Genetic Identification of POU1F1/PstI gene of Lakor Goat from Lakor Island, Southwest Maluku Regency

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Abstract. Lakor goat is a domestic animal from Lakor island in South West Maluku Regency. They successfully survive and reproduce with limited water, grass and high temperature. Growth traits is an economic trait which important for breeding strategy (productivity improvement). Pituitary Specific Transcription Factor 1 (POU1F1) is otherwise known as PIT1 gene plays a role of growth and carcass traits. The objective of this study was to identify the POU1F1 gene in Lakor goat from Lakor island in Southwest Maluku Regency. Sixty-three samples of hair tail were collected from three different locations: Ketti Letpey (18), Werwawan Jamluli (26) and Letoda (19). DNA was extracted by DNA isolation kit. POU1F1 gene was amplified using PCR technique while genotyping used PCR-RFLP with PstI restriction enzyme. Result showed that only one genotype (TT) was found and monomorphic. It may be caused remote area and limited of ram that indicated inbreeding has occurred. Inbreeding rate or coefficient study and introgression of ram from out of Lakor island by Artificial Insemination (AI) or natural mating is needed.

1. Introduction
Lakor island is one of the small islands which were formed from coral at Southwest Maluku regency. Local goat has been successfully survived and reproduced with limited water, grass and high temperature. They are known as Lakor goat. Government has protected their genetic resource through SK 2912/Kpts/OT.140/6/2011. Body color of Lakor goat has a combination of solid color with black and white, grey, brown, and spot black white while head color is dominant of black. Lakor goat mature has body weight reached 43 kg (ram) and 34 kg (ewe) with chest circumference of 79 cm (ram) and 63 cm (ewe) [1]. Body weight of Lakor goat was higher than Kacang goat reaching 25kg (ram) and 22kg (ewe) [2].

Pituitary specific transcription factor 1 (POU1F1) is a member of the tissue-specific POU-containing transcription factor family which otherwise named Pit1 or GHF-1. POU1F1 was located at chromosome 1q21-22 with six exons including the POU domain. The location is same as bovine and ovine [3, 4]. It is expressed in anterior pituitary lobe and binds with several genes encoding growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone (TSH) [5, 6].

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Several mutations were found in \( \text{POU1F1} \) gene i.e C>T at the 256\(^{th}\) nucleotide (C256T) of CDS in exon 3, C>T at the 53\(^{rd}\) nucleotide (C53T) and T>G at the 123\(^{rd}\) nucleotide (T123G) in intron 3, G>T at the 682\(^{nd}\) nucleotide (G682T), T>G at the 723\(^{rd}\) nucleotide (T723G) in exon 6 [4], c.876+110T>C (3’UTR_110T>C) in partial of exon 6 and 3’UTR [7]. Previous studies found that \( \text{POU1F1} \) gene effect economic traits in goat. SNP 3’UTR_110T>C effected cashmere yield in Inner Mongolia White Cashmere goats [8]. Whereas, this SNP influenced the growth trait and body weight in Chinese goats [9].

Genetic identification of Lakor goat is important. Information in molecular base could be used to early information of their status and genetic diversity. Based on that information, breeder or government can build a breeding strategy to increase of genetic quality and performance. Moreover, location of Lakor island, including Leti islands in Southwest Maluku regency is interesting to explore. So, the objective of this study was to identify of \( \text{POU1F1} \) gene in Lakor goat from Lakor island, Southwest Maluku regency.

2. Material and methods

2.1. Material
Total of samples of Lakor goat in this study was 63 samples from Lakor island in Southwest Maluku Regency which were collected from three locations i.e. Ketti Letpey (18), Werwawan Jamluli (26) and Letoda (19). Hair follicles from goat tail were collected and saved in envelops to keep them dry. DNA was extracted using DNA Isolation Kit (gSYNC\(^{TM}\) DNA Extraction Kit, Geneaid). DNA products were stored at -20\(^{\circ}\)C for future analysis.

2.2. Methods

2.2.1. Amplification of \( \text{POU1F1} \) gene. The amplification of \( \text{POU1F1} \) gene used Polymerase Chain Reaction (PCR) technique. A pair of primers, Forward: 5’-CCATCATCTCCCTTCTT-3’ and Reverse: 5’-AATGTACAATGTGCCTTCT-3’ was used to get the specific fragment target [7]. A total volume of PCR mixture is 10 μl consisting of 5 μl PCR mastermix (Bioline, Biosystems), 1 μl of each of primers (10 pmol/μl), 2 μl water free nuclease and 1 μl DNA template. This mixture was run in thermal cycler machine (Techne Plus, USA) with following program: pre-denaturation 94\(^{\circ}\)C for 5 minutes, 35 cycles of denaturation 94\(^{\circ}\)C for 45 second; annealing at 56.4\(^{\circ}\)C for 1 minute; extension at 72\(^{\circ}\)C for 1 minute and final extension at 72\(^{\circ}\)C for 5 minutes with 10\(^{\circ}\)C hold. PCR products were checked by agarose 1% (electrophoresis 100 Voltage, 1 hour). Gel was stained with Ethidium bromide and visualized under UV light (MajorScience, USA).
2.2.2. Genotyping. Genotyping of POU1F1 gene in this study used Restriction Fragment Length Polymorphism (RFLP) method. PstI restriction enzyme cut CTGCA→G sites in PCR product. Volume total of mixture was 15 μl consisting 3 μl of PCR product, 1.5 μl 10x Buffer, 0.3 μl PstI enzyme (10U/μl), and 10.2 μl water free nuclease and then digested at 37°C overnight. RFLP products were electrophoresed using 2% gel agarose and run on 100 voltage for 1 hour. Gel was stained with Ethidium bromide for 1 hour and visualized under UV light light (MajorScience, USA).

3. Results and discussion
A 450 bp specific fragment of POU1F1 gene was successfully amplified using PCR technique (Figure 1). Those DNA fragment located at partial exon 6 and its flanking 3’UTR region [7] and Figure 2 showed location of primer according to POU1F1 mRNA sequences (GenBank Accession No. MH892432.1). Mutation at c.876+110T>C (3’UTR_110T>C) was recognized by PstI (CTGCAG) endonuclease restriction enzyme. Therefore, pattern of genotypes would be produce. There were CC (370 and 80 bp), CT (450, 370 and 80 bp) and TT (450 bp) [7, 8]. The mutation Thymine (T) to Cytosine (C) did not change of amino acid Serine (Ser279Ser) and included silent mutation (Feng et al., 2012). In this study, only TT genotype was observed in all of populations which means that mutation at c.876+110T>C was not occurred. This finding was similar with several previous studies, in Shaanbei White Chasmere goats [10], Barbari goats [11], Assam Hill goats [12] and Boer [4].
allele was found highest in Barki (0.840) while Zaribi, Ardi and Masri were found in 0.351, 0.512 and 0.250, respectively [13]. Comparing with Indonesian local goat populations (Kacang goat, Ettawa Grade goat and Marica goat) two genotypes i.e TT and CT with T allele frequency of 0.71, 0.67, and 0.95, respectively was found. Genetic diversity of *POU1F1* gene in Marica goat was low [14].

TT genotype has the highest body weight (29.11 ±6.24 kg) (P<0.05) of among of genotypes (CT (26.31±7.05 kg) and CC (28.37±7.86 kg)) and the other traits i.e chest circumference, chest width, huckle-bone width, Cannon circumference and trunk index [9]. TT genotype also influenced in cashmere traits which TT genotype has average cashmere yield 558.0 ± 8.0g and CT genotype has 514.5± 28.7g [8].

This study found that *POU1F1* gene is monomorphic at region c.876+110T>C (3'UTR_110T>C) could be early genetic information. Exploration of the other regions of *POU1F1* gene is needed. Moreover, Lakor island geographically is small island with limited area and goat population. Non-random mating and inbreeding may be occurred. Based on mitochondrial study of Lakor goat, has reported low genetic variation (data not showed and published). Study of inbreeding coefficient through microsatellite is needed. Phenotype study such as qualitative (body colour), quantitative (body measurement) and reproduction traits is important to evaluate the Lakor goat’s performance.

4. Conclusions

Monomorphic allele was found in *POU1F1* genes in Lakor goat with T allele. This SNP (c.876+110T>C (3'UTR_110T>C)) did not apply in breeding program of Lakor goat. Exploration the other regions in *POU1F1* gene is needed. On the other hand, remote area and limited of rams in Lakor island may be the cause. Study of inbreeding coefficient and rotation or introgression of rams is important. Increasing the genetic diversity reduce the inbreeding within populations

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