ISSN: 0852-3681
E-ISSN: 2443-0765

Jurnal Ilmu-Ilmu Peternakan 33(3): 361-371
Available online at http://jiip.ub.ac.id

Identification of Genetic Diversity of *P21-Activated Kinases* (PAK1) Gene in Senduro Goats and Boerawa Goats using PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism)

Heri Damayanti[1)], Tri Eko Susilorini[1)], Guruh Prasetyo[1)], Nur Hidayah[1)], Ahmad Zarkasi Efendi[1)], Ari Ardiantoro[1)], Wike Andre Septian[1)] and Suyadi*[2)]

[1)] Departement of Animal Production, Faculty of Animal Sciences, Brawijaya University, Malang, 65145, Indonesia

[2)] Departement of Animal Reproduction and Breeding, Faculty of Animal Sciences, Brawijaya University, Malang, 65145, Indonesia

Submitted: 22 June 2023, Accepted: 15 December 2023

ABSTRACT: This study aimed to detect genetic diversity associated with *litter size* traits of the P21-activated kinases (PAK1) gene in Senduro and Boerawa goats using the PCR-RFLP method. The restriction enzyme used is MSP1 (C*CGG). A pair of primers used in this study, namely Forward ((5'-GCTCAGTGTTGTATTAGCAG-3') and Reverse (5'-CAAGATATAAAGCACAGCCG-3') product length 713 bp (access code ENSCHIG00000003071). The samples used in this study consisted of 80 samples of Senduro goats and 20 samples of Boerawa goats from UPT PT-HMT Singosari, Malang Regency. The results showed that the PAK1 exon 9 gene in Senduro goats and Boerawa goats UPT PT-HMT Singosari was uniform (monomorphic), it was because all samples had GG genotype and G allele frequency of 100%. In conclusion, the PAK1 exon 9 / MSP1 gene is monomorphic and does not associate with litter size in Senduro goats and Boerawa goats, so it cannot be used as a genetic marker.

Keywords: Senduro goat; Boerawa goat; PAK1; PCR-RFLP; Genetic diversity

*Corresponding Author: suyadi@ub.ac.id

INTRODUCTION

Senduro breed goats are dairy-type goats resulting from a cross between Etawah breed Jamnapari goats and Menggolo goats (local Lumajang goats). The Litter size of Senduro goats is 1.50±0.59 heads, with a birth weight of 3.20±0.44 kg (Yulianto, 2019). Senduro goat milk production is 1.3 ± 0.5 liters per day (Susilorini and Kuswati, 2019) and the highest milk production is achieved at 4th parity at the age of 5.5-7 years (Filian et al., 2016).

Boerawa goat is a crossed between male Boer and Etawah Crossbreed goat. The average litter size of Boerawa goat is 1.21 (Kostaman and Sutama (2005). According to Nasution et.al (2014), the average birth weight of Boerawa goats is 3.23±0.70 kg while the body weight of an adult Boerawa goat weighs 19.40 kg and 20.15 kg. Meanwhile, the birth weight of Etawah Crossbreed goat is 2.57±0.72 kg and Boer goats are 2.71±0.70 kg.

The genetic quality of Senduro goats and Boerawa goats can be improved using molecular selection. This is because DNA characterizers can detect superior traits of livestock in a relatively faster time and on an accurate laboratory scale Huda, et al (2015). Litter Size can be used as a parameter to determine the productivity of livestock and it is controlled by several genes. According to Etherton and Bauman (1998), Growth Hormone (GH) is one of the most important genes that affect growth, this protein hormone is centesis and secreted by the pituitary gland (Ilham, et al., 2016). Furthermore, Amiri et al (2018) stated that the Growth Hormone gene plays a role in regulating tissue growth, reproduction, lipid metabolism, lactation, and normal body growth in mammals. Another gene that affects litter size is PAK1 gene.

The PAK1 gene is a protein-coding gene. PAKs (P21-activated kinases) are threonine /serine kinases that are effector proteins for Rho GTPases Cdc42 and Rac downstream which plays an important role integrin and receptor-type kinases PAK1 activity is regulated by various downstream signaling molecules dependent on phosphoinositides (PDK1) (King, et al, 2000), Protein kinase A (PKA) (Howe et al, 2000), phosphoinositides-3-kinases (Tsakiridis et al, 1996), and AKT through phosphorylation and interacting proteins (Tang et al (2000). The results of Wang, et al (2014) stated that the candidate genes that played the highest selective role for litter size 2 were KIT, KCNH7, and KMT2E, while for litter size 3 were PAK1, PRKAA1, and SMAD9.

This study aims to identify the genetic diversity of the PAK1 gene exon 9 using the PCR-RFLP method in Senduro goats and Boerawa goats and to examine the association of the genetic diversity of PAK1 genes with litter size in Senduro goats and Boerawa goats.

MATERIALS AND METHODS

Time and place of research

The study was conducted from August 2022 to March 2023. 80 blood samples of Senduro goats and 20 blood samples of Boerawa goats from UPT PT-HMT Singosari, Malang Regency were collected. DNA amplification was carried out at the Biotechnology Laboratory, Faculty of Animal Science, Brawijaya University, Malang.

Materials and Tools

The research materials include 70% alcohol, Ice gel and cotton, DNA Genomics Mini Kit (Geneaid Lot No.FF15503), agarose (1stBase Lot1A1019FH9934), 70% alcohol, Diamond Nucleic Acid Dye (Promega Lot 0000358902), ethanol absolute, TBE buffer, Green Master Mix (Promega Lot 0000385484), blue dye, Nuclease Free Water (Promega Lot 0000385484) and a pair of PAK1 gene primers (P21-activated kinases) (access code ENSCHIG00000003071) with Forward (5'-GCTCAGTGTTGTATTAGCAG-3') and Reverse (5'CAAGATATAAAGCACAGCCG-3') along 713 bp.

The following research tools were used: Ethylene Diamine Tetraacetic Acid (EDTA) anti-coagulant vacuum tube, tube holder, cool box, clear insulation, label paper and waterproof pen, 1.5 ml tube, beaker glass (IWAKI), micropipette (Select BioProducts), GD Column, tip (Axygen scientific 301-03-051), centrifuge (HETTICH Mikro 185), tube rack, PCR machine (BioRad T100 Thermal Cycler), microcentrifuges (Forcemini SBC-140-3), microwave (Panasonic NN-SM32HM), electrophoresis device (Bio-Rad Mupid-Exu), Gel Doc (Gite 965GW).

Research methods

Research methods are survey methods (Purposive sampling) and laboratory research that uses experimental without treatment including DNA isolation, DNA amplification, genotyping of PCR-RFLP results of the PAK1 gene, and PCR-Sequencing of the BMP15 gene.

Blood sampling

Blood samples of Senduro goats are taken in the jugular vein.

DNA isolation

DNA isolation using the procedure from the Genomic DNA Mini Kit for Blood.

Table 1. Primer Information and Annealing Temperature

Primer Name	Primer sequence (5' →3')	Annealing temperature (°C)	Product Size (bp)
PAK1	F (5'-GCTCAGTGTTGTATTAGCAG-3')	57°C	713 bp
	R (5'-CAAGATATAAAGCACAGCCG-3')		

DNA amplification

PCR-RFLP PAK1 gene has a premix composition of 15 µl consisting of sample 1 µl, primer forward 0.25µl, primer reverse 0.25µl, go tag 7µl, and DW 6.5 µl. Amplification for the PAK1 gene stage I initial denaturation at 95 ° C for 5 minutes, stage II denaturation at 95 ° C for 15 seconds, annealing at 57 ° C for 30 seconds, and elongation at 72 ° C for 45 seconds carried out for 35 cycles, stage III is post-elongation at 72 ° C for 5 minutes.

PCR Product Visualization of PAK1 gene Exon 9

Genotyping of the PAK1 gene was carried out using the RFLP (Restriction Fragment Length Polymerization) method. The length of the band that appears can determine the genotype of the PAK1 gene, referring to the sequence of the Capra hircus gene with the access code ENSCHIG00000003071. The AA genotype was determined from 1 DNA band (713 bp). The AG genotype was determined from 3 DNA bands with lengths of 194 bp, 520 bp, and 713 bp. The GG genotype was determined from 2 bands i.e. 194 bp, 520 bp).

Data analysis

Allele frequency and genotype frequency of the PAK1 gene

Allele frequency and genotype frequency of the PAK1 gene according to Nei and Kumar (2000) as follows:

Allele Frequency Formula:

$$X_i = \frac{2n_{ii} + \sum n_{ij}}{2N}$$

Information:

X_i = I-th allele frequency

n_{ii} = Number of individuals with genotype I

n_{ij} = Number of individuals with the IJ genotype

N = number of individual samples

Genotive Frequency Formula:

$$X_{ii} = \frac{n_{ii}}{N}$$

Information:
X_{ii} = I-th genotypic frequency
n_{ii} = Number of individuals with genotype I
N = number of individual samples

Heterozygosity Value
The value of observed heterozygosity (Ho) can be calculated by the following formula:

$$Ho = \sum_{i \neq j} \frac{n_{ij}}{N}$$

Information:
H_o = frequency of heterozygosity of observations
n_{ij} = number of heterozygosity individuals at the 1st locus
N = number of individuals analyzed

The value of expectation heterozygosity (He) can be calculated by the following formula:

$$He = 1 - \sum_{i \neq j}^{n} {x_i}^2$$

Information:
He = heterozygosity and expectation among populations
X_i^2 = Allele frequency
n = number of alleles

Polymorphic Informative Content (PIC) Value
The information level of an allele is calculated by the Polymorphic Informative Content (PIC) value approach:

$$PIC = 1 - \sum_{i=1}^{n} pi^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2pi^2pj^2$$

Information:
pi = Allele frequency to i
pj = allele frequency to j
n = Number of alleles per marker
Hardy-Weinberg balance

$$Hardy - weinberg\ equilibrium\ tested\ with\ X2\ test\ (Hart\ and\ Clark, 1997)$$

$$x^2 = \sum \frac{(obs - \exp)^2}{exp}$$

Information:
X2 = chi-squared test
Obs = number of genitive observations to i
Exp = Number of Expectations of the Genotype to I

Association of genetic diversity of BMP15 gene with litter size

The association of the genetic diversity of BMP15 genes with litter size was performed by T-test analysis.

Mathematical models used:

$$t_{hit} = \frac{M_1 - M_2}{\sqrt{\frac{ss_1 + ss_2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Information:

M_1 = average of group 1
M_2 = average of group 2
SS_1 = Sum of Square Group 1
SS_2 = Sum of Square Group 2
N_1 = Number of Subject/Sample Group 1
N2 = Number of Subject/Sample Group 2

RESULTS AND DISCUSSION

PAK1 gene amplification

The success of DNA amplification depends on the interaction between PCR components at appropriate concentrations. PCR optimization depends on the primary attachment temperature (annealing), DNA concentration, and Mg2+ concentration. The annealing temperature that is often used is 5°C below Tm / melting temperature (Muladno, 2000).

The annealing temperature in this study was 57°C for 30 seconds. Each gene has a different annealing temperature to produce targeted DNA bands/fragments. Based on the formula Tm = 4 (G+C)+2(A+T), Tm = 4 (9)+2(11), Tm = 36+22=58, so Tm in this study is 58°C for primary Tm and reverse Tm. The annealing temperature in this study was 57°C which means 1°C below Tm.

Identification of genetic diversity of PAK1 genes

Identification of genetic diversity of PAK1 genes through analysis of Restriction Fragment Length Polymorphism (RFLP), the process of cutting the PAK1 gene using restriction enzyme MSP1. Gene diversity related to economic traits such as milk production and quality is usually identified using RFLP (Sumantri, et al., 2007), due to DNA multiplication through PCR quickly and being able to identify genotypes using restriction enzymes (Jakaria, et al. 2007). The restriction enzyme MSP1 recognizes the cutting site (C*CGG) located in exon 9 of the PAK1 gene. Restriction enzymes will recognize a particular sequence, break down the DNA it recognizes, and produce many DNA fragments that are useful in identifying gene diversity both between individuals and between populations.

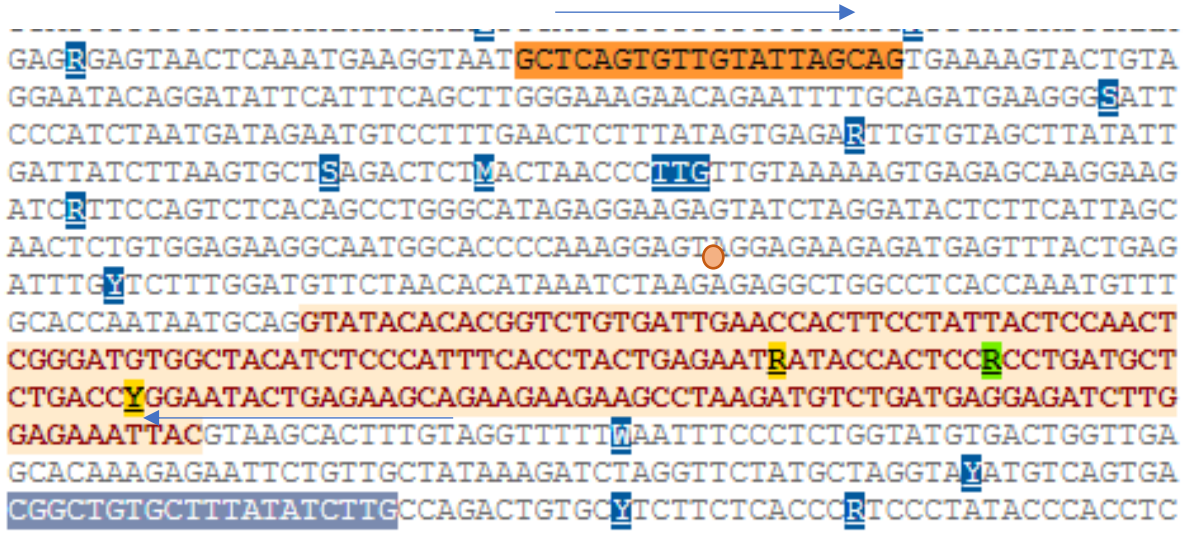
The restriction enzyme cutting PCR products in Senduro goats and Boerawa goats had 2 fragments namely 194 bp and 520 bp for all samples of this study. From the results of the cutting, one type of genotype was produced, namely the GG genotype. PAK1 gene diversity was not found in Senduro goats or Boerawa goats in this study. This is because the PAK1 gene in Senduro goats and Boerawa goats is in a uniform state (monomorphic).

PAK1 Gene Allele Frequency

According to Nei and Kumar (2000), allele frequency is the relative frequency of an allele in a population or the number of alleles to the total number of alleles present in a population. The results of the analysis on Senduro goats and Boerawa goats showed that the GG genotype frequency was 100%.

This is because the G allele in the PAK1 gene of Senduro goats and Boerwa goats has a G allele frequency with a value of 1. The A allele is not found in the population of Senduro goats and Boerawa goats, therefore the PAK1 gene is uniform

(monomorphic) in both Senduro and Boerawa goats. The uniform PAK1 gene in the goat G allele of this study indicates that there has been a selection process for the G allele. Reproductive management of Senduro goats and Boerawa goats of UPT PT-HMT Singosari was carried out by natural mating, the male: female ratio was 1:7. There are 4 Senduro males prepared to marry Senduro females, while for Boerawa there are 2 males. This controlled mating is most likely what causes the PAK1 gene in Boerawa and Senduro goats to be monomorphic. Selection is a process that uses force in determining which livestock will breed in the next generation (Noor, 2010). This is in line with Putri, et al (2021) who stated that through the selection process and marriage arrangements, the chances of the emergence of the desired genotype can be arranged and also reduce the chance of the emergence of unwanted genotypes



Information:

: Primer Forward

: Primer Reverse

Y : MSP1 enzyme cutting site (C*CGG)

Figure 1. The position of the primary attachment to the gene sequence PAK1 exon 9 (GenBank access number ENSCHIG00000003071). Source: Ensemble

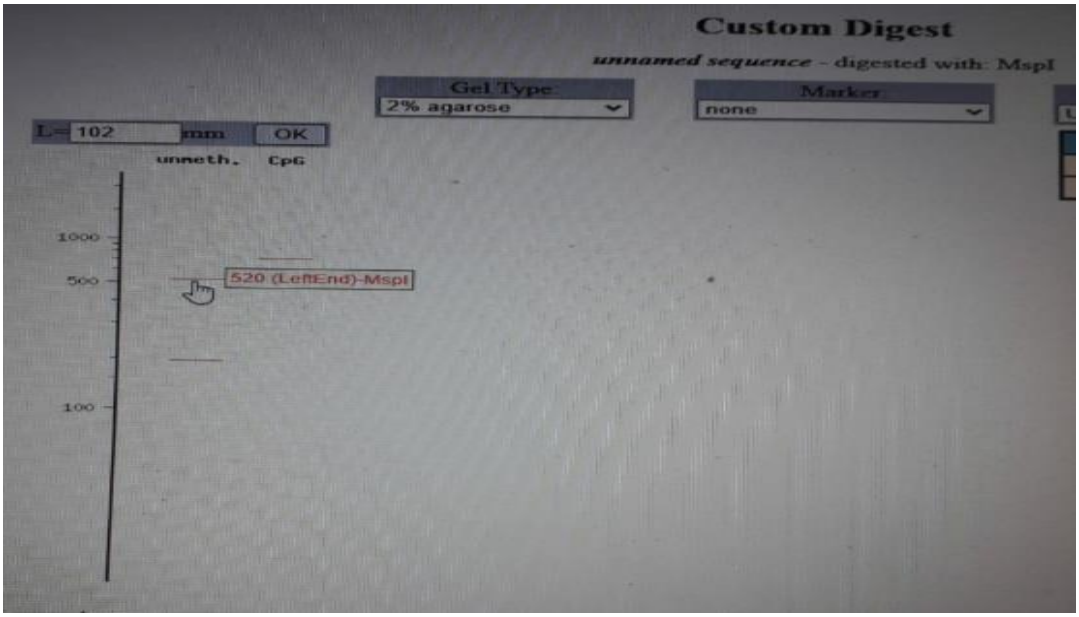


Figure 2. The cutoff point of the PAK1 gene exon 9 sequence with the enzyme MSP1 (there are 2 cutoff points, namely: 194 bp and 520 bp with a product point of 713 bp. Source: Nebcutter

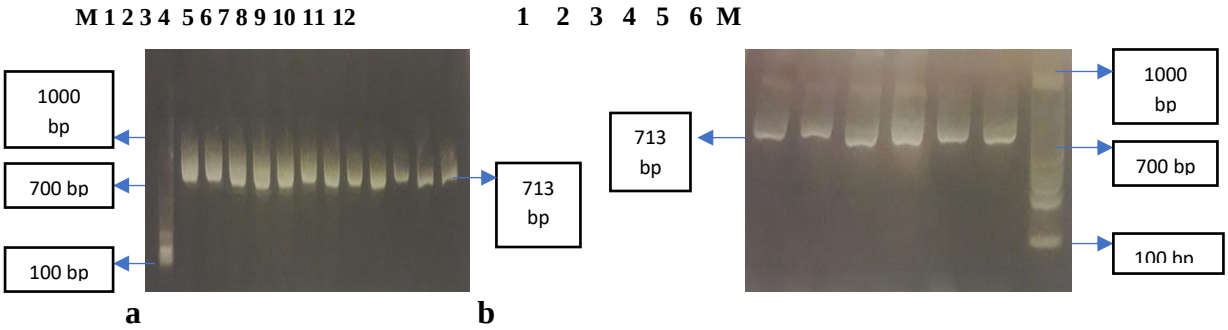


Figure 3. Amplification of PAK1 gene in Senduro goat samples (1-12) (a) and Boerawa goat samples (1-6) (b) in agarose gel 1.5%, M (marker)

Tabel 2. Allele frequency of the PAK1|MSP1 gene in Senduro goats and Boerawa goats

Livestock	Number of Samples	Allele Frequency		Source
		A	G	
Senduro goats	80	0	1	Research
Boerawa goats	20	0	1	Research

Hardy-Weinberg Degree of Balance, Heterozygosity, and Polymorphic Informative Content (PIC)

From Table 2 above, it can be seen that the number of Senduro goats that have been successfully amplified is 80 females, and 20 females in Boerawa goats. The results of statistical analysis showed that the genotype frequency of the PAK1|MSP1 gene in Senduro goats and Boerawa goats was in Hardy-Weinberg equilibrium (HWE). From Table 3 can be seen the results of chi-square (X2), observation heterozygosity value (Ho), expectation heterozygosity value (He), and Polymorphic Informative Content (PIC) value shows that the genotype frequency of the PAK1 gene fragment exon 9 whose value is constant is 0. The genetic diversity of the PAK1 gene is monomorphic with only one genotype found, namely GG

in Senduro goats and Boerawa goats. The population for Senduro goats and Boerawa goats in UPT PT-HMT Singosari is in Hardy-Weinberg equilibrium. Intensive livestock rearing and controlled mating patterns may be the cause of the population of Senduro goats and Boerawa goats in UPT-PT HMT Singosari Malang Regency in a balanced state according to Hardy-Weinberg balance. Senduro females mate naturally with only 4 males Senduro and Induk Boerawa (PE mate naturally only with 2 Boer cross males) in UPT PT-HMT Singosari.

The frequency of genotypes and alleles in HWE will always be constant from generation to generation unless there are selection events, mutations, genetic drift (Noor, 2010), non-random mating, limited population size, and gene flow (Vogel and Motulsky, 1997). The provisions of Hardy-Weinberg's law are that in a population, allele frequency and genotype frequency will remain constant (in equilibrium) from generation to generation, The conditions of the validity of Hardy-Weinberg's law are that the number of individuals or chromosomes of a population is always large (Prime, 2015) and eliminates the selection and mutation process in genetic algorithms (Panggabean, 2016), then the population is in equilibrium (Allendorf et al, 2013).

The way to determine the base length of each sample is by comparing the electrophoresis result of PCR-RFLP samples with markers. RFLP results of the PAK1 gene in Senduro goat and Boerawa goat were cut with MSP1 restriction enzyme at point 194 bp and 521 bp with a DNA product length of 713 bp (Figure 4 and Figure 5).

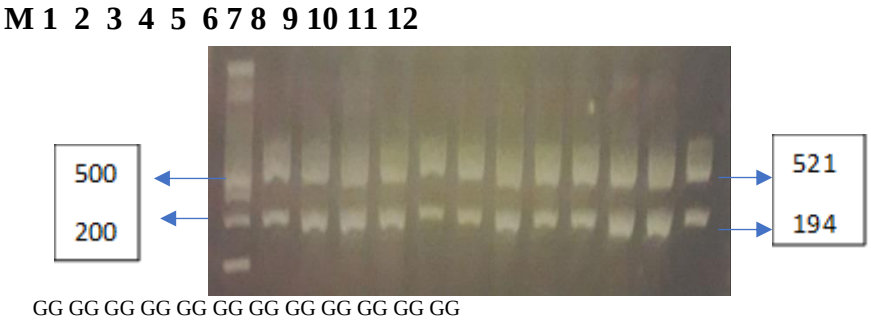


Figure 4. RFLP results of the PAK1 gene in Senduro goats were cut with MSP1 restriction enzyme at points 194 bp and 521 bp with DNA product length of 713 bp (M: marker; Senduro goat samples: 1-12).

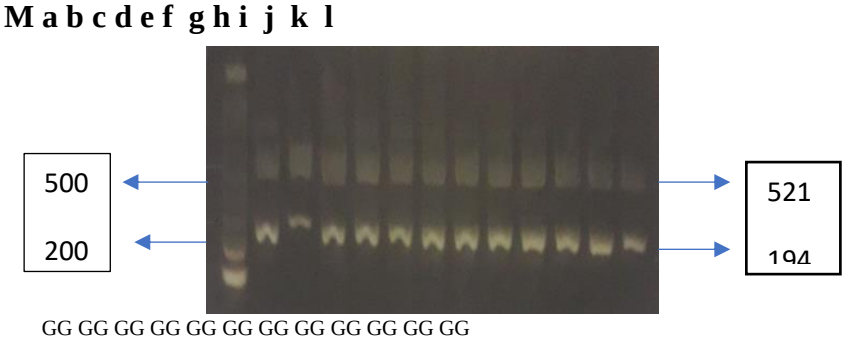


Figure 5. RFLP results in Boerawa goats were cut with MSP1 repression enzyme at 194 bp and 521 bp with a DNA product length of 713 bp (M: marker; Boerawa goat samples: a, b, c, d, e, f, g, h, I, j, k, l).

Association of PAK1 gene genotype to *litter size*

The association of the PAK1 gene with litter size in Senduro goats and Boerawa goats in UPT PT-HMT Singosari was analyzed by T-test using SPSS Window IBM software version 26.0 (IBM Corporation). The lowest litter size of Senduro goats in this study was 1.00 at parity 1, while the highest litter size was 2.13 at parity 8. The highest litter size of Boerawa goats was 1.50 at parity 4 and the lowest litter size was 1.00 at parity 3, parity 5, and parity 6. The average litter size of Senduro goats was 80 samples of 1.54 ± 0.22, while the average litter size of Boerawa goats was 20 samples of 1.26 ± 0.16 (Table 4).

The results of the T-test (independent samples test) showed that the genetic diversity of the PAK1 gene was uniform (monomorphic), and did not have a significant effect on the litter size of Boerawa goats and Senduro goats (P>0.05). Although statistically insignificant, the GG genotype in Senduro goats has a higher average litter size than the GG genotype of Boerawa goats.

Table 4. Average Litter Size of Senduro goats and Boerawa goats by Genotype

PAK1 gene	Number of goats	Average Litter Size
Senduro		
GG	80	1,54±0,22
Boerawa		
GG	20	1,26±0,16

According to Nei (1975) and Allendorf and Luikart (2007), a locus is said to be polymorphic if the frequency of the allele that generally appears (most common allele) is less than 0.99 (99%) while monomorphic if only one allele is known at a locus or the presence of the most common allele that has a high frequency (> 99%). The results of the calculation of the allele frequency of the PAK1 exon 9 gene in the population of Senduro goats and Boerawa goats are monomorphic (uniform), all samples of Senduro goats and Boerawa goats have the GG genotype, with a G allele frequency value of 1.00 and cannot be associated with litter size.

The results of this study are different from the results of Wang, et al (2014) stated that the candidate gene that plays the highest selective role for litter size 3 is PAK1. Furthermore, Wang, et al (2014) stated that in addition to PAK1, genes that play a selective role for litter size 3 are PRKAA1 and SMAD9. The discovery of new candidate genes involved in litter size regulation in goats can broaden the understanding of the basic genetics of reproductive ability so that reproductive performance can be improved when the candidate genes are applied. However, the results of this study did not find any polymorphic PAK1 gene in Senduro goats and Boerawa goats, so it cannot be used as a DNA marker.

ACKNOWLEDGMENTS:

Research project Principal Investigator Grant (HPU) Prof. Dr. Sc. Agr. Ir. Suyadi, MS., IPU., ASEAN Eng who has funded this research.

CONCLUSION:

Identification of the PAK1 exon 9 gene in Senduro UPT PT-HMT Singosari goats, Malang Regency with an average litter size as big 1,54, and Boerawa goats with an average litter size as big 1,26 by PCR-RFLP method using MSP1 restriction enzyme is uniform (monomorphic), this is because all samples have the GG genotypes and G allele frequency is 100%.

REFERENCES:

Allendorf, F. W., Luikart, G., & Aitken, S. N. (2007). Conservation and the genetics of populations. *Mammalia, 2007*, 189-197.

Etherton, T. D., & Bauman, D. E. (1998). Biology of somatotropin in growth and lactation of domestic animals. *Physiological reviews*, *78* (3), 745-761.

Filian, B. V., Santoso, S. A. B., Harjanti, D. W., & Prastiwi, W. D. (2016). Hubungan paritas, lingkar dada dan umur kebuntingan dengan produksi susu Sapi Friesian Holstein di BBPTU-HPT Baturraden. *Jurnal Agripet*, *16*(2), 83-89. https://doi.org/ 10.179 69/agripet.v16i2.5102.

Hartati & Bayu. (2020). Detection of Growth Hormone (GH|MSP1) gene polymorphism in Madurese cattle raised in the Beef Cattle Research Workshop Group Barn. ISBN: 978-602-52203-2-6

Hartati, H., Soewandi, B. D. P., Hapsari, A. A. R., Anwar, S., & Pamungkas, D. (2019). Identification of GH| MspI and GHR| AluI gene polymorphism and its association with calf birth weight of Grati-PO cattle. *Jurnal Ilmu Ternak dan Veteriner*, *24*(2), 49-55.

Howe, A. K., & Juliano, R. L. (2000). Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. *Nature cell biology*, *2*(9), 593-600. https:// doi.org/10.1038/35023536

Huda, N., Made Sriasih, S., & Maskur, M. (2015). Polymorphism of Growth Hormone Receptor Gene with Restriction Enzyme MBO II (GHR| MBO II) in Bali Cattle. *Jurnal Ilmu dan Teknologi Peternakan Indonesia*, *1*(1), 31-39. https://doi.org/ 10.29303/jitpi.v1i1.7

Ilham, F., Dako, S., Rachman, A. B., Dagong, M. I. A., Rahim, L., Yulianty., & 1. (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 Research for Rural Development, 28(9).

Rachman, A. B. (2017). Genetic polymorphisms of growth hormone (GH) gene in Kacang goat population based on polymerase chain reactionrestriction fragment length polymorphism (PCRRFLP) identification. *ARTIKEL*, *1*(822).

Jakariaa , D. Duryadib , R.R. Noora , B. Tappac & H. Martojo. 2007. Evaluasi Keragaman Genetik Gen Hormon Pertumbuhan (GH) pada Sapi Pesisir Sumatera Barat Menggunakan Penciri PCR-RFLP .Media Peternakan.30(1) :1-10.

King, C. C., Gardiner, E. M., Zenke, F. T., Bohl, B. P., Newton, A. C., Hemmings, B. A., & Bokoch, G. M. (2000). p21-activated kinase (PAK1) is phosphorylated and activated by 3-phosphoinositide-dependent kinase-1 (PDK1). *Journal of Biological Chemistry*, *275*(52), 41201-41209. https://doi.org/10.1074/jbc.m006553200

Maylinda, S. 2010. Introduction to Livestock Breeding. Malang: UB Press.

Muladno. 2010. Genetic Engineering Technology. Bogor (ID): IPB Press.

Nei M, Kumar S. (2000). Molecular Evolution and Phylogenetics. New York (US): Columbia University Pr.

Nei, M. 1975. Molecular Population Genetics and Evolution North Holl PubIlo.

Noor, R.R. 2010. Livestock Genetics. Jakarta (ID): Self-help Spreader.

Panggabean, T. N. (2016). Analisis tingkat optimasi algoritma genetika dalam hukum ketetapan Hardy-Weinberg pada bin packing problem. *CESS (Journal of Computer Engineering, System and Science)*, *1*(2), 12-18. https://doi.org/10.24114/cess.v1i2.4062

Putri, R. F., Nugraha, C. D., Furqon, A., Septian, W. A., & Suyadi, S. (2021). Identifikasi Keragaman Genetik Gen Inhibin Subunit Alpha (INHA) dan Inhibin Subunit Beta A (INHBA)

Pada Sapi Peranakan Ongole (PO). *TERNAK TROPIKA Journal of Tropical Animal Production*, *22*(1), 69-76. https://doi.org/10.21776/ub.jtapro.2021.022.01.9

Rahmadhan, D. (2019). Pengaruh suhu annealing terhadap amplifikasi gen tem menggunakan primer dengan% gc rendah. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, *4*(1). 1-7

Sumantri, C., Herdiana, D., Farajallah, A., & Rahmat, D. (2009). Polymorphism of Pituitary-Specific Transcription Factor-1 (Pit-1) Gene at Locus (Pit-1-Hinf1) and its effects on dam body weight and milk production of local sheeps. *Jurnal Ilmu Ternak dan Veteriner*, *14*(3), 212-229.

Susilorini, T. (2013). *Polimorfisme Gen B-Lactoglobulin Serta Hubungannya Dengan Produksi Dan Protein Susu Kambing Peranakan Etawah (Pe)* (Doctoral dissertation, Universitas Brawijaya).

Susilorini, T.K, & Kuswati. 2019. Goat & Sheep Farming. Malang: UB Press.

Tang, Y., Zhou, H., Chen, A., Pittman, R. N., & Field, J. (2000). The Akt proto-oncogene links Ras to Pak and cell survival signals. *Journal of Biological Chemistry*, *275*(13), 9106-9109. https://doi.org/10.1074/jbc.275.13.9106

Tsakiridis, T., Taha, C., Grinstein, S., & Klip, A. (1996). Insulin activates a p21-activated kinase in muscle cells via phosphatidylinositol 3-kinase. *Journal of Biological Chemistry*, *271*(33), 19664-19667. https://doi.org/10.1074/jbc.271.33.19664

Tse, E. Y. T., & Ching, Y. P. (2014). The role of p21-activated kinases in hepatocellular carcinoma metastasis. *Journal of molecular signaling*, *9*(1), 1-9. https://doi.org/10.1186/1750-2187-9-7

Vega, F. M., & Ridley, A. J. (2008). Rho GTPases in cancer cell biology. *FEBS letters*, *582*(14), 2093-2101.

Vogel, F., Motulsky, A. G., Vogel, F., & Motulsky, A. G. (1997). The human genome: chromosomes. *Human Genetics: Problems and Approaches*, 25-82.

Wang, J. J., Zhang, T., Chen, Q. M., Zhang, R. Q., Li, L., Cheng, S. F., ... & Lei, C. Z. (2020). Genomic signatures of selection associated with litter size trait in Jining Gray goat. *Frontiers in Genetics*, *11*, 286. https://doi.org/10.3389/fgene.2020.00286