



Polymorphisms of IGF-1 Gene in Indonesian Local Goat Reared Under Smallholder Farmers in Sulawesi Region

(Polimorfisme gen IGF-1 pada populasi kambing lokal Indonesia yang dipelihara oleh peternak kecil di daerah Sulawesi)

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ABSTRACT. The objective of this study was to determine the allele frequency of IGF-1 in Kacang and Peranakan Ettawa goats in Indonesia. The DNA samples were extracted from the blood of 105 heads of goats and collected from the South Sulawesi and West Sulawesi provinces. The IGF-1 target was amplified using the PCR-RFLP method. Two allele variants (A and B) and three genotypes of the IGF-1 gene (AA, AB, and BB) have been identified in the local goat population. Allele frequencies of IGF-1 were A (0.81) and B (0.19) in the total population of local goats. Allele A is the most common allele in both the Kacang and Peranakan Ettawa goat populations, with the highest frequency found in the Kacang population (0.87). The most common genotype is genotype AA, with the highest frequency in the Kacang population (0.75). The observed and expected heterozygosity were 0.276 and 0.303, respectively. The population of local goats in these regions was in Hardy-Weinberg equilibrium. The conclusion of this research is that the IGF-1 gene in local goats in the Sulawesi region is polymorphic and this diversity information can be used for association studies with growth traits, litter size, and twinning rate.

Keywords: Ettawa crossbreed goats, genetic performance, IGF-1, Kacang goats, polymorphism

ABSTRAK. Penelitian ini bertujuan untuk mengetahui frekuensi alel IGF-1 pada kambing Kacang dan Peranakan Ettawa di Indonesia. Sampel DNA diambil dari darah 105 ekor kambing dan dikumpulkan dari Provinsi Sulawesi Selatan dan Sulawesi Barat. Target IGF-1 diamplifikasi menggunakan metode PCR-RFLP. Dua varian alel (A dan B) dan tiga genotipe gen IGF-1 (AA, AB, dan BB) telah diidentifikasi pada populasi kambing lokal. Frekuensi alel IGF-1 adalah A (0,81) dan B (0,19) pada total populasi kambing lokal. Alel A merupakan alel yang paling banyak ditemukan baik pada populasi kambing Kacang maupun kambing Peranakan Ettawa, dengan frekuensi tertinggi terdapat pada populasi Kacang (0,87). Genotipe yang paling umum adalah genotipe AA, dengan frekuensi tertinggi pada populasi Kacang (0,75). Heterozigositas yang diamati dan diharapkan adalah 0,276 dan 0,303, masing-masing. Populasi kambing lokal di wilayah ini berada dalam keseimbangan Hardy-Weinberg. Kesimpulan yang dihasilkan adalah bahwa gen IGF-1 pada kambing lokal yang dipelihara oleh peternak di wilayah Sulawesi bersifat polimorfik dan informasi keragaman ini dapat digunakan untuk studi asosiasi dengan sifat pertumbuhan, litter size dan kelahiran kembar.

Kata kunci: gen IGF-1, kambing Kacang, kambing PE, performa genetik, polimorfisme

INTRODUCTION

The characteristics of goat farming in many areas of Sulawesi in Indonesia are generally characterized by traditional systems with an uneconomic business scale and not applying good breeding practices yet. That condition causes the degradation of genetic quality, especially body performance. The diversity of body performance in goats is caused by several factors, including genetic differences. Improving the genetic quality of local goats is one of the strategies needed to increase local goat productivity. One strategy that can be taken is through the characterization of functional genes associated with productivity traits.

One of the important functional genes is the IGF-1 gene. Insulin-like growth factor 1 (IGF-1) is an important growth factor involved in various physiological processes including reproduction, fetal development, and growth (Monte *et al.*, 2019; Sankhyan *et al.*, 2020; Thomas *et al.*, 2016). IGF-1 plays an important role in mammalian fertility as a reproductive trigger when the nutritional condition is ready (Velazquez *et al.*, 2008). The IGF-1 system also plays a key role in the bone, muscle, and cartilage growth (Duclos *et al.*, 1999; Yakar *et al.*, 2002; Zapf and Froesch, 2010).

The gene encoding IGF-1 appears to be a promising candidate gene for marker-assisted selection in various economic traits in domestic livestock. The polymorphism of IGF-1 gene is known associated with growth traits in chicken (Amills *et al.*, 2003; Bennett *et al.*, 2006; Seo *et al.*, 2001; Zhou *et al.*, 2005) and in buffalo (El-Magd *et al.*, 2017), and wool traits and litter size

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in sheep (Darwish *et al.*, 2017; He *et al.*, 2012), respectively. The IGF-1 gene in goats was encoded by a single gene located on chromosome 5 (Schibler *et al.*, 1998), consisting of three leader exons (1w, 1, and 1a) and three other exons (3, 4, and 6), where exon 3 and exon 4 encode the maturity of IGF-1 peptide (Mikawa *et al.*, 1995). The objective of this study is to investigate the polymorphism of the IGF-1 gene in local goats (Kacang and Peranakan Ettawa) reared by smallholder farmers in the South and West Sulawesi regions in Indonesia.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Blood samples were collected randomly from a total of 105 heads of local goats (56 heads of Kacang goats collected in South Sulawesi Province and 49 heads of Peranakan Ettawa goats in West Sulawesi Province). Samples were taken from the jugular vein using a tube including EDTA and stored at -20°C before DNA extraction. DNA was extracted and isolated with DNA isolation kit (GeneJET genomic DNA purification kit, Thermo Scientific®).

PCR Amplification

A 25 μL aliquot mixture was made for PCR reaction that contained ~ 100 ng of genomic DNA, 1 mM MgCl_2 , 200 μM of the dNTPs mix (Fermentas), and 25 pmol of each primer, 1x buffer, and 0.5 U Taq DNA polymerase (Dreamtaq, Fermentas). The PCR condition was performed with an initial denaturation process at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. The final step of an extension was at 72°C for 5 min. To amplify the exon 4 region of the IGF-1 gene, the nucleotide sequence for primers was used based on Wu-Jun *et al.* (2010) ie. (Forward: 5'-CACAGCGTATTATCCCAC-3') and (Reverse: 5'-GACACT ATGAGCCAGAAG-3'). All PCR processes were performed in a thermal cycler machine (SensoQuest, Germany). The PCR products were electrophoresed on 1.5% agarose gels in 1x TBE buffer (tris borate EDTA) containing 10% of Ethidium Bromide (EtBr) at 100 volts for 45 min and visualized under UV-transilluminator.

IGF-1 Genotyping with PCR-RFLP Method

The PCR products were digested with the restriction endonucleases: *BsuRI* (*HaeIII*)

(Thermo Scientific) that recognized the GG|CC restriction site. Restriction products were electrophoresed on 2% agarose gel stained with EtBr and visualized under UV-transilluminator. Genotypes were determined based on the length of DNA fragments, AA (363 bp), AB (363 bp, 264 bp, and 99 bp), and BB (264 bp and 99 bp).

Data Analysis

The genotype and allele frequencies were calculated based on Nei and Kumar, (2000) formulation. The test of Hardy-Weinberg equilibrium (HWE) was carried out with a chi-square test (Kaps and Lamberson, 2017). Observed (H_o) and Expected heterozygosity (H_e) based on Nei's heterozygosities (1973) and computed using PopGene32 software version 1.31 (Yeh *et al.*, 1999).

RESULTS AND DISCUSSION

Insulin like growth factor 1 (IGF-1) gene was successfully amplified using PCR machine at annealing temperature 60°C . The IGF-1 gene amplification yielded a length of 363 bp PCR product. After digestion with the *HaeIII* restriction enzyme, fragments of 363, 264, and 99 bp were obtained by electrophoresis (Figure 1). This cut resulted in the identification of two alleles, namely allele A (363 bp) and allele B (264 and 99 bp).

The results of this study indicate that the IGF-1 gene was polymorphic in both Kacang and Peranakan Ettawa goat populations. The genotype and allele frequency of the IGF-1 gene are presented in Table 1. In this study, three genotypes were found in Kacang and Peranakan Ettawa goat populations, ie. AA (363 bp), AB (363 bp, 264 bp, and 99 bp), and BB (264 bp and 99 bp). This result differs from the study reported by Alakilli *et al.* (2012) on Zaribi goat that only found two genotypes (AA and BB). Allele A was the most common allele in both the Kacang and Peranakan Ettawa goat populations. The Kacang goat population has the highest frequency of the allele A (0.87), and the most common genotype is genotype AA with the highest frequency in the Kacang goat population (0.75). The same results as those reported by Wu-Jun *et al.* (2010) which showed the highest AA genotype frequencies were also found in Nanjiang Cashmere goat populations, but a different result was reported in Xinjiang goat, where the B allele and genotype BB were more common.

The diversity of the IGF-1 gene was also reported by Qiong *et al.* (2011). They reported the

variation of alleles and new mutations found in exon 4 regions in the IGF-1 gene in three local goat breeds in China. This variation is also reported to be related to differences in cashmere production and body weight in goats. Other studies reported that the IGF-1 gene variant has an association with twinning rate, growth traits, and yearling fleece weight in the Markhoz goat (Kurdistani *et al.*, 2013; Rasouli *et al.*, 2017),

prolificacy trait (Thomas *et al.*, 2016), growth traits in the Nanjiang Huang goat (Zhang *et al.*, 2008), milk yield and body size in Chinese dairy goat (Deng *et al.*, 2010), and reproductive performances and milk yield in Sarda dairy sheep (Luridiana *et al.*, 2020). The IGF-1 genetic variation was also reported to have an association with the Cashmere fiber trait in Changthangi goats (Shanaz *et al.*, 2020).

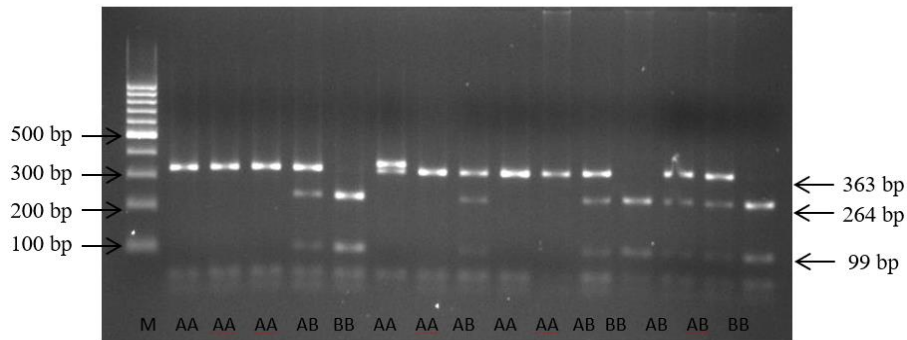


Figure 1. The genotype of PCR-RFLP *Bsu*RI (*Hae*III) analysis of the IGF-1 gene. Line M= Marker 100 bp, Line AA= genotype AA with 363 bp, Line AB= genotype AB with 363, 264, and 99 bp, and Line BB= genotype BB with 264 and 99 bp fragment in length.

Table 1. Genotype and allele frequency of IGF-1 gene in local goat population

Breed Population	n	Genotype Frequency			Allele Frequency	
		AA	AB	BB	A	B
Kacang	56	0.75	0.23	0.02	0.87	0.13
Peranakan Ettawa	49	0.60	0.32	0.08	0.75	0.25
Total	105	0.67	0.28	0.05	0.81	0.19

Note: n= individual number

The results of the chi-square (X^2) test showed the distribution of three genotypes in the local goat population it was in Hardy-Weinberg Equilibrium (Table 2). This show that the local goat population in this study was enormous, there was no mutation, selection, genetic drift or migration (Noor, 2008). Genotype BB was the rare genotype that was only found in the allele

frequency of 0.02 to 0.08 in the Kacang and Peranakan Ettawa goat populations, respectively. According to Nei and Kumar, (2000), genetic diversity can be measured using heterozygosity value. All values of heterozygosity (expected heterozygosity, Nei's expected heterozygosity, and average heterozygosity) of the IGF-1 gene in local goats were moderate (Table 3).

Table 2. Observed and expected genotype frequency of IGF-1 gene in local goat

Genotype	Observed Freq. (O)	Expected Freq. (E)	X^2 (Chi Square)	p value (0.05;1)
AA	71	69.55	0.89 ^{ns}	3.841
AB	29	31.90		
BB	5	3.55		
Total	105	105		

Note; ns= not significant (p value 0.05)

Table 3. Observed and expected heterozygosity value of IGF-1 gene in local goat.

Breed Population	n	Heterozygosity			
		H_o	H_e	Nei*	Average
Kacang	56	0.232	0.234	0.232	
Peranakan Ettawa	49	0.326	0.373	0.369	
Total	105	0.276	0.303	0.302	0.302

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

The results of the IGF-1 gene characterization show that genetic diversity in the local goat population is quite high. This suggests that genetic variation in the IGF-1 gene could be utilized in local goat selection and breeding programs to improve the genetic quality of the goat that is maintained by smallholder communities in the Sulawesi region. Selection programs related to the genetic diversity of the IGF-1 gene can be directed at selecting productive traits such as litter size, growth and twinning rate.

CONCLUSIONS

IGF-1 gene condition of Indonesian local goats which is reared under smallholders in the Sulawesi region showed polymorphism. Allele A was the most common allele in both the Kacang and Peranakan Ettawa Goat populations. The highest frequency was found in the Kacang goat population (0.87), whereas the most common genotype was the AA genotype, with the highest frequency in the Kacang goat population (0.75). The level of heterozygosity was found to be moderate. Variation in the IGF-1 gene could be used as a future genetic marker in local goat selection for better genetic performance and used for the next study to find any association between IGF-1 polymorphism with litter size or twinning rate and growth traits in Indonesian local goats.

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REFERENCES

- Alakilli, S.Y.M., Mahrous, K.F., Salem, L.M. and Ahmed, E.S. (2012), "Genetic polymorphism of five genes associated with growth traits in goat", *African Journal of Biotechnology*, Academic Journals (Kenya), Vol. 11 No. 82, pp. 14738–14748.
- Amills, M., Jiménez, N., Villalba, D., Tor, M., Molina, E., Cubiló, D., Marcos, C., *et al.* (2003), "Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits", *Poultry Science*, doi: 10.1093/ps/82.10.1485.
- Bennett, A.K., Hester, P.Y. and Spurlock, D.E.M. (2006), "Polymorphisms in vitamin D receptor, osteopontin, insulin-like growth factor 1 and insulin, and their associations with bone, egg and growth traits in a layer - Broiler cross in chickens", *Animal Genetics*, doi: 10.1111/j.1365-2052.2006.01439.x.
- Darwish, H.R., El-Shorbagy, H.M., Abou-Eisha, A.M., El-Din, A.E. and Farag, I.M. (2017), "New polymorphism in the 5' flanking region of IGF-1 gene and its association with wool traits in Egyptian Barki sheep", *Journal of Genetic Engineering and Biotechnology*, doi: 10.1016/j.jgeb.2017.08.001.
- Deng, C., Ma, R., Yue, X., Lan, X., Chen, H. and Lei, C. (2010), "Association of IGF-I gene polymorphisms with milk yield and body size in Chinese dairy goats", *Genetics and Molecular Biology*, doi: 10.1590/S1415-47572010005000034.
- Duclos, M.J., Beccavin, C. and Simon, J. (1999), "Genetic models for the study of insulin-like growth factors (IGF) and muscle development in birds compared to mammals", *Domestic Animal Endocrinology*, doi: 10.1016/S0739-7240(99)00040-5.
- El-Magd, M.A., Saleh, A.A., Nafeaa, A.A., El-Komy, S.M. and Afifi, M.A. (2017), "Polymorphisms of the IGF1 gene and their association with growth traits, serum concentration and expression rate of IGF1 and IGF1R in buffalo", *Journal of Zhejiang University: Science B*, doi: 10.1631/jzus.B1600573.
- He, J.N., Zhang, B.Y., Chu, M.X., Wang, P.Q., Feng, T., Cao, G.L., Di, R., *et al.* (2012), "Polymorphism of insulin-like growth factor 1 gene and its association with litter size in Small Tail Han sheep", *Molecular Biology Reports*, doi: 10.1007/s11033-012-1846-y.
- Kaps, M. and Lamberson, W.R. (2017), *Biostatistics for Animal Science*, Cabi.
- Kurdistani, Z.K., Rostamzadeh, J., Rashidi, A. and Davis, M.E. (2013), "Evaluation of insulin-like growth factor-I gene polymorphism on

- growth traits and yearling fleece weight in goats”, *Small Ruminant Research*, Elsevier B.V., Vol. 111 No. 1–3, pp. 10–15, doi: 10.1016/j.smallrumres.2012.08.003.
- Levene, H. (1949), “On a matching problem arising in genetics”, *The Annals of Mathematical Statistics*, Institute of Mathematical Statistics, Vol. 20 No. 1, pp. 91–94.
- Luridiana, S., Mura, M.C., Di Stefano, M.V., Pulinas, L., Cosso, G., Nehme, M. and Carcangiu, V. (2020), “Polymorphism of insulin-like growth factor 1 gene and its relationship with reproductive performances and milk yield in Sarda dairy sheep”, *Veterinary and Animal Science*, Elsevier B.V., Vol. 9, doi: 10.1016/j.vas.2019.100084.
- Mikawa, S., Yoshikawa, G. ichi, Aoki, H., Sakai, H., Komano, T. and Yamano, Y. (1995), “Dynamic Aspects in the Expression of the Goat Insulin-Like Growth Factor-I (IGF-I) Gene: Diversity in Transcription and Post-Transcription”, *Bioscience, Biotechnology, and Biochemistry*, doi: 10.1271/bbb.59.87.
- Monte, A.P.O., Barros, V.R.P., Santos, J.M., Menezes, V.G., Cavalcante, A.Y.P., Gouveia, B.B., Bezerra, M.E.S., *et al.* (2019), “Immunohistochemical localization of insulin-like growth factor-1 (IGF-1) in the sheep ovary and the synergistic effect of IGF-1 and FSH on follicular development in vitro and LH receptor immunostaining”, *Theriogenology*, Elsevier Inc., Vol. 129, pp. 61–69, doi: 10.1016/j.theriogenology.2019.02.005.
- Nei, M. (1973), “Analysis of gene diversity in subdivided populations”, *Proceedings of the National Academy of Sciences*, National Acad Sciences, Vol. 70 No. 12, pp. 3321–3323.
- Nei, M. and Kumar, S. (2000), *Molecular Evolution and Phylogenetics*, Oxford university press.
- Noor, R.R. (2008), “Genetika ternak”, *Penebar Swadaya, Jakarta*.
- Qiong, W., Chao, F., Wu-Jun, L., Yi, F. and Shi-Gang, Y. (2011), “A Novel Mutation at Exon 4 of IGF-1 Gene in Three Indigenous Goat Breeds in China”, *Asian Journal of Animal and Veterinary Advances*, doi: 10.3923/ajava.2011.627.635.
- Rasouli, S., Abdolmohammadi, A., Zebarjadi, A. and Mostafaei, A. (2017), “Evaluation of Polymorphism in IGF-I and IGFB-3 Genes and their Relationship with Twinning Rate and Growth Traits in Markhoz Goats”, *Annals of Animal Science*, De Gruyter Open, Vol. 17 No. 1, pp. 89–103.
- Sankhyan, V., Thakur, Y.P. and Dogra, P.K. (2020), “Genetic polymorphism in IGF-1 gene in four sheep and goat breeds and its association with biometrical traits in migratory Gaddi goat breed of western Himalayan state of Himachal Pradesh, India”, *Indian Journal of Animal Research*, Agricultural Research Communication Centre, Vol. 54 No. 4, pp. 508–512, doi: 10.18805/ijar.B-3795.
- Schibler, L., Vaiman, D., Oustry, A., Giraud-Delville, C. and Cribru, E.P. (1998), “Comparative gene mapping: A fine-scale survey of chromosome rearrangements between ruminants and humans”, *Genome Research*, doi: 10.1101/gr.8.9.901.
- Seo, D.S., Yun, J.S., Kang, W.J., Jeon, G.J., Hong, K.C. and Ko, Y. (2001), “Association of Insulin-Like Growth Factor-I (IGF-I) Gene Polymorphism with Serum IGF-I Concentration and Body Weight in Korean Native Ogol Chicken”, *Asian-Australasian Journal of Animal Sciences*, doi: 10.5713/ajas.2001.915.
- Shanaz, S., Shamas Ganai, T.A., Hussain, I., Ahmad, S.F., Sheikh, F.D., Shabir, N., Nabi, N., *et al.* (2020), “IGF-1 Gene Polymorphism and its Association with Cashmere Fiber Trait in Changthangi Goats”, *Journal of Natural Fibers*, Bellwether Publishing, Ltd., Vol. 17 No. 12, pp. 1819–1826, doi: 10.1080/15440478.2019.1604283.
- Thomas, N., Venkatachalapathy, T., Aravindakshan, T. and Raghavan, K.C. (2016), “Molecular cloning, SNP detection and association analysis of 5’ flanking region of the goat IGF1 gene with prolificacy”, *Animal Reproduction Science*, Elsevier B.V., Vol. 167, pp. 8–15, doi: 10.1016/j.anireprosci.2016.01.016.
- Velazquez, M.A., Spicer, L.J. and Wathes, D.C. (2008), “The role of endocrine insulin-like

- growth factor-I (IGF-I) in female bovine reproduction”, *Domestic Animal Endocrinology*, doi: 10.1016/j.domaniend.2008.07.002.
- Wu-Jun, L., Guang-Xin, F., Yi, F., Ke-Chuan, T., Xi-Xia, H., Xin-Kui, Y., Mou, W., *et al.* (2010), “The polymorphism of a mutation of IGF-1 gene on two goat breeds in China”, *Journal of Animal and Veterinary Advances*, doi: 10.3923/javaa.2010.790.794.
- Yakar, S., Rosen, C.J., Beamer, W.G., Ackert-Bicknell, C.L., Wu, Y., Liu, J.L., Ooi, G.T., *et al.* (2002), “Circulating levels of IGF-1 directly regulate bone growth and density”, *Journal of Clinical Investigation*, doi: 10.1172/JCI0215463.
- Yeh, F.C., Yang, C. and Boyle, T. (1999), “POPGENE version 1.31: Microsoft Window-based Freeware for Population Genetic Analysis”, University of Alberta Canada. Edmonton, AB.
- Zapf, J. and Froesch, E.R. (2010), “Insulin-like growth factor I actions on somatic growth”, *Comprehensive Physiology*, Wiley Online Library, pp. 663–699.
- Zhang, C., Zhang, W., Luo, H., Yue, W., Gao, M. and Jia, Z. (2008), “A new single nucleotide polymorphism in the IGF-I gene and its association with growth traits in the Nanjiang Huang goat”, *Asian-Australasian Journal of Animal Sciences*, doi: 10.5713/ajas.2008.70673.
- Zhou, H., Mitchell, A.D., McMurtry, J.P., Ashwell, C.M. and Lamont, S.J. (2005), “Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens”, *Poultry Science*, doi: 10.1093/ps/84.2.212.