Polymorphism of GH Exon 2 (c.100A>G) and GH Exon 4 (c.68A>C) in Sapera Goat

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Abstract. Growth hormone gene plays a key role in regulating body growth and in developing mammary gland. Saanen and Etawah Grade (Peranakan Etawah) are widely cultivated in Indonesia to produce milk. Sapera goat is the crossing between Saanen goat and Etawah Grade goat. Sapera goats are used in Indonesia because they are more adaptive to the tropical climate compared to Saanen and more milk yield compared to Etawah Grade. This study aimed to identify on two single nucleotide polymorphisms of the growth hormone gene, namely GH exon 2 (SNP: c.100A>G) and GH exon 4 (SNP: c.68A>C). These two SNPs were identified by the PCR-RFLP method. The GH exon 2 showed three genotypes (AA, AG, and GG). All breeds of dairy goat exhibited the highest AG genotype frequency (0.600-0.100). On the other hand, GH exon 4 was monomorphic. GH exon 2 could be employed as a useful marker to assist selection related to the growth and milk traits in dairy goat breeds. Further analysis is needed to investigate GH exon 4 (SNP: c.68A>C) in a larger sample size.

1. Introduction
Goat has an essential role to produce milk and meat in Indonesia. Goat has a tremendous contribution to poor society. In regarding data from livestock and animal health statistics, the goat population were 18,720,706 heads in 2018. This number exceeded the total cattle population of 17,600,000 heads in 2018 [1]. Therefore, dairy goat farming businesses should be developed by utilize a large population. Milk of dairy goat has a very good price at the market, but Goat milk has not been widely consumed by the public. An increase in milk production can increase the profits of the farmers. There are various ways for increasing milk production, one of which is by a genetic improvement. Dairy goat genetic improvement by Indonesian Research Institute for Animal Production (IRIAP) has been done either by maintaining pure local goat of Peranakan Etawah (PE) or Etawah Grade and introducing exotic goat such as Saanen breed or by crossbreeding such as Sapera (50% PE, 50% Saanen) and Saanpe (25% PE, 75% Saanen).

A key systemic regulatory hormone is a growth hormone (GH), which has a developmental role in all tissues and organs. GH function is influenced by perturbation in early development and impact of
GH on tissue programming [2]. Growth and carcass traits are economically important traits in livestock and are controlled by multiple genes. Selection of animals with higher growth rates and better carcass composition is of great importance to breeders and consumers. It is more realistic to focus on only few candidate genes that have significant effects on the genetic variation on the growth traits [3]. Therefore, genetic polymorphisms of the Growth hormone (GH) gene that are significantly associated with growth traits are very useful. The GH gene also contributes to the development of mammary glands and has a significant effect on milk production. In Holstein cows, GH has a significant effect on 305-day milk yield [4]. Therefore, it is important to identify genetic variation of the GH gene in dairy goat breeds that associated with growth and lactation traits.

2. Materials and methods

2.1. Animals and DNA
A total of 108 animals consisted of Etawah grade (6 heads), Saanen (9 heads), Saanpe (3 heads), and Sapera (90 heads). Two mL of blood drawn from the jugular vein into tubes containing K2 EDTA anticoagulant. DNA was isolated using the Phenol-Chloroform protocol with minor modification [5].

2.2. PCR amplification and PCR-RFLP
The primer used in this research in accordance [6]. The mixture for amplifying DNA in a thermal cycler machine consists of 2 µl DNA sample; 0.3 µL Primer; 7.5 µL 2X GoTaq Green Master Mix (Promega Co., Madison, WI) and 5.2 µL Nuclease Free Water (NFW) for final volumes of 15 µL. The amplification process occurred in 30 cycles consisting of initial denaturation at 94 °C for 5 minutes, final denaturation at 94 °C for 30 seconds, attachment of primer (annealing) at 61 °C and 62 °C at for 30 seconds, elongation of DNA (initial extension) at 72 °C for 1 minute and a final extension at 72 °C for 2 minutes. The PCR product is mixed with 0.7 µl enzyme buffer, 0.4 µl HaeIII enzyme (exon 2) or PstI (exon 4), and 0.9 µl NFW. The mixture was incubated at 37 °C overnight. Identification of allele and genotype was done through electrophoresis analysis on agarose gel 2% (v/w) which stained by Flurosafé (1st Base) and photographed by Alpha Imager® EP.

2.3. Statistical analysis
The data was analyzed using POPGENE version 1.32 program [7] to calculate the observed heterozygosity value (H0), the expected heterozygosity value (He), allele frequency, and genotype frequency. The data were also processed using CERVUS version 3.0.7 program [8] to obtain the Polymorphism Information Content (PIC) value andprovean score was analyzed using Provean Web Server[9]. The allele frequency data were analyzed using FactoMineR [10] and Factoextra [11] package in R version 4.0.0 [12].

3. Results and discussion
The amplified product (amplicon) of the GH exon 2 had a fragment length of 198 bp (figure 1 A), and mutation site at 100 bp. The following DNA restriction fragments were generated by the GH-HaeIII polymorphisms (see figure 1 B): 186 and 12 bp for AA genotype; 186, 97, 89, and 12 bp for AG genotype; 97, 89 and 12 bp for GG genotype.
Figure 1. Visualization of the amplified GH exon 2 (A) on 1.5% agarose gel and PCR-RFLP (B) on 2% agarose gel. Marker: 100 base pairs (bp).

The frequency of AG genotype was very high (0.8377) in Boer goat, whilst the frequency of GG genotype was very low (0.1623)[13]. This is the same as found in this study (see table 1). But in this study found frequencies of GG genotype were 0.333 and 0.344, respectively, in Saanen and Sapera goat. In another study, Mahrous et al. [14] found AG genotype (frequency ranged from 0.90 to 0.95) and AA genotype (frequency ranged from 0.05 to 0.10) in Barki, Damascus, and Zaraibi goat. Hua et al. [13] also found a tendency that AG genotype individuals had better performance in other aspects such as body weight and size at birth and growth rate than GG genotype although no significant differences appeared. Because of the important roles of GH, it is an interesting phenomenon that deserves further investigation of mutations that influence growth variation.

The amplified product (amplicon) of the GH exon 4 had a fragment length of 200 bp (figure 2 A), and mutation site at 68 bp. This mutation site is found according to accession number EF583944. The following DNA restriction fragments were generated by the GH-HaeIII polymorphisms (figure 1 B): 200 bp for AA genotype; 200, 131, and 69 bp for AC genotype; 131, 69 bp for CC genotype. This study only found CC genotype for GH exon 4 (table 2).
Table 1. Frequencies of GH exon 2 | HaeIII on the breed of diary goat.

| Breed       | Genotype | Genotype frequency | Allele | Allele frequency | Ho  | He   | PIC  |
|-------------|----------|--------------------|--------|------------------|-----|------|------|
| Etawah Grade| AA       | 0.000              | A      | 0.500            |     |      |      |
|             | AG       | 1.000              | G      | 0.500            | 1.00| 0.546| 0.358|
|             | GG       | 0.000              |        |                  |     |      |      |
| Saanen      | AA       | 0.000              | A      | 0.667            |     |      |      |
|             | AG       | 0.667              | G      | 0.333            | 0.667| 0.471|
|             | GG       | 0.333              |        |                  |     |      |      |
| Saanpe      | AA       | 0.333              | A      | 0.333            |     |      |      |
|             | AG       | 0.667              | G      | 0.667            | 0.667| 0.533|
|             | GG       | 0.000              |        |                  |     |      |      |
| Sapera      | AA       | 0.056              | A      | 0.356            |     |      |      |
|             | AG       | 0.600              | G      | 0.644            | 0.600| 0.461|
|             | GG       | 0.344              |        |                  |     |      |      |

Figure 2. Visualization of the amplified GH exon 4 (A) on 1.5% agarose gel and PCR-RFLP (B) on 2% agarose gel. Marker: 100 base pairs (bp).

Based on information from accession number EF583944, A allele can be found in Nanjiang goats which are meat-type goats. Perhaps this is the reason for not finding A allele in the dairy goat breeds. This mutation cause changes in amino acids from Glutamine to Lysine.
Table 2. Frequencies of GH exon 4 | PstI on the breed of dairy goat.

| Breed (n) | Genotype | Genotype frequency | Allele | Allele frequency | Ho  | He  | PIC |
|-----------|-----------|--------------------|--------|------------------|-----|-----|-----|
| Etawah (6) | AA        | 0.000              | A      | 0.000            | 1.000 | 0.000 | 0.000 |
|           | AC        | 0.000              | C      | 1.000            | 0.000 | 0.000 | 0.000 |
|           | CC        | 1.000              |        |                  |      |      |      |
| Saanen (9) | AA        | 0.000              | A      | 0.000            | 1.000 | 0.000 | 0.000 |
|           | AC        | 0.000              | C      | 1.000            | 0.000 | 0.000 | 0.000 |
|           | CC        | 1.000              |        |                  |      |      |      |
| Saanpe (3) | AA        | 0.000              | A      | 0.000            | 1.000 | 0.000 | 0.000 |
|           | AC        | 0.000              | C      | 1.000            | 0.000 | 0.000 | 0.000 |
|           | CC        | 1.000              |        |                  |      |      |      |
| Sapera (90) | AA       | 0.000              | A      | 0.000            | 1.000 | 0.000 | 0.000 |
|           | AC       | 0.000              | C      | 1.000            | 0.000 | 0.000 | 0.000 |
|           | CC       | 1.000              |        |                  |      |      |      |

The SNPs in this study were analyzed using provean (table 3). Provean predicts the functional impact for all classes of protein sequence variations not only single amino acid substitutions but also insertions, deletions, and multiple substitutions [15]. Provean scores will show neutral or deleterious. If the result is neutral, the changes in amino acids is common. But if the result is deleterious, the changes of amino acids will give an effect [15]. GH exon 4 had a deleterious effect according to provean score. It is necessary to study the effects of A allele on the goat phenotype. The use of protein function prediction tools and protein structure prediction tools can help understand the effects of an SNP.

Table 3. Provean score of SNPs in this study.

| Gene | Amplified region | SNP    | Provean score | Prediction (cutoff = -2.5) |
|------|------------------|--------|---------------|---------------------------|
| GH   | Exon 2           | S19G¹  | -1.975        | Neutral                   |
|      | Exon 4           | Q20K²  | -2.988        | Deleterious               |

Accession number: 1: EU048226.1, 2: EF583945.1.

The PIC value for GH exon 2 is 0.38. The maximum PIC value for biallelic markers is 0.5 [16]. Based on this information, the PIC value of GH exon 2 is very informative because it is higher than the average of maximum PIC value for biallelic markers. Therefore, GH exon 2 could be employed as useful markers for helping the selection of Sapera goat. Using PCA, Etawah Grade and Sapera have a close genetic relationship because Sapera is the result of Saanen bucks mate with Etawah Grade does (figure 3).
Figure 3. Principle Component Analysis of Four Breed Using Allele Frequency Data (1: Etawah Grade, 2: Saanen, 3: Saanpe, 4: Sapera).

4. Conclusions
In this study, GH exon 2 had 3 genotypes. Frequencies of AG genotype were very high in four breeds (ranged 0.600 – 1.000). On the other hand, GH exon 4 is monomorphic in dairy goat. The possible Allele A in exon 4 can be found in meat-type goat.

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