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Short Communication: Identification of Leptin gene in crossbred beef cattle

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Abstract. *Anugratama LE, Hartatik T. 2020. Short Communication: Identification of Leptin gene in crossbred beef cattle. Biodiversitas 21: 226-230.* Leptin is a gene that affects animal weight. Leptin gene is known to control body weight, feed intake, energy expenditure, immune function, and reproduction. This study aims to identify the diversity of the Leptin gene in crossbred beef cattle, Sumba Ongole cattle, Brahman cross cattle, Bali cattle, buffalo, sheep, and goat by comparing with four GenBank data of cattle. Crossbred beef cattle obtained from Klaten, Central Java, Indonesia. Leptin nucleotide sequences were analyzed using BioEdit to identify Single Nucleotide Polymorphism (SNP). To create amino acid change in Leptin gene, the coding sequence of exon 2 was established using BioEdit ver. 7.0.5. Phylogenetic tree and genetic distance have been analyzed based on the Leptin gene using MEGA 10.1.1 program. The result shows that eight variations of SNP were found in exon 2. The phylogenetic tree represents that crossbreed beef cattle, Sumba Ongole cattle, Brahman cross cattle, Bali cattle, *Bos taurus*, *Bos indicus*, *Bos frontalis*, *Bos grunniens*, *Bubalus bubalis* are in the same cluster with various genetic distance. The results of this study are expected to provide genetic information that will be used for further research on the relationship between Leptin gene polymorphisms to animal weight.

Keywords: GenBank, Leptin gene, phylogenetic, single nucleotide polymorphism

Abbreviations: DNA: Deoxyribo Nucleic Acid, MAS: Marker-Assisted Selection, mRNA: messenger Ribonucleic Acid, NCBI: National Center for Biotechnology Information, SNP: Single Nucleotide Polymorphism

INTRODUCTION

One of the genes that affect animal weight is the Leptin / LEP gene (Hernandez et al., 2016). Leptin is a hormone that is produced in white adipose tissue and secreted into the bloodstream as a 16 KD protein. It plays important roles in the control of body weight, feed intake, energy expenditure, immune function and reproduction (Fruhbeck et al., 1998). The versatile hormone Leptin is one of the key factors regulating meat quality traits. Numerous studies show close relationships between Leptin gene polymorphisms and carcass and meat quality traits of beef cattle (Buchanan et al., 2002). Leptin single nucleotide polymorphisms (SNPs) in cattle have been significantly associated with serum leptin concentration as well as with backfat thickness, marbling score and live and hot weight at slaughter (Nkrumah et al., 2005). Chung et al. (2008) have also shown a close relationship between Leptin gene polymorphisms and carcass and meat quality traits of Korean beef cattle. Markers in the Leptin gene are already part of commercial genotyping panels designated for Marker-Assisted Selection (MAS) in beef cattle. Barzehkar et al. (2009) have reported associations of A113G polymorphism in the Leptin gene with marbling in Iranian sheep. Buchanan et al. (2002) reported changes in the mutation of the C1180T nucleotide sequence that caused changes in the coding of proteins from Arginine to Cysteine (Arg25Cys), these changes affect the physiological function of Leptin in the metabolic process.

To identify the diversity of Leptin gene can use bioinformatics. Bioinformatics can be defined as the application of computer technology to the management of biological information. Bioinformatics is the science of storing, extracting, organizing, analyzing, interpreting and utilizing information from biological sequences and molecules. Bioinformatics tools that can be used for analysis Leptin gene such as sequence alignment, multiple sequence alignment, gene finding, protein domain analysis, pattern identification, genomic analysis, motif finding (Raza 2012). The program that usually uses deoxyribonucleic acid (DNA) alignment and compares two or more sequences is the BioEdit program (offline) and Mega 10. Mega 10 also can be used to construct phylogenic tree and genetic distance.

This study aims to identify the diversity of the Leptin gene in crossbred beef cattle (Bos Taurus x Bos indicus), Bali cattle (*Bos sondaicus* syn. *Bos javanicus javanicus*), Sumba Ongole (*Bos indicus*), Brahman cross (*Bos indicus*), buffalo, sheep, and goat by comparing the DNA sequence of Leptin gene from DNA samples of this study and GenBank data. The results of this study are expected to provide genetic information that will be used for further research on the relationship between Leptin gene polymorphisms to animal growth, feed conversion efficiency, and health.

MATERIALS AND METHODS

Study area

This research was done at the Laboratory of Genetic and Animal Breeding, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. The study was conducted from April to August 2019. Analysis of sequencing was performed at LPPT, Universitas Gadjah Mada. The materials used in this study were blood samples of crossbred beef cattle, Sumba Ongole cattle, Brahman cross cattle, Bali cattle and nine sequences of Leptin gene from *Bos taurus* (U50365.1), *Bos indicus* (EU313203.1), *Bos grunniens* (EU603265.1), *Bos frontalis* (EU642566.1), *Bubalus bubalis* (AH013754.2), *Capra hircus* (JQ739232.1, JQ739233.1, GU944974.2), *Ovis aries* (HE605296.1) which is obtained from NCBI GenBank data.

Procedures

Sample collection

Five blood samples were collected from crossbred beef cattle, one sample from Sumba Ongole cattle, and three samples from Bali cattle. The blood samples were taken from the jugular vein using venoject connected to a vacutainer containing an anticoagulant K_3EDTA. K_3EDTA serves to prevent blood clots from forming in the vacutainer. The samples were stored in a freezer at -20ºC.

DNA extraction

Isolation of DNA was performed using Extraction Kit (Geneaid, Taiwan) in the Laboratory of Genetic and Animal Breeding, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

DNA amplification and sequencing

Five genomic DNA samples from Crossbred beef cattle, one sample from Sumba Ongole cattle, and three samples from Bali cattle were amplified used PCR method to get targeted sequence. The primer that used for PCR amplification of targeted sequence of Leptin gene is forward F: 5'-AGCGGTTATGGGATATGCC-3' and reverse is R: 5'-AATGCCCCAAGAGACACTGA-3' with 961 bp DNA fragments gene located in the part of intron 1, exon 2, and part of intron 2. Polymerase Chain Reaction performed in a total reaction of 25,5 µl, containing 12,5 µL PCR Kit (KAPA BIOSYSTEMS, USA), 10 µL aquabidest, 1.5 µL of DNA, 1.5 µL of both forward and reverse primers. The reactions were performed using a thermal cycle (PEQLAB Primus 25 advanced, Germany) with a pre-denaturation temperature at 94ºC for 1 minute, followed by 30 cycles of reaction; denaturation at 94°C for 1 minute, annealing at a temperature of 57°C for 1 minute and extension at 72°C for 1 minute, then the last step was a final extension at 72°C for 5 minutes. The quality of the PCR product was determined using gel electrophoresis (1%), the thick and DNA bands were the preferred result (Fathoni et al., 2018). The targeted sequence was sequencing analyzed by LPPT UGM.

Comparison analysis

Single nucleotide polymorphism identification, comparison sequences, and amino acid change were performed using BioEdit software. A total of 18 sequences of Leptin gene (four Crossbred beef cattle, one sample Sumba Ongole cattle, one sample Brahman cross cattle, three sample form Bali cattle and nine GenBank sequences were aligned using ClustalW on BioEdit ver. 7.0.5 to reveal the SNPs and to perform the amino acid change. Phylogenetic tree and genetic distance have been analyzed based on the Leptin gene. The genetic distance and phylogenetic tree were displayed based on Kimura method (Kimura, 1980) using Mega 10.1.1 program.

RESULTS AND DISCUSSION

The target DNA in this study was Leptin gene fragment in the intron 1, exon 2, and intron 2 regions based on GenBank acc no. U50365.1, but only exon 2 was analyzed for identification of amino acid change in crossbred beef cattle (Figure 1, Table 2). Exon 2 was located at sequence no 1108 to 1251 of GenBank acc no. U50365.1. This study was the first to identify SNPs in the Leptin gene on several sequences from DNA sample of crossbred beef cattle, Sumba Ongole cattle (*Bos indicus*), Brahman cross cattle (*Bos indicus*), Bali cattle (*Bos sondaicus*), and GenBank of *Bos taurus* (U50365.1), *Bos indicus* (EU313203.1), Bos grunniens (EU603265.1), *Bos frontalis* (EU642566.1), *Bubalus bubalis* (AH013754.2), *Capra hircus* (JQ739232.1, JQ739233.1, GU944974.2), *Ovis aries* (HE605296.1) sequences were aligned using BioEdit program. As the result, eight SNPs were identified in exon 2 region: g.1120 C>T, g.1128 T>C, g.1130 G>A, g.1155 G>C, g.1158 T>C, g.1180 C>H, g.1181G>A, g.1218 A>G. The SNPs presented in Table 1. Four individual genotype of crossbred beef cattle, Sumba Ongole, and Bali cattle base on sequence analysis has been submitted to the GenBank with the accession number MN709606 (Sumba Ongole, SO_01), MN709607 (Brahman cross, BX392), MN 709608 (Belgian Blue F2020), and MN709609 (Bali cattle, B2013-160).

Based on the result of sequence alignment, 8 SNPs have been found in the CDS region. One SNP was heterozygous for Sumba Ongole and crossbred beef cattle at SNP g.1180 C>Y. Single nucleotide polymorphism g.1180 C>Y was identified by the presence of "double peak". The SNP was resulting in two alleles, C and T (Figure 2). Single nucleotide polymorphism can cause changes in amino acids. These changes can be either silent or missense. A silent mutation occurs if the SNP only changes the DNA but does not change the amino acid. Missense mutation will occur if the SNP not only change the DNA but also an amino acid. The results of this study found 4 SNPs were silent and 4 SNPs missense which can be seen in Table 2.

AGCGGTTATGGGATATGCCTGCAGTCGTACAGCTATTAAATGTCTGGATTCAAACCAGACCTTGAAAGCCC
GCCGTCCACCCGCTCGTGCCCTGGCTCACTGCTGCGTGGTCTACAGCACACCTCCTGTGGTTTTCTTGATT
CCGCCGCACCTCTCCCCAGGGGAGTGCCTTTCATTACTGTCATTTCTAGACAATGAATTGTCTTTGAGGAG
ATGATAGCCATGGCAGACAGCAAATCTCGTTGTTATCCGCATCTGAAGACCTGGATGCGGGTGGTAACGGA
GCACGTGGGTGTTCTCGGAGATCGACGATGTGCCACGTGTGGTTTCTTCTGTTTTCAGGCCCCAGAAGCCC
ATCCCGGGAAGGAAAATGCGCTGTGGACCCCTGTATCGATTCCTGTGGCTTTGGCCCTATCTGTCTTACGT
GGAGGCTGTGCCCATCYGCAAGGTCCAGGATGACACCAAAACCCTCATCAAGACAATTGTCACCAGGATCA
ATGACATCTCACACACGGTAGGGAGGGACTGGGAGACGAGGTAGAACCGTGGCCATCCCGTGGGGGACCCC
AGAGGCTGGCGGAGGAGGCTGTGCAGCCTTGCACAGGCCCCAGTGGCCTGGACGCCCCCCTGGCATAAAGA
CAGCTCCTCTCCTCCTCCACTTCCCTTGCCTCCCGCCTTCTCACTCTCCTCCCTCCCAGACCGGAATCCTA
GTGCCCAGGCCCAGAAGGAGTCACAGAGGTCCTGGGGTCCCCTTGGCAGGTGGCCAGAACCCCAGCAGCAG
TCCCTCTGGGCCTCCATCTCATTTCTAGAATGTTTTAGTCGTTAGGCATTCTTCCTGCCTGGTAACTGAGC
TTAGACCCTGCGAGCTCATTACTCATTACTGCCAGCCCTGCCTGTCAAGCCCTCTTCAGATACAACCCTCT
GTGTTTTTGTAAATAGTTATCAGTGTCTCTTGGGGCATT

Figure 1. Sequence target of Leptin gene in crossbred beef cattle. (Gray shadow=exon 2, H=C/T/A)

Table 1. Alignment of Leptin gene in *Bos taurus, Bos indicus, Bos grunniens, Bos frontalis, Bubalus bubalis, Capra hircus, Ovis aries,* Brahman cross cattle, Sumba Ongole cattle, Bali cattle, crossbred beef cattle

SNP (U50365.1)	*Bos taurus*	*Bos indicus*	*Bos grunniens*	*Bos frontalis*	*Bubalus bubalis*	*Capra hircus*	*Ovis aries*	Brahman cross cattle (*Bos indicus*)	Sumba Ongole (*Bos indicus*)	Bali cattle (*Bos sondaicus*)	Crossbred beef cattle
1120 C>T	C	C	T	C	C	C	C	C	C	C	C
1128 T>C	T	T	T	T	C	C	C	T	T	T	T
1130 G>A	G	G	G	G	A	G	G	G	G	G	G
1155 G>C	G	G	G	G	G	C	C	G	G	G	G
1158 T>C	T	T	C	C	C	C	C	T	T	T	T
1180 C>H	C	C	A	A	C	C	C	C	Y	C	Y
1181 G>A	G	G	G	G	G	G	G	G	G	A	G
1218 A>G	G	G	G	G	G	G	G	G	G	A	G

Note: A is Adenin, C is Cytosine, G is Guanine, T is Thymin, Y is pYrimidine that base (C/T), H that base (C/T/A)

Table 2. Amino acid analysis in CDS exon 2 of Leptin gene

SNP (U50365.1)	Codon	Amino Acid	Mutation
1120 C>T	CCC	Proline (Pro)	Missense
	TCC	Serine (Ser)	
1128 T>C	TAT	Tyrosine (Tyr)	Silent
	TAC	Tyrosine (Tyr)	
1130 G>A	GGA	Arginine (Arg)	Missense
	CAA	Glutamine (Gln)	
1155 G>C	CTG	Leucine (Lys)	Silent
	CTC	Leucine (Lys)	
1158 T>C	TCT	Serine (Ser)	Silent
	TCC	Serine (Ser)	
1180 C>H	CGC	Arginine (Arg)	Missense
	YGC	Arginine/Cysteine	
	AGC	Serin (Ser)	
	TGC	Cysteine (Cys)	
1181 G>A	CGC	Arginine (Arg)	Missense
	CAC	Histidine (His)	
1218 A>G	ACA	Threonine (Thr)	Silent
	ACG	Threonine (Thr)	

Note: Coding Sequences (CDS) are sequences of DNA that encode proteins from the start codon to the stop codon. This study only identifies CDS because mutations that occur in CDS can cause changes in amino acids. Changes in amino acids affect the phenotype.

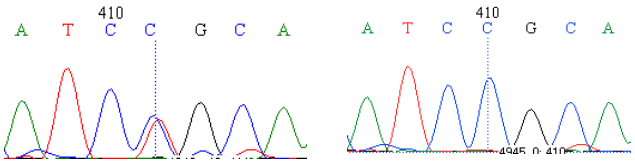

Figure 2. The Electropherogram indicated the polymorphism of Leptin gene in crossbred beef cattle (SNP g. 1180 C>Y). Heterozygote CT (*left*) and homozygote CC (*right*). Homozygote TT was not available in crossbred beef cattle.

Missense mutation SNPs founded on g. 1120 C>T, g. 1130 G>A, g. 1180C>H, g. 1181G>A. The first SNP g. 1120 C>T changes amino acid from Proline to Serine. The second SNP g. 1130 G>A has changed the amino acid Arginine to Glutamine. The third SNP g. 1180 C>H changes the amino acid Arginine to Cysteine or Serin. The fourth SNP g.1181 G>A also a missense mutation that changes the amino acid from Arginine to Histidine

A phylogenetic tree is a diagram that represents evolutionary relationships among organisms. The phylogenetic tree was constructed based on the Kimura 2-parameter model with 1,000 bootstrap replications (Figure 3). As a result of Figure 3, it showed that there were 2 big groups. The Crossbred beef cattle, Sumba Ongole cattle, Brahman cross cattle, Bali cattle, *Bos frontalis*, *Bos grunniens* and Buffalo were clustered in one big group, whereas the sheep and goat in other clusters. The cattle groups were separated from sheep and goats.

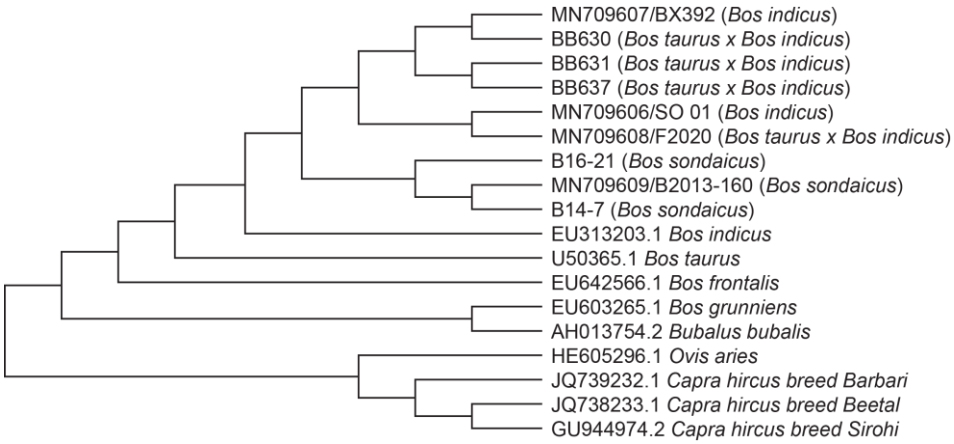

Figure 3. Phylogenetic tree based on Leptin gene

Discussion

Total of 8 SNPs was presented in Table 1. There were only 4 SNPs that affect the encoded amino acid. SNPs g. 1120 C>T (Pro/Ser), g. 1130 G>A (Arg/Gln), g. 1180 C>H (Arg/Cys/Ser), and g. 1181G>A (Arg/His) was confirmed by BioEdit. SNP g. 1180 C>Y in crossbred beef cattle was confirmed by electropherogram (Figure 2). It has two alleles C and T. Hernández et al. (2016); Orrù et al. (2011); and Fathoni et al. (2018) also reported that in Brahman; Simmental bulls; and Kebumen Ongole Grade cattle has SNP g. 1180 C>T (Arg/Cys). Fathoni et al. (2018) reported a change in mutation g. 1180 C>T has significantly associated with high weaning chest circumference in Kebumen Ongole Grade cattle. Javanmard et al. (2010); Buchanan et al. (2002); Konfortov et al. (1999) also reported SNP g. 1180 C>T change amino acid from arginine to cysteine. Buchanan et al. (2002) reported SNP g.1180 C>T has significant associations between the SNP genotype and carcass fat levels in cattle. Schenkel et al. (2005) reported that this SNP was significantly associated with carcass quality and composition in cattle. Furthermore, Leptin gene had been reported to have an effect on body weight and body size in Chinese cattle (Yang et al., 2007), growth traits in Nellore cattle (Silva et al., 2013).

The phylogenetic relationship of the Leptin gene (Figure 3) explained that the crossbred beef cattle, which belonged to BB630, BB631, BB637, F2020 (MN709608) has a close relationship, Brahman cross cattle (BX392) with GenBank no MN709607, Sumba Ongole cattle (SO 01) with GenBank no MN709606 and Bali cattle (B14-7, B2013-160 [genbank no MN709609], B16-21). The constructed tree demonstrated that our studied sample (Crossbred beef cattle, Brahman cross cattle, Sumba Ongole cattle, and Bali cattle) have a close relationship with *Bos indicus* than *Bos taurus.* Also, it showed that the studied sample has a very far relationship with *Bos frontalis*, *Bos grunniens*, and Buffalo. The sheep and goats were out of the group of cattle.

Finally, this study has provided information regarding the identification of the Leptin gene in Crossbred beef cattle. Also, we performed a phylogenetic analysis of all samples with other cattle and goat/sheep breeds. 8 SNPs have found in our study, but only 5 SNPs were missense mutations. SNPs g. 1120 C>T (Pro/Ser), g. 1130 G>A (Arg/Gln), g. 1180 C>H (Arg/Cys/Ser), and g. 1181G>A (Arg/His) may have potential as a Marker-Assisted Selection (MAS) because missense mutations may have affect to traits of cattle. Crossbred beef cattle were distantly related to *Bos taurus*, *Bos frontalis*, and Buffalo. The Crossbred beef cattle may have a unique gene pool compared to these mentioned breeds, so it very interesting for future research.

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