Polymorphism of Growth Hormone (GH) Gene in Lakor Goat from Lakor Island of Southwest Maluku Regency

Rony Marsyal Kunda[1*], Slamet Diah Volkandari[2], Maman Rumanta[3], dan Pieter Kakisina[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Pattimura, Ambon, 97233, Indonesia
[2]Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI) Bogor, 16911, Indonesia
[3]Department of Biology, Faculty of Teacher Training and Education, Universitas Terbuka, Tangerang Selatan, 15418, Indonesia

ABSTRACT

Lakor goat survive in Lakor island in Southwest Maluku with high temperature and limited water. Growth trait in goat is interest to explore cause related with economic trait that encoded by growth hormone (GH) gene. The aim of this study was identify of polymorphism GH gene of Lakor goat in Lakor island. A total of 63 samples were collected from three locations (village) i.e Ketti Letpey (18), Werwawan-Yamluli (26), and Letoda (19). DNA was extracted from hair follicles. A 422 bp specific DNA fragment was successfully amplified and genotyped by PCR-RFLP method using *Hae*III enzyme. Results showed that polymorphism was found with two variant of genotypes (AA and AB) and two alleles (A and B). AB genotype was dominant in all of populations (93.7%) with A and B alleles were 0.53 and 0.47, respectively. Heterozygosity observed and expected value reached 0.502 and 0.498, respectively while Polymorphic Information Content was in moderate values (0.374). All of populations were in disequilibrium genetic. It maybe caused limited buck and nonrandom mating in population that effect of low genetic variation. Inbreeding study are needed to explore it. The introgression of bucks from other families in several locations within Lakor island can be an alternative solution to increase the genetic diversity of the lakor goat population.

Keywords: Growth hormone gene, Lakor goat, Polymorphism, Southwest Maluku Regency

Introduction

Goat is one of the livestock of the principal meat producing animals in Indonesia and has immense contribution to the resource poor section of society for their livelihood. Several goat breeds that have adapted to the environment and geography in the territory of Indonesia, they are Marica, Bengal, Etawah, Kosta, Kacang, Gembrong, Muara, Samosir (Batubara *et al.*, 2013), and Lakor goats (Volkandari *et al.* 2019; Rumanta *et al.* 2020). Budisatria *et al.* (2008), stated that goats are known as livestock that produce meat, milk, and fur, but are also used in traditional and religious ceremonies. Lakor goat is a domestic goat (*Capra hircus*) have been successfully bred and propagated in habitats with high temperature, limited rainfall and forage, but have very good adaptability. They represent a unique genetic resources by virtue of their adaptability, resistance to many infectious diseases and prolificacy in the tropics of Indonesia. Lakor goat has protected based on the Minister of Agriculture Decree No. 2912/Kpts/OT.140/6/2011 with a very limited distribution, only on Lakor Island. Salamena *et al.* (2014) reported that, Lakor goat is suspected to be a cross-breed between the Etawah goat descendant and the Kacang goat (Indonesian native goat), in which genetically Etawah is dominant. Based on morphological characters, color of Lakor goat is varies from white, black, brown, grey, crème, and combination of those five colors. Volkandari *et al.* (2019), reported that 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.

Growth hormone (GH) plays a vital role in different biological processes, such as growth, metabolism, lactation, and reproduction of livestock species (Katoh *et al.* 2008; Seevagan *et al.* 2015). Physiologically, GH is released from the anterior lobe of pituitary gland, where its main effects are associated with the stimulation of growth of bones and skeletal muscles, through the action of insulin-like growth factor (IGF-1) (An *et al.* 2011), as well as its great role in milk production (Akers, 2006). Lakor goats have been

successfully bred and propagated in habitats with high temperature, limited rainfall and forage, but have very good adaptability. They represent a unique genetic resources by virtue of their adaptability, resistance to many infectious diseases and prolificacy in the tropics of Indonesia.

Livestock exhibiting high genetic merit in growth and body measurements receive high priority inbreeding programmers for meat purpose (Susilorini *et al.* 2017). Although, lot of progress has been achieved in animal improvement using conventional breeding methods, environmental influences limits accuracy of such methods for improving polygenic inheritance like body measurements (Bin *et al.* 2009). However, the genetic improvement of such traits can be enhanced by marker assisted selection, which is highly accurate in estimating breeding value of livestock (Dekkers, 2004). In view of the pivotal role of growth hormone in livestock growth and development, GH gene may be used as a candidate gene for studying its polymorphism and association in relation to growth. Supakorn (2009), asserted that GH gene may be used as a candidate gene for studying its polymorphism and association in relation to growth.

Recently, genetic polymorphism in candidate genes has affecting role in production traits, have encouraged considerable research interest due to their ability for utilization as an aid to genetic determination and to demarcate evolutionary relationships in different livestock breeds (Sodhi *et al.*, 2007). Polymerase Chain Reaction" (PCR) and Restriction Fragment Length Polymorphism" (RFLP), assist to study the specified genes and to amplify them in vitro (Liu and Cordes, 2004). Many researchers studied an association of polymorphism of growth hormone gene with body weight and growth like. Genetic identification of the lakor goat population based on the identification of the GH gene polymorphism is very important. This Information in can be used to early information of their status and genetic diversity. The present study was undertaken to identify GH gene polymorphism of Lakor goat in Lakor island from Southwest Maluku Regency. The results of this study will serve as scientific information for the government of Southwest Maluku regency regarding the conservation of native livestock in the future.

Materials and Methods

Ethical approval

This study does not require ethical approval because using non-invasive samples (hair follicles)

Samples and DNA extraction

A total of 63 samples of Lakor goat (Figure-1) from Lakor island in Southwest Maluku Regency (Figure-2) were collected from May to June 2019 from four locations: Ketty Letpey (18 samples), Werwawan and Yamluli (26 samples), and Letoda (19 samples). Hair follicles from goat tails were collected and stored in envelopes to keep dry. DNA was extracted using DNA Extraction Kit (gSYNC ™ DNA Extraction Kit, Geneaid). DNA samples were stored at -20ºC before future analysis.

Genotyping Procedure

Genotyping of Growth Hormone (GH) gene used PCR-RFLP method. GH gene was amplified by a pair primer from Hua *et al.* (2009), Forward: 5'-CTCTGCCTGCCCTGGACT-3' and Reverse: 5'-GGAGAAGCAGAAGGCAACC-3'. Total volume of mixture was 10µl containing of 5µl PCR Mastermix (Bioline, Biosystems), 1µl (10 pmol/µl) of each primer, 2µl water free nuclease and 1µl DNA template. PCR mixture was run by thermal cycler machine (Techne Plus, United State) following program: pre-denaturation at 94ºC for 5 minutes, 35 cycles of 95ºC for 30 seconds, 65ºC for 30 seconds, 72ºC for 45 seconds and a final extension at 72ºC for 7 minutes (Hua *et al.*, 2009). PCR products were checked using 1% agarose gel (100V, 1 hour) and then stained by Ethidium

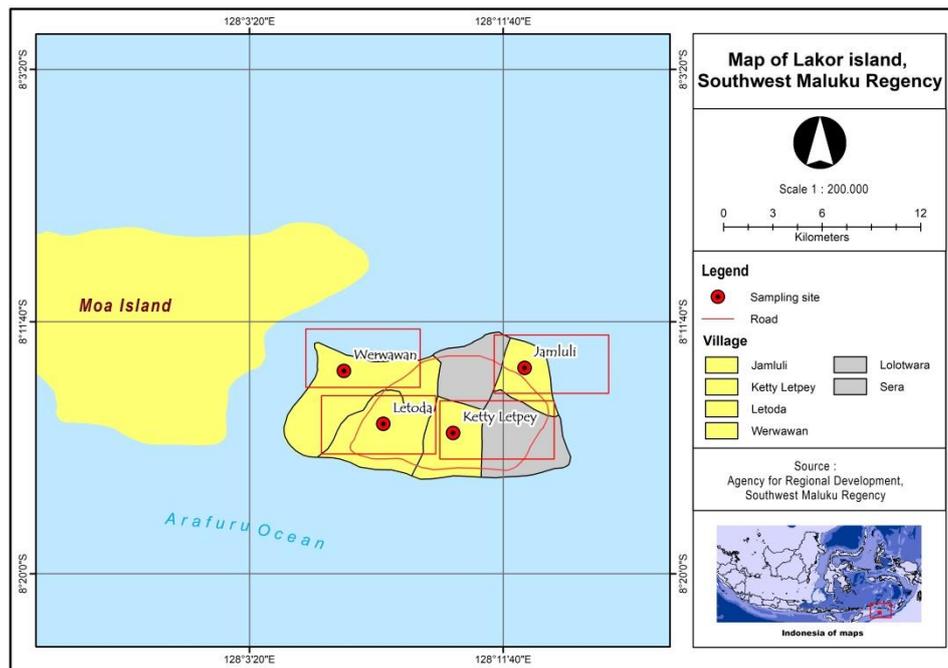Figure 1. Phenotype of Lakor goat from Soutwest Maluku Regency; (A) doe; B (buck).

Figure-2. Sampling sites in Lakor Island, Southwest Maluku Regency [Source: Agency for Regional Development, Southwest Maluku Regency, Indonesia].

Bromide. Gel were visualized under UV light (UV Transimullator, USA) and taken of picture by camera.

For genotyping, *Hae*III restriction enzyme was used. PCR products were digested overnight at 37ºC in waterbath. Fifteen microliter of RFLP mixture was prepared containing 3µl of PCR product, 1.5µl buffer tango, 0.3µl (10U/µl) of *Hae*III enzyme, and 10.2µl water free nuclease. RFLP product was electrophoresed by 2% agarose gel and run with 100 Voltage for 1 hour. Gel was visualized under UV light (UV Transimullator, USA) and taken of picture by camera. Genotype of each samples were identified using genotype patterns from Hua *et al*. (2009).

Data analysis

Genotype and allele frequencies, heterozygosity observed and expected, polymorphic information content (PIC) were analyzed by GenAlex 7.0 (Kalinowski *et al.* 2007) while Hardy Weinberg Equilibrium (HWE) was directly calculated by Nei and Kumar (2000).

Results and Discussion

A 422 bp specific target of Growth Hormone (GH) gene was successfully amplified in all of samples (Figure 2). The DNA fragment was spanned from 727 to 1148 according GenBank Accession No. D00476.1 where located at exons 2 and 3. Transition mutation at 781 Adenine to Guanine (A781G) that caused amino acid change from Serine to Glycine at the residue 35. The mutation was recognized by *Hae*III (GG^CC) restriction enzyme (Figure 3). Three genotype patterns was detected i.e AA, AB and BB with two alleles (A and B). Genotype AA was detected with 366 and 56 bp; AB genotype is 422, 366 and 56 bp while BB genotype is 422 bp (Hua *et al*. 2009). In this study, only two genotypes were found, AA and AB (Figure 4) while BB genotype was absence.

Genotype AB of Lakor goat was dominant in all of population (Ketti Letpey, Werwawan Jamluli and Letoda, 94.4%; 92.3% and 94.7%, respectively). Frequency of A allele higher than B (Table 1). The result of this study has similar with previous studies, in Chengdu-Ma goat from China (Bai *et al*. 2005), Boer goat (Hua *et al*. 2009), Savana and Kalahari goat from Malaysia (Marini *et al*. 2012), Kacang goat from Indonesia (Ilham *et al*. 2016), and Saburai goat from Indonesia (Sulastri *et al*., 2017). Conversely to the other studies, Mahrous *et al*. (2018), reported that BB genotype was detected 5-10% and absence of AA genotype of three breed goat (Barki, Damascus and Zaraibi) from Egypt. In India, Barbari and Sirohi goat were detected AB (82% and 90%, respectively) and BB (18% and 10%, respectively) genotypes (Singh *et al*. 2015). These data suggest that there has been a significant amount of inbreeding depression within populations. This is supported by the fact that buck with strong genetic quality have not been introduced to Lakor Island, so the breeding process that occurs in the population takes place naturally and has the potential to result in inbreeding depression.

Polymorphic Information Content (PIC) value in this study was 0.374 that mean was reasonably informative. Bostein *et al*. (1980), classified of PIC value became 3 groups, slightly
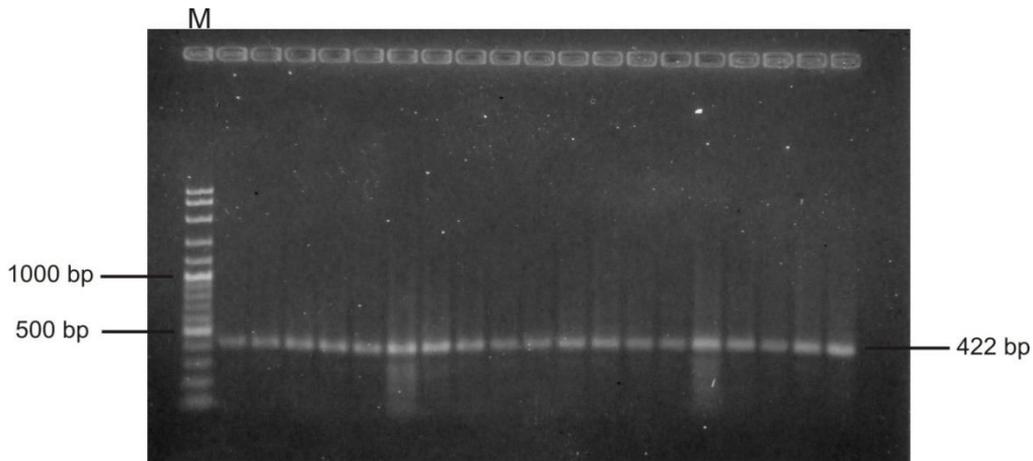
196

Figure 3. PCR product of Growth Hormone (GH) gene (422 bp) of Lakor goat. M: Ladder 100 bp.

Forward

```
 721 accctgctct gcctgccctg gactcaggtg gtgggcgcct tcccagccat gtccttgtcc
 781 ggcctgtttg ccaacgctgt gctccgggct cagcacctgc atcaactggc tgctgacacc
HaeIII (gg^cc)
 841 ttcaaagagt ttgtaagctc cccagagatg tgtcctagag gtggggaggc aggaaggggt
 901 gaatccgcac cccctccaca caatgggagg gaactgagga cctcagtggt attttatcca
 961 agtaaggatg tggtcagggg agtagaaatg ggggtgtgtg gggtggggag ggttccgaat
1021 aaggcagtga ggggaaccac acaccagctt agacccgggt gggtgtgttc tcccccagg
1081 agcgcaccta catcccggag ggacagagat actccatcca gaacacccag gttgccttct
1141 gcttctccga aaccatcccg gcccccacgg gcaagaatga ggcccagcag aaatcagtga
```
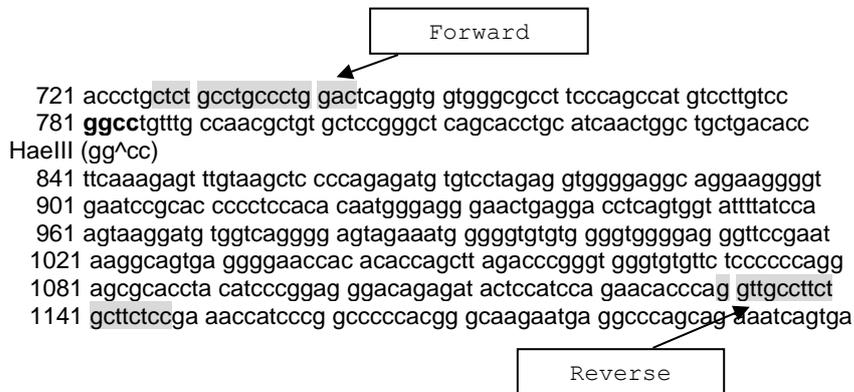
Reverse

Figure 4. Location of Primer and Restriction Site of *Hae*III (GG^CC) based on GenBank Accession No. D00476.1
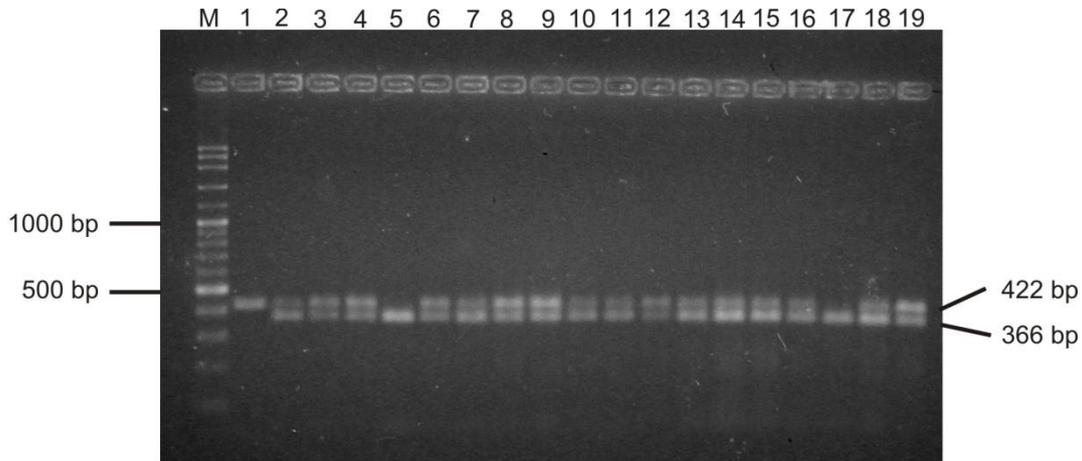

Figure 5. Representative genotyping of GH gene in Lakor goat by agarose gel electrophoresis. Strands with 366 for AA genotype, 422 and 366 for AB genotype appeared at this locus. M represented a marker with 100 bp DNA ladder. The 56 bp fragments were invisible in the gel.

(0-0.25), reasonably (0.25-0.5), and highly (>0.5) informative. Guo and Elston (1999), PIC value is often used to measure the informativeness of genetic markers. Loci with many alleles and PIC near 1 are most desirable (Bostein *et al.* 1980).

Based on analysis, all of the populations of Lakor goat were in disequilibrium genetic (HWE).

Equilibrium genetic in population can be disturbed of several factor i.e. number of forces, nonrandom mating, mutation, natural selection, genetic drift, introgression, and gene flow (Falconer and Mackay 1996). Limited of buck in Lakor island may be cause of low genetic diversity that proven with heterozygosity observed (Ho) value higher

197

Table 1. Genotype and Allele Frequencies, Heterozygosity Observed and Expected, HWE and PIC values of Lakor Goat in GH gene

| Locations | N | Genotypes frequencies | | | Allele frequencies | | Heterozygosity | | PIC | HWE (X^2) |
		AA	AB	BB	A	B	Observed (Ho)	Expected (He)		
Ketti Letpey	18	1 (5.6%)	17 (94.4%)	0 (0.0%)	0.53	0.47	0.513	0.498	0.374	14.410
Werwawan-Yamluli	26	2 (7.7%)	24 (92.3%)	0 (0.0%)	0.54	0.46	0.507	0.497	0.374	19.102
Letoda	19	1 (5.3%)	18 (94.7)	0 (0.0%)	0.53	0.47	0.512	0.499	0.374	15.390
Total	63	4 (6.3%)	59 (93.7%)	0 (0.0%)	0.54	0.46	0.502	0.498	0.374	48.853

$X^2 = 3.841$.

than expected (He) (Table 1). Nonrandom mating related with inbreeding (mating between close relatives). Inbreeding increase offsprings homozigosity and usually result in reduce fitness (Keller and Waller 2002; Hardjosubroto 2004; Charlesworth and Willis 2009). For decreasing of inbreeding rate, we suggest two alternatives: 1) identify of inbreeding rate is needed; and 2) rotation of buck from other locations or village in Lakor island.

Research by Volkandari *et al.* (2019) and Rumanta *et al.* (2020) reported that there is low genetic variation within the Lakor goat population after few generations of selection and domestication. This condition is due to improper breeding mechanisms and broodstock management. Conventional breeding practices lead to a loss of genetic where the contribution of parent is practically unknown. Moreover, this practice makes it difficult for selection of the next generation, where the relationships and genetic lineages of the resulting offspring are unclear. This will lead to inbreeding which, in turn, contributes to a loss of genetic variation within the Lakor goat population. In this study, we have found that genetic variation in populations is reduced, and it is important to protecting genetic loss so that it is not significant.

Currently, the policy being socialized related to the use of genetic information from the GH gene to farmers on Lakor island is controlling breeding mechanisms and broodstock management may be done by selecting buck from neighboring villages and not from the same village as doe. Rumanta *et al.* (2020), reported that on Lakor Island, respective villages have a permanent fence composed of rocks (local name is *Lutur*) to protect the mixing of livestock between villages. We assume that, in this way, the rate of genetic loss in the Lakor goat population can be reduced. This is very important for the traditional livestock industry on Lakor Island and to maintain the future sustainability of this endemic species. If no mitigation measures are taken, the loss of genetic variation in the population may result in severe effects in the future.

Conclusions

This research found the highly significant Chi-square values for Lakor goat breed revealed that not was in Hardy–Weinberg equilibrium for this region of GH gene. Results showed that polymorphism was found with two variant of genotypes (AA and AB) and two alleles (A and B). AB genotype was dominant in all of populations (93.7%) with A and B alleles were 0.53 and 0.47, respectively. We asserted that The Lakor goat population has a high level of inbreeding depression as a result of geographical isolation and not have been the introduction of buck from other breeds. However, as the study was based on a limited sample size, further study on larger sample is suggested.

References

Akers, R. M. 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. J. Dairy Sci. 89: 1222–1234. http://dx.doi. org/10.3168/jds.S0022-0302(06)72191-9.

An, X. P., L. X. Wang, J. X. Hou, G. Li, Y. X. Song, J. G. Wang, M. M. Yang, Y. Cui, and B. Y. Cao. 2011. Novel polymorphisms of goat growth hormone and growth hormone receptor genes and their effects on growth traits. Mol. Biol. Rep. 38: 4037–4043.

Marini, A., A. B., K. Aslinda, R. M. Hifzan, A. B. M. Faisal, and K. Musaddin. 2012. HaeIII-RFLP Polymorphism of growth hormone gene in Savanna and Kalahari goats. Malaysian. J. Anim. Sci.15: 13-19.

Batubara, A., R. R. Noor, A. Farajallah, and B. Tiesnamurti. 2013. Genetic Diversity of Y-chromosomal DNA in Six Local Indonesian

Goat Breeds. Proceedings IAARD Press, Indonesian. p316-325.

Bai, W. L., J. Wang, and R. Y. Yin. 2005. Study on genetic polymorphism of HaeIII site of GH gene in Chengdu-Ma goat and Boer goat. Heilongjiang. Anim. Sci. Vet. Med. 8: 13-14.

Bin, L. X., Y. X. Ling, S. H. Wang, T. M. Chao, W. T. Gu, and L. C. Zhong. 2009. Environmental influence on precision and accuracy estimating gene effect by molecular marker regression model. Inform. Comput. 2: 132-135.

Bostein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Budisatria, I. G. S., H. M. J. Udo, A. J. van der Zijpp, E. Baliarti, and T. W. Murti. 2008. Religious festivities and marketing of small ruminants in central Java-Indonesia. Asian. J. Agric. Dev. 5: 57-73.

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10: 783-796.

Dekkers, J. C. M. 2004. Commercial application of marker and gene assisted selection in livestock: Strategies and lessons. J. Anim. Sci. 82: E313-E328.

Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Fourth edition. Longman Group, England.

Guo, X. and R. C. Elston. 1999. Linkage information content of polymorphic genetic markers. Hum. Hered. 49: 112-118.

Hardjosubroto, W. 2004. Aplikasi Pemuliabiakan Ternak di Lapangan. Grasindo, PT Gramedia Widiasarana Indonesia, Jakarta.

Hua, G. H., S. L. Chen, J. N. Yu, K. L. Cai, C. J. Wu, Q. L. Li, C. Y. Zhang, A. X. Liang, L. Han, L. Y. Geng, Z. Shen, D. Q. Xu, and L. G. Yang. 2009. Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. Meat. Sci. 81: 391-395.

Ilham F., S. D. A. B. Rachman, M. I. A. Dagong, L. Rahim, and Yulianty. 2016. Genetic polymorphisms of growth hormone (GH) gene in Kacang goat population based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) identification. Livestock. Res. Rur. Dev. 28: 1-7.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16: 1099-1106.

Katoh, K., S. Kouno, A. Okazaki, K. Suzuki, and Y. Obara. 2008. Interaction of GH polymorphism with body weight and endocrine functions in Japanese black calves. Domest. Anim. Endocrinol. 34: 25–30.

Keller, L. F. and D. M. Waller. 2002. Inbreeding effects in wild populations. TRENDS. Ecol Evol. 17: 230-241.

Liu, Z. J. and J. F. Cordes. 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture. 238: 1-37.

Mahrous, K. F., S. H. Abdel-Aziem, M. A. M. Abdel-Hafez, M. Abdel-Mordy, and H. E. Rushdi. 2018. Polymorphism of growth hormone gene in three goat breeds in Egypt. Bulletin of the National Research Centre. 42: 35.

Nei, M. and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford University Press. New York.

Rumanta, M., R. M. Kunda, S. D. Volkandari, Indriawati, and P. Kakisina. 2020. Genetic characterization and phylogenetic study of Lakor goat from Southwest Maluku Regency based on mitochondrial COI gene. Vet. World. 13: 1209-1220.

Salamena, J. F., D. Malle, C. C. E. Latupeirissa, and I. P. Siwa. 2014. Potential development of local animal genetic resources in Maluku. Occas. Pap. 54: 17-25.

Singh, P. P., S. S. Tomar, M. S. Thakur, and A. Kumar. 2015. Polymorphism and association of growth hormone gene with growth traits in Sirohi and Barbari breeds of goat. Vet. World. 8(3):382-387.

Sodhi, M., M. Mukesh, B. Prakash, B. P. Mishra, R. C. Sobti, K. P. Singh, and S. P. S. Ahlawat. 2007. MspI allelic pattern of bovine growth hormone gene in Indian Zebu cattle (*Bosindicus*) breeds. Biochem. genet. 45: 145-153.

Sulastri, Siswanto, and K. Adhianto. 2017. Correlation of polymorphism of growth hormone to the pre-weaning Saburai goat growth. Proceedings of PERIPI. Pages 254-261.

Susilorini, T. E., Kuswati, and S. Maylinda. 2017. Polymorphism of Growth Hormone Gene in Selecting Etawah Crossbred (PE) Goats. Res. J. Life. Sci. 04: 153-158.

Supakorn, C. 2009. The important candidate genes in goats-A review. Walailak. J. Sci. Technol. 6: 17-36.

Seevagan, M., V. Jeichitra, R. Rajendran, and K. G. Tirumurugaan. 2015. Detection of lethal SNP (A781G) in growth hormone (GH) gene of Indian sheep. Small. Rumin. Res. 126: 13–15.

Volkandari, S. D., P. Kakisina, M. Rumanta, and R. M. Kunda. 2019. Genetic Identification of POU1F1/PstI Gene of Lakor Goat from Lakor Island, Southwest Maluku Regency. IOP Conference Series: Earth and Environmental Science, Moscow. p012123.