Identification of Single Nucleotide Polymorphism on Growth Hormone Gene in Aceh Cattle

E. M. Sari*, R. R. Noor, C. Sumantri, M. Yunus, Han J. L., & Muladno

*Department of Animal Production, Faculty of Agriculture, Syiah Kuala University, Jln. Teuku Nyak Arief Banda Aceh 23111, Indonesia
A Department of Animal Production and Technology, Bogor Agricultural University, Jln. Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia
B CAAS (Chinese Academy of Agricultural Sciences) - ILRI (International Livestock Research Institute), JLLFGR (Joint Laboratory on Livestock and Forage Genetic Resources) Beijing-China

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ABSTRACT

This research was aimed to identify the changes of nucleotide (Single Nucleotide Polymorphism) growth hormone gene in the population of Aceh cattle. There were 44 samples of DNA sequenced, and a few samples from Gen Bank (M57764). Based on the analysis using MEGA program, it was identified one new mutation on exon five on 2230 bp in which C nucleotide turned into T nucleotide, and this was called Silent Mutation (Leusine–Leusine/ CTC–CTT). The frequency of Single Nucleotide Polymorphism (SNP) genotype on 2230 bp (C/T) was CC (0.36), TT (0.14) and CT (0.50). The genotype TT was not possessed by Aceh cattle from Saree, but possessed by those from Banda Aceh and Indrapuri. Chi-square test showed not significant differences in allele frequencies for three population. The frequency of genotype SNP on 2291 bp (A/C) was AC (0.11) and CC (0.89). The frequency of allele C was higher than allele A and T.

Key words: Aceh cattle, SNPs, GH gene

INTRODUCTION

Maintain the local livestock resources are important, because it can achieve the sustainable food security for millions of human beings. Besides, maintaining the livestock resources is also able to increase the list of germ plasma as the sources of animal protein. Thus, in order to reduce the extinction speed, especially on the local Indonesian livestock, it is necessary to find the gene information through the efforts of knowing the genetic diversity towards the local livestock.

Aceh cattle is one of Indonesian beef cattle which is suitable to be bred in Indonesia. This type of cattle adapts well to Indonesian environment, tropical climate, and is able to live on the local water and food (Sari et al., 2010). Even though Aceh cattle is able to adapt well, their productivity is still lower than imported ones. By improving the productivity of Indonesian local cattle, it is hoped that the breeders’ interest to breed local cattle will increase, so that the population of local cattle is increasing and able to reduce Indonesian dependency on beef and cattle from other countries.
Bovine growth hormone (bGH) is a single peptide of molecular weight equal to 22-kD secreted from pituitary gland, composed of 190 or 191 amino acids residues and containing Ala or Phe at the N-terminus due to alternative processing of bGH precursor (Dybus, 2002). This gene is approximately 2800 bp with five exons and four introns, and also a part of multiple gene family that contains prolactin and placental lactogens and is located on BTA19, 66cM from centromeric marker BM9202 (Zakizadeh et al., 2006). Allelic variations in the structural or regulatory sequences would be interesting from several points of view. Firstly, genetic polymorphism could have possible direct or indirect effect on growth performance. Secondly, variations in introns or flanking sequences have potential usefulness as genetic marker and help the genetic improvement of populations (Ge et al., 2003).

In farm animals, many polymorphisms have been identified in the GH gene, but only a few of these have been precisely characterized for nucleotide changes and positions in the DNA sequence. In bovine, a single nucleotide polymorphism (SNP) in exons 5 (at codon 127) changes Leusine to Valine (CTG to GTG) in GH molecule (Lucy et al., 1993). Ge et al. (2003) used sequencing method on Angus cattle and found three new SNP on promoter area. Zakizadeh et al. (2006) also reported in Iranian cattle. The research of GH gene AluI in Indonesian cattle have been reported in Bali, Madura, and Benggala cattle (Sutarno et al., 2002), West Sumatra Pesisir cattle (Jakaria et al., 2007); Bali cattle (Jakaria & Noor, 2011), but the research of GH gene AluI in exon 5 in Aceh cattle have never been reported. Based on the description above, it is necessary to carry out a research to identify clearly the position of nucleotide changes of DNA sequence on GH gene exon 5 and to identify the number and SNP allele frequency on Aceh cattle.

**MATERIALS AND METHODS**

**Blood Sampling, Time, and Place of Research**

The DNA sample used for sequencing was taken from various region in Indonesia: Banda Aceh (9), Saree (12), and Indrapuri (23). The blood sample of Aceh cattle was taken using venoject (EDTA) 5 mL on vena jugulars, and then it was kept in an ice box for later laboratory analysis. DNA isolation, extraction, and purification were all carried out in Genetics and Animal Breeding Laboratory, Department of Animal Production and Technology, Bogor Agricultural University. GH gene analysis was conducted in Molecular and Genetics Laboratory CAAS (Chinese Academy of Agricultural Sciences) Beijing-China.

**Genome DNA Extraction**

Genome DNA extraction was carried out using Sambrook et al. (1989) method which was modified using buffer lysis cell (400 µL 1 x STE, and 40 µL 10% SDS and 10 µL proteinase-K). The DNA was then purified using phenol-chloroform method, in which 40 µL 5 M NaCl and 400 µL phenol and chloroform iso amyl alcohol (CIAA) was added. The DNA was precipitated using 40 µL 5 M NaCl and 800 µL ethanol absolute. The precipitate was washed once by adding 800 µL 70% ethanol, centrifuged with the speed of 12,000 rpm for 5 min, the ethanol was discarded and evaporated. Then, the DNA precipitate was dissolved in 10 µL 80% TE (Elution buffer).

**DNA Amplification**

The DNA was amplified with Polymerase Chain Reaction (PCR). Each PCR reaction was made with the volume of 50 µL with the composition of 5 µL 1x buffer PCR; 4 µl dNTP; 1 µL Taq DNA Polymerase; 1 µL Primer Forward and Reverse; 3 µL DNA; and 35 µL dH2O. PCR machine used was Gene Amp PCR System 9700 Applied Biosystem. The PCR program was performed for 34 cycles in which the first cycle was at 94 °C for 5 min followed by 33 subsequent cycles of denaturation step on 94 °C for 30 s, and annealing on 59 °C for 30 s, and extension on 72 °C for 5 min and the last cycle extension on 72 °C for 5 min. The PCR product was kept on the temperature of 4 °C for 25 min. The forward primer was 5'-TAGGGGAGGTGGAAAAATGGA-3' and the reverse primer was 5'-GACACCTACTCTGACATGCG-3' (Yao et al 1996). The position of primer Forward and Reverse in PCR product of GH gene shows in Figure 1.

**Data Analysis**

The result of sequence of GH gene fragment of Aceh cattle was analyzed by Molecular Evolutionary Genetic Analysis (MEGA4) software program with alignment explorer/clustal method (Kumar & Tamura, 2006), also compared to the sequence in GenBank (M57764). The analysis was to ensure that the analyzed sequence is the GH gene fragment, and to find out the existence of mutation in GH gene and the sequence of nucleotide. Data was analyzed by allele frequency (Nei, 1987). The gene frequencies were calculated by counting methods as:

\[
p = \frac{[2(AA) + (Aa)]}{2N}; q = \frac{[2(aa) + (Aa)]}{2N}
\]

The genotype frequency was analyzed by Hardy-Weinberg:

\[
x^2 = \frac{\sum_{i=1}^{n} (O - E)^2}{E}
\]

Note: X² = Chi-square test
O = total of genotype observed -i
E = total of genotype expected -i

![Figure 1: The position of primer Forward and Reverse in PCR product of GH gene](image)

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RESULTS AND DISCUSSION

The Frequency Allele and SNPs Sequence Analysis

The samples which were managed to be sequenced were forty four samples from Banda Aceh (9), Saree (12), and Indrapuri (23). The failure to sequence was caused by unsuccessful amplification, limited number of DNA, and too many peak duplication on sequence graphs. The analysis on the diversity of nucleotide sequence was conducted using MEGA programs after the sequence of Aceh cattle DNA was paralleled with the sample of comparison sequence from Gen Bank (M57764). The PCR product of GH Alu locus (404 bp) detected by agarose gel and the alignment of Aceh cattle can be seen in Figure 3 and 4, respectively.

The result of GH gene nucleotide sequence in Aceh cattle, the change from C to G nucleotide on position 2141 bp was not found. Jakaria & Noor (2011) also found that there was no mutation on position 2141 bp in Bali cattle, and Jakaria et al. (2003) on Angus cattle.

Hecht & Geldermann (1996) compared the sequence from exon 5 areas which clamped GH gene on eight cattle offspring and found an insert/an omission sequence from exon 5 areas which clamped GH gene on that of Ge et al. (2003) on Angus cattle.

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change of C-G nucleotide on the fifth exon from GH gene, which yields two alleles on GH gene called leucine and valine in the position of 127. Lucy et al. (1993) reported that the cattle produced less milk when it was related to GH valine variant on Holstein cattle.

The population of Aceh cattle from Banda Aceh only had CC genotype, whereas AA, AC, and CC genotype were possessed by those from Saree and Indrapuri. CC genotype has higher value than AA and AC genotype in the whole sample of Aceh cattle population (Banda Aceh, Saree, and Indrapuri), so that the frequency of C allele is higher than that of A allele (Table 2). Chi-square test showed no significant differences in genotypic frequencies for three populations of Aceh cattle in the mutation position on 2291 bp. It means that the genotypic frequencies were still in the Hardy-Weinberg equilibrium. This condition was caused by both allele and genotype frequencies in a population remain constant. Mutation and selection did not occur in this population.

CONCLUSION

There is one new SNP (C>T) in exon five of Aceh cattle on the position of 2230 bp. The genotypic frequencies in the position 2230 bp and 2141 bp are still in the Hardy-Weinberg equilibrium. It is necessary to conduct a further research on other local cattle populations to see polymorphisms on the existing local cattle in Indonesia especially in Aceh cattle as well as the effects of the other polymorphism in the growth hormone exon five.

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REFERENCES


Nei, M. 1987. Molecular Evolutionary Genetics. Columbia Uni -
versity Press, New York.


