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Diversity Gen Growth Hormone (Gh) of Kacang Goat In Kota Gorontalo and Regency Of Bone Bolango (Province Of Gorontalo)

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ABSTRACT

Growth Hormone (GH) is a hormone produced by cells in the anterior lobe of the pituitary somatrotop and formation process under the control of GH gene. One important function of this hormone is to help the process of tissue formation and metabolism of fat to meat forming. The purpose of this study was to determine the genetic diversity of genes GH Kacang goat in sub-populations of Kota Gorontalo and Regency of Bone Bolango. Blood samples were used for DNA extraction process in Centre of Biotechnology Laboratory University of Hasanuddin is 41 samples of Kacang goats with 21 samples from Kota Gorontalo city and 20 samples from Regency of Bone Bolango. Genomic DNA was extracted using a kit DNA extraction Genjet Genomic DNA Extraction (Thermo Scientific) following standard protocol phenol-chloroform, amplified by the technique of Polymerase Chain Reaction (PCR), and genotyping was done by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) using the restriction enzyme Hae III. Data were analyzed descriptively by calculating the frequency of genotype, allele frequency, and degree of heterozygosity. The results showed GH genotype frequencies for the genotypes AA and AB were 2.45 and 97.5% respectively and the frequency of alleles A and B were 51.2 and 48.7% per cent respectively and the observed heterozygosity (Ho) and expected heterozygosity (He) were 0.97 and 0.50 respectively. Based on the sub-population genotype frequencies obtained GH gene of Kacang goat from Kota Gorontalo is 95.25 % for AA and 4.76% for AB, the frequency of allele A and B was 52.3% 47.6%, observated heterozygosity (Ho) 0.95 and expected heterozygosity (He) 0.51. GH gene genotype frequencies in Kacang goat from Regency of Bone Bolango is AB 100%, the frequency of allele A and allele B 0.5 0.5, observation heterozygosity (Ho) of 1.00 and expectation heterozygosity (He) 0.51. Based on the results concluded GH gene Kacang goat from Kota Gorontalo and Regency of Bone Bolango is polymorphic so that it can be used as the basis for the implementation of the selection.

Key Words: Genetic Diversity, Growth Hormone, Kacang Goat

INTRODUCTION

Kacang Goat as one of the local goat native to Indonesia which has advantages among others like able to adapt and survive on land with low quality forage conditions, resistance to local diseases is quite good, and the reproductive rate is high. Kacang Goat have smaller body size and body weight better than goats of Peranakan Etawah (PE) that is preferred by traditional farmers because they does not require high costs in the supply of feed during the production process. Along with population growth, the demand for meat in Indonesia is increasing as well so the goats are increasingly required not just for the main products (meat, milk, and feathers), but as one of the main requirements in various religious rituals such as animal sacrifices or the procession akiqah in Islam.

Existence of Kacang goat in some areas of Indonesia today is quite alarming and increasingly threatened by the onslaught of interbreeding with imported goat breeds. This is done with a desire

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Existence of Kacang goat in some areas of Indonesia today is quite alarming and increasingly threatened by the onslaught of interbreeding with imported goat breeds. This is done with a desire

to accelerate the increase in productivity, but not accompanied by efforts to make the preservation of breed of Kacang goat as Animal Genetic Resources locally. Based on data from the Directorate General of Animal Science and Veterinary Republic of Indonesia (2013) up to the year 2013, the population of goats in Indonesia is 18.576.192 of which, 9.864.157 (56.42%) spread across the island of Java, 4.108.439 (23.59%) on the island of Sumatra, and 3.510.127 (19.99%) are scattered on other islands in Indonesia. Specifically in the provzince of Gorontalo, total population of goats owned 76.982 is dominated of Kacang goat and small portion of breed of Etawa goat and derived from crosses both.

Kacang goat as one of the germplasm and has been designated by the government as clumps of Kacang goat through a decree of the Minister of Agriculture No. 2840 / Kpts / LB.430 / 8/2012 need to be followed through purification, development, and sustainable use in the context of preservation. Research results of Ilham (2014) in the local goats in regency of Bone Bolango expressed that goats found in phenotype is Kacang goat, breed of Etawa goat and derived from crosses both.

The diversity of phenotypes are often in different levels with the level of genetic diversity (genes) of each goat. One gene that is often used to determine the genetic diversity in goats is genes Growth Hormone (GH). These genes function to control the process of formation of GH in the cell somatotrop the anterior lobe that plays a role in tissue growth and fat metabolism (Burton *et al*, 1994), increasing the efficiency of the use of feed, increasing the growth of organs and bone in animals growing (Etherton and Bauman, 1998), and setting the development of mammary glands in ruminants (Akers, 2006). Detection of genetic diversity has better accuracy than phenotypically because is not affected by the environment. Under these conditions, this study aims to determine the genetic diversity of genes GH Kacang goat in Kota Gorontalo and Bone Bolango Regency, Gorontalo Province.

MATERIAL AND METHODS

Blood Collection

Collection of blood samples obtained from goat of Kacang had 21 numbers from Kota Gorontalo and Bone Bolango Regency have 20 number so the total samples analyzed were 41. The determination is based on the region of origin of most populated goat of Kacang in each of the districts. Blood collected using vacumtainer tubes from the jugular vein (about 3 ml) using a needle and tubing vacuttainerberisi venojet EDTA. Blood from every region were subsequently collected and stored in a refrigerator temperature of 4 ° C prior to extraction of genomic DNA. The process of extracting and GH gene diversity analysis was carried out in the Laboratory of Integrated Biotechnology, Faculty of Animal Science, University of Hasanuddin.

Extraction of DNA

Blood DNA extraction procedure was based on standard phenol-chloroform method (Sambrook *et al*., 1989). DNA was isolated and purified using a DNA extraction kit Genjet Genomic DNA Extraction (Thermo Scientific) extraction by following the protocols provided. A total of 200 ml of blood samples were lysed by adding 400 ml of lysis buffer solution and 20 ml proteinase K (10 mg / ml) was then incubated at 56°C for 60 minutes in water bath shaker. After incubation the solution was added 200 mL of 96% absolute ethanol and centrifuged at 6,000 xg for 1 minute. Purification of DNA was conducted using spin column by adding 500 µl wash solution wash buffer I, followed by centrifugation at 8000 × g for 1 minute. After the supernatants were discarded, the DNA re-washed with 500 mL wash buffer II and centrifuged at 12,000 xg for 3 minutees. Furthermore, the DNA was dissolved in 200 mL of elution buffer and disetrifugation at 8,000 x g. DNA extracted was collected and stored at -20 ° C. DNA quality testing conducted qualitatively by electrophoresis on a 1.5% agarose gel with 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na 2 EDTA) containing 100 ng / ml ethidium bromide and visualized on a UV transiluminator (gel documentation system).

Amplification of target DNA by the Polymerase Chain Reaction (PCR)

Composition of the PCR reaction was conditioned at 25 µl reaction volume consisting of 100 ng of DNA, 0:25 mM each primer, 150 uM dNTP, 2.5 mM Mg 2+, 0.5 and 1x Taq DNA polymerase buffer. Primers used for gene amplification GH consists of forward primer with the DNA sequence 5'-CTCTGCCTGCCCTGGACT-3'dan reverse primer with a DNA sequence 5'-GGAGAAGCAGAAGGCAACC-3' (Hua *et al.*, 2009). Proses amplification preceded by initial denaturation at 94 ° C for 2 min, followed by the second stage has 35 cycles, each cycle consisting of denaturation at 94 ° C for 45 seconds, annealing (annealing) on temperature 65°C for 30 seconds, and elongation (extension) of the cycle ending at a temperature of 72 ° C for 5 minutes. Process of amplification target of DNA using PCR machine (SensoQuest, Germany). Detection of the success of the amplification process is carried out by means of PCR products in electrophoresis on 1.5% agarose gel with 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na 2 EDTA) containing 100 ng / ml of ethidium bromide, and visualized on a UV transiluminator (gel documentation system).

Genotyping Gene Fragment GH

PCR products obtained from each of the target genes was analyzed by the method of Restriction Fragment Length polymorphisms (RFLP) through cuts GH gene using HaeIII restriction enzyme cutting sites which have GG | CC. A total of 4 µl DNA PCR products added 0.5 µl restriction enzyme (5U); 0.7 µl enzyme buffer; and 1 µl milique water to a volume of 7 µl further incubated for 17 h at 37 ° C. PCR-RFLP products later in electrophoresis on 2% agarose gel with 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na 2 EDTA) containing 100 ng/ml ethidium bromide. Then visualized on a UV transiluminator (gel documentation system). Allele is determined by interpreting the ribbon (band) formed the most distant migration of the anode (-) to the cathode (+) as the A allele, allele B next, and so on.

Data analysis

The data were then analyzed by calculating the value of each of the genotype frequency, allele frequency, the degree of heterozygosity, and hardy-Weinberg equilibrium by chi-square test with the formula Nei and Kumar (2000).

The formula used to calculate the frequency of genotype and allele frequencies among others:

$$X_{ii} = \frac{n_{ii}}{N} \text{ x } 100\%$$

$$X_i = \frac{(2n_{ii} + \sum_{j \neq i} n_{ij})}{2N}$$

Description:

Xii = Frequency Genotype
Xi = allele frequencies - i
Nii = number of samples ii genotype
Nij = number of samples ij genotype
n = number of samples

The formula used to calculate the value of the observation heterozygosity (Ho) and expectations of heterozygosity (He), among others:

$$H_o = \sum_{k}^{s} w_k \sum_{i \neq j}^{q} X_{kij}$$

$$H_e = 1 - \sum_{k}^{s} w_k \sum_{i}^{q} x_{ki}^2$$

Description :

Ho = heterozygosity observation among the population;
He = heterozygosity expectations among the population;
wk = the relative size of the population;
Xkij (i ≠ j) = frequency in the population to AiAj-k

RESULTS AND DISCUSSION

GH Gene Amplification

GH genes in Kacang goats were successfully amplified by PCR using a 1.5% agarose gel. Long GH which successfully amplified gene fragment in this study was 422 bp (Figure 1). This is similar to research conducted by Hua *et al* (2009) that the length of the GH gene amplification product by using the base pair primer was 422 base pairs (bp). Long fragment amplification product can be determined by matching the attachment site on the primer pair GH gene sequences (GenBank access number JN012229.1).

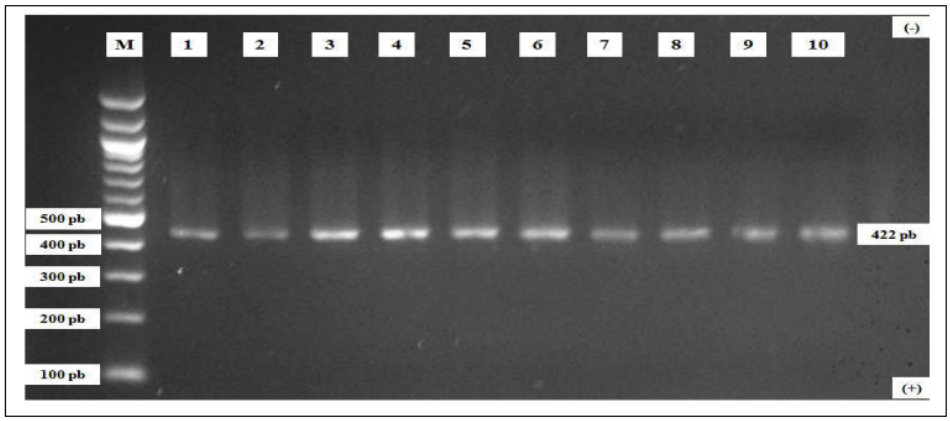

Figure 1. Growth Hormone Gene Amplification results Kacang Goat with PCR Technique

GH gene amplification is a process of propagation or multiplication component of GH gene DNA extracted using the aid of PCR. Temperature annealing in Kacang goats in this study is 65°C for 30 seconds. This condition is different from the temperature annealing in goats of Peranakan Etawah (PE), Saanen, and Peranakan Etawah-Saanen (PESA) which is 60° C for 45 seconds (Yuniarsih *et al*, 2011), in cattle Bali temperature of 60ºC (Sumantri *et al*, 2011), but similar to the results of research on Kacang goat in Jeneponto (Yuliyanty, 2013). Several factors can affect the success rate of amplification in livestock among other interaction components of the mixture of PCR (Palumbi, 1996), the temperature annealing (Yuniarsih *et al*., 2011), the purity of the extraction, the accuracy of the primaries used is the content of G / C is 50%, as well as the accuracy of PCR conditions (Rahayu *et al*., 2006).

The diversity of genes GH / HaeIII with RFLP

Genetic diversity can be calculated using several measuring devices including genotype frequency, allele frequency, and degree of heterozygosity. Genotype frequency is the proportion of individuals of each genotype, gene frequency involves the identification of alleles at each locus were analyzed and the calculation of the proportion of different types of alleles (Zein *et al*, 2012). Allele frequency is relatively from allele frequency in the population or the number of total alleles contained in the population (Nei and Kumar, 2000) The value of heterozygosity is the most appropriate way to measure the genetic diversity of a population (Nei, 1987).

The frequency of genotype and allele frequencies

Based on the results of the analysis of genotype frequency in the DNA fragment GH genes in Kacang goats of the 41 samples the results of two kinds of genotypes AA and AB was obtained while genotype B was not found (Table 1). AB genotype frequency (97.5%) was higher than the AA genotype frequency (4.76%). AB genotypes in this study is characterized by the formation of three

fragments with a length of each 422 bp, 366 bp, and 56 bp fragment while AA 2 fragment with a length of 366 bp and 56 bp (Figure 2). This result does not vary much with the results Yuliyanty (2013) in Kacang goats that received genotypes AB (0.723) high, while the AA genotype (0.276) The results of the other studies obtained frequency of Boer goat genotype AB (0.837) higher than the AA genotype (0.162) but not found BB genotype (Hua *et al.*, 2009).

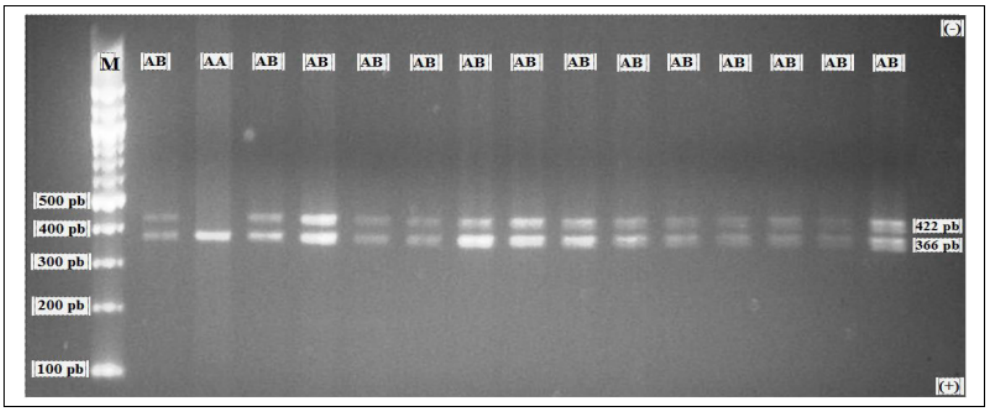

Figure 2. Visualization of PCR-RFLP gene segments GH|HaeIII goat beans in Gorontalo city and Bone Regency Bolango

Results of analysis of gene segments GH|HaeIII Kacang goat on both sub-populations indicated that the A allele frequency was 0.512 higher than the frequencies of alleles B 0.487. A allele frequencies were higher due to the discovery of genotype AA in the sub-population of the Kota Gorontalo though only one tail so that the effect on the results of the A allele frequency calculation higher than allele frequencies of alleles obtained B. is not much different from the results Yuliyanti (2013) who discovered the A allele frequency was 0.638 and the B allele was 0.36. Allele frequency values of both A and B lower than 0.95 indicates a polymorphic gene GH|HaeIII in Kota Gorontalo and Regency of Bone Bolango. Nei (1987) says that an allele is said to be polymorphic allele if it has a frequency equal to or less than 0.99. Nei and Kumar (2000) stated that the genetic diversity occurs when there are two or more alleles in a population (typically more than 1%).

The degree of heterozygosity and Hardy-Weinberg Equilibrium

Based on the analysis, the value of observation heterozygosity (Ho) was 0.97 and expectation value of heterozygosity (He) is 0.50 (Table 1). Heterozygosity value is slightly different from the results of Yuliyanti (2013) in Kacang goats in Jeneponto stating He Ho amounted to 0.533 and 0.461. Heterozygosity values influenced the amount by the number of samples, the number of alleles and allele frequencies. According to Tambasco *et al* (2003), the difference between the value of the observation heterozygosity (Ho) and the expectation value of heterozygosity (He) may be an indicator of lack of balance genotypes in the population observed. Nei (1987) stated that the value of heterozygosity ranged between 0 (zero) to 1 (one), if the value of heterozygosity equal to 0, then among the population measured have a genetic relationship is very close and if the value of heterozygosity equal to 1 then among the population as measured there genetic or genetic linkage relationships altogether. Observation heterozygosity values is higher than the expectations of heterozygosity indicates a relatively high genetic diversity in the population of Kacang goats in Kota Gorontalo and Regency of Bone Bolango District. Some factors that may affect the high level of diversity is the mating of females to males who are not pure breeds of Kacang goat.

Table 1. Frequency of genotypes, allele frequencies and heterozygosity Value Kacang Goat in Kota Gorontalo and Regency of Bone Bolango, Gorontalo Province

Region	n (goat)	Genotype	Frequency Genotype	Allele Frequency		Heterozygosity		X^2
				A	B	Ho	He	
Kota Gorontalo	21	AA	1 (0,047)	0,523	0,476	0,95	0,51	16,4
		AB	20 (0,952)					
		BB	0					
Bone Bolango	20	AA	0	0,50	0,50	1.00	0,51	19,0
		AB	20 (1,00)					
		BB	0					
Kota Gorontalo and Bone Bolango	41	AA	1 (0,024)	0,512	0,487	0,97	0,50	36,23
		AB	40 (0,975)					
		BB	0					

Description: degrees of freedom (db) = 1; X 20.05 = 3.84 and = 6.64

Balance X20,01 alleles within a population (Hardy-Weinberg equilibrium) be based on the value of chi squared (X2) are calculated based on differences in genotype frequency of observations with genotype frequencies expectations (Misrianti *et al* , 2011). Based on the analysis of gene GH|HaeIII chi squared on the results obtained in the two sub-populations of Kacang goats in Kota Gorontalo and Bone Bolango in an unbalanced state (X2 count 36.3> X2 Table 3.84) of provisions expected by Hardy-Weinberg law. This imbalance indicates that there has been a relationship that is not random in both subpopulations are caused by several factors. Hardy Weinberg equilibrium is closely related to the frequency of genotype and allele frequencies. Based on Hardy-Weinberg law that dominant and recessive gene frequencies in a population large enough will not change from generation to generation if there is no selection, migration, mutation, genetic drift (Hardjosubroto, 1998). The results of research conducted by Ilham (2014) declared a local goat in Bone Bolango phenotypically characterized qualitatively like those of Kacang and Peranakan Etawa goats beans(?genes). Introductions Peranakan Etawah (PE) goat in the past in order to improve the genetic quality of Kacang goat in Gorontalo bit much has altered the genetic composition of Kacang goat in Kota Gorontalo and Bone Bolango Regency. Artificial selection that is not directed has caused the Kacang and PE goats with a pattern that is not directed also to produce offspring goat-PE composite but without clear genetic composition percentage causing an imbalance of Hardy-Weinberg.

CONCLUSION

Based on the results of the study concluded GH gene genotype frequencies of the whole sub-populations observed was AA and AB 2.45% and 97.5%, the frequency of allele A and allele B 51.2% and 48.7%, observations heterozygosity (Ho) 0, 97 and hope heterozygosity (He) 0.50. Kacang goat population in Kota Gorontalo and Bone Bolango Regency in an unbalanced condition and are polymorphic so that it can be used as the basis for selection in order to improve the quality of genetic relationship.

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