Polymorphisms of IGF-1 Gene in Indonesian Local Goat Reared Under Smallholder Farmers in Sulawesi Region

(Polimorpisme gen IGF-1 pada populasi kambing lokal Indonesia yang dipelihara oleh peternak kecil di daerah Sulawesi)

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ABSTRACT. The objective of this study was to determine the allele frequency of IGF-1 in Kacang and Peranakan Ettawa goats in Indonesia. The DNA samples were extracted from the blood of 105 heads of goats and collected from the South Sulawesi and West Sulawesi provinces. The IGF-1 target was amplified using the PCR-RFLP method. Two allele variants (A and B) and three genotypes of the IGF-1 gene (AA, AB, and BB) have been identified in the local goat population. Allele frequencies of IGF-1 were A (0.81) and B (0.19) in the total population of local goats. Allele A is the most common allele in both the Kacang and Peranakan Ettawa goat populations, with the highest frequency found in the Kacang population (0.87). The most common genotype is genotype AA, with the highest frequency in the Kacang population (0.75). The observed and expected heterozygosity were 0.276 and 0.303, respectively. The population of local goats in these regions was in Hardy-Weinberg equilibrium. The conclusion of this research is that the IGF-1 gene in local goats in the Sulawesi region is polymorphic and this diversity information can be used for association studies with growth traits, litter size, and twinning rate.

Keywords: Ettawa crossbreed goats, genetic performance, IGF-1, Kacang goats, polymorphism

INTRODUCTION

The characteristics of goat farming in many areas of Sulawesi in Indonesia are generally characterized by traditional systems with an uneconomic business scale and not applying good breeding practices yet. That condition causes the degradation of genetic quality, especially body performance. The diversity of body performance in goats is caused by several factors, including genetic differences. Improving the genetic quality of local goats is one of the strategies needed to increase local goat productivity. One strategy that can be taken is through the characterization of functional genes associated with productivity traits.

One of the important functional genes is the IGF-1 gene. Insulin-like growth factor 1 (IGF-1) is an important growth factor involved in various physiological processes including reproduction, fetal development, and growth (Monte et al., 2019; Sankhyan et al., 2020; Thomas et al., 2016). IGF-1 plays an important role in mammalian fertility as a reproductive trigger when the nutritional condition is ready (Velazquez et al., 2008). The IGF-1 system also plays a key role in the bone, muscle, and cartilage growth (Duclos et al., 1999; Yakar et al., 2002; Zapf and Froesch, 2010).

The gene encoding IGF-1 appears to be a promising candidate gene for marker-assisted selection in various economic traits in domestic livestock. The polymorphism of IGF-1 gene is known associated with growth traits in chicken (Amills et al., 2003; Bennett et al., 2006; See et al., 2001; Zhou et al., 2005) and in buffalo (El-Magd et al., 2017), and wool traits and litter size...
in sheep (Darwish et al., 2017; He et al., 2012), respectively. The IGF-1 gene in goats was encoded by a single gene located on chromosome 5 (Schibler et al., 1998), consisting of three leader exons (1w, 1, and 1a) and three other exons (3, 4, and 6), where exon 3 and exon 4 encode the maturity of IGF-1 peptide (Mikawa et al., 1995). The objective of this study is to investigate the polymorphism of the IGF-1 gene in local goats (Kacang and Peranakan Ettawa) reared by smallholder farmers in the South and West Sulawesi regions in Indonesia.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Blood samples were collected randomly from a total of 105 heads of local goats (56 heads of Kacang goats collected in South Sulawesi Province and 49 heads of Peranakan Ettawa goats in West Sulawesi Province). Samples were taken from the jugular vein using a tube including EDTA and stored at -20 °C before DNA extraction. DNA was extracted and isolated with DNA isolation kit (GeneJET genomic DNA purification kit, Thermo Scientific).

PCR Amplification

A 25 µL aliquot mixture was made for PCR reaction that contained ~100 ng of genomic DNA, 1 mM MgCl2, 200 µM of the dNTPs mix (Fermentas), and 25 pmol of each primer, 1x buffer, and 0.5 U Taq DNA polymerase (Dreamtaq, Fermentas). The PCR condition was performed with an initial denaturation process at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 30 s, and extension at 72 °C for 60 s. The final step of an extension was at 72 °C for 5 min. To amplify the exon 4 region of the IGF-1 gene, the nucleotide sequence for primers was used based on Wu-Jun et al. (2010) ie. (Forward: 5’-CACACGTTATTATCCCCAC-3’) and (Reverse: 5’-GACACT ATGAGCCAGAAG-3’). All PCR processes were performed in a thermal cycler machine (SensoQuest, Germany). The PCR products were electrophoresed on 1.5% agarose gels in 1x TBE buffer (tris borate EDTA) containing 10% of Ethidium Bromide (EtBr) at 100 volts for 45 min and visualized under UV-transilluminator.

IGF-1 Genotyping with PCR-RFLP Method

The PCR products were digested with the restriction endonucleases: BsaRI (HaeIII) (Thermo Scientific) that recognized the GG|CC restriction site. Restriction products were electrophoresed on 2% agarose gel stained with EtBr and visualized under UV-transilluminator. Genotypes were determined based on the length of DNA fragments, AA (363 bp), AB (363 bp, 264 bp, and 99 bp), and BB (264 bp and 99 bp).

Data Analysis

The genotype and allele frequencies were calculated based on Nei and Kumar, (2000) formulation. The test of Hardy-Weinberg equilibrium (HWE) was carried out with a chi-square test (Kaps and Lamberson, 2017). Observed (H0) and Expected heterozygosity (He) based on Nei’s heterozygosities (1973) and computed using PopGene32 software version 1.31 (Yeh et al., 1999).

RESULTS AND DISCUSSION

Insulin like growth factor 1 (IGF-1) gene was successfully amplified using PCR machine at annealing temperature 60 °C. The IGF-1 gene amplification yielded a length of 363 bp PCR product. After digestion with the HaeIII restriction enzyme, fragments of 363, 264, and 99 bp were obtained by electrophoresis (Figure 1). This cut resulted in the identification of two alleles, namely allele A (363 bp) and allele B (264 and 99 bp).

The results of this study indicate that the IGF-1 gene was polymorphic in both Kacang and Peranakan Ettawa goat populations. The genotype and allele frequency of the IGF-1 gene are presented in Table 1. In this study, three genotypes were found in Kacang and Peranakan Ettawa goat populations, ie. AA (363 bp), AB (363 bp, 264 bp, and 99 bp), and BB (264 bp and 99 bp). This result differs from the study reported by Alakilli et al. (2012) on Zaribi goat that only found two genotypes (AA and BB). Allele A was the most common allele in both the Kacang and Peranakan Ettawa goat populations. The Kacang goat population has the highest frequency of allele A (0.87), and the most common genotype is genotype AA with the highest frequency in the Kacang goat population (0.75). The same results as those reported by Wu-Jun et al. (2010) which showed the highest AA genotype frequencies were also found in Nanjiang Cashmere goat populations, but a different result was reported in Xinjiang goat, where the B allele and genotype BB were more common.

The diversity of the IGF-1 gene was also reported by Qiong et al. (2011). They reported the
variation of alleles and new mutations found in exon 4 regions in the IGF-1 gene in three local goat breeds in China. This variation is also reported to be related to differences in cashmere production and body weight in goats. Other studies reported that the IGF-1 gene variant has an association with twinning rate, growth traits, and yearling fleece weight in the Markhoz goat (Kurdistani et al., 2013; Rasouli et al., 2017), prolificacy trait (Thomas et al., 2016), growth traits in the Nanjiang Huang goat (Zhang et al., 2008), milk yield and body size in Chinese dairy goat (Deng et al., 2010), and reproductive performances and milk yield in Sarda dairy sheep (Luridiana et al., 2020). The IGF-1 genetic variation was also reported to have an association with the Cashmere fiber trait in Changthangi goats (Shanaz et al., 2020).

Figure 1. The genotype of PCR-RFLP BsuRI (HaeIII) analysis of the IGF-1 gene. Line M= Marker 100 bp, Line AA= genotype AA with 363 bp, Line AB= genotype AB with 363, 264, and 99 bp, and Line BB= genotype BB with 264 and 99 bp fragment in length.

Table 1. Genotype and allele frequency of IGF-1 gene in local goat population

<table>
<thead>
<tr>
<th>Breed Population</th>
<th>n</th>
<th>Genotype Frequency</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Kacang</td>
<td>56</td>
<td>0.75</td>
<td>0.23</td>
</tr>
<tr>
<td>Peranakan Ettawa</td>
<td>49</td>
<td>0.60</td>
<td>0.32</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>0.67</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Note: n= individual number

Table 2. Observed and expected genotype frequency of IGF-1 gene in local goat

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed Freq. (O)</th>
<th>Expected Freq. (E)</th>
<th>X²(Chi Square)</th>
<th>p value (0.05;1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>71</td>
<td>69.55</td>
<td>0.89</td>
<td>3.841</td>
</tr>
<tr>
<td>AB</td>
<td>29</td>
<td>31.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>5</td>
<td>3.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>105</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: ns= not significant (p value 0.05)

Table 3. Observed and expected heterozygosity value of IGF-1 gene in local goat.

<table>
<thead>
<tr>
<th>Breed Population</th>
<th>n</th>
<th>H₀</th>
<th>Hₑ</th>
<th>Nei*</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kacang</td>
<td>56</td>
<td>0.232</td>
<td>0.234</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td>Peranakan Ettawa</td>
<td>49</td>
<td>0.326</td>
<td>0.373</td>
<td>0.369</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>0.276</td>
<td>0.303</td>
<td>0.302</td>
<td>0.302</td>
</tr>
</tbody>
</table>

Noted: n= individual number, H₀= Observed Heterozygosity, Hₑ= Expected Heterozygosity according to Levene (1949) and Nei’s (1973)

Polymorphisms of IGF-1 Gene in Indonesian Local Goat Reared Under Smallholder Farmers in Sulawesi Region … (Lellah Rahim, et al..)
The results of the IGF-1 gene characterization show that genetic diversity in the local goat population is quite high. This suggests that genetic variation in the IGF-1 gene could be utilized in local goat selection and breeding programs to improve the genetic quality of the goat that is maintained by smallholder communities in the Sulawesi region. Selection programs related to the genetic diversity of the IGF-1 gene can be directed at selecting productive traits such as litter size, growth and twinning rate.

CONCLUSIONS

IGF-1 gene condition of Indonesian local goats which is reared under smallholders in the Sulawesi region showed polymorphism. Allele A was the most common allele in both the Kacang and Peranakan Ettawa Goat populations. The highest frequency was found in the Kacang goat population (0.87), whereas the most common genotype was the AA genotype, with the highest frequency in the Kacang goat population (0.75). The level of heterozygosity was found to be moderate. Variation in the IGF-1 gene could be used as a future genetic marker in local goat selection for better genetic performance and used for the next study to find any association between IGF-1 polymorphism with litter size or twinning rate and growth traits in Indonesian local goats.

ACKNOWLEDGMENT

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