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Diversity of Insulin Growth Factor-1 (Igf-1) Gene of Kacang Goat in Kota Gorontalo and Regency of Bone Bolango Province of Gorontalo

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Abstract

41 samples of DNA genome of kacang goat blood in the Kota Gorontalo (21) and Bone Bolango Regency (20) has been extracted in the Faculty of Animal Science, Biotechnology Laboratory of Integrated Hasanuddin University. Amplification and Genotyping is applied by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using the restriction enzyme *Hae*III. The analysis of IGF-1 gene from both locations shown two kinds of alleles ($A = 0.951$, $B = 0.048$) and two kinds of genotype ($AA = 0.902$, $AB = 0.975$), observed heterozygosity (H_o) = 0.097 and expected heterozygosity (H_e) = 0.093. Partially gene IGF-1 in the Kota Gorontalo has two kinds of alleles ($A = 0.952$ and $B = 0.047$) and two genotypes ($AA = 0.904$, $AB = 0.095$), H_o = 0.095 and H_e = 0.092. Instead IGF-1 gene in Bone Bolango Regency have two kinds of alleles ($A = 0.95$, $B = 0.05$) and two genotypes (AA and $AB = 0.90 = 0.10$) with H_o = 0.10 and H_e = 0.097. Based on the results, it can be concluded that IGF-1 gene in kacang goat of Kota Gorontalo and Bone Bolango regency are polymorphic so it can have opportunity for doing selection.

Keywords: Genetic Diversity, Insulin-Like Growth Factor-1, Kacang Goat

Introduction

Livestock growth (prenatal and postnatal) is the change in body size (shape and size) due to changes in organs and tissues until it reaches the size and shape characteristics of each animal. Growth and development of the body in the field of animal husbandry is very important and it can be an indicator of the success of the management of maintenance.

The rate of growth and development of livestock affected by many factors, both internal and external. Growth is internally regulated by a group of growth hormones, directly and indirectly, including Growth Hormone (GH), Growth Hormone Receptor (GHR), Insulin Like Growth Factor - I (IGF-I), and Pituitary Specific Transcription Factor - I (PIT-I). IGF-I is one of the hormones that are often used in studying candidate genes to be used as a genetic marker for selection (Sumantri et al 2009). IGF-1 is a small peptide of 70 amino acids with a molecular mass of 7649 Da (Laron, 2001). IGF-1 is a mediator of a wide range of biological effect, for example, increase the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participate in genetic activation of the cell cycle, increase lipid synthesis, stimulates the production of progesterone in granular cells, and intervention in the synthesis of DNA, proteins, RNA, and in cell proliferation (Etherton, 2004). IGF-1 gene controls the

formation of the hormone IGF-1 and are often used to detect genetic diversity in sheep and cattle, but the goats especially kacang goats still lacking.

Kacang goat is a Indonesian native goat are cultivated by small and medium farmers with the main aim to get benefit from the sale of the meat. Kacang goat is essential to preserve its existence as one of the Animal Genetic Resources (AGR). During the maintenance period, kacang goat do not require significant costs because they are able to adapt to various environments with a low quality feed and this is causing a lot of kacang goat breeders maintained by the people.

Genetic improvement towards increasing the quality and quantity of mutton kacang goat can be initiated by a selection based on the phenotype and genetic. Selection is based on the appearance of genetic information can be done using IGF-1 gene diversity based on a particular method so that it can be used as Marker Assisted Selection (MAS) later. This study aims to determine the genetic diversity of IGF-1 gene of kacang goat in Gorontalo city and Bone Bolango regency, Gorontalo province.

Methodology

Collection of blood samples obtained from the Kota Gorontalo (21) and Bone Bolango Regency (20) so that the total sample was 41 goats. Blood from the jugular vein (about 3 ml) accommodated using venojet needles and tubes containing EDTA vacuttainer subsequently collected and stored in a refrigerator temperature of 4° C prior to extraction of genomic DNA.

The procedure and the process of extracting genomic DNA, DNA amplification target, and Genotyping Fragment IGF-1|HaeIII gene by the method of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was conducted at the Laboratory of Biotechnology Integrated, Faculty of Animal Husbandry, University of Hasanuddin according to research Tunnisia (2013). Primers used for amplification of the gene IGF-1 consists of a forward primer with the DNA sequence 5'-CACAGCGTATTATCCAC-3 'and reverse primer with a DNA sequence 5'-GACACTATGAGCCAGAAG-3' (Liu, et al 2010).

Genotype and alleles frequencies were calculated by using Nei and Kumar (2000). The Hardy-Weinberg (HWE) equilibrium were tested by chi-square test (χ^2). The value of observed Heterozygosity (H_o) and Expected Heterozygosity (H_e) were based on heterozygosity formulas by Nei and Kumar (2000) and counted with PopGene 2 version 1.31 software (Yeh et al 1999).

Results and Discussion

Amplification and Genotyping IGF-1 Gene

The long of IGF-1 gene fragment were successfully amplified is 363 bp (Figure 1) according to research conducted by Liu, et al (2010) that the PCR amplification products for goats in IGF-1 gene exon 4 is 363 bp.

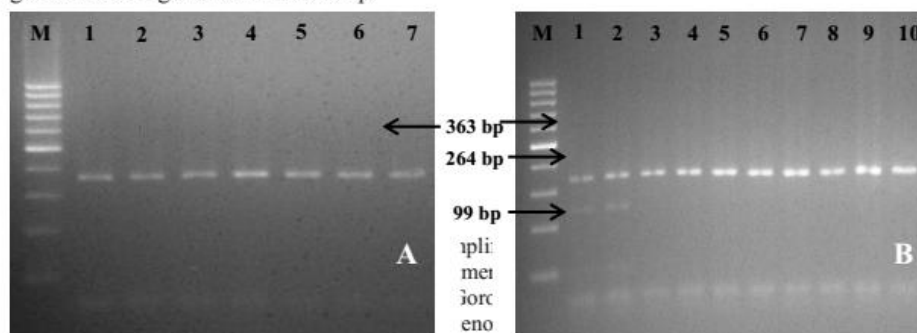


Table 1. Frequency of genotype, allele frequencies, and heterozygosity value of IGF-1|HaeIII gene on Kacang Goat in Kota Gorontalo and Bone Bolango Regency, Gorontalo Province

Area	n	Genotype	Genotype Frequency	Allele Frequency		heterozygosity		X ²
				A	B	Ho	He	
Kota gorontalo	21	AA	19 (0,904)	0,952	0,047	0,095	0,092	0,025
		AB	2 (0,095)					
Bone Bolango	20	AA	18 (0,90)	0,95	0,05	0,10	0,097	0,027
		AB	2 (0,10)					
Kota Gorontalo dan Bone Bolango	41	AA	37 (0,902)	0,951	0,048	0,097	0,093	0,079
		AB	4 (0,097)					

Description: degrees of freedom (df) = 1; $X^2_{0.05} = 3.84$ and $X^2_{0.01} = 6.64$

Results of the IGF-1 gene analysis on 41 samples of kacang goat, obtained two kinds of genotypes AA and AB while genotype B was not found (Table 1). The frequency of AA genotype (0,902) higher than genotype AB (0,097). AA genotype had one fragment size 363 bp, AB genotype 3 each fragment size 363 bp, 264 bp, and 99 bp (Figure 1). This result does not vary much with the research of Tunnisia (2013) in kacang goats in Jeneponto who obtained the AA genotype (0,914) and genotype AB (0,860), but in contrast to the study of Liu et al (2010) who obtained three kinds of genotype namely AA (0,487 and 0,277), AB (0,239 and 0,236), and BB (0,274 and 0,486) on the IGF-1 gene xinjiang goat and nanjiang cashmere goat.

Based on the value of genotype frequencies, the number of alleles found is 0.951 higher than the B allele is 0.048. Although the B allele is low, but these results have indicated their IGF-1|HaeIII polymorphic genes in Kacang Goat in Kota Gorontalo and Bone Bolango Regency. Nei (1987) said that an allele is said to be polymorphic if it has an allele frequency is equal to or less than 0.99. Nei and Kumar (2000) states that genetic diversity occurs when there are two or more alleles in a population (typically more than 1%).

Heterozygosity and Hardy-Weinberg Equilibrium

Analysis of the value of observation heterozygosity (Ho) was 0.097 and the value expectations of heterozygosity (He) was 0.093 (Table 1). These results (Ho closer to 0) indicates the diversity of the IGF-1|HaeIII gene kacang goat in Kota Gorontalo and Bone Bolango Regency quite low. Nei (1987) states that the value of heterozygosity ranged between 0-1, heterozygosity value equal to 0 means that measured between populations that have a genetic relationship is very close and if it is equal to 1 then the population have no relationship or genetic linkage at all.

The results of chi-square analysis of IGF-1|HaeIII gene in 41 samples that was obtained shows that Kacang Goat in Kota Gorontalo and Bone Bolango Regency is in equilibrium ($X^2_{count} 0.079 > X^2_{table} 3.84$) based on the law of Hardy-Weinberg as a result there is no selection, mutation, migration, and genetic drift. Hardy Weinberg law states that dominant and recessive gene frequencies in a population large enough will not change from generation to generation if no selection, migration, mutation, genetic drift (Hardjosubroto, 1998).

Conclusion

IGF-1|HaeIII Gene in Kacang Goat Kota Gorontalo and Bone Bolango Regency is polymorphic. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods in the IGF-1|HaeIII gene generates allele A (0.951) and allele B (0.048) with

AA genotype (90.2%) and AB (9.75%). Genotype frequency of IGF-1 gene in equilibrium by the law of Hardy Weinberg.

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