



Genetic Diversity Analysis of the (IGF1|HaeIII) Gene in PE, Kacang, and Senduro Goats in Indonesia Based On PCR-RFLP

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Abstract. This study aimed to analyze the genetic diversity of the IGF1 gene among three local goat varieties in Indonesia: Peranakan Etawah (PE), Kacang, and Senduro. DNA samples from 120 goats were collected and evenly distributed among the three breeds. The DNA samples were extracted and amplified using PCR. The samples were analyzed with the HaeIII enzyme using RFLP to identify the polymorphisms. The results revealed monomorphism at the IGF1 locus across all sampled populations indicated with GG genotype with no genetic variation detected. This finding suggests a lack of genetic diversity at this specific gene in the local goat breeds studied. While the monomorphic nature of IGF1 limits its uses as a genetic marker for selective breeding in these populations. The study contributes to the understanding of genetic resources in Indonesian goat breeds and highlights the need for broader genetic assessments.

Keywords: Genetic polymorphism, HAEIII, Local Goat, IGF1, PCR-RFLP.

1 Introduction

Genetic diversity is important for livestock breeding as it has an impact to the adaptability and productivity of the animal. Higher genetic diversity is associated with improved growth rates, disease resistance, and reproductive efficiency, which are essential traits for livestock in varying environmental conditions [1]. Goats are an essential part of the agricultural economy in Indonesia, providing meat, milk, and other products. From various local goat breeds, PE, Kacang, and Senduro goats are the widely known breeds known from their distinctive characteristics and adaptability with Indonesia's tropical conditions. By understanding the genetic structure among these breeds is important to improve breeding strategies and enhancing genetic potential to increase in yield in farm animals [2].

Insulin-like growth factors (IGF) are a family of multifunctional cytokines involved in cell proliferation and differentiation. They play a crucial role in regulating the growth and development of animals. The IGF family is essential for various biological processes related to growth and tissue development across different animal species [3,4]. IGF1 or Insulin-like Growth Factor 1 gene is a key regulator of growth

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and development in mammals, influencing factors such as body size, muscle growth, and reproductive traits. In goats, polymorphisms in the IGF1 gene may have significant effects on growth traits, making it a target of interest for genetic diversity studies [3]. Hafez observed genetic polymorphisms in various loci across goat breeds, such as IGF-I and IGFBP-3 genes in Egyptian goats [5]. Widayanti also observed the prion protein (PRNP) gene in Indonesian goats demonstrating the presence of genetic diversity relevant to growth and disease-related traits [6]. The PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method is widely used to detect gene polymorphisms due to its simplicity and reliability [7].

This study focuses on analyzing the genetic diversity of the IGF1 gene, specifically using the HaeIII restriction enzyme, in PE, Kacang, and Senduro goats from Indonesia, especially East Java.

2 Materials and Methods

2.1 Sample Collection

Blood samples were collected from a total of 120 goats, consisting of 40 Kacang goats from Sidoarjo, 40 PE goats from UPTPT Singosari Malang, and 40 Senduro goats from Lumajang. The blood samples were collected using Vacutainer tubes and stored at -20°C.

2.2 DNA Extraction

Genomic DNA was extracted from the blood samples using GeneAid Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan). Blood samples were added with RBC Lysis Buffer, GB Buffer, and Elution Buffer. After the Cell Lysis step, the second step is DNA Binding by adding absolute ethanol and GD Column in a 2ml Collection tube. The third step is washing by adding 400 µL of W1 Buffer to the GD Column and 600 µL of wash Buffer. The last step is DNA Elution by adding 100 µL of pre-heated Elution Buffer. This method is aligned with the fundamental principles of DNA extraction methods as described in the standard molecular biology protocols [8].

2.3 Amplification and genotyping of DNA target (PCR-RFLP)

PCR amplification of the 121 – 345 Bp region in the exon 4 with the total length of 466 Bp in the IGF-1 gene. DNA sequences of the forward and reverse primer used were F = 5'-GAA TGT GAC TGG GAA GTG TG -3' and R=5'-CCT TTC TTG GCT GTG TTC AG -3'. The DNA was amplified by PCR technique. Extracted DNA samples of as much as 0.5- 1 µL were put into a 0.2 mL Eppendorf tube, then 14 µL of premix solution was added. The premix is composed of 0.4 µL of primer, 6.2 µL of DW, and 7.0 µL Green Master Mix. The amplification process begins with the denaturation stage at 95°C for five minutes. The second stage consisted of 35 cycles, each cycle consisting of denaturation at 95°C for 10 seconds, annealing at 60°C for 20

seconds, DNA extension at 72°C for 30 seconds, and DNA extension at 72°C for 30 seconds. The last stage is primer elongation at 72°C for 5 minutes.

RFLP analysis was performed by HaeIII restriction enzyme which cuts the DNA at the GGCC sequence. The RFLP premix solution were put into 0.2 mL Eppendorf tube that consisted of 0.4 μ L of HaeIII enzyme, 0,7 μ L 10x Buffer and 0.9 μ L of DW Buffer. 5 μ L of PCR products were added into the RFLP premix solution and incubated at 370C overnight. The resulting DNA fragments were then analyzed by gel electrophoresis to determine the DNA fragment.

3 Results and Discussion

3.1 Results

The analysis of the IGF1 gene using PCR-RFLP with the HaeIII enzyme revealed that all three goat breeds (PE, Kacang, and Senduro) were monomorphic at the target locus (Table 1). After digestion of the PCR product with HaeIII, no variation in restriction fragment patterns was observed across the 120 samples from the three breeds. Upon visualizing the restriction digestion products, two bands were consistently observed across all samples, with the first band located at approximately 345 bp and the second band at 121 bp (Figure 1). The presence of these two bands in all 120 samples from the PE, Kacang, and Senduro breeds suggests a conserved genetic structure at this specific locus, with no sequence variation that would disrupt the HaeIII restriction site.

Table 1. The total of IGF-1/ HaeIII polymorphisms in terms of genotype and allele In 3 different breeds of local Indonesian goats.

Breeds	N	Genotype			Alel	
		GG	GC	CC	G	C
Kacang	40	40	0	0	40	0
PE	40	40	0	0	40	0
Senduro	40	40	0	0	40	0
Total	120	120	0	0	120	0

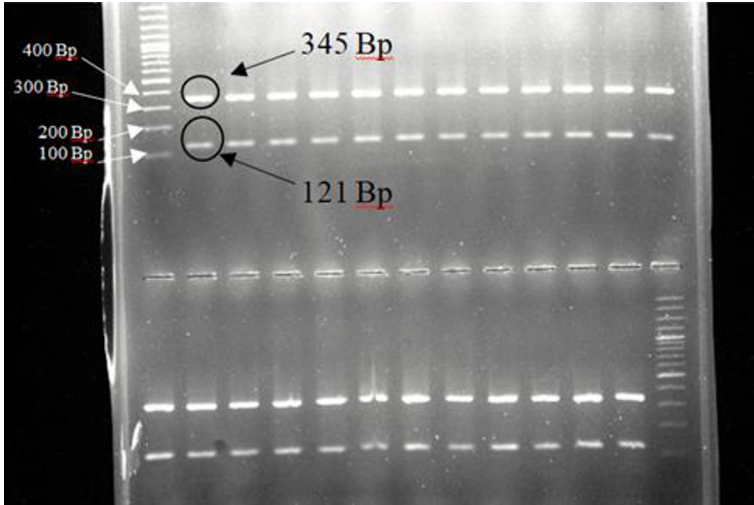


Fig. 1. PCR-RFLP banding pattern of IGF1 gene digested by HaeIII in PE, Kacang, and Senduro goats.

The genotypic profile for all individuals in the PE, Kacang, and Senduro goats was identical, with no alternative alleles detected. The frequency of the double allele was 1.0 in all breeds, and the populations were fixed for this allele at the IGF1 locus. There were no observed heterozygotes, and the observed and expected heterozygosity values were both zero. These results suggest that the IGF1 gene in the studied goat breeds is monomorphic.

3.2 Discussion

The monomorphic state observed in the PE, Kacang, and Senduro goats at the IGF1 locus raises important considerations regarding the genetic diversity of these breeds. Several potential factors could explain this lack of variation according to Hardy-Weinberg Equilibrium. Scientists routinely use HWE tests as an effective quality control method to identify major genotyping errors in their data. However, deviations from HWE aren't always due to technical mistakes. Other biological and population factors can cause these deviations, such as when a population contains distinct genetic subgroups or when there is frequent breeding between closely related individuals or inbreeding. Therefore, while HWE testing is valuable for detecting technical errors, researchers must carefully consider multiple possible explanations when they find HWE deviations in their genetic data [9].

The findings of this genetic study align with previous research that has documented similar patterns of genetic uniformity in other goat breeds namely Barki, Habsi and Damascus x Barki Crossbreed. The study revealed significant allele frequency variations among different goat breeds in Eastern Europe [10]. Similar patterns of monomorphism have also been reported in the growth hormone gene among Egyptian goat breeds, highlighting the potential impact of selective breeding on genetic variation

[7]. Furthermore, A study on Indonesian goat breeds indicated that genetic distances among local breeds were minimal, suggesting a shared genetic heritage that may contribute to the observed homozygosity in the current study [11].

4 Conclusion

The study of IGF1 gene polymorphisms using PCR-RFLP analysis with the HaeIII enzyme in three Indonesian goat breeds (PE, Kacang, and Senduro) revealed a monomorphic pattern at the IGF1 locus. All samples across these breeds showed a single genotype and allele, with a frequency of 1.0, indicating a fixed genetic state with no observed heterozygosity. This lack of polymorphism suggests low genetic diversity at this locus within these local Indonesian breeds. Several factors, including the founder effect and selective breeding practices may have contributed to this monomorphic state. Selective breeding for growth traits associated with the IGF1 gene likely played a significant role in reducing allelic variation, potentially due to the prioritization of favorable growth-related traits over generations.

Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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