Identification of IGF1 Gene Polymorphisms Using PCR-RFLP in Indigenous Goats of East Java

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Abstract. The objective of this study was to identify insulin growth factor (IGF-1) gene polymorphism using PCR RFLP technique in order to improve growth evaluation. The insulin-like growth factor 1 (IGF1) gene is a critical factor in the growth and development of goats. It influences a range of traits, including body size, milk production, and reproductive performance. This gene was therefore identified as a potential candidate gene. Blood samples were collected from 120 local goats of East Java, namely 43 Cross Etawa goats, 43 Senduro goats, and 43 Kacang goats. The DNA was extracted using the Geneaid DNA Purification Kit. The IGF-1 polymorphisms were determined by the PCR-RFLP method, employing the HaeIII restriction enzyme. The primers for the 398 bp long IGF1 gene are forward 5' GGAATCGTGGATGAGTGC 3' and reverse 5' CACAGCAGTAGAAGGGAG 3'. The results demonstrated that the local goat population in East Java is in Hardy-Weinberg equilibrium. The conclusion of this study is that the IGF-1 gene in local goats in East Java is polymorphic, and this diversity information can be employed for association studies with growth traits, litter size, and twinning rates.

Keywords: IGF1 gene, Indigenous Goats, PCR-RFLP.

1 Introduction

The livestock sector is fundamental to Indonesia's agricultural economy, significantly contributing to the provision of animal protein and the sustenance of rural livelihoods. Indigenous goats possess considerable nutritional, economic, and cultural significance among various livestock species, especially in areas such as East Java. These goats significantly enhance food security by providing a consistent supply of meat and milk, which are crucial for augmenting overall protein consumption vital for public health and development [1].

Indigenous goats in East Java exhibit exceptional adaptation to local agro-ecological circumstances, showcasing significant resilience and necessitating few resources. Their capacity to flourish on indigenous forage and transform low-quality feed into high-quality protein renders them a compelling choice for smallholder farmers, particularly

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in regions where alternative livestock may be inappropriate owing to climatic or economic limitations [1]. Goat meat is comparatively thinner than other red meats and is abundant in key amino acids, rendering it a healthier choice for customers [2]. Goat milk is a superior source of calcium and several minerals, enhancing a balanced diet and mitigating nutritional deficits.

Notwithstanding their significance, the productivity of indigenous goats is constrained relative to their genetic potential. Improving productivity is essential to satisfy the increasing demand for animal protein resulting from population increase and urbanization in Indonesia [1]. Genetic enhancement procedures provide a sustainable method to augment the productivity and resilience of these goats, therefore facilitating sustainable livestock development and enhancing food security.

The insulin-like growth factor 1 (IGF1) gene is essential for the regulation of growth, development, and metabolic functions in animals. The IGF1 gene, situated on chromosome 5 in goats, is an essential element of the somatotropic axis, encompassing growth hormone (GH), and is crucial for postnatal growth and metabolism [3] [4]. The expression of IGF1 is affected by multiple factors, including genetic polymorphisms that influence growth traits and reproductive efficiency among diverse goat breeds [5] [6] [7]. Prior research has discovered particular single nucleotide polymorphisms (SNPs) in the IGF1 gene linked to favorable characteristics. A new SNP in the promoter region of IGF1 has been associated with increased kidding numbers in Yunshang Black goats via augmenting transcriptional activity [8]. Polymorphisms in the IGF1 gene have been associated with body weight and diverse phenotypic characteristics in Indian goat breeds, indicating its potential as a genetic marker for selective breeding [7] [5]. These findings highlight the importance of the IGF1 gene in goat physiology and its prospective use in genetic enhancement initiatives.The physiological effects of IGF1 are facilitated by many signaling pathways, including the PI3K/Akt and MAPK pathways, which govern anabolic processes like protein synthesis and cell survival [6] [8]. Furthermore, IGF1 engages with other hormones and growth factors, complicated its regulation network and underscoring its significance in growth and reproduction [9] [10]. Comprehending the genetic variants in the IGF1 gene may facilitate better informed breeding decisions, enhancing herd performance and resilience to environmental problems [11].

Despite extensive global study on IGF1 gene variants, there is a paucity of studies examining indigenous goats in East Java. The genetic diversity and distinct adaptations of these goats provide an opportunity to investigate the polymorphisms of the IGF1 gene and their correlation with growth and reproductive characteristics. Recognizing these genetic variances is crucial for formulating targeted breeding plans to improve the productivity and sustainability of goat husbandry in the area. The primary objective of this study is to identify polymorphisms in the IGF1 gene of indigenous goats in East Java utilizing the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique.

2 Materials and Methods

2.1 Material

Whole blood samples were collected from a total of 129 indigenous goats comprising three breeds: 43 Peranakan Etawah (PE) goats from Tulungagung, 43 Kacang goats from Sidoarjo, and 43 Senduro goats from Lumajang, East Java, Indonesia. The goats were randomly selected and represented the typical population of each breed in their respective regions. Blood samples were obtained via jugular vein puncture using vacutainer tubes containing EDTA as an anticoagulant to prevent clotting. All samples were kept at 4°C during transportation to the laboratory and stored at –20°C until genomic DNA extraction for further analysis.

2.2 Methods

DNA Extraction. Genomic DNA was extracted from whole blood samples using the Geneaid Blood DNA Mini Kit, with slight modifications to enhance yield and purity. The extraction principle involved lysing red and white blood cells to release DNA into solution. Proteins and other cellular contaminants were degraded and removed through incubation with specialized buffers at elevated temperatures. DNA was then precipitated using absolute ethanol and selectively bound to a silica membrane within a spin column. Impurities were washed away with specific buffers, and the DNA was thoroughly dried to eliminate residual ethanol. Finally, the purified DNA was eluted from the membrane using a pre-warmed elution buffer. The extracted DNA was stored at –20°C and assessed for concentration, purity, and integrity, confirming its suitability for downstream applications such as PCR amplification and genotyping analyses essential for identifying IGF1 gene polymorphisms.

PCR Amplification of the IGF1 Gene. The IGF1 gene segment was amplified with particular primers aimed at the target area. The forward primer sequence is 5' GGAATCGTGGATGAGTGC 3', while the reverse primer sequence is 5' CACAGCAGTAGAAGGGAG 3'. The Polymerase Chain Reaction (PCR) was conducted in a reaction volume adjusted for effective amplification. The criteria for PCR were as follows: A preliminary pre-denaturation phase at 95°C for 5 minutes to guarantee thorough denaturation of the genomic DNA. This was succeeded by 35 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 20 seconds to facilitate specific primer binding to the target sequence, and extension at 72°C for 20 seconds to synthesis the new DNA strands. A concluding extension phase was performed at 72°C for 5 minutes to guarantee the complete extension of any residual single-stranded DNA. The anticipated PCR product measured 343 base pairs in length.

Restriction Fragment Length Polymorphism (RFLP) Analysis. The PCR products were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis using the restriction enzyme HaeIII, which recognizes and cuts at the GGCC sequence. The digestion reaction was set up in a total volume of 7 μl, comprising 5 μl of PCR product, 0.4 μl of HaeIII enzyme, 0.7 μl of the appropriate 10X buffer provided by the enzyme manufacturer, and 0.9 μl of distilled water to adjust the final volume. The reaction mixtures were gently mixed and incubated at 37°C for 16 hours to ensure complete digestion of the PCR products. The HaeIII enzyme is expected to cut the 343 bp PCR product into fragments of 195 bp and 80 bp if the restriction site is present, allowing for the detection of polymorphisms within the IGF1 gene.

Electrophoresis. Agarose gel electrophoresis separated DNA fragments by size after PCR and RFLP amplification and digestion. Dissolve agarose powder in 1X TAE buffer and heat until melted to make a 1.5% agarose gel for PCR products. Ethidium bromide (0.5 μg/ml) was added to the gel for DNA visualization. PCR samples were put into gel wells using loading dye and a DNA ladder for size reference. Electrophoresis was done at steady voltage until the dye front moved far enough. For RFLP products, a higher-resolution 2.5% agarose gel separated enzyme-digested DNA fragments. Similar gel preparation and electrophoresis procedures were employed for PCR products. A gel documentation method detected and recorded DNA bands on the gels after electrophoresis under UV transillumination. Comparing DNA fragment sizes to molecular weight markers allowed genotypes to be identified based on IGF1 gene restriction sites. This methodological approach detected IGF1 gene polymorphisms using PCR and RFLP analysis, revealing genetic heterogeneity in indigenous goat populations.

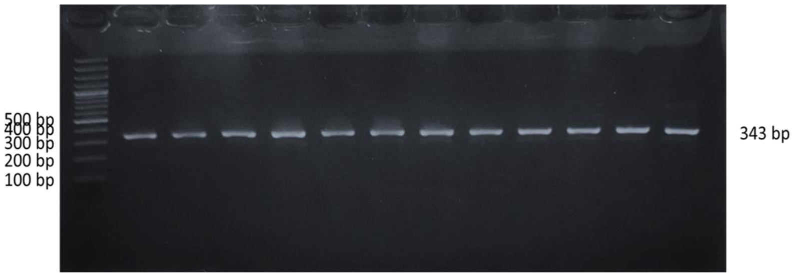

Fig. 1. PCR Amplification Results of IGF1 Gene in Samples Visualized.

3 Results and Discussion

Goat growth, development, and reproduction depend on the insulin-like growth factor 1 (IGF1) gene polymorphism. Using PCR-RFLP, this study found IGF1 gene polymorphisms in three East Java goat breeds: Peranakan Etawa (PE), Kacang, and Senduro. Allele and genotype frequencies, Hardy-Weinberg equilibrium (HWE), and heterozygosity levels reveal these populations' genetic dynamics and their implications on cattle performance and breeding methods.

Table 1. Allele and Genotype Frequencies of IGF1 Gene in Three Indigenous Goat Breed.

Breeds	Allele Frequencies		n	Genotype Frequency		
	C	G		CC	CG	GG
PE	0.67	0.33	43	0.51	0.33	0.16
Kacang	0.69	0.31	43	0.47	0.44	0.09
Senduro	0.77	0.23	43	0.63	0.28	0.09

In all three goat breeds, the C allele predominated over the G allele in the allele frequency analysis. The C gene was most common in Senduro goats (0.77), followed by Kacang (0.73) and PE goats (0.67). The C allele's predominance suggests a gene-related selection advantage. In mammals, the IGF1 gene regulates cell proliferation and differentiation, mainly in skeletal muscle and other tissues [6] [8]. In goat breeding, the C allele is associated with desirable development traits including increased body size and milk supply, which may explain its high occurrence. G allele frequency decreased, especially in Senduro and Kacang goats. The lower frequency may indicate that the G allele is neutral or associated with disadvantages in certain groups. Natural and artificial selection may have favored the C allele, increasing its prevalence. In numerous goat breeds, IGF1 gene polymorphisms were linked to advantageous phenotypes [5] [7].

Table 2. Allele and Genotype Frequencies of IGF1 Gene in Three Indigenous Goat Breeds.

Breeds	n	Allele Frequencies				Genotype Frequency					
		n	C	n	G	n	CC	n	CG	n	GG
PE	43	29	0.67	14	0.33	22	0.51	14	0.33	7	0.16
Kacang	43	30	0.69	13	0.31	20	0.47	19	0.44	4	0.09
Senduro	43	33	0.77	10	0.23	27	0.63	12	0.28	4	0.09

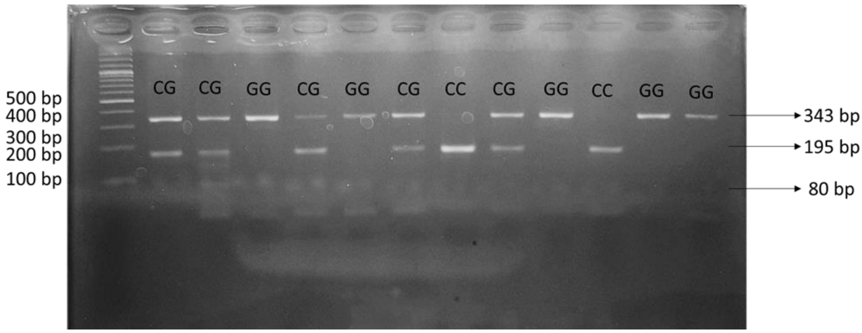

Fig. 2. PCR-RFLP Gel Electrophoresis Profile Showing IGF1 Gene Genotypes (CC, CG, and GG) in Samples.

Genotype frequencies reveal population genetics. The most prevalent genotype among PE goats was CC (51%), followed by CG (33%), and GG (16%). Kacang goats have roughly similar distributions of CC (47%) and CG (44%), with GG being the least common at 9%. A majority of Senduro goats were CC (63%), with CG (28%), and GG (9%). The predominance of the CC genotype, notably in Senduro goats, suggests a genetic advantage. The deviation from Hardy-Weinberg equilibrium in the PE goat population ($\chi^2 = 13.05$, above 3.842) suggests evolutionary effects such selection, mutation, migration, or non-random mating. The PE breed is known for dairy production, and selective breeding to boost milk yield and quality may have influenced gene frequencies (Yin et al., 2017). As evidenced in the increased predominance of the CC genotype, selective breeding of favorable individuals may boost homozygosity for certain alleles.

Table 3. The Hardy-Weinberg Equilibrium of IGF1 Gene in Three Indigenous Goat Breeds.

	The Hardy-Weinberg Equilibrium								
	Kacang			**Senduro**			**PE**		
	CC	**CG**	**GG**	**CC**	**CG**	**GG**	**CC**	**CG**	**GG**
Observation	20	19	4	27	12	4	22	14	7
Expectation	20,47	18,39	4,1	25,49	15,23	2,27	43,44	19,01	4,68
O - E	-0,47	0,61	-0,1	1,51	-3,23	1,73	-21,4	-5,01	2,32
(O -E)²	0,22	0,37	0	2,28	10,43	2,99	459,7	25,1	5,38
(O -E)²/E	0,01	0,02	0	0,089	0,684	1,317	10,58	1,32	1,15

Both the Kacang and Senduro goat populations showed Hardy-Weinberg equilibrium, with χ^2 values of 0.033 and 2.09, respectively, below the critical threshold. This equilibrium suggests random mating or balanced selection pressures, sustaining genetic stability. The Kacang goat has a higher observed heterozygosity (Ho = 0.44) than expected (He = 0.43), indicating genetic variety. Genetic variability increases a population's responsiveness to selection forces, making it more flexible and resistant to environmental changes (Agus & Widi, 2018). Heterozygosity levels reveal group genetic variability. Selective breeding for certain traits may have reduced genetic variety in PE goats, since the observed heterozygosity (Ho = 0.33) was lower than the expected heterozygosity (He = 0.44). If not treated carefully, reduced heterozygosity might lead to inbreeding depression. In Senduro goats, Ho (0.28) was lower than He (0.36), but the population remained stable, showing that reduced heterozygosity has not yet affected genetic stability. These findings are important for breeding operations. The predominance of the C allele and CC genotype in Senduro goats shows that this breed is genetically promising for growth performance and milk output selection programs. To take advantage of the IGF1 gene's role in development and lactation via PI3K/Akt and MAPK [8] [12], breeding approaches may target CC genotypes.

The balanced genotype distribution and high heterozygosity of kacang goats make them a valuable genetic resource for variation and adaptability. Crossbreeding can add beneficial traits and genetic variety to other breeds [1]. Kacang goats' genetic stability suggests minimal artificial selection, maintaining a genetic variation needed for long-term survival. The PE goat population's deviation from equilibrium and decreasing heterozygosity may require careful management to avoid genetic diversity losses. Genetic diversity and beneficial feature enhancement must be balanced in breeding tactics. Controlled mating systems and new genetic material may reduce inbreeding and diversity loss [13].

IGF1 gene polymorphisms are useful for marker-assisted selection in goat breeding. Breeders can improve herd performance by comparing genotypes to growth and reproductive phenotypes. IGF1 polymorphisms have been linked to increased body weight, kidding rates, and reproductive efficiency [8] [7]. Molecular markers speed genetic improvement and enable sustainable livestock production. Limitations must be addressed in this investigation. The 43-person sample size per breed may restrict generalizability. A larger sample size would better portray these communities' genetic diversity. Environmental variables and management methods, not studied in this study, may impact allele frequencies and phenotypes. These components should be studied together to better understand their effects on genetic dynamics.

This study identified IGF1 gene variations but did not examine their effects on behavior. Phenotypic association studies would prove the variations' functional effects. The growth rates, milk production, reproductive effectiveness, and other features of individuals with different IGF1 genotypes may be examined [14]. Identifying IGF1 gene polymorphisms improves our understanding of East Java's indigenous goat breeds' genetic dynamics. The predominance of the C allele and CC genotype, notably in Senduro goats, suggests using these genetic markers in selective breeding to optimize growth and output. Kacang goats provide genetic diversity, which is essential for flexibility and sustainability. The findings suggest animal breeding should use molecular genetics. Breeders can accelerate genetic improvement using genetic markers like IGF1 polymorphisms, increasing productivity, food security, and smallholder farmers' economic benefits. These technologies improve cattle growth and rural Indonesian livelihoods [15]. To maximize goat breeding programs' IGF1 gene polymorphism potential, more research is needed. Larger and more diverse populations are needed to confirm these findings and better understand the genetic landscape. Functional research on how IGF1 alleles affect gene expression and protein activity may help us understand their development and reproduction roles [9].

4 Conclusion

Polymorphisms of the IGF1 gene in East Javan goats demonstrate their genetic dynamics. Senduro goats exhibit a high frequency of the C allele and CC genotype, rendering them ideal for selective breeding aimed at enhancing growth and production. The genetics of Kacang goats enhance sustainability and adaptability. These findings illustrate the significance of molecular genetics in bovine production. Genetic markers such as IGF1 polymorphisms assist breeders in accelerating genetic enhancement, hence improving productivity, food security, and the incomes of smallholder farmers. The advancement of livestock contributes positively to rural communities in Indonesia. Research is required to enhance goat breeding using IGF1 gene variants. To understand the genetic landscape, bigger and more varied populations must corroborate these findings. Functional analyses of IGF1 alleles elucidate growth and reproductive mechanisms. Employing molecular genetics in goat breeding enhances productivity and genetic diversity while contributing to global sustainable livestock management initiatives, hence improving food security and rural economies. This approach might enhance the global competitiveness of goat farming.

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