

Original Research Paper

Genetics Polymorphisms of *INHA* in Local Goat Populations in South-West Sulawesi Region of Indonesia

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Abstract: Inhibin subunit alpha (*INHA*) is one important gene that control reproductive characteristics of animal. The aim of this research was to identify the genetic diversity of *INHA* gene of Kacang and Peranakan Etawa (PE) goat as local goat breed which were maintained by farmers at goat development centers in Sulawesi region, Indonesia. DNA samples were collected from 137 heads Kacang breed from Jeneponto, South Sulawesi and 119 heads of Peranakan Etawa (PE) from Polewali Mandar regency, West Sulawesi. The PCR-RFLP technique with *Hae*II restriction enzyme was used to identify the genetic diversity base on the *INHA* gene. A and G alleles and AA, AG and GG genotypes of *INHA* gene were could identified in the local goat populations. Frequency of G allele, as predominant allele, were found in both populations (0.9343 of Kacang and 0.9160 of Peranakan Etawa goats). Both of local goat populations were shown low genetic diversity with average observed heterozygosity (H_o) of *INHA* gene were 0.13. Nevertheless, the data obtained in this study can be used as preliminary information to determine some development and improvement strategies of genetic quality of local goats that exist in this region.

Keywords: *INHA* Gene, Prolificacy, Local Goat, Litter Size, Genetic Diversity

Introduction

In Sulawesi region, Indonesia, common goat breeds kept by rural communities are Kacang and Peranakan Etawa (PE) goats. Kacang goats are generally maintained extensively by farmers in the South Sulawesi region, while the Peranakan Etawa goats are generally maintained intensively in the area of West Sulawesi. The different types of goats are preserved due to the geographical, natural and socio-cultural differences of the farmers in the area.

Kidding rate is one of the main economic aspects that need to be considered in goat farming business (Ling *et al.*, 2014), because the main income of goat's business depends on the height of litter size (Abraham and Thomas, 2012). Goat is a small ruminant animal that is prolific. *INHA* gene (inhibin subunit alpha) is one of the genes known to be related to reproductive traits (He *et al.*, 2010). Inhibin is a glycoprotein hormone present in gonads that inhibits of FSH (follicle-stimulating hormone) synthesis and release (Bhardwaj *et al.*, 2012). Besides that, inhibin also serves as a negative feedback on control of FSH secretion by pituitary (Yang, 1988). Inhibin consist of three genes namely *INHA*, *INHβA* and

INHβB. The *INHA* gene is located on the 2q41-42 chromosome in goats and there are 3 exons in the *INHA* goat gene (Goldammer *et al.*, 1995).

Several mutations in the *INHA* gene have been reported to study the association with reproductive traits, such as premature ovarian failure (Dixit *et al.*, 2004; Marozzi *et al.*, 2002). It is believed that the *INHA* gene could be used as a candidate gene for reproductive traits. A high correlation between the *INHA* variant and litter size has been reported in Chinese local goat breed (Hou *et al.*, 2012; Wu *et al.*, 2009).

The genetic diversity of functional genes in local goats has been reported by several previous studies, such as the diversity of *IGF-1* gene (Tunnisa *et al.*, 2013), *GH* (Ilham *et al.*, 2016) and *Pit-1* (Dagong *et al.*, 2016), as well as genes associated with prolific properties such as the *BMP15* and *BMPRIIB* genes (Hidayat *et al.*, 2015; Hasan, 2011). There has been no report of the *INHA* gene related to genetic variability of the gene in local goat populations in Indonesia. Therefore, the objective of this study is to estimate the genetic diversity of *INHA* gene in Kacang and Peranakan Etawa (PE) goat populations in the South and West region of Sulawesi, Indonesia.

Materials and Methods

Blood Collection and DNA Extraction

A total of 256 heads of local goats which consists of Kacang (137 heads) originated from Jeneponto regency of South Sulawesi Province and Peranakan Etawa goats (119 heads) from Polewali Mandar regency of West Sulawesi Province was used as samples in this study. Blood samples were collected from *jugular vein* with vacutainer with EDTA tube and then stored at -20°C for further DNA analysis. DNA was isolated and extracted by using DNA isolation kit (Geneaid™) according to the protocol provided.

PCR Amplification and Genotyping of INHA Gene

INHA gene fragment identified by using PCR-RFLP method. Fragments of *INHA* gene was amplified with forward and reverse primer sequences as follow F: 5'-CCACACAGGACTGGACAGACA-3' and R: 5'-GCAGGAACAGAGAGGACAACG-3' (Agaoglu *et al.*, 2015) with a predicted 217 bp length of amplicon product.

The PCR reaction was setup at 25 µl volume consisting of 1 µl DNA template (50-100 ng), 0.25 µM of both forward and reverse primer, 150 µM dNTPs, 2.5 µM MgCl₂, 0.5 U enzyme Taq DNA polymerase and 1x buffer. The PCR condition was start with an initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 sec, primer annealing at 58°C for 45 sec, extension at 72°C for 45 sec, with a final extension at 72°C for 5 min on Labcycler SensoQuest. The PCR products were separated on 1.5% agarose gel containing 200 ng/ml of Ethidium bromide and then visualized on the UV Transilluminator.

Based on PCR-RFLP method, the PCR products were digested using *HaeII* restriction enzymes to identify the *INHA* alleles. The alleles were then determine based on the length of RFLP DNA fragment products, A allele with 217 bp, while G allele with 190 and 27 bp.

Data Analysis

INHA molecular data analysis, include allele and genotype frequencies, observed (H_o) and expected (H_e) heterozygosity, Hardy-Weinberg equilibrium of local goat population were calculated by POPGENE program (version 1.31) (Yeh *et al.*, 1999).

Results and Discussion

PCR product of *INHA* gene of local goat population at South and West region of Sulawesi has amplification length of 217 bp. The PCR product was then followed by RFLP analysis using *HaeII* restriction enzyme. Based on the variant, two alleles can be identified in the local goat populations. Namely A allele with a fragment length of 217 bp and

G allele with a fragment length of 190 and 27 bp, but 27 bp fragment is not visible in agarose gel because its size is too small to be identified (Fig. 1).

Percentages of genotype and allele frequencies of *INHA* gene in local goat populations in South and West Sulawesi were shown at Table 1. The genotype frequency of *INHA* gene in Kacang goat population in this study were 86.86% and 13.14% for GG and AG genotypes, respectively, while the AA genotype was not found in Kacang goat population due to low allele frequency of A allele. The frequency of predominant G allele is 93.43% in Kacang goat. In Peranakan Etawa population, GG, AG and AA genotypes frequencies were 84.87%, 13.45% and 1.68%, respectively (Table 1). The results of Peranakan Etawa are similar to those previously reported by Agaoglu *et al.* (2015) which identifies the genetic diversity of the *INHA* genes in the Honamli and Hair goat populations in Turkey. Agaoglu *et al.* (2015) report that GG and AG are common genotypes, whereas AA genotype and A allele are found in relatively small frequencies.

Variations of *INHA* gene are known to have association with reproductive traits in dairy cattle and litter size in sheep (Tian *et al.*, 2010; Tang *et al.*, 2010). The effect of *INHA* gene on litter size in goats has been reported by Hou *et al.* (2012) who identified polymorphism at the locus 5' promoter region of the *INHA* gene in three breed of goats (Xinong Saanen, Guanzhong and Boer). The results showed that the AA genotype has greater of litter size than the AB and BB genotypes. The same effect was also reported by Liu *et al.* (2017) using the SSCP method to identify polymorphism in 5' flanking and exon 1 of *INHA* gene in Jining Grey goats. The results showed that Bb genotype has a larger litter size than BB genotype (2.80 vs 2.01 kg). Similar results were also reported by Isa *et al.* (2017) which identified a significant association of locus g.3234 C>T with higher litter size in West African Dwarf goats. The results showed that the CT genotype was significantly higher than that of CC genotype.

Gene equilibrium in a population may change in the event of selection, mutation, migration and genetic drift (Falconer and Mackay, 1996). Chi-square test results showed that genotype and allele frequency of *INHA* gene distribution in local goat population at south and west Sulawesi were in equilibrium condition of Hardy-Weinberg ($P>0.05$) (Table 2). The Hardy-Weinberg equilibrium condition in both goat populations provides an understanding that the frequency of alleles and genotypes in both populations will constant at every generation as long as there is no selection, no mutations, no migration and mating between individuals in that population occurs randomly.

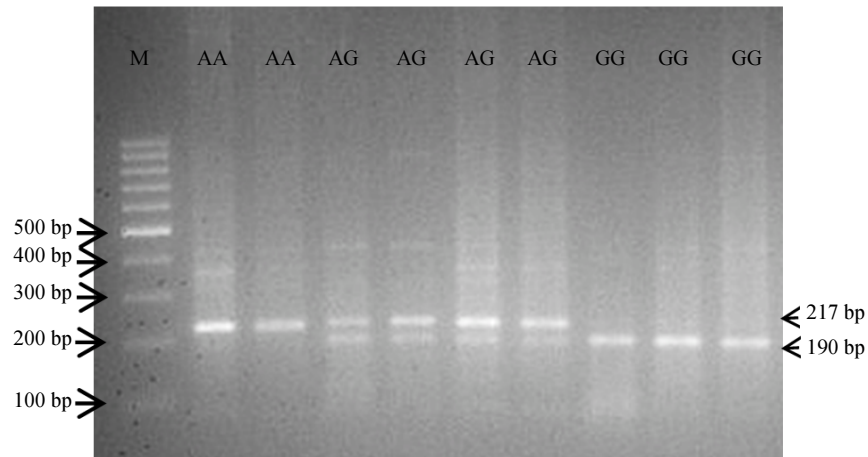


Fig. 1: The genotype of PCR-RFLP (*Hae*II) analysis of *INHA* gene. Lane M = molecular marker (100 bp DNA Ladder), Lane AA = genotype AA with 217 bp, Lane AG = genotype AG with 217, 190 and 27 bp, Lane GG = genotype GG with 190 and 27 bp. Fragments of 27 bp not appear on the gel photo

Table 1: Allele frequencies of *INHA* gene in local goat populations

Breed population	n	Genotype frequency (%)			Allele frequency (%)	
		GG	AG	AA	G	A
Kacang	137	86.86	13.14	0	93.43	6.57
PE	119	84.87	13.45	1.68	91.60	8.40
Total	256	85.94	13.28	0.78	92.58	7.42

Note: n = individual number, PE = Peranakan Etawa

Table 2: Expected and observed genotype frequencies of *INHA* gene in local goat population

Genotype	Observed Freq. (O)	Expected Freq. (E)	X ² (Chi Square)	p value (0.05;1)
GG	220	219.37	0.329 ^{ns}	0.587
AG	34	35.24		
AA	2	1.37		
Total	256	256		

ns = not significant (p value 0.05)

Table 3: Heterozygosity value of *INHA* gene in goat population

Breed population	n	Heterozygosity			
		H _o	H _e	Nei*	Average
Kacang	137	0.131	0.123	0.122	
PE	119	0.134	0.154	0.153	
Total	256	0.132	0.137	0.137	0.138

Noted: n = individual number, H_o = Observed Heterozygosity, H_e = Expected Heterozygosity according to Levene (1949) and Nei's (1973)

The genetic diversity of the *INHA* gene could be seen from the observed and expected heterozygosity (Table 3). The allele diversity index was categorized low if the observed heterozygosity value was less than 0.3 (Isa *et al.*, 2017). The heterozygosity value in two local goats is about 0.13. This result indicates that the genetic diversity of *INHA* gene in local goat at South and West Sulawesi were low (H_o<0.3). Genotype GG was observed as predominant genotype (Table 2). Genetic variation data of *INHA* gene can be used as a source of information on

genetic diversity of local goats in Sulawesi. It can be utilized in determining its genetic development and strategy improvement beside the potential utilization to increase income and welfare of rural farmers.

Conclusion

The *INHA* gene in Kacang and Peranakan Etawa populations showed the genetic diversity through identification of two alleles A and G. G allele was the

predominant allele in Kacang and Peranakan Etawa populations with frequencies of 93.43% and 91.60% respectively. AA genotype only found in Peranakan Etawa population with frequencies of 1.68%. Kacang and Peranakan Etawa population have low genetic diversity value (Heterozygosity of the *INHA* gene = 0.13). Nevertheless, the data obtained in this study can be used as preliminary information to determine some development and improvement strategies of genetic quality of local goats that exist in this region.

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Author's Contributions

Muhammad Ihsan Andi Dagong: Contributed on the original ideas of the research, data collection, analysis and interpretation and manuscript writing.

Sri Rachma Aprilita Bugiwati: Contributed on the original ideas of the research, data collection and manuscript preparation.

Lellah Rahim: Gave scientific input, data collection and manuscript preparation.

Nurul Purnomo: Data collection and analysis, laboratory and field work.

Ethics

This article is original and all of the other authors have approved and read the manuscript. The corresponding author confirm that no ethical issues involved.

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