bp of 4th exon, 4th intron, and 99 bp of 5th exon (Balogh *et al*., 2009).

Identification of GH Gene by Using PCR-RFLP Method

Determination of GH gene genotypes in this study was done by PCR-RFLP method using AluI which have cutting site AG|CT. Based on DNA sequences of GH genes amplified segment there were three sites AluI cutting, which produced fragments of length 20, 51, 96, and 265 bp, known as the leucine allele (L). This results also supported by Andreas *et al* (2010) and Sumantri *et al* (2013). There was a substitution from C to G at position 1758 (Lucy *et al*., 1993), so the produces fragments of length 20, 147, and 265 bp, known as the valine allele (V) (Balogh *et al*., 2009). Visualization on 2% agarose gel showed that the GH|AluI locus the three buffalo population was monomorphic. The LL genotype was found in a total sample (Figure 1).

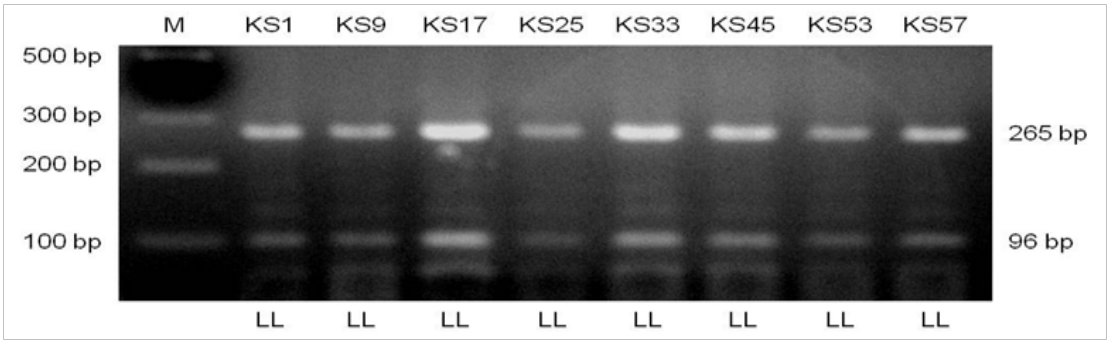

Figure 1. Visualization of the GH|AluI locus on 2% Agarose Gel. M: DNA Ladder 100 bp, 1-16: Buffalo Samples Genotype LL

Genetic Diversity of GH|AluI Genes within Indonesian Simeulue Buffalo

Level of diversity within populations can be drawn from the allele frequency. Allele frequency is a ratio of one allele relative to the overall allele found in one population. Information on genetic diversity of a population using multiple loci can be described by the value of heterozygosity (Nei and Kumar, 2000).

Genetic diversity based on molecular marker

GH|AluI loci in buffalo were very low. This was indicated by the value of one genotype frequency and allele which had a value of 1, which marks the fixation process. Low diversity in buffalo can be caused by a limited number of males in the population

CONCLUSION

Based on this research, it can be identified that the use of GH/AluI only resulted in LL genotype and monomorphic, so that it cannot be used as a marker. This phenomenon is likely due to the limited number of samples and the existence of natural selection towards LV and VV genotype as the consequence of Simeulue buffalo adaption's to the local environment. Thus, a further research is still necessary, by using more samples and if diversity is found, sequencing needs to be done so that the results are more accurate.

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