Genetic Analysis of The Leptin Gene in Goats Based on GenBank DNA Sequences

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Abstract

The Leptin gene is the gene that produces the leptin hormone, which is released from adipose tissue and can increase the productivity of animals. This study aimed to identify polymorphic nucleotides, changes in amino acid components, and species of goats based on GenBank Leptin DNA sequence data. A total of five goat leptin DNA sequences were extracted from NCBI GenBank data. The leptin DNA sequence was aligned with Bioedit to locate SNPs and amino acid changes. The tree produces cultivars grown using Clustal Omega Ver. 1.2.4. Based on the DNA sequencing results of leptin genes in five goats, five SNPs were located in the coding sequence (CDS), SNPs g.17T/A, g.43T/A, g.74G/A, g.93C/A. and d. 386A/G. SNP was a missense mutation and a silent mutation. The analysis of phylogenetic trees of Leptin showed that there were three breeds of goats in one branch and two breeds of goats in different branches. These results provided the first report for further studies on the genetic diversity of leptin genes in different local goat breeds.

Keywords: genetic diversity, goat, leptin gene, phylogenetic tree, SNPs

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INTRODUCTION

Indonesia has a rich biodiversity, including goats. Goats are raised by farmers for the purpose of meat and milk production. For example, the Peranakan Etawa (PE) goat is one of the local goat breeds favored by Indonesian breeders because it is a dual-purpose animal for both meat and milk production, in addition to other local goat breeds. including Kacang goat, Marica goat, Sapera goat, Jawa Randu goat, (Meydilasari *et al*., 2020; Nanda *et al*., 2020; Pahlevy *et al*., 2022). Local goat productivity can be increased through breeding with molecular selection through growth genes, one of which is the leptin gene. Polymorphisms in growth hormone coding and regulatory genes have great potential as genetic markers for phenotypic traits with high economic value (Utomo and Safitri, 2018).

Leptin comes from the Greek word leptos meaning "thin", this refers to the leptin gene. Leptin is a product of the OB gene that has a weight of 16 KD and codes for 167 amino acids. This gene has 3 exons and 2 introns, but only 2 exons are translated into proteins. The leptin gene is located on chromosome 4 of cattle, sheep, and goats and on chromosome 8 of river buffalo. This gene has endocrine effects in the brain and in various peripheral tissues as well as transducing autocrine/endocrine signals in tissues where it is expressed. This suggests that leptin is expressed in ruminant adipose tissue, fetus, breast, rumen, small intestine, and pituitary gland (Gregorio *et al*., 2014). Leptin is expressed proportionally to body fat, suppressing food intake and preventing low metabolism, thus promoting overall weight loss. Thus, this negative energy balance suppresses leptin levels, allowing for weight gain (Münzberg and Heymsfield, 2019).

The goat leptin gene is 4955 bp long with positions for exon 2 at 1 at 1128 bp, intron 2 at 1129 at 2967 bp, exon 3 at 2968 at 4955 bp, and coding sequences (CDS) at 985 at 1128 bp and 2968 at 3327 bp. manufactures leptin products (NCBI, 2022a). The use of bioinformatics tools in sequencing, gene search, and gene pattern search has been widely practiced. Bioedit (offline) and NCBI (online) are commonly used in DNA

sequence analysis. This program contains many easy-to-use features. Many studies on candidate genes in goats use BioEdit as a genetic analysis which includes DNA sequencing, detection of single nucleotide polymorphisms (SNPs), restriction enzyme analysis, and analysis of variation. DNA modification of amino acids (Latifah *et al*., 2020). The single nucleotide polymorphism (SNP) method was used as a genome mapping tool to determine the relationship between quantitative traits and DNA variants allowing livestock selection based on genetic markers or so-called selection support markers (MAS) (Sutikno *et al*., 2020). Study on genetic analysis of goat leptin gene to identify single nucleotide polymorphisms (SNPs), changes in amino acid composition, and phylogenetic tree of goat based on DNA sequence data for leptin gene from Bank gene. This study was a preliminary study that will be used for further studies, in particular, to study the correlation between the leptin gene SNP and quantitative traits in goats.

MATERIALS AND METHODS

Samples

A total of five CDS of the leptin DNA gene from five goats were extracted from NCBI GenBank data. Capra hircus DNA sequence used GenBank Acc. No. AM114392.2 (Garganita breed), GU944974.2 (Sirohi breed), JQ739232.1 (Barbari breed), JQ739233.1 (Beetroot breed) and MH716185 (Osmanabadi breed).

Genetic Analysis

The position of the sequence used in this study was the CDS. CDS Leptin gene data from GenBank (https://www.ncbi.nlm.nih.gov/) downloaded in fasta format. The leptin gene DNA sequence was aligned using ClustalW Multiple Alignment analysis included in the BioEdit version 7.2.5 program to identify SNPs in the goat leptin gene. Naming SNPs based on GenBank Acc. Number AM114397.2. Amino acid changes were also analyzed using BioEdit version 7.2.5. Phylogenetic trees were analyzed based on the leptin genes of goats and sheep. Reconstruction of the phylogenetic tree on the Leptin gene was performed using the Neighbor-Joining (NJ) method based on nucleotide sequences. Phylogeny based on nucleotides using the Clustal Omega ver.1.2.4 program (Medeira *et al*., 2022).

RESULTS AND DISCUSSION

The DNA sequences of leptin genes in different cultivars were aligned based on CDS position using BioEdit ver 7.2.5 (Figure 1). Based on the association results, five SNPs were found in the leptin gene (Table 1). SNP detection in Osmanabadi goats from India (GenBank Acc. MH716185 (NCBI, 2022b)) found SNPs g.17T/A, g.43T/A, g.74G/A, g.93C/A, and g. 386A/G. Studying the leptin gene in Bligon goats detected four SNPs at positions g.758G/A, g.864C/T, g.1171G/A, and g.1454G/A (Hartatik *et al*., 2020). In the study of Anugratama and Hartatik (2020), leptin gene SNPs in some DNA sample sequences from crossbred beef cattle, Sumba Ongole (*Bos indicus*), Brahman hybrid (*Bos indicus*), Bali cow (*Bos sondaicus*) and GenBank *Bos oxen* (U50365.1), *Bos indicus* (EU313203.1), *Bos grunniens* (EU603265.1), *Bos frontalis* (EU642566.1), *Bubalus bubalis* (AH013754.2), *Capra hircus* (JQ739232.1, JQ7392233.1, GU944974.2), *Ovis aries* (HE605296.1), eight SNPs were detected in exon 2: g.1120C→T, g.1128T→C, g.1130G→A, g.1155G→C, g.1158T→C, g.1180C→H, g.1181G→A, g.1218A→G, based on the sequence alignment results, 8 SNPs were found in the CDS region. Three SNPs (128G/T, 270C/T, and 166T/C) were also found in goat breeds from southwest China (White Chuandong, Black Jintang, Banjiao, Black Dazu, White Guizhou, Nanjiang Huang, Chengdu Ma), Gullin Ma, Matou, and Boer goat) can then be used in selective support programs in goat production (Wang *et al*., 2011).

Study on the location of 170G→A leptin gene polymorphism affected sperm motility and scrotal circumference, and 332G→A polymorphism location affected sperm viability and affected Sanjabi ram's dermis circumference, thus the potential association of leptin gene

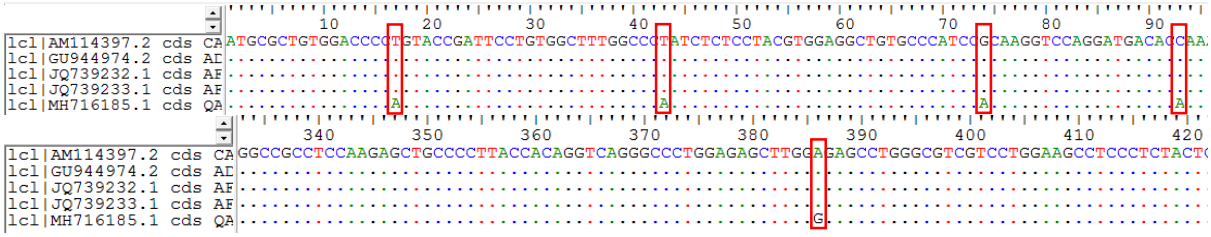

Figure 1. Goat leptin sequence alignment.

Table 1. Five SNPs of the leptin gene in goats based on GenBank

SNPs	Location	DNA sequence reference
g.17T/A g.43T/A g.74G/A g.93C/A g.386A/G	CDS	AM114397.2

Table 2. Amino acid changes based on SNPs

SNPs	Amino Acid Change
g.17T/A	AM114397.2 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 GU944974.2 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 JQ739232.1 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 JQ739233.1 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 MH716185.1 1 ATG CGC TGT GGA CCC CAG TAC CGA TTC CTG TGG CTT TGG CCC AAT 45 1 Met Arg Cys Gly Pro Gln Tyr Arg Phe Leu Trp Leu Trp Pro Asn 15
g.43T/A	AM114397.2 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 GU944974.2 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 JQ739232.1 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 JQ739233.1 1 ATG CGC TGT GGA CCC CTG TAC CGA TTC CTG TGG CTT TGG CCC TAT 45 1 Met Arg Cys Gly Pro Leu Tyr Arg Phe Leu Trp Leu Trp Pro Tyr 15 MH716185.1 1 ATG CGC TGT GGA CCC CAG TAC CGA TTC CTG TGG CTT TGG CCC AAT 45 1 Met Arg Cys Gly Pro Gln Tyr Arg Phe Leu Trp Leu Trp Pro Asn 15
g.74G/A	AM114397.2 46 CTC TCC TAC GTG GAG GCT GTG CCC ATC CGC AAG GTC CAG GAT GAC 90 16 Leu Ser Tyr Val Glu Ala Val Pro Ile Arg Lys Val Gln Asp Asp 30 GU944974.2 46 CTC TCC TAC GTG GAG GCT GTG CCC ATC CGC AAG GTC CAG GAT GAC 90 16 Leu Ser Tyr Val Glu Ala Val Pro Ile Arg Lys Val Gln Asp Asp 30 JQ739232.1 46 CTC TCC TAC GTG GAG GCT GTG CCC ATC CGC AAG GTC CAG GAT GAC 90 16 Leu Ser Tyr Val Glu Ala Val Pro Ile Arg Lys Val Gln Asp Asp 30 JQ739233.1 46 CTC TCC TAC GTG GAG GCT GTG CCC ATC CGC AAG GTC CAG GAT GAC 90 16 Leu Ser Tyr Val Glu Ala Val Pro Ile Arg Lys Val Gln Asp Asp 30 MH716185.1 46 CTC TCC TAC GTG GAG GCT GTG CCC ATC CAC AAG GTC CAG GAT GAC 90 16 Leu Ser Tyr Val Glu Ala Val Pro Ile His Lys Val Gln Asp Asp 30

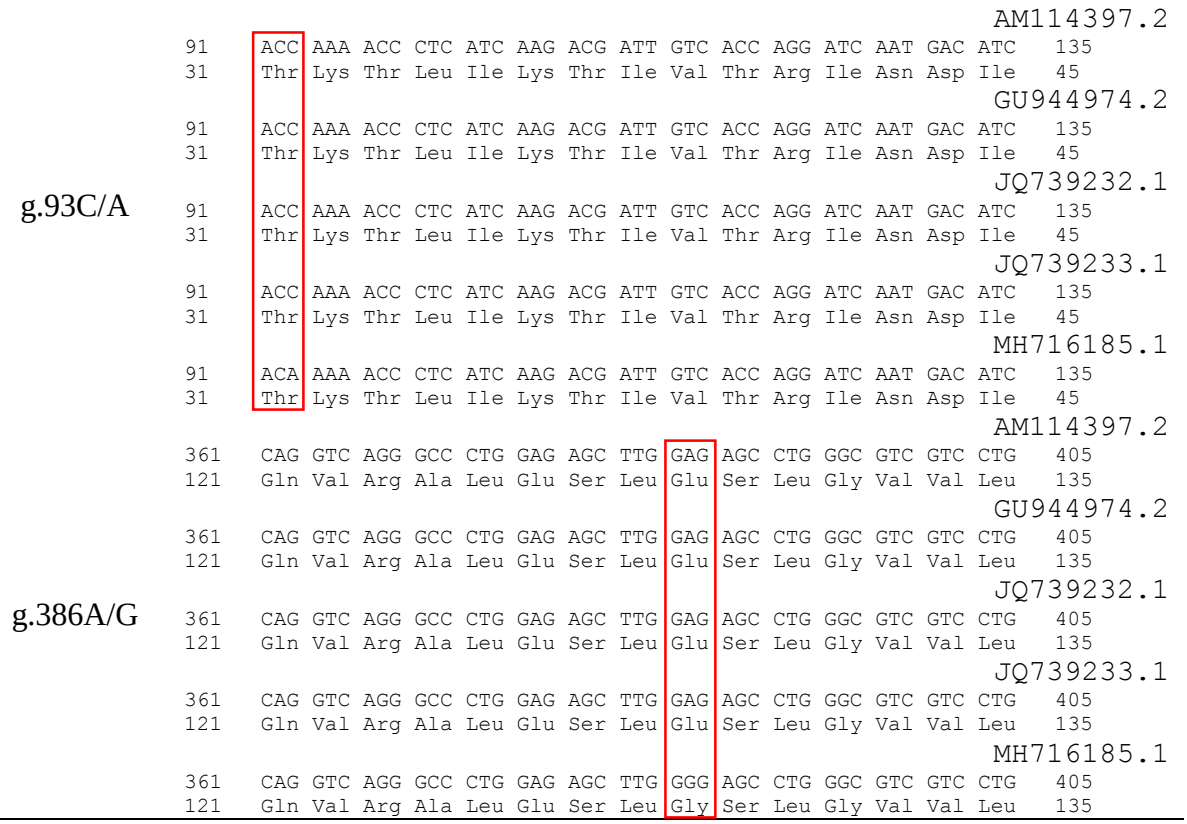

Figure 2. Phylogenetic tree of the leptin gene in goats.

polymorphisms on testicular size and sperm fertility could be used in a breeding program to increase male fertility (Bakhtiar) *et al*., 2017). Meanwhile, the study of LEP C528T and LEP C73T polymorphisms affects fat formation levels in Aberdeen Angus heifers and first calves. Maximum internal fat weight, back fat thickness, and maximum fat content of longissimus dorsi muscle in heifers heterozygous for the LEP C528T and LEP C73T polymorphisms (Dzhulamanov *et al*., 2022). A study using Madrasin cattle (a cross between Madura cattle and Limousin cattle) amplified the leptin gene by polymerase chain reaction (PCR) and determined the fragment length-cutting polymorphism by restriction enzyme l BsaA1, at position 2793 with the ACGT dot position, the results of polymorphism of the leptin gene divided into three bands were obtained. i.e. AA with one fragment (522 bp), CG with two fragments (441 bp and 81 bp), and AG with three fragments (522 bp, 441 bp, and 81 bp) (Utomo *et al*., 2021).

Four SNPs in the CDS region are missense mutations and 1 SNP is a silent mutation (Table 2). 4 SNPs are missense mutations, including SNPs g.17T/A, which code for a different amino acid, namely leucine (CTG) to glutamine (CAG), SNPs g.43T/A, which code for amino acid tyrosine (TAT) to asparagine (AAT) coding, SNPs g.74G/A encode amino acid arginine (CGC) in histidine (CAC), SNPs g.386A/G encode amino acid glutamic acid (GAG) in glycine (GGG), while SNPs are silent mutations, SNPs.. g.93C / Code for the same amino acid, namely threonine (ACC/ACA). Amino acid changes were determined using BioEdit version 7.2.5. A study by Wang *et al*. (2011) stated that the G/T mutation occurs in exon 13: C/T and T/C are located in exon 18 with no amino acid changes. It is assumed that any mutation in the gene affects gene expression, the rate and

regulation of gene transcription, or the amino acid sequence of the leptin gene product (Alim *et al*., 2019).

SNPs that result in amino acid mutations can affect certain productivity in livestock. Studies on Tyrosine Kinase (TEK) gene polymorphisms show that the relationship between semen concentration and mutations is not unidirectional, meaning that the higher the cement concentration, the fewer mutations will occur, on the contrary, the lower the semen concentration, the more mutations will increase (Hernawati *et al*., 2021). Mutations or base changes in the FSHR gene in Madrasin cattle cause changes in the amino acid serine to glycine (Utomo, 2018). The g.10054T>C polymorphism of the Osteopontin Promoter gene in Holstein Friesian Breeds caused mutations that correlated with post-thawing semen quality. Thymine (T) base change to Cytosine (C) is a transition mutation. Transition mutations are mutations that occur when a pyrimidine base in a DNA nucleotide chain is replaced by another pyrimidine base or a purine base by another purine base (Hernawati *et al*., 2019).

Phylogenetic tree formation in candidate genes has been performed extensively in goats (Hartatik *et al*., 2019; Jiyanto *et al*., 2014). The leptin gene was also analyzed in a phylogenetic tree (Vazir *et al*., 2020). In this study, a phylogenetic analysis of the DNA sequences of the leptin gene was performed in 5 goat breeds registered at the NCBI GenBank. Based on Figure 2, the Garganica goat breeds (AM114397.2), Sirohi goat breeds (GU944974.2), and Barbarian goat breeds (JQ739232.1) belong to the same clade, while the Beetal goat breeds (JQ739233.1) and Osmanabadi Goat (MH716185.1) is on another branch.

CONCLUSION

Based on NCBI GenBank data, five SNPs (SNPs g.17T/A, g.43T/A, g.74G/A, g.93C/A, and g.386A/G) were found in the leptin gene. SNPs are a missense mutation and a silent mutation. The analysis of the phylogenetic tree of the leptin gene showed that there are three breeds of goats in one branch and two breeds of goats in different branches.

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