BIODIVERSITAS
Volume 24, Number 12, December 2023
Pages: 6567-6573

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241218

Comparison of genetic diversity of LEP gene between Indonesian domestic goats: Etawa Cross and Senduro Goats

MUHAMMAD FAJAR AMRULLAH[1], BUDI UTOMO[2], SUZANITA UTAMA[2], TITA DAMAYANTI LESTARI[1,♥], TRI WAHYU SUPRAYOGI[2], TJUK IMAM RESTIADI[2], RISTAQUL HUSNA BELGANIA[1], SUHENDRA PAKPAHAN[3], ASWIN RAFIF KHAIRULLAH[4], SHENDY CANADYA KURNIAWAN[5], OTTO SAHAT MARTUA SILAEN[6], ABDULLAH HASIB[7]

[1]Graduate Program of Reproductive Biology, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia. Tel.: +62-31-5992785, ♥email: titadlestari@fkh.unair.ac.id
[3]Research Center for Applied Zoology, National Research and Innovation Agency. Jl. Jakarta-Bogor Km. 46, Bogor 16911, West Java, Indonesia
[4]Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[5]Graduate Program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule, Cell and Organ Functioning, Wageningen University and Research. Wageningen 6708 PB, Netherlands
[6]Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia. Jl. Salemba Raya No. 6, Central Jakarta 10430, Jakarta, Indonesia
[7]School of Agriculture and Food Sustainability, University of Queensland. Gatton, QLD, 4343, Queensland, Australia

Manuscript received: 16 November 2023. Revision accepted: 20 December 2023.

Abstract. *Amrullah MF, Utomo B, Utama S, Lestari TD, Suprayogi TW, Restiadi TI, Belgania RH, Pakpahan S, Khairullah AR, Kurniawan SC, Silaen OSM, Hasib A. 2023. Comparison of genetic diversity of LEP gene between Indonesian domestic goats: Etawa Cross and Senduro Goats. Biodiversitas 24: 6567-6573.* The leptin (LEP) gene is a gene that functions to produce the hormone leptin secreted by fat tissue that can increase livestock productivity. This study aimed to identify the profile of the LEP gene between Etawah Cross goats and Senduro goats. Gene profiling includes single nucleotide polymorphisms (SNPs) and phylogeny tree reconstruction. Furthermore, 10 whole blood samples from 5 Etawah Cross goats and 5 Senduro goats were collected from goat farms in Lumajang District, Lumajang Regency, Indonesia. The Research procedure involved collecting blood samples, extracting DNA, amplification using the polymerase chain reaction (PCR) method using specific primers, and nucleotide sequencing method. The edited DNA sequences were aligned with some DNA sequences (n=32) of the *Capra hircus* group published in Genbank. The results of this study obtained the alignment of the LEP gene sequences of Etawah Cross goats and Senduro goats, including T723A, G729A, G758A, A763T, G774C, T1100C, and G1454A. Based on the LEP gene phylogeny tree, Etawah Cross goats and Senduro goats belong to the same clade and have a close kinship with the Bligon goats. The profile of the LEP gene between Etawah Cross goats and Senduro goats varies so that further studies can be carried out to look for the relationship between SNPs and the productivity traits of the LEP gene.

Keywords: Etawa cross, genetic diversity, LEP gene, Senduro, quality

INTRODUCTION

Indonesia is rich in livestock genetic resources (Widyas et al. 2022). The diversity of genetic resources for livestock represents the heritable variation within and between livestock populations, including different goat breeds. Breeders in Indonesia farm various breeds of goats; each breed has unique and distinct characteristics based on the location or area where they are farmed (Ilham et al. 2023). The most commonly farmed type of goat by breeders is the Kacang goat (Rahmawati et al. 2022), which is exceptionally well-suited to tropical weather and regions. Additionally, a native breed of goat known as the Etawa cross (PE) goat is typically dual-purpose (meaning it can provide both milk and meat) (Hariyono and Endrawati 2022). Other native goats found in Indonesia that have flourished are the Bligon (a hybrid between PE and Kacang), Gembrong, Boer, Boerja (a cross between Boer and Bligon), and Kejobong (Kusminanto et al. 2020).

A goat known as an Etawa cross is produced by crossing native Indonesian goats, Kacang goats, with the Indian Etawa goats (Susilorini et al. 2022). The Etawa cross is frequently utilized in projects to enhance the quality of goat breeds since it has a higher body percentage than other local goats (Guntoro et al. 2023). For a century, people have thought that a combination of Etawa, Kacang, and Jawarandu goats produced the Senduro goat breed (Susilorini et al. 2022). It has long been preserved in the Lumajang Regency, East Java's Senduro District. Since breeders have chosen Senduro goats from the Etawa cross for many years, their physical characteristics are generally similar to those of the Etawa cross (Fatmawati et al. 2022). Additionally, according to the Decree of the Minister of Agriculture of the Republic of Indonesia, Senduro goats are recognized as a local Indonesian breed (Almaida et al. 2020).

Cross-breeding causes the development of new strains, so genetic quality improvement is needed to continue producing superior livestock (Widyas et al. 2022).

Improving the genetic quality of livestock is an activity of livestock breeding to obtain good livestock productivity (Kurnianto 2022). At the molecular level, selection can be done by evaluating the nucleotide sequence profile of the genes in the DNA that affect livestock productivity, one of which is the LEP gene (Kuswati et al. 2022). LEP or Leptin comes from the Greek word Leptos, which means thin and is described as a hormone that reduces body weight (Obradovic et al. 2021). Leptin promotes general weight loss by suppressing food intake, preventing low metabolism, and is expressed proportionately to body fat (Münzberg and Heymsfield 2019). The obesity epidemic has explained a pathway of leptin resistance, with too much Leptin in both sexes leading to infertility (Childs et al. 2021).

Leptin is encoded by the LEP gene, of about 20 kb. It consists of three exons separated by two introns, and the exonic portion of the LEP gene covers about 15 kb of the bovine genome (Işık et al. 2022). The first exon is truncated in the mature blood circulating hormone. Meanwhile, the other two exons produce fully mature 167 residues by excising the first 24 signal-aminoacid residues to produce 16 kDa of blood-circulating Leptin (Obradovic et al. 2021). Leptin contains a distinctive three-dimensional (3D) four-α-helix bundle structure of an A-B-C-D pattern (Al-Shuhaib 2019). This structure is arranged in four sequentially similar, antiparallel, left-hand twisted α-helical bundles connected by two crossover links alongside one short loop (Greco et al. 2021).

However, under conditions of balanced nutrition, leptin secretion is timed and regulated within a narrow range of levels that optimize its trophic effect (Childs et al. 2021). Each species can inherit the LEP gene (Yupanqui-Lozno et al. 2019). LEP gene variants are highly identified to boost goat productivity, impact intramuscular deposition and body weight, and can be employed as a marker-assisted selection (MAS) approach (Sycheva et al. 2023). Etawa Cross and Senduro goats come from the same maternal, namely Jamnapari. Due to the mating with local goats and the isolation in their area by the breeders, this goat breed was formed (Pakpahan et al. 2022). Both breeds require a parallel genetic analysis, especially the LEP gene, to determine the gene profile. This is the background of this research regarding comparing LEP gene profiles between Etawa cross and Senduro goats. The results of this study are expected to be used as a guide in preserving the genetic resources of Indonesia's local goats.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee of Veterinary of Medicine Faculty approved the study, Universitas Airlangga (No:1.KEH.085.07.2022).

Sample collection and DNA extraction

This research was conducted from January to April 2023. Etawa cross and Senduro goat blood samples were brought from Burno Village, Senduro District, Lumajang Regency, East Java, Indonesia. The total blood samples used were 10 blood samples from 5 Etawah Cross goats and 5 Senduro goats. Blood sampling was done by collecting about 3 mL of blood through the jugular vein, one-third of the head. Blood samples were collected using a 3 mL syringe with a vaccumtainer tube containing EDTA and then stored in coolbox. Extraction of DNA was performed using an isolation DNA Extraction Kit from Thermo Purelink Genomic DNA Mini kit (Invitrogen K-182001, USA).

Primary design and amplification of the LEP gene

Primary design and LEP gene amplification using the Polymerase chain reaction (PCR) procedure were carried out based on the method of Hartatik (Hartatik et al. 2020). The target LEP gene sequence was retrieved from GenBank *Capra hircus*/AM114397.2 as a reference. The pair primers used in this study were forward (5'-AGCGGTTATGGGATATGCC-3') and reverse (5-AATGCCCCAAGAGACA CTGA-3'). The PCR product that will be obtained has a total length of 967 bp.

PCR amplification with a total reaction volume of 30 µL master mix (containing 1.5 µL genomic DNA (approx. 100 ng), 1.5 µL (10 µM) forward primer, 1.5 µL (10 µM) reverse primer, 15 µL PCR kit, and 10.5 µL aquabidest). PCR conditions were started with 5-minute denaturation at 94°C, carried out for 30 cycles, annealing at 54°C for 1 minute, and extension at 72°C for 5 minutes.

Visualization of amplification products using electrophoresis method based on the method of Utomo et al. (2021). Next, 3 µL PCR Product sample in 1 µL Loading dye (SYBR dye). In the first well, 1000 bp of Leader DNA was used. The PCR products were then analyzed by electrophoresis on 1% agarose gel at constant voltage (100 volts) for 2 hours with ethidium bromide and SYBR dye (Invitrogen S7563, USA) added for staining, and the agarose was photographed using ultra-violet light. Then, the results were recorded using the documentation system Geldoc UV Transilluminator (Bio-Rad, USA).

Nucleotide sequencing

Subsequently, 10 samples (5 from Etawah cross goats and 5 Senduro goats) were collected for sequencing. Clean-up PCR product samples were packaged using a microtube. For the success of the DNA Sequencing reaction, the sample needs to be quantified to estimate the purity of the DNA template using agarose gel electrophoresis. Nucleotide sequencing used the services of a sequencing company (1st BASE through PT. Genetika Science Indonesia). The primer used for nucleotide sequencing in the LEP gene is the same as that used for the amplification step.

Data analysis

Data obtained from nucleotide base sequences were then edited with the Bio Edit Version 7.0.0 program. The edited DNA sequences were aligned with some DNA sequences of the *Capra hircus* group published in Genbank (http://ncbi.nlm.nih.gov). The data used for comparison is *Capra hircus* with accession number AM114397.2. The synchronization process with ClustalW2 is included in Bio Edit Version 7.0.0. After matching the data, analysis was

performed to determine the position of single nucleotide polymorphisms (SNPs).

The neighbor-joining (NJ) approach was utilized to reconstruct the phylogenetic tree of the LEP gene by utilizing the nucleotide sequence. Molecular Evolutionary Genetic Analysis (MEGA ver.11.0.13) employs nucleotide-based phylogeny with bootstrap value of 1000x repetitions, utilizing Kimura's 2-parameter model (Prasetya et al. 2011). Genbank LEP data Accession No. JQ739233.1 (*Capra hircus* breed Beetal), JQ739232.1 (*Capra hircus* breed Barbari), GU944974.2 (*Capra hircus* breed Sirohi) and MN635656.1 (*Capra hircus* Bligon goats).

RESULTS AND DISCUSSION

Results

PCR products were obtained from 10 leptin gene (5 samples from Etawah cross goats and 5 samples from Senduro goats) using a PCR protocol, the results of ClustalW multiple alignments of LEP gene Etawa cross and Senduro goat, originally 860 bp. After performing ClustalW multiple alignment analysis, SNPs were obtained. Based on multiple alignment results, several SNPs were found in the LEP gene sequences from Etawa cross goats and Senduro goats. SNPs are based on changes in nitrogenous bases (adenine, guanine, cytosine, thymine) between the corresponding sequences. The position of the SNP in each sample is shown in Table 1.

Multiple alignment results of the LEP gene in Etawa cross goats and Senduro goats show changes at several positions. Changes in this nucleotide sequence have the effect of a mutation; mutations in nitrogen bases can be transition and transversion mutations. Transition mutations are mutations in which one type of base is replaced (for example, a purine base is replaced by a purine base). In transversion mutations, conversely, nitrogenous bases are replaced by identical nitrogenous bases (for example, pyrimidine bases are replaced by purine bases).

A phylogenetic tree is a two-dimensional diagram that shows the relationships between organisms, or more specifically, the gene sequences of organisms. The relationship between the linkage distance of the LEP genes from samples from Etawa cross goats and Senduro goats with the Genbank LEP gene target was described using the MEGA program version 11.0.13. Leptin gene sequence data used by Genbank from *Capra hircus* (Acc No. AM1143972), Beetal breed (JQ739233.1), Barbari breed (JQ739232.1), Sirohi breed (GU944974.2) and Bligon breed (MN635656.1) are included (Figure 1).

Based on Figure 1, the LEP gene Etawa cross goats (LPE1) and Senduro goats (LS1) are in the same clade and are a branch of the *Capra hircus* breed Bligon (MN635656.1), so the two goats have a close kinship with the Bligon goat close relationship with the goat Bligona. The root tree were an arrangement of LEP gene relationships among goats from *Capra hircus* (AM114397.2), then Beetal breed (JQ739233.1), then Barbari breed (JQ739232.1), then Sirohi breed (GU944974.2) and Bligon breed (MN635656.1), which is a close relative of the LEP gene with Etawa cross goats and Senduro goats. The ecological conditions of a species and the life history of a species affect the genetic variation of that species.

Discussion

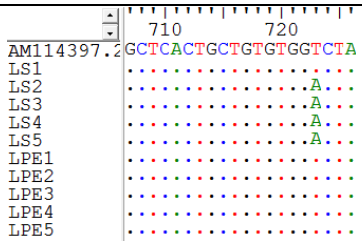
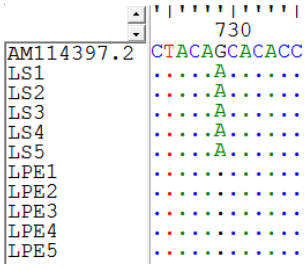
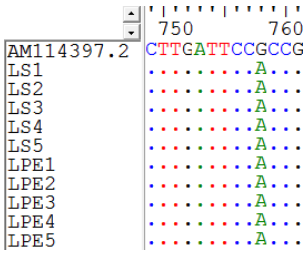
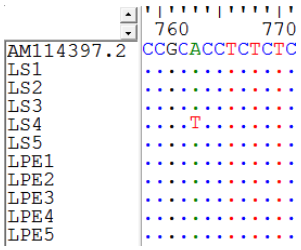
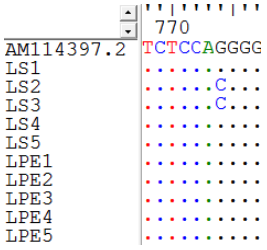
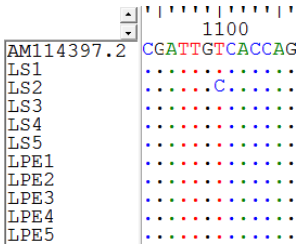
Analysis of 10 sequences obtained from 5 Etawa cross goats and 5 Senduro goats were compared with the sequences *Capra hircus* (AM113497.2), and 7 SNPs were found. A related study on goat breeds in India reported that the Indian goat LEP gene sequence was 4842 bp long, consisting of 913 bp 5'UTR, two exons (exons 2 and 3) with corresponding introns, and 1587 bp 3'. UTR. Analysis of 42 sequences from 7 Indian goat breeds revealed 22 variants compared to sequences from Italian Garganica goats (AM114397.2), and there are seven SNPs distributed throughout the LEP gene. Each SNPs was located in exon 2 and intron 2, while five were observed in the 3'UTR. Information about potential mutations in the promoter region of the LEP gene may be useful for elucidating the regulatory mechanisms of this gene that affect body fat mass, fat storage, and obesity (Maitra et al. 2014).

Moreover, seven SNPs were detected in the LEP gene of Etawa cross goats and Senduro goats. SNPs were found in exon 2 (g.723 T>A, g.729 G>A, g.758 G>A, g.763 A>T, g.774 G>C, and g.1100 T>C) and intron 2 (g.1454 G>A). In the LS1 sample, there are changes at position 729 (G→A), 758 (G→A), and position 1454 (G→A). The LS2 sample has changes at positions 723 (T→A), 729 (G→A), 729 (G→A), 774 (G→C), and 1100 (T→C). The LS3 sample has changed in positions 723 (T→A), 729 (G→A), 758 (G→A), 774 (G→C), and 1454 (G→A). The LS4 sample has changes at positions 723 (T→A), 729 (G→A), 758 (G→A), and 763 (A→T). The LS5 sample has changed at positions 723 (T→A), 729 (G→A), and 758 (G→A). The LPE1, LPE2, LPE3, and LPE4 samples had changed at positions 758 (G→A) and 1454 (G→A). The LPE5 sample has only 1 SNP at position 758 (G→A).

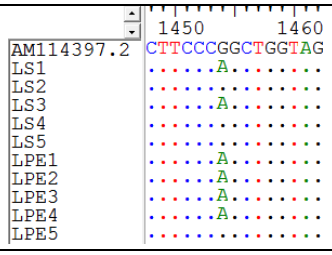
Bioinformatic analysis of LEP gene sequences of several Indian goats compared to exotic goats identified 22 variants (AM114397.2) of which seven are SNPs. SNPs were found in exon 2 (g.1029 T>C), introns 2 (g.1621 G>A), and 3'UTR (g.3968 T>C, g.3971 C>T, g.4026 G>A, g.4105 G>A, and g.4225 T>C). This LEP gene variant can be used as a reference in a larger goat population for association studies with meat quality traits and selection with the help of marker-assisted selection (Tian et al. 2013).

There were transversion mutations at SNPs 723 (T>A), 763 (A>T), and 774 (G>C), and transition mutations at SNPs 729(G>A), 758 (G>A), 1100 (T> C), and 1454 (G>A). According to Yaari et al. (2013), substitution-based mutations replace one base with another base, resulting in a change in the location of the sequence. Substitution consists of transitions and transitions. The substitution of one purine base (A↔G) for another purine base or one pyrimidine base (T↔C) for another pyrimidine base is known as a transition substitution (Arabnejad et al. 2018). A purine base becomes a pyrimidine base or vice versa through transposition substitution (Luo et al. 2016).

Table 1. The results of detecting leptin gene SNPs in Etawa Cross goats and Senduro goats were compared with the Genbank target of the Leptin gene in *Capra hircus* No.Acc AM114397.2 exons 2-3

Sample	Base position	Multiple alignment sequence	Locus location	Mutation	Mutation type
LS2 LS3 LS4 LS5	723		Exon 2	T > A	Transversion
LS1 LS2 LS3 LS4 LS5	729		Exon 2	G > A	Transition
LS1 LS2 LS3 LS4 LS5 LPE1 LPE2 LPE3 LPE4 LPE5	758		Exon 2	G > A	Transition
LS4	763		Exon 2	A > T	Transversion
LS2 LS3	774		Exon 2	G > C	Transversion
LS2	1100		Exon 2	T > C	Transition

LS1 LS3 LPE1 LPE2 LPE3 LPE4	1454	Intron 2	G > A	Transition



Note: LS1-LS5: Leptin Gene Senduro Goats, LPE1-LPE5 : Leptin Gene Etawa Cross, A: Adenine, G: Guanine, C: Cytosine, T: Thymine

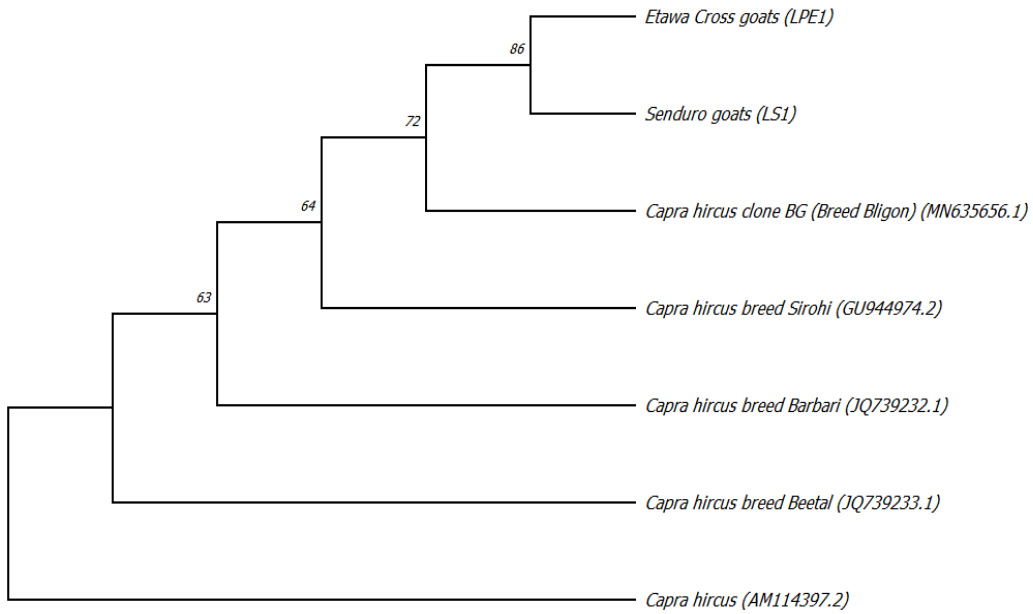


Figure 1. Reconstruction of a nucleotide-based leptin gene phylogeny tree using the Neighbor-Joining (NJ) method, the Kimura-2-Parameter model, and bootstrap 1000x repetitions

The location of transition and transversion mutations within the protein-coding gene determines their effects (Guo et al. 2017). The activity of protein enzymes is not significantly affected by an event if it takes place in a location that does not significantly alter the structure of the protein that is used to preserve the structural integrity of the cell or if it does not alter the structure of the enzyme or its active site (Seok 2021). However, a protein's enzymatic activity decreases when transition and transversion mutations occur in a protein region that dramatically alters the structure of the protein's active site (Stoltzfus and Norris 2016). These mutations have detrimental effects.

The phylogenetic tree of the LEP gene shows that Etawa cross goats and Senduro goats form the same clade; a bootstrap score of 86% supports the clade. The bootstrap score is a measure used to determine the validity of a family tree. The bootstrap analysis tests how well the bootstrap model and dataset are supported by the testing software and how reliable the branches can be in Phylogenetic tree construction using the Kimura 2-parameter (K2P) model. Sequence alignment and evaluation of genetic differences are important steps in molecular evolutionary studies and DNA barcoding studies (Nishimaki and Sato 2019). The K2P model is the most widely used of all nucleotide substitution models to estimate genetic differences or distances and phylogenetic relationships (Su et al. 2014).

Based on the LEP gene sequence, Etawa cross goats and Senduro goats are very close to *Capra hircus* clone BG (Breed Bligon, No. Acc MN635656.1). This proves that Etawa cross goats and Senduro goats are very closely related to Bligon goats, with a bootstrap value of 72%. Based on genetic similarity from this study are Senduro goats and Etawa Cross goats has a close kinship, instead there is a distant kinship with the comparison of *Capra hircus*, but it has a close kinship between individuals (Ciptadi et al. 2021).

In conclusion, the LEP gene profile of Etawa cross and Senduro goats can be identified. Alignment/alignment of the LEP gene sequence between Etawa cross goats and Senduro goats has a base length of 860 bp, which can be aligned with the LEP target gene *Capra hircus* No. Acc AM114397.2. Several single nucleotide polymorphisms (SNPs) were obtained in the LEP gene sequence of Etawa cross goats and Senduro goats. These SNPs include T723A, G729A, G758A, A763T, G774C, T1100C, and G1454A. The SNPs formed are the result of transversion and

transition mutations. Based on the LEP gene phylogeny tree reconstruction, Etawa crosses goats, and Senduro goats are in the same clade and have a close kinship with Bligon goats.

ACKNOWLEDGEMENTS

The authors are thankful to the Faculty of Veterinary Medicine, Airlangga University, and the National Research and Innovation Agency (BRIN) Indonesia for providing the necessary facilities for the study.

REFERENCES

Al-Shuhaib MBS. 2019. A comprehensive in silico prediction of the most deleterious missense variants in the bovine LEP gene. Biotechnol 100 (4): 429-439. DOI: 10.5114/bta.2019.90244.

Almaida RG, Oktanella Y, Ciptadi G. 2020. Genetic variation of Senduro and Peranakan Etawa goats based on Cyt-B (Cytochrome-B) gene sequence using polymerase chain reaction. J Trop Anim Prod 21 (2): 102-110. DOI: 10.21776/ub.jtapro.2020.021.02.3.

Arabnejad M, Dawkins BA, Bush WS, White BC, Harkness AR, McKinney BA. 2018. Transition-transversion encoding and genetic relationship metric in ReliefF feature selection improves pathway enrichment in GWAS. BioData Min 11 (1): 23. DOI: 10.1186/s13040-018-0186-4.

Childs GV, Odle AK, MacNicol MC, MacNicol AM. 2021. The importance of Leptin to reproduction. Endocrinology 162 (2): bqaa204. DOI: 10.1210%2Fendocr%2Fbqaa204.

Ciptadi G, Budiarto A, Nasich M, Rahayu S, Putri AI, Karima HN, Almaida RG. 2021. Use of genetic similarity analysis for identification and study of the origin of Indonesian local goats based on XY sex chromosome karyotyping and cytochrome-b genes sequence. IOP Conf Ser Earth Environ Sci 743 (1): 012001. DOI: 10.1088/1755-1315/743/1/012001.

Fatmawati M, Suwanti LT, Mufasirin, Hambarruk W, Sukesi LE, Rofiah, Novianto E, Wahyuningtyas BK. 2022. A comparative study among dairy goat breeds in Lumajang and Malang, East Java based on milk organoleptic and milk composition. Biodiversitas 23 (6): 2899-2903. DOI: 10.13057/biodiv/d230616.

Greco M, De Santo M, Comandè A, Belsito EL, Andò S, Liguori A, Leggio A. 2021. Leptin-activity modulators and their potential pharmaceutical applications. Biomolecules 11 (7): 1045. DOI: 10.3390/biom11071045

Guntoro B, Setiawan A, Haryadi FT. 2023. Development of Kaligesing etawah cross-breed goat in Purworejo Central Java Province. IOP Conf Ser Earth Environ Sci 1183 (1): 012104. DOI: 10.1088/1755-1315/1183/1/012104.

Guo C, McDowell IC, Nodzenski M, Scholtens DM, Allen AS, Lowe WL, Reddy TE. 2017. Transversions have larger regulatory effects than transitions. BMC Genom 18 (1): 394. DOI: 10.1186/s12864-017-3785-4.

Hariyono DNH, Endrawati E. 2022. Indigenous goat genetic resources in Indonesia: current status and future improvement. J Adv Vet Res 13 (1): 141-149.

Hartatik T, Latifah L, Yuliana R, Kustantinah A. 2020. Genotyping and chi-square analysis of 967 Bp leptin gene in bligon goat. IOP Conf Ser Earth Environ Sci 478 (1): 012019. DOI: 10.1088/1755-1315/478/1/012019.

Ilham F, Ciptadi G, Susilorini TE, Putra WPB, Suyadi S. 2023. Morphology and morphometric diversity of three local goats in Gorontalo, Indonesia. Biodiversitas 24 (3): 1366-1375. DOI: 10.13057/biodiv/d240305.

Işık R, Ünal EÖ, Soysal Mİ. 2022. Polymorphism detection of DGAT1 and Lep genes in Anatolian water buffalo (*Bubalus bubalis*) populations in Turkey. Arch Anim Breed 65 (1): 1-9. DOI: 10.5194/aab-65-1-2022.

Kurnianto E. 2022. Improving genetic quality of local livestock for achieving food security. IOP Conf Ser Earth Environ Sci 1041 (1): 012081. DOI: 10.1088/1755-1315/1041/1/012081.

Kusminanto RY, Alawiansyah A, Pramono A, Sutarno, Cahyadi M. 2020. Body weight and body measurement characteristics of seven goat breeds in Indonesia. IOP Conf Ser Earth Environ 478 (1): 012039. DOI: 10.1088/1755-1315/478/1/012039.

Kuswati K, Furqon A, Septian WA, Susilawati T. 2022. Polymorphism of leptin gene (single nucleotide polymorphisms c.73T>C) and its association with body weight and body measurements in Madura cattle. Vet World 15 (3): 775-781. DOI: 10.14202/vetworld.2022.775-781.

Luo GH, Li XH, Han ZJ, Zhang ZC, Yang Q, Guo HF, Fang JC. 2016. Transition and transversion mutations are biased towards GC in transposons of *Chilo suppressalis* (Lepidoptera: Pyralidae). Genes (Basel) 7 (10): 72. DOI: 10.3390/genes7100072.

Maitra A, Sharma R, Pandey AK, Singh LV, Mandakmale SD, Mishra BP. 2014. Preliminary identification and characterisation of leptin gene polymorphism in Indian goats. J Appl Anim Res 42 (1): 118-122. DOI: 10.1080/09712119.2013.795895.

Münzberg H, Heymsfield SB. 2019. New insights into the regulation of leptin gene expression. Cell Metab 29 (5): 1013-1014. DOI: 10.1016/j.cmet.2019.04.005.

Nishimaki T, Sato K. 2019. An extension of the Kimura two-parameter model to the natural evolutionary process. J Mol Evol 87 (1): 60-67. DOI: 10.1007/s00239-018-9885-1.

Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, Gojobori T, Isenovic ER. 2021. Leptin and obesity: Role and clinical implication. Front Endocrinol (Lausanne) 12 (1): 585887. DOI: 10.3389/fendo.2021.585887.

Pakpahan S, Widayanti R, Artama WT. 2022. Selection signatures and formation of the Samosir goat breed through the cultures of the Batak Toba Tribe in Samosir Island, Indonesia. Vet World 15 (4): 1044-1050. DOI: 10.14202/vetworld.2022.1044-1050.

Prasetya H, Saefuddin A, Muladno. 2011. Performance comparison between kimura 2-parameters and jukes-cantor model in constructing phylogenetic tree of neighbour joining. Indonesian J Stat Appl 16 (1): 8-16.

Rahmawati RD, Atmoko BA, Budisatria IGS, Ngadiyono N, Panjono. 2022. Exterior characteristics and body measurements of Bligon goat on the different agro-ecological zones in Bantul District, Yogyakarta, Indonesia. Biodiversitas 23: 143-150. DOI: 10.13057/biodiv/d230118.

Seok SH. 2021. Structural insights into protein regulation by phosphorylation and substrate recognition of protein kinases/phosphatases. Life (Basel) 11 (9): 957. DOI: 10.3390/life11090957.

Stoltzfus A, Norris RW. 2016. On the causes of evolutionary transition: Transversion bias. Mol Biol Evol 33 (3): 595-602. DOI: 10.1093/molbev/msv274.

Su Z, Wang Z, López-Giráldez F, Townsend JP. 2014. The impact of incorporating molecular evolutionary model into predictions of phylogenetic signal and noise. Front Ecol Evol 2 (1): 11. DOI: 10.3389/fevo.2014.00011.

Susilorini TE, Wulandari D, Furqon A, Septian WA, Saputra F, Suyadi S. 2022. Genetic diversity of various goat breeds in east java based on DNA microsatellite markers. Trop Anim Sci J 45 (3): 247-254. DOI: 10.5398/tasj.2022.45.3.247.

Sycheva I, Latynina E, Mamedov A, Tsibizova O, Kozak Y, Svistounov D, Bystrenina I, Orishev A. 2023. Effect of TG5 and LEP polymorphisms on the productivity, chemical composition, and fatty acid profile of meat from Simmental bulls. Vet World 16 (8): 1647-1654. DOI: 10.14202/vetworld.2023.1647-1654.

Tian J, Zhao Z, Zhang L, Zhang Q, Yu Z, Li J, Yang R. 2013. Association of the leptin gene E2-169T>C and E3-299T>A mutations with carcass and meat quality traits of the Chinese Simmental-cross steers. Gene 518 (2): 443-448. DOI: 10.1016/j.gene.2012.11.071.

Widyas N, Widi TSM, Prastowo S, Sumantri I, Hayes BJ, Burrow HM. 2022. Promoting sustainable utilization and genetic improvement of indonesian local beef cattle breeds: A review. Agriculture 12 (10): 1566. DOI: 10.3390/agriculture12101566.

Yaari G, Heiden JAV, Uduman M, Gadala-Maria D, Gupta N, Stern JN, O'Connor KC, Hafler DA, Laserson U, Vigneault F, Kleinstein SH. 2013. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. Front Immunol 4 (1): 358. DOI: 10.3389/fimmu.2013.00358.

Yupanqui-Lozno H, Bastarrachea RA, Yupanqui-Velazco ME, Alvarez-Jaramillo M, Medina-Méndez E, Giraldo-Peña AP, Arias-Serrano A, Torres-Forero C, Garcia-Ordoñez AM, Mastronardi CA, Restrepo CM, Rodriguez-Ayala E, Nava-Gonzalez EJ, Arcos-Burgos M, Kent

JW, Cole SA, Licinio J, Celis-Regalado LG. 2019. Congenital Leptin deficiency and Leptin gene missense mutation found in two colombian sisters with severe obesity. Genes (Basel) 10 (5): 342. DOI: 10.3390/genes10050342.