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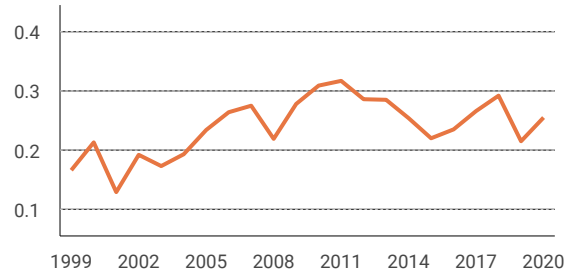

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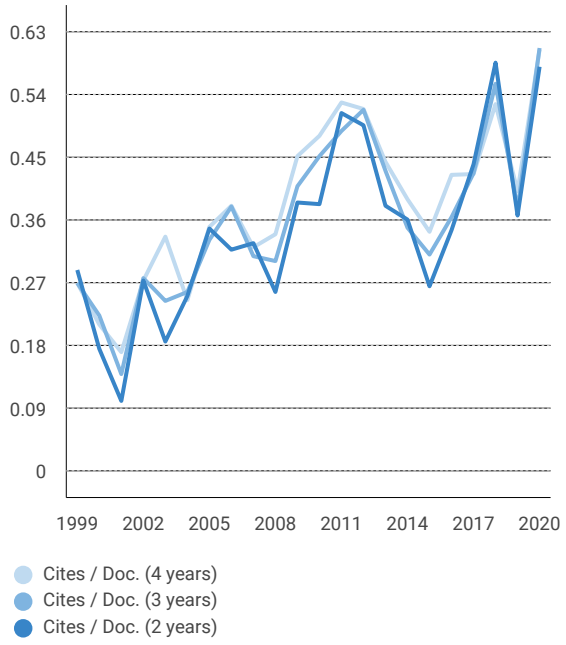

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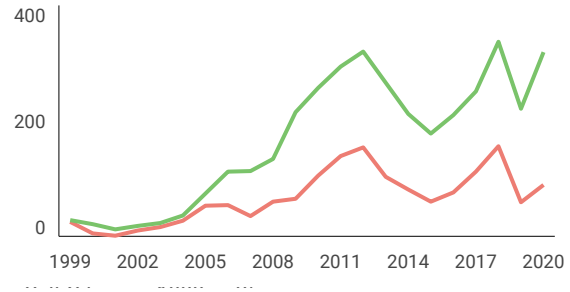

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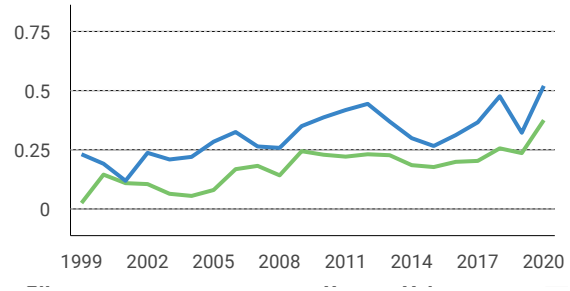

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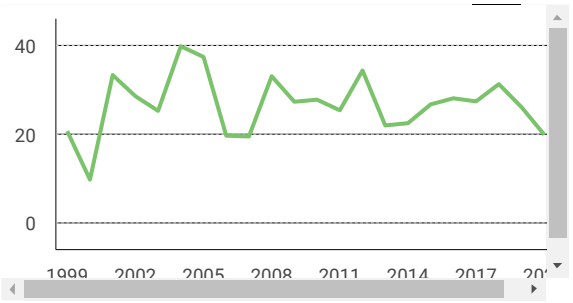

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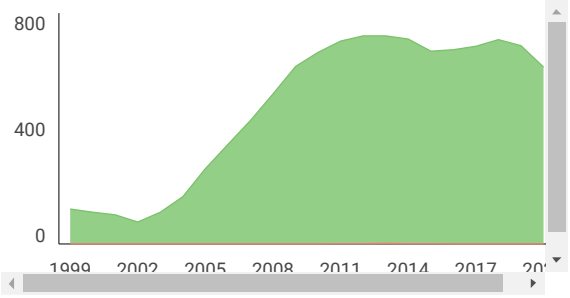

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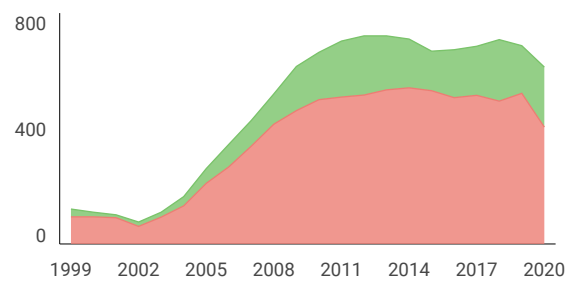

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Genetic polymorphisms of growth hormone (GH) gene in Kacang goat population based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) identification

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Abstract

Kacang goat is a small ruminant native from Indonesia and spread widely in several Southeast Asian countries, and has become part of the world's biodiversity. Growth Hormone (GH) in goat is a protein secreted from anterior hipophyse by somatrotop cells and its formation process under the control of GH gene. Its main function is to stimulate the growth of bone, muscle and fat metabolism.

The purpose of this study is to identify the GH gene polymorphism in Kacang goat with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods. A total of 168 blood samples from Kacang goat in two regions of Indonesia (Gorontalo and South Sulawesi province) were extracted by using DNA extraction kit to collect whole DNA genome. Amplification and genotyping of GH gene was performed using PCR-RFLP using the H*ae*III restriction enzyme. The results obtained two kinds of genotypes, with the AA genotype frequencies (0.095) and AB (0.904). The frequency of allele A (0.547) and B (0.452) indicate a polymorphism in the GH A781G locus in Kacang goat. The observed (Ho) and expected (He) heterozygosity value were respectively 0.0904 and 0.496. GH alleles distribution in Kacang goat populations were not in Hardy-Weinberg equilibrium.

Based on statistical analysis, there is no difference between body size of genotype AA and AB. The results of this study can be used as initial information in formulating a strategy for Kacang goat breeding by utilizing Marker Assisted Selection (MAS).

Keywords: *body size, breeding strategy, frequency of allele, heterozygosity*

Introduction

Local goats are the genetic resources that have great potential to be utilized in formulating policies for superior goat breeding that can be adapted to local conditions in Indonesia (Batubara et al 2011). As one of Indonesian native goat, Kacang goat have the advantage to adapt and survive on land with low quality forage conditions, resistance to local diseases and high reproductive rate. The size of Kacang goat is small (relative) with compact body, erect ears and small horns in both sexes and mostly maintained by farmers in traditional farming systems with the main objective for meat production (Sodiq et al 2010). The size and weight of Kacang goat smaller than Peranakan Etawah (PE). These characteristics preferred by local farmers because it does not require high cost of feed supply during the production process.

For most of Asian people, goat have a role not only as a source of animal protein (meat and milk), but as one of the main requirements in religious rituals such as animal sacrifices (qurban) or celebrates of a newborn child (aqiqah) particularly in Islamic tradition.

Growth and development is a complex process involving a wide variety of genes in the endocrine glands and regulated in coordination, that lead the metabolic processes in the body to run smoothly. One of the genes that control the growth of livestock and are often used to determine the genetic diversity both in cattle and goats are Growth Hormone (GH) gene. Growth hormone is a peptide encoded by a single gene about 2.5 kb in length and consists of five exons and four introns (Wickramaratne et al 2010). This gene function controls the process of formation of GH in somatotrof cells in the anterior lobe plays a role in tissue growth and fat metabolism (Burton et al 1994), improve feed efficiency, increase organ and bone growth in the developing animal (Etherton and Bauman 1998) setting the mammary gland development in ruminants (Akers 2006) and also associated with prolific trait and superovulation effects (Zhang et al 2011).

GH gene polymorphism especially on A781G locus has been detected by several investigators including in the Boer goat (Hua et al 2009), several domestic goat in China (Li et al 2004, Bai et al 2005, Zhang et al 2011) Indians sheep (Seevagan et al 2015), and Egypt goat and sheep (Othman et al 2015). The purpose of this research was to identify GH gene polymorphism in Kacang goat population with PCR - RFLP methods.

Material and methods

Blood sampling

A total of 168 blood samples obtained from 121 heads from Gorontalo and 47 heads from South Sulawesi province, Indonesia. Blood sample was collected from jugular vein by using vacutainer tubes containing with Ethylene Diamine Tetra Acetic (EDTA) Acid and then stored in refrigerator temperature condition set to 4°C before DNA extraction.

DNA Extraction

DNA was isolated and purified using GeneJet Genomic DNA extraction kit (Thermo Scientific). A total of 200 µl blood sample was lysed by adding 400 µl of lysis buffer solution and 20 µl proteinase K (10 mg/ml), the mixture was then incubated at 56 ^{O}C for 60 minutes in water bath shaker. After incubation, the solution was then added 200 µl of 96% absolute ethanol and centrifuged at 6.000x g for 1 minute. DNA purification was conducted using spin column by adding 500 µl wash buffer I solution followed by centrifugation at 8000x g for 1 minute. After the supernatants were discarded, the DNA was washed again with 500 µl of wash buffer II and centrifuged at 12.000x g for 3 mins. After the supernatants were discarded, the DNA was dissolved in 200 µl of elution buffer and centrifuged at 8.000x g for 1 min and for subsequent DNA extraction 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 and genotyping of DNA target (PCR-RFLP)

PCR reaction were conditioned with a reaction volume of 25 ul of 100 ng of template DNA, 0.25 mM of each primer, 150 uM dNTP, 2.5 mM Mg^{2+}, 0.5 U Taq DNA polymerase, and 1x buffer. DNA sequences of the forward and reverse primer used were F= 5'-CTCTGCCTGCC CTGCCCTGGACT-3'and R=5'-GGAGAAGCAGAAGGCAACC-3' (Hua et al 2009).

The PCR condition starting with initial denaturation temperature at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 65 °C for 30 seconds, followed by one cycle of final extension at 72 °C for 5 minutes with using a PCR machine (SensoQuest, Germany). The PCR products were then electrophoresis on 1.5% agarose gel and visualized on UV transiluminator. To identify the allele variation, PCR products obtained from the target gene was then analyzed by using RFLP with HaeIII restriction enzymes with the cutting site on the GG|CC.

Statistic analysis

Genotype and alleles frequencies were calculated by using Nei and Kumar (2000). The Hardy–Weinberg (HWE) equilibrium were tested by chi-square test (X^2) with formula:

$$X^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where χ^2 = Chi-square value; O_i = Total observed genotypes; and E_i = Total expected genotypes

The value of observed Heterozygosity (Ho) and Expected Heterozygosity (He) were based on heterozigosity formulas by Nei and Kumar (2000) and counted wih PopGene 2 version 1.31 software (Yeh et al 1999).

$$H_o = \sum_k^s w_k \cdot \sum_{i \neq j}^q x_{kij} \qquad H_e = 1 - \sum_k^s w_k \cdot \sum_i^q x^2_{ki}$$

Where H_O = observed heterozigosity between population; He = expected heterozigosity between population, W_k =

relative population size; X_{kij} (i≠j) = frequency A_iAj at to-k population.

Phenotypes between these two genotypes are distinguished by using comparative test Mann-Whitney U-test.

Results and discussion

Amplification GH gene

GH gene fragments were successfully amplified is 422 bp in length (Figure 1), these results are consistent with studies conducted by Hua et al (2009). Fragment length amplification product known by matching the primers set alignment with GH gene sequence (GenBank access number JN012229.1).

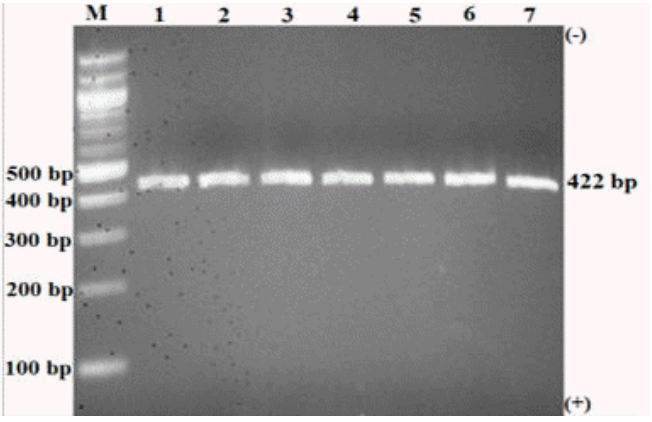

Figure 1. Visualization of Growth Hormone Gene Amplification on 1.5% agarose gel.
Lane M = 1000 bp DNA Marker, Lanes 1-7 = Kacang goat samples

GH gene structure observed in this study consisted of exon 2 (126 bp), intron 2 (227 bp) and part of exon 3 (69 bp) and these results were consistent with the studies that have been reported by Seevagan et al (2015) in Indian sheep.

Genotype and alleles frequency

Genotype frequency is the proportion of individuals of each genotype in which the description of gene frequencies involves identifying alleles at each locus were analyzed and calculated the proportion of different types of alleles (Zein et al 2012). Allele frequency is the relative frequency of an allele in a population or an allele of the total number of alleles present in a population (Nei and Kumar 2000). Based on the results of RFLP analysis in the whole sample were observed, obtained two kinds of genotypes, AA and AB while genotype BB was not found (Table 1). The AB genotype frequencies (0.904) was higher than the AA (0.095). AB genotype obtained in this study was characterized by the formation of three fragments with a length of each fragment was 422, 366, and 56 bp while AA with 2 fragments of 366 bp and 56 bp (Figure 2). The same results were also found in Boer goat that reported by Hua et al (2009), as well as in 9 breeds of Indian local sheep (Kumari et al 2014), Vembur sheep (Seevagan et al 2015), Matou goat (Zhang et al 2011), and Egyptian goat (Othman et al 2015).

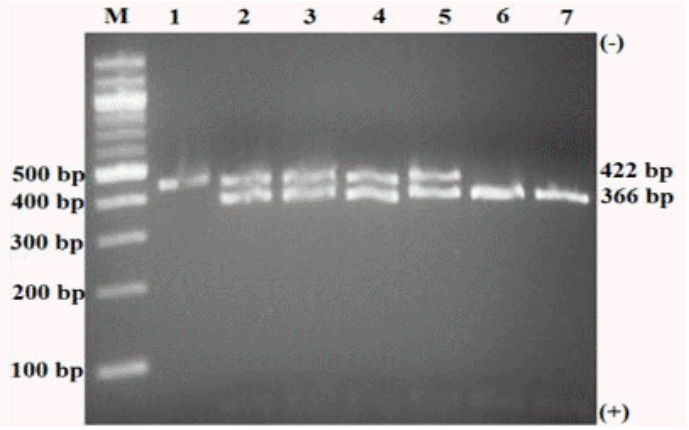

Figure 2. Visualitation PCR-RFLP of GH Gene. Lane M = DNA Marker, Lane 1 = Uncutting PCR product (422 bp), Lanes 2-5 = HaeIII cutting size 422, 366, and 56 bp (AB genotype), Lane 6-7 = HaeIII cutting size of 366 bp 56 (AA genotype)

Based on the results of this study which did not found BB genotype in Kacang goat on A781G locus probably caused by natural selection. Seevagan et al (2015) reported the absence of BB genotype on GH gene locus A781G

indicating that mutations in this locus was a recessive lethal. Lethal gene expression marked by the death of the gene carriers before birth or shortly after birth. In the heterozygous state, lethal recessive gene will not appear on the phenotype because overshadowed by the dominant allele and the new will arise in the offspring such has been mated with individuals who have the same genotype.

GH gene locus A781G known polymorphic by obtaining the two alleles (A and B alleles) with each frequency in the population was 0.547 and 0.452 (Table 1). Similar results were also reported by Hua et al (2009) who reported higher frequency of allele A in Boer goat population, as also reported in Indian Vembur sheep by (Seevagan et al 2015), and Indian local goat (Kumari et al 2014). An allele known to be polymorphic if it has frequency equal to or less than 0.99 (Nei 1987) or genetic diversity occurs when there were two or more alleles in population (Nei and Kumar, 2000).

Sirotkin et al (2003) stated that the GH gene has an important role in oogenesis, follicular development and embryogenesis during the reproductive process. Growth hormone has a role in postnatal growth and development, tissue growth, lactation, reproduction, and metabolism of proteins, fats, and carbohydrates (Akers, 2006; Ayuk and Sheppard, 2006; Thidar et al 2008). Mutations in the GH gene has considerable influence, especially during the formation of tissues and organs of the embryo in the womb.

The ddegree of heterozygosity and Hardy-Weinberg equilibrium

The degree of heterozygosity obtained in Kacang goat from these two research location for observed heterozygosity (Ho) value were 0.97, while for the expected heterozygosity (He) 0.50 (Table 1). The degree of heterozygosity represents the mean percentage of heterozygosity individuals in a population and describes level of polymorphism. High heterozygosity shows high genetic diversity within a population (Nei and Kumar 2000). Heterozygosity value ranged from 0 to 1, 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 being measured there is no genetic relationship or linkage genetic altogether (Nei, 1987). Heterozygosity value is influenced by the number of samples, number of alleles and their frequency.

Table 1. Heterozygosity Value, Genotype and Allele Frequencies of Growth Hormone (GH) Gene in Kacang Goats from two region in Sulawesi Island, Indonesia.

Location	N	Genotype	Genotype Frequencies	Allele Frequencies		Heterozygosity		X^2
				A	B	Ho	He	
		AA	3 (0.025)					
Gorontalo Province	121	AB	118 (0.975)	0.512	0.487	0.97	0.50	108*
		BB	0					
		AA	13 (0.276)					
South Sulawesi Province	47	AB	34 (0.723)	0.638	0.361	0.723	0.466	14.5*
		BB	0					
		AA	16 (0.095)					
Total	168	AB	152 (0.904)	0.547	0.452	0.904	0.496	113*
		BB	0					

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

Observed heterozygosity (Ho) value higher than expected heterozygosity (He) indicates a relatively high genetic diversity in the population of Kacang goat in Gorontalo and South Sulawesi province. One of the factors that affect the high genetic diversity in these goat were crossbreeding performed by local farmers between Kacang goat and Peranakan Etawah (PE) without a systematic breeding patterns. The result of this cross has produced offspring with the genetic composition of combination of the two breeds of goat so that the value of heterozygosity obtained quite high.

Based on the chi squared test result of GH gene in total Kacang goat population in these two location were not in Hardy-Weinberg equilibrium. The balance of alleles in a population (Hardy-Weinberg equilibrium) viewed based the value of chi squared (X^2) which is calculated based on differences in observed genotype frequency with the expected (Misrianti et al, 2011). This imbalance indicates that the non-random mating has occurred on the these goat population and can also be caused by several factors including mutations, lethal alleles, migration or selection. Hardy-Weinberg equilibrium is closely related to the frequency of genotype and allele frequencies. Hardy-Weinberg equilibrium states dominant and recessive gene frequencies in a population large enough will not change from generation to generation if no selection, migration, mutation and genetic drift (Hardjosubroto 1994). GH gene locus A781G in the Kacang goat were polymorphic and has the genetic diversity based on the value of heterozygosity were high enough to be the basis for genetic improvements to increase the Kacang goat quality as a part in the preservation of local goat that exist in various countries around the world. Local government support in the form of

regulation on local livestock breeding model especially Kacang goat, with procedures for the selection and mating of livestock is needed. Such support is important in order Kacang goat not diminishing the level of purity as a result of crossbreeding and superior seed stock can always be available at any time when needed.

The association of genotype and body size

Result from this analysis was used Mann Whitney U-Test shows there is no different of body size of adult female kacang goat that significant between genotype AA and AB (Table 2). This research have a same result with Singth et al (2005) that there is no significant association between GH genotype and chest girth and paunch girth could be established but comparatively higher chest girth and paunch girth were observed for AB genotype across the breeds. Malewa et al (2014) said GH gene of sheep (Donggala and East Java sheep breeds) to segment intron 2, complete exon 3, complete intron 3, complete exon 4, and a part of intron 4, have been found Genotype AB did not show significant differences in weaning weight compared to genotypes AA and BB both in Donggala and East Java sheep.

Table 2. The influence of Genotipe Gen Growth Hormone to Body Size of Mature Female Kacang Goat

Phenotype	Genotype (Loci A781G)		*p*
	AA	AB	
Height Shoulder (cm)	58.02±5.64	56.99±6.31	0.407
Chest Circumference (cm)	71.93±6.72	69.42±8.53	0.167
Body Length (cm)	60.62±5.4	62.46±7.4	0.108

$p < 0.05$ show there's have significant different of body size between this two genotype

The different result has found by Hua et al (2009) said goats with AB genotype weighed about 2 kg heavier than those with AA genotype at weaning, and measured about 1.4 cm greater than those with AA genotype in chest girth at birth ($P < 0.05$). The Goats Matou dan Boer breed, two loci of GH gene (A781G and A1575G) are highly associated with abundant prolificacy and superovulation response in goat breeds (Zhang et al 2011).

Conclusions

- This study has demonstrated that GH gene locus A781G in Kacang goat was polymorphic, with the frequency of allele A (0.547) higher than the B allele (0.452). Distribution of GH gene alleles in Kacang goat populations is not balanced based on Hardy-Weinberg rule.
- There is no statistical difference in body size between the adult female goat kacang genotypes AA and AB.
- The results of this study could be used as the initial information in formulating a strategy for Kacang goat breeding by utilizing of DNA marker-based selection.

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