This is an open-access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.112

Diversity of Leptin Gene Exon 2 in Pesisir Cattle using Sequencing Method

Yurnalis[2], Tinda Afriani[1]*, Jaswandi[2], Adisti Rastosari[1] and Mylaufa Asyraf

[1]Departement of Livestock Production, Faculty of Animal Science, Andalas University, Padang, 25163 West Sumatera, Indonesia
[2]Departement of Biotechnology Reproduction, Faculty of Animal Science, Andalas University, Padang, 25163 West Sumatera, Indonesia
***Corresponding author:** tindaafriani@ansci.unand.ac.id

Article History: 23-298 Received: 19-Sep-23 Revised: 28-Oct-23 Accepted: 29-Oct-23

ABSTRACT

This research aims to determine the genetic diversity of Leptin exon 2 and intron 2 in Pesisir cattle using sequencing methods. The samples used in this study consist of 96 blood samples from Pesisir cattle raised at BPTU Padang Mengatas, Payakumbuh, West Sumatra. Blood samples were isolated using the genomic DNA Purification Kit (Promega) protocol. The isolated DNA was then amplified using a pair of forward primers 5'- ATC TGA AGA CCT GGA TGC GG -3' and reverse primers 5'- TGG TTG CAG GTC TGA GCT TA -3' resulting in a Leptin gene exon 2 and intron 2 fragment with a length of 744 bp. Then the amplification products were sent for sequencing with the assistance of 1st Base Singapore's services. The research results indicate that there are 13 diversities in the intron 2 region of the Pesisir cattle samples, with the discovery of 12 mutations occurring at positions +327 G>A, +398 C>T, +399 A>G, +439 C>T, +441 C>A, +547 G>C, +565 C>G, +622 G>A, +629 C>T, +647 C>T, +693 T>C, +712 G>T and 1 deletion at position +751 G>del. In the Hardy-Weinberg equilibrium test, there are 8 mutations that are in Hardy-Weinberg equilibrium, which are at positions +327, +399, +439, +547, +565, +622, +693, +712 while 4 mutations are not in Hardy-Weinberg equilibrium, which are at positions +398, +441, +629 dan +647. In this research, a check of restriction enzymes revealed the presence of 12 restriction enzymes that can be used in studies utilizing the PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) method.

Key words: Pesisir cattle, Leptin gene, Sequencing, Diversity.

INTRODUCTION

Local cattle play a pivotal role in enhancing economic progress, creating employment opportunities, and meeting the demand for animal protein. Local cattle also contribute to the agricultural enterprise system and have been traditionally maintained by farmers. Pesisir cattle, including one of the local cattle breeds, are widely distributed in the South Coastal region of West Sumatra Province, Indonesia. Pesisir cattle possess remarkable attributes, including exceptional body resilience and adaptability to their environment, which confer distinct advantages compared to other cattle breeds (Zaituni et al. 2022). These cattle have a small-sized body compared to other cattle breeds such as PO cattle, Bali cattle, Madura cattle, and Aceh cattle. Despite their small size, these cattle exhibit a relatively high carcass percentage.

The selection occurring in Pesisir cattle is oriented towards negative selection, where there is a tendency for farmers to retain cattle with smaller body sizes. Meanwhile, cattle with larger bodies are sold at higher prices. The high demand for Pesisir cattle is primarily due to the upcoming Muslim festival of Eid al-Adha. Wang et al. (2020) stated that enhancing both the quantity and quality of meat production has consistently been the central objective of breeding in the cattle industry, and the animal's genotype is another critical element in assessing effective fattening for cattle production. Scientific evidence suggests that a diverse array of candidate genes has a profound impact on both the fattening performance and carcass characteristics of cattle (Ardicli et al. 2019). There is a need for genetic improvement programs that have the potential to enhance productivity and increase the population of Pesisir cattle. However, information regarding Pesisir cattle, particularly their biological and genetic aspects, remains limited.

The advancing technology in the field of molecular genetics allows for selection at the DNA level. Molecular livestock selection is carried out by evaluating the nucleotide sequence profiles of genes within DNA that

Cite This Article as: Yurnalis, Afriani T, Jaswandi, Rastosari A and Asyraf M, 2024. Diversity of leptin gene exon 2 in Pesisir cattle using sequencing method. International Journal of Veterinary Science 13(4): 427-434. https://doi.org/10.47278/journal.ijvs/2023.112

influence livestock productivity, one of which is the Leptin gene. The Leptin gene produces the Leptin hormone synthesized by adipose (fat) tissue. Anugratama and Hartatik (2020) stated that the Leptin gene markers are already included in genotyping panels available for Marker-Assisted Selection (MAS) in the beef cattle industry. The Leptin gene stands out as a significant genetic factor with economic potential, making it a viable candidate for use as a genetic marker in Marker Assisted Selection (MAS) for the direct identification and selection of breeding stock (Sharifzadeh and Doosti 2010).

Based on GenBank (accession number: NC_037331.1), the Leptin gene is located on chromosome 4 and has a length of 16,824 base pairs (bp), consisting of 3 exons and 2 introns. Exon 1 is 104 bp long, intron 1 is 12,044bp long, exon 2 is 172bp long, intron 2 is 1,760bp long, and exon 3 is 2,744bp long. Taniguchi et al. (2002) explained that the Leptin gene consists of 167 amino acids and has a molecular weight of 16kDa. Leptin / LEP Gene is one of the genes that affect animal weight (Hernandez et al. 2016). The target DNA amplified in this study is a fragment of the Leptin gene located in the region of exon 2 and intron 2.

Several studies have been conducted to identify genetic diversity using the Leptin gene in the exon 2 and intron 2 regions. Kong et al. (2006) reported Leptin gene polymorphism in exon 2 associated with backfat thickness in Hanwoo cattle. Furthermore, Lagonigro et al. (2003) found Leptin gene polymorphism in exon 2 associated with feed intake in Hereford and Aberdeen Angus cattle. Kaygisiz et al. (2011) reported Leptin gene polymorphism in exon 2 associated with chest circumference in Brown Swiss cattle. Hilmia et al. (2015) also reported that genetic diversity in Leptin exon 2 is related to fat deposition, which can affect meat quality in local cattle in Ciamis. Meanwhile, Leptin gene polymorphism in the intron 2 region has been reported to be associated with body weight in Sistani cattle (Nobari et al. 2010). Oprzadek et al. (2003) reported Leptin gene polymorphism in intron 2 associated with carcass weight in Friesian Holstein cattle. Mandefro et al. (2021) reported that the Single Nucleotide Polymorphism (SNP) associated with a missense mutation found in exon 2 has the potential to serve as a marker for enhancing the selection and overall improvement of livestock production and productivity.

Molecular genetic techniques that can be employed to identify gene diversity (polymorphism) include DNA sequencing. Sequencing is a method used to determine the sequence of nucleotide bases such as adenine, thymine, guanine and cytosine in a DNA sequence or sequence. This sequence contains fundamental information of a gene as it contains the instructions required for the formation of living organisms. The aim of this study is to assess the genetic diversity of Leptin exon 2 and intron 2 in Pesisir cattle using sequencing methods.

MATERIALS AND METHODS

The materials used in this study consisted of 96 blood samples from Pesisir cattle, comprising 74 females and 22 males, which were raised at Balai Pembibitan Ternak Unggul (BPTU) Padang Mengatas, Payakumbuh City, West Sumatra, Indonesia.

DNA Isolation

The isolation of DNA was performed from blood samples using the Genomic DNA Purification Kit from Promega. To visualize the results, the isolated DNA was subjected to electrophoresis (Thermo Scientific) on a 1% agarose gel.

Leptin Gene Amplification (PCR)

The primers used to amplify the Leptin gene in this study were a pair of primers, Forward 5'- ATC TGA AGA CCT GGA TGC GG -3' and Reverse 5'- TGG TTG CAG GTC TGA GCT TA -3', with a primer length of 744bp (Yurnalis 2015). These primers were designed using Primer3 based on the DNA sequence information of the Leptin gene in GenBank (accession number: NC_037331.1), as shown in Fig. 1.

The DNA amplification procedure commenced with the addition of 2µL of DNA sample, 3µL of a mixture of reverse and forward primers, 12.5µL of master mix, and 7.5µL of Nuclease-Free Water into a PCR tube. Subsequently, the PCR machine was programmed with the following steps: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 45s, annealing at 58°C for 45s, initial extension at 72°C for 1min, and final extension at 72°C for 5min. This process aims to amplify the target DNA, making it suitable for further analysis.

To analyze the results of Leptin gene amplification, electrophoresis was conducted using 1.5% agarose gel (ThermoSCIENTIFIC® TopVision Agarose #R0491) stained with Ethidium Bromide Solution (MP Blomedicals®), and the outcomes were observed using a UV transilluminator (SynGENE® G:BOX). The success of Leptin gene amplification was deemed achieved when bands appeared at positions or sizes corresponding to the target length of 744 bp on the agarose gel. This determination was made by comparing the positions of the formed bands with those of the DNA ladder. Subsequently, the visualization results from electrophoresis were documented using a camera. The amplified Leptin gene products were purified before being sent to Singapore for sequencing.

Data Analytics

The acquired data were then processed. Genotype and phenotype frequencies were determined using the Nei and Kumar (2000) formula. The Hardy-Weinberg test was performed using a chi-square test.

RESULTS AND DISCUSSION

DNA Isolation

The isolation of DNA from 96 blood samples of Pesisir cattle was subjected to electrophoresis using 1% agarose gel and visualized using a UV Transilluminator, resulting in the outcomes shown in Fig. 2.

Based on Fig. 2, it can be observed that Pesisir cattle blood samples exhibit DNA fragments that appear clean and distinct within the agarose wells. The DNA isolation results, showing thicker and brighter DNA, indicate better quality DNA obtained. If the DNA isolation results in thinner DNA, the quality may be somewhat compromised, but the DNA can still be utilized in the Polymerase Chain Reaction (PCR) process.

```
ATGAATTGTC TTTGAGGAGA TGATAGCCAT GGCAGACAGC AAATCTTGTT GTTATCCGCA
TCTGAAGACC TGGATGCGGG TGGTAACGGA GCACGTGGGT GTTCTCGGAG ATCGACGATG
TGCCACGTGT GGTTTCTTCT GTTTTCAGGC CCCAGAAGCC CATCCCGGGA AGGAAAATGC
GCTGTGGACC CCTGTATCGA TTCCTGTGGC TTTGGCCCTA TCTGTCTTAC GTGGAGGCTG
TGCCCATCTG CAAGGTCCAG GATGACACCA AAACCCTCAT CAAGACAATT GTCACCAGGA
TCAATGACAT CTCACACACG GTAGGGAGGG ACTGGGAGAC GAGGTAGAAC CGTGGCCATC
CCGTGGGGGA CCCCAGAGGC TGGCGGAGGA GGCTGTGCAG CCTTGCACAG GGCCCCAGTG
GCCTGGACGC CCCCCTGGCA TAAAGACAGC TCCTCTCCTC CTCCACTTCC CTTGCCTCCC
GCCTTCTCAC TCTCCTCCCT CCCAGACCGG AATCCTAGTG CCCAGGCCCA GAAGGAGTCA
CAGAGGTCCT GGGGTCCCCT TGGCAGGTGG CCAGAACCCC AGCAGCAGTC CCTCTGGGCC
TCCATCTCAT TTCTAGAATG TTTTAGTCGT TAGGCATTCT TCCTGCCTGG TAACTGAGCT
TAGACCCTGC GAGCTCATTA CTCATTACTG CCAGCCCTGC CTGTCAAGCC CTCTTCAGAT
ACAACCCTCT GTGTTTTTGT AAATAGTTAT CAGTGTCTCT TGGGGCATTT TTTCTGAGGT
CCATGGTTGC AGGTCTGAGC TTACAGATGA GGTCTGTATT TAGAATGAGG GAGATGTCTG
```

Fig. 1: The Nucleotide Sequence of Leptin Gene Exon 2 and Intron 2 Based on GenBank No. NC_037331.1.

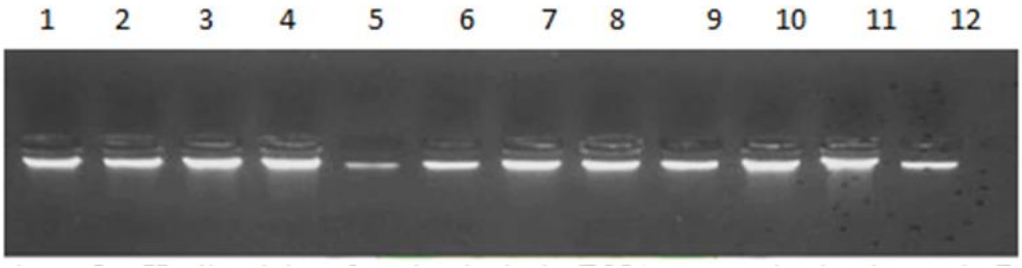

Fig. 2: The results of electrophoresis for the isolation of DNA from Pesisir cattle blood samples (No. 1-12 = Individual DNA isolation samples).

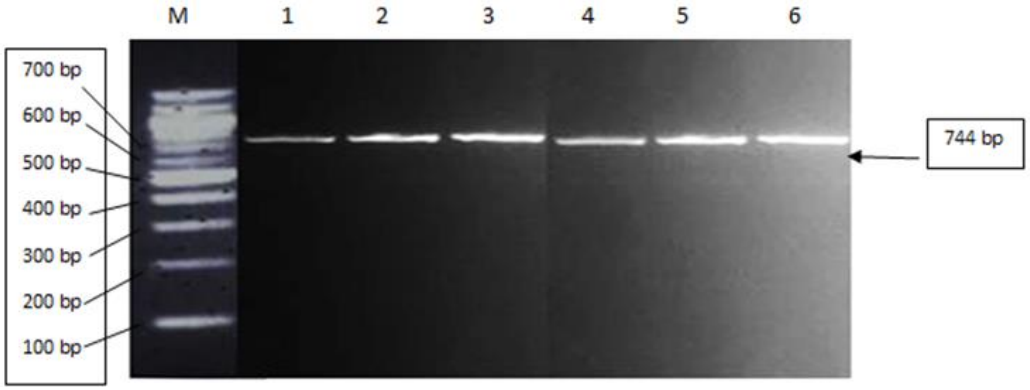

Fig 1: The results of Leptin gene amplification in the exon 2 and intron region in Pesisir cattle (M= marker, No. 1-6= Individu sampel).

In Fig. 2, there is a band that appears less intense, specifically in sample number 5. This may be attributed to the low concentration of DNA obtained. Sambrook and Russell (2001) stated that as the DNA concentration increases, the resulting DNA bands become thicker and brighter. According to Faatih (2009), a clear appearance of DNA bands indicates a high quantity of detected DNA. The success of DNA isolation can be determined by the absence of impurities such as proteins and RNA (Hidayati et al. 2016).

Leptin Gene Amplification

The results of Leptin gene amplification in Pesisir cattle are presented in Fig. 3. Based on Fig. 3, it can be observed that the amplification of the Leptin gene using these primers can be considered successful. This is because during electrophoresis, only one DNA band was found in each well (gel block). The amplification process can be deemed successful when there is one DNA band of the expected fragment size in each well.

Handoyo and Rudiretna (2001) stated that in the DNA amplification process, primers serve as the boundaries for the target DNA fragments to be amplified. The length of the amplified fragments can be determined by matching the primer binding sites to the Leptin gene sequence. The primer binding conditions to the target DNA (genome) significantly influence the success of Leptin gene amplification. This aligns with the view of Viljoen et al. (2005) who stated that the success of the PCR process depends on precise factors such as primer annealing temperature, primer concentration and DNA concentration.

The optimal band formation for the Leptin gene PCR product occurs when using a primer concentration of 10µM and an annealing temperature of 58°C (Setyawati and Zubaidah 2021). From the Fig. 3, it is evident that the primer pair used aligns with the designed sequence, resulting in a PCR product length of 744bp, located in the exon 2 and intron 2 regions of Pesisir cattle.

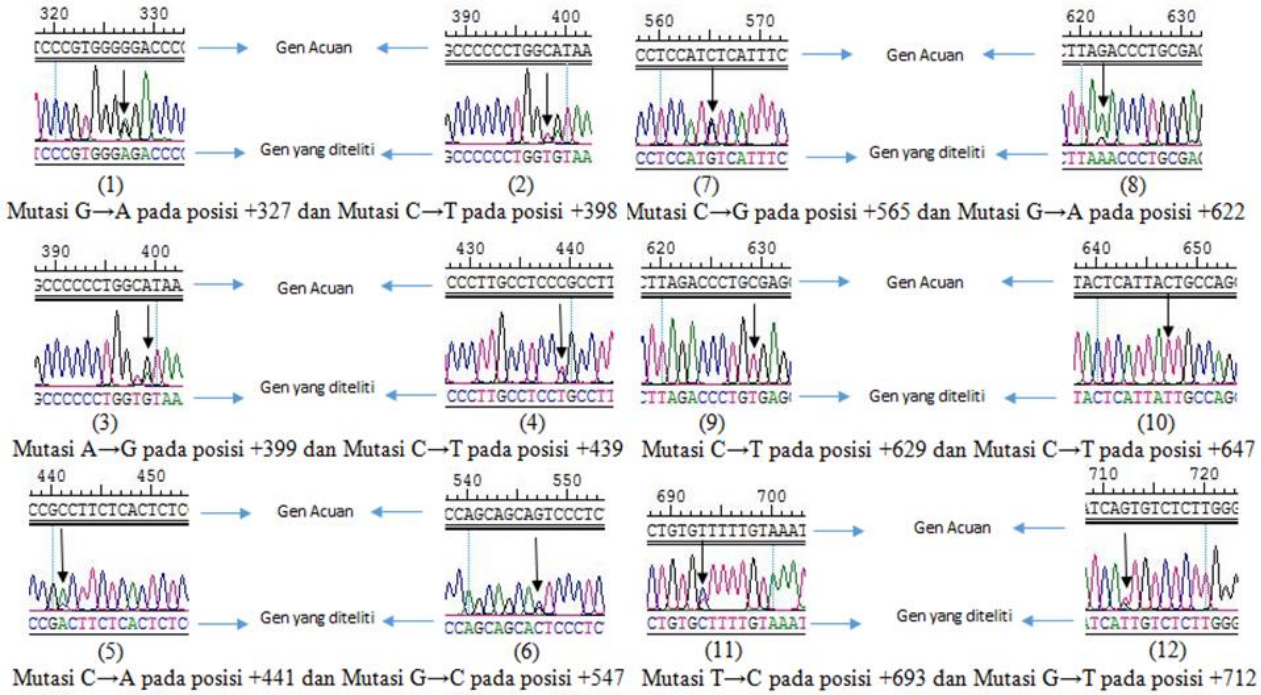

Fig. 4: Mutations in the Leptin gene.

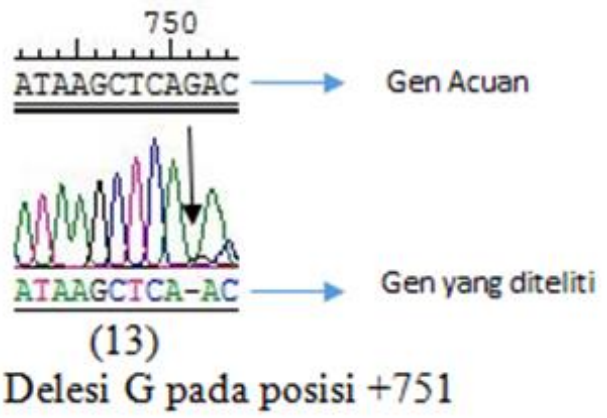

Fig. 2: Deletions in the Leptin gene.

Table 1: Mutations in the Leptin gene of Pesisir cattle

No.	Mutation	SNP Position	Mutations
1.	G→A	+327	Transition
2.	C→T	+398	Transition
3.	A→G	+399	Transition
4.	C→T	+439	Transition
5.	C→A	+441	Transversion
6.	G→C	+547	Transversion
7.	C→G	+565	Transversion
8.	G→A	+622	Transition
9.	C→T	+629	Transition
10.	C→T	+647	Transition
11.	T→C	+693	Transition
12.	G→T	+712	Transversion
13.	G→del	+751	Deletion

Note: SNP = *Single Nucleotide Polymorphism,* (+) = Intron 2 region Del = Deletion

Leptin Gene Diversity

Ninety-six PCR products were sequenced using the services of 1st Base in Singapore. All samples sent for sequencing were successfully sequenced and analyzed using the SeqManTM 4.00 DNA STAR program. The reference sequence used was the Leptin gene sequence obtained from GenBank with accession number NC_037331.1. Fig. 3 and 4 display the electropherograms from the sequencing results, revealing mutations and deletions in the Leptin gene.

The data in ABI file format visualize the electropherogram, where each type of nucleotide is represented by a distinct color. For instance, adenine (A) is displayed in green, guanine (G) in black, thymine (T) in red, and cytosine (C) in blue. It's crucial to acknowledge that even though intron regions are not directly involved in protein synthesis, the translation process can be affected by changes in introns. This phenomenon is influenced by several factors that remain unidentified at this point (Afriani et al. 2022).

The differences or changes that occur in DNA nucleotide bases between two individuals at a specific location within the genome result from a substitution process. Vignal et al. (2002) added that with the occurrence of substitutions, there are typically two possibilities at the same position in the DNA sequence. Changes in the Leptin gene in exon 2 and intron 2 can be observed in Table 1.

Based on Table 1, it can be observed that there are 13 variations in the intron 2 region among the Pesisir cattle samples. From the conducted research, diversity was only found in the intron 2 region, while no variations were detected in the exon 2 region. From Table 1, it can be seen that at position +327, a transition mutation occurred, changing the sequence from GGGGA to GGAGA. At position +398, a C→T mutation occurred, and this type of mutation is the same as at position +327, which is a transition. Mutations at position +399 A→G and position +439 C→T also fall into the transition category. At position +441, a C→A mutation and at position +547, a G→C mutation occurred, which are categorized as transversion mutations. Transversion also occurred at position +565,

where there was a base change from C→G. Transition mutations occurred at positions +622, +629, +647, and +693, resulting in G→A, C→T, C→T, and T→C mutations, respectively. At position +712, a transversion occurred where the guanine (G) base changed to thymine (T). Furthermore, at position +751, there was a deletion of guanine (G). Ablondi et al. (2021) stated that the common method for identifying genetic diversity relies on the detection of mutations or alterations in nucleotide bases. Changes in the amino acids in the area caused by mutations in the exon region of the Leptin gene can impair livestock productivity (Ermawati et al. 2022).

The PCR products that have been sequenced with the assistance of 1st base in Singapore reveal the presence of 10 variable sites in the intron 2 region. The accumulation of base differences among 96 individuals yields 10 haplotypes, as shown in Table 2, which depicts the base differences among Pesisir cattle individuals and the diversity within the population.

From Table 2, it can be explained that the composition of base differences (haplotypes) in this study indicates variations among cattle individuals, making the Leptin gene region in this research a molecular marker for Pesisir cattle and supporting taxonomic studies. Different haplotype types in each cattle individual can be used as references in identifying Pesisir cattle individuals. According to Akbar et al. (2014), the greater the diversity of composite haplotype types within a population, the higher the level of genetic diversity, and vice versa. Haplotype (haploid genotype) is a group of alleles inherited together from a single parent organism. Polymorphic variations in multiple segments of the leptin gene exhibit the capacity to be harnessed for molecular-based selection strategies, with reported associations to traits encompassing body and carcass weights, milk protein content, backfat thickness, and rib-eye areas (Casarini and Crepiex 2019).

Genotype and Allele Frequency

The genotype diversity of each cattle individual can be determined through the DNA patterns identified. Utomo et al. (2021) stated that Genotypic findings are commonly utilized for elucidating the allelic distribution in a population through the examination of genetic diversity. According to Nei and Kumar (2000), genotype frequency is the ratio of the number of a specific genotype to the total population. Meanwhile, allele frequency is the relative ratio of a specific allele to the total alleles at a locus within a population. The values of allele frequency and genotype frequency determine the diversity of the Leptin gene in Pesisir cattle, as presented in Table 3.

Based on the analysis in Table 3, it is found that there is genetic diversity in the Leptin gene of Pesisir cattle, evidenced by the variation in the target fragment region. Overall, the results of the analysis are polymorphic in nature. According to Falconer and Mackay (1996), an allele is considered polymorphic if its allele frequency is equal to or less than 0.99. This aligns with the view of Nei and Kumar (2000), who state that an allele is declared polymorphic or diverse if the frequency of one allele of a gene is equal to or less than 0.99 or more than 1%.

Polymorphic or diverse fragments occur due to the influence of breed differences, inadequate selection of livestock, and mutations. Yonatika et al. (2021) stated that, genetic diversity is positively correlated with geographical distance or closely related to geographical conditions. The research conducted by Foote et al. (2015), demonstrated a noteworthy correlation between Leptin levels and parameters such as 12th-rib fat thickness, yield grade, and marbling score. The Leptin gene's genetic diversity and the carcass quality are significantly associated (Uemoto et al. 2012).

Hardy-Weinberg Equilibrium Test

Testing for Hardy-Weinberg equilibrium in Pesisir cattle was conducted using the chi-square test to determine whether the observed data deviate from the expected values for the number and types of genotypes or alleles (Table 4).

Based on Table 4, it can be explained that from the observed genotype and allele frequencies, there are 8 mutations that are not significantly different, and 4 mutations that are significantly different from the expected genotype and allele frequencies. Thus, it can be concluded that the genotype and allele frequencies of the Leptin gene in the Pesisir cattle population under study exhibit only 8 mutations that are in Hardy-Weinberg equilibrium, while the other 4 mutations studied are not in Hardy-Weinberg equilibrium.

A population can be considered to be in Hardy-Weinberg equilibrium if the genotype and allele frequencies under investigation remain constant from one generation to the next. According to Vasconcellos et al. (2003), a population is considered to be in Hardy-Weinberg equilibrium when genotype and allele frequencies remain constant from one generation to the next due to random gamete combination in a large population.

Genotypic equilibrium in a sufficiently large population occurs in the absence of selection, mutation, migration, and genetic drift (Syarifulaya et al. 2015). Genetic drift is the change in genotype frequencies caused by random fluctuations due to the likelihood of mating patterns, sampling errors, and sudden frequency changes caused by environmental factors (such as natural disasters). Syarifulaya et al. (2015) explain that the accumulation of genotypes, population subdivision, mutations, selection, migration, and mating within the same group (endogamy) can lead to genotypic imbalance.

Restriction Enzymes

Diversity information can be clearly observed using DNA sequence alignment, allowing the determination of genotype and allele frequencies from this information. This alignment can also be utilized to identify the potential restriction enzymes with the assistance of various bioinformatics software. Cheng et al. (2016) stated that the usage of restriction enzymes for genotyping is an effective cost method and it is a fundamental way of how PCR-RFLP works. In accordance with European standards for molecular microbiology and the detection of genetically modified organisms, it is crucial to confirm the PCR products produced (Prihandini et al. 2021).

The software used to assess the potential restriction enzyme sites in DNA sequences is CLC MainWorkbench 8. The potential restriction enzyme sites from the CLC MainWorkbench application can be seen in Table 5. Information about the enzymes with potential for use as

Table 2: Polymorphism of Leptin gene intron 2 aligned with GenBank reference (accession number NC_037331.1) in the Pesisir cattle population

HAPLOTYPE	Sequence numbering of the Control region.											
	+327	+398	+399	+439	+441	+547	+565	+622	+629	+647	+693	+712
Ref	G	C	A	C	C	G	C	G	C	C	T	G
A	A	T	G	.	.	C	G	.	.	.	.	T
B	A	.	G	T	.	C	G	.	.	.	.	T
C	.	.	.	T	.	.	.	.	.	.	C	.
D	.	.	.	.	A	C	.	.	.	.	.	.
E	.	.	.	T	A	C	.	.	.	.	.	.
F	.	.	.	.	.	.	.	A	.	.	.	.
G	.	.	.	.	A	C	.	.	T	T	.	.
H	.	.	.	.	.	.	.	.	.	.	C	.
I	.	.	.	T	.	.		.	.	.	.	.
J	.	.	.	.	.	.	.	.	.	T	.	.

Ref=Reference, (+)=intron 2 region

Table 3: Genotype and allele frequencies in the Leptin gene

No	SNP Position	N	Genotype Frequency			Allele Frequency	
1.	+327	96	GG	GA	AA	G	A
	G→A		0.98	0.02	0.00	(0.99)	(0.01)
2.	+398	96	CC	CT	TT	C	T
	C→T		0.99	0.00	0.01	(0.99)	(0.01)
3.	+399	96	AA	AG	GG	A	G
	A→G		0.98	0.02	0.00	(0.99)	(0.01)
4.	+439	96	CC	CT	TT	C	T
	C→T		0.85	0.14	0.01	(0.92)	(0.08)
5.	+441	96	CC	CA	AA	C	A
	C→A		0.84	0.13	0.03	(0.90)	(0.10)
6.	+547	96	GG	GC	CC	G	C
	G→C		0.82	0.15	0.03	(0.90)	(0.10)
7.	+565	96	CC	CG	GG	C	G
	C→G		0.98	0.02	0.00	(0.99)	(0.01)
8.	+622	96	GG	GA	AA	G	A
	G→A		0.97	0.03	0.00	(0.99)	(0.01)
9.	+629	96	CC	CT	TT	C	T
	C→T		0.97	0.00	0.03	(0.97)	(0.03)
10.	+647	96	CC	CT	TT	C	T
	C→T		0.96	0.00	0.04	(0.96)	(0.04)
11.	+693	96	TT	TC	CC	T (0.92)	C
	T→C		0.83	0.17	0.00		(0.08)
12.	+712	96	GG	GT	TT	G	T
	G→T		0.98	0.02	0.00	(0.99)	(0.01)
13.	+751	96	-	-	-	-	-
	G→del						

SNP=Single Nucleotide Polymorphism, (N)=Number of sample, Del=Deletion.

Table 4: Chi-square test for Hardy-Weinberg equilibrium of the Leptin gene in Pesisir cattle.

No	SNP Position	Mutation	X^2_h	X^2_t 0.05	Conclusion
1.	+327	(G→A)	0.0149	5,9914	Not significantly different
2.	+398	(C→T)	104,0859	5,9914	Significantly different
3.	+399	(A→G)	0.0149	5,9914	Not significantly different
4.	+439	(C→T)	0.3393	5,9914	Not significantly different
5.	+441	(C→A)	6,0833	5,9914	Significantly different
6.	+547	(G→C)	4,9774	5,9914	Not significantly different
7.	+565	(C→G)	0.0615	5,9914	Not significantly different
8.	+622	(G→A)	0.6578	5,9914	Not significantly different
9.	+629	(C→T)	103,9194	5,9914	Significantly different
10.	+647	(C→T)	103,8336	5,9914	Significantly different
11.	+693	(T→C)	0.8809	5,9914	Not significantly different
12.	+712	(G→T)	0.0149	5,9914	Not significantly different

SNP=Single Nucleotide Polymorphism, (+)= Intron 2 region; $X^2_h > X^2_{t\,(0.05)}$=Significantly different, $X^2_h < X^2_{t\,(0.05)}$ =Not Significantly different.

restriction enzymes can be applied in further research, such as studying the genetic diversity of Pesisir cattle using the PCR-RFLP method.

DNA fragments cut using restriction enzymes will exhibit the genetic characteristics of an organism. These fragments will generate two different haplotype types, namely polymorphic and monomorphic types. In polymorphic types, the cut DNA fragments will result in fragments at different sequence positions. Meanwhile, in monomorphic types, the cut DNA fragments produce

Table 5: Potential Restriction Enzymes

No	SNP Position	Potential Restriction Enzymes
1	+327 G>A,	Sth132I (cccg↓), BssIMI (gggt↓c)
2	+398 C>T	Not found
3	+399 A>G	Not found
4	+439 C>T	MnlI (cc↓tc)
5	+441 C>A	Sth132I (cccg↓), MnlI (cc↓tc)
6	+547 G>C	Lsp1109I (gcag↓c), Bsp423I (gcag↓c)
7	+565 C>G	MnlI (cc↓tc), BfaI (ctag↓)
8	+622 G>A	SaqAI (ttaa↓), Tru9I (ttaa↓)
9	+629 C>T	Psp124BI (ga↓gctc), SacI(ga↓gctc)
10	+647 C>T	Not found
11	+693 T>C	MnlI (cc↓tc)
12	+712 G>T	Not found
13	+751 G >del	AluBI (agct↓), AluI (agct↓)

SNP=Single Nucleotide Polymorphism, (+) =Intron 2 region, Del=Deletion

fragments at the same sequence position, identifying a low level of genetic variation. Molecular markers employed to elucidate disparities in genotype among individuals as a result of discrepancies in marker sequences are denoted as polymorphic markers. In contrast, DNA markers that do not possess the capability to discriminate between or among genotypes are labeled as monomorphic markers (Amiteye 2021).

Conclusion

In the studied Leptin gene of Pesisir cattle, 13 variations were identified in intron 2, including 8 transition mutations, 4 transversion mutations, and 1 deletion, all of which are polymorphic and have the potential to be used as genetic markers for further testing as marker candidates. In the Hardy-Weinberg equilibrium test, 8 mutations were found to be in Hardy-Weinberg equilibrium at positions +327, +399, +439, +547, +565, +622, +693, +712, while 4 mutations were not in Hardy-Weinberg equilibrium at positions +398, +441, +629, and +647. The examination of restriction enzymes in this study identified 12 restriction enzymes, including Sth132I, BssIMI, Mn1I, Lsp1109I, Bsp423I, BfaI, SaqAI, Tru9I, Psp124BI, SacI, AluBI, and AluI.

Acknowledgement

The authors wish to thank the rector and Institute Of Research and Community Services of Andalas University Padang, Indonesia who funded this research with grant number
001.w/UN.16.06.D/PT.01/SPP.RD/FATERNA/2023.

Author's Contribution

All authors contributed equally to this work

Conflict of Interest

The authors declare no conflict of interest

REFERENCES

Ablondi M, Sabbioni A, Stocco G, Cipolat-Gotet C, Dadousis C, Van Kaam JT, Finocchiaro R and Summer A, 2021. Genetic diversity in the Italian Holstein dairy cattle based on pedigree and SNP data prior and after genomic selection. Frontiers in Veterinary Science 8: 773985. https://doi.org/10.3389/fvets.2021.773985

Afriani T, Purwati E, Hellyward J, Jaswandi, Mundana M, Farhana A and Rastosari A, 2022. Identification of single nucleotide polymorphism (SNP) in early exon 10 of follicle stimulating hormone receptor (FSHR) gene in Pesisir cattle. Jurnal Ilmiah Terpadu 10(3): 264-276. http://dx.doi.org/10.23960/jipt.v10i3.p264-276.

Akbar N, Zamani NP and dan Maddupa HH, 2014. Genetic diversity of yellowfish tuna (Thunnus albacares) from two populations in the Moluccas Sea, Indonesia. Depik 3(1): 65-73. https://doi.org/10.13170/depik.3.1.130

Amiteye S, 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon 7(10): e08093. https://doi.org/10.1016/j.heliyon.2021.e08093

Anugratama LE and Hartatik T, 2020. Short Communication: Identification of Leptin gene in crossbred beef cattle. Biodiveritas 21(1): 226-230. https://doi.org/10.13057/biodiv/d210129

Ardicli S, Samli H, Vatansever B, Soyudal B, Dincel D and Balci F, 2019. Comprehensive assessment of candidate genes associated with fattening performance in Holstein-Friesian bulls. Arch Anim Breed 62(1): 9–32. https://doi.org/10.5194/aab-62-9-2019

Casarini L and Crepieux P, 2019. Molucular mechanisms of action of FSH. Frontiers in Endocrinology (Lausanne) 10(1): 305-315. https://doi.org/10.3389/fendo.2019.00305

Cheng YH, Kuo CN and Lai CM, 2016. Effective natural PCR-RFLP primer design for SNP genotyping using teaching learning based optimalization with Elite Strategy. IEEE Trans Nanobioscience 15 (7): 657-665. https://doi.org/10.1109/TNB.2016.2597867

Ermawati D, Panjono, Bintara S, Kurlyana T and Hartatik T, 2022. Diversity of partial sequence leptin gene (Exon 3) in crossbred cattle compared to GenBank database. Biodiversitas 23(11): 5606-5612. https://doi.org/10.13057/biodiv/d231109

Faatih M, 2009. Isolasi dan digesti DNA Kromosom. Jurnal Penelitian Sains dan Teknologi 10(1): 61-67.

Falconer DS and Mackay TFC, 1996. Introduction to Quantitive Genetics. Fourth Edition. Longman, New York.

Foote AP, Hales KE, Kuehn LA, Keisler DH, King DA, Shackelford SD, Wheeler TL and Freetly HC, 2015. Relationship of leptin concentrations with feed intake, growth, and efficiency in finishing beef steers. Journal of Animal Science 93(9): 4401–4407. https://doi.org/10.2527/jas.2015-9339

Handoyo D dan Rudiretna A, 2001. Prinsip umum dan pelaksanaan polymerase chain reaction (PCR). Pusat Studi Bioteknologi. Universitas Surabaya 9(1): 17-29.

Hernandez N, Gonzalez JCM, Bracamonte GMP, Rincon AMS, Villalobos NL, Morris ST, Encinia FB, Rivas EO, Contreras VIP and Garcia LAM, 2016. Association of polymorphisms in growth hormone and leptin candidate genes with live weight traits of Brahman cattle. Genetics Molecular Research 15: 1-9. https://doi.org/10.4238/gmr.15038449

Hidayati E, Saleh and Aulawi T, 2016. Identifikasi keragaman gen BMPR-1B (Bone Morphogenetik Protein Receptor IB) pada ayam araba yam kampung dana yam ras petelur menggunakan PCR-RLFP. Jurnal Peternakan 13(1): 1-12. http://dx.doi.org/10.24014/jupet.v13i1.2383

Hilmia N, Noor RR, Sumantri C and Priyanto R dan Gurnadi E, 2015. Hubungan keragaman gen Leptin dengan kualitas fisik daging sapi lokal di Ciamis. Jurnal Ilmu Ternak 15(2). https://doi.org/10.24198/jit.v15i2.9528

Kaygisiz A, Bengi C and Cilek S, 2011. Investigation of leptin gene polymorphisms in East Anatolian Red Anatolian and Black cattle and determination of genetic distance from Brown Swiss cattle. Journal Animal Plant Science 21: 121-125.

Kong HS, Oh JD, Lee SG, Hong YS, Song WI, Lee SJ, Kim HC, Yoon BH, Lee HK and Jeon GJ, 2006. Association of polymorphisms in the bovine leptine gene with ultasound

measurements for improving in Korean cattle. Asian-Australian Journal Animal Science 19(12): 1691-1695. https://doi.org/10.5713/ajas.2006.1691

Lagonigro R, Wiener P, Pilla F, Woolliams JA and Williams JL, 2003. A new mutation in coding region of the bovine leptine gene associated with feed intake. Animal Genetics 34: 371-374. https://doi.org/10.1046/j.1365-2052.2003.01028

Mandefro A, Sisay T, Kim KS, Edea Z, Konwarh R and Dadi H, 2021. Single nucleotide polymorphisms of leptin gene in five Ethiopian indigenous cattle breeds and the Korean Hanwoo breed. Tropical Animal Health and Production 53: 202. https://doi.org/10.1007/s11250-021-02642-1

Nei M and Kumar S, 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nobari K, Ghazanfari S, Nassiry MR, Tahmoorespur M and Jorjani E, 2010. Relationship between leptin gene polymorphism with economical traits in Iranian Sistani and Brown Swiss cows. Journal of Animal and Veterinary Advances 9(22): 2807-2810. https://doi.org/10.3923/javaa.2010.2807.2810

Oprzadek J, Flisikowski K, Zwierzchowski L and Dymnicko E, 2003. Polymorphism at loci of leptin (LEP), Pit1 an STA-T5A and their association with growth, feed conversion and carcass quality in Black-and-White bulls. Animal Science Papers and Reports 21: 135-145.

Prihandini PW, Primasari A, Luthfi M, Pamungkas D, Sari APZNL, Dina TB and Maharani D, 2021. Identification of restriction enzyme in the FSHR gene of indonesian local cattle. IOP Conference Series: Earth and Environmental Science 888: 12-24. https://doi.org/10.1088/1755-1315/888/1/012024

Sambrook J and Rusell D, 2001. Molecular cloning Third Edition. CSHL Press, New York.

Setyawati R dan Zubaidah S, 2021. Optimasi Konsentrasi Primer dan Suhu Annealing dalam Mendeteksi GenLeptin pada Sapi Peranakan Ongole (PO) Menggunakan Polymerase Chain Reaction (PCR). Indonesian Journal of Laboratory 4(1): 36-40. https://doi.org/10.22146/ijl.v4i1.65550

Sharifzadeh A and Doosti A, 2010. Genetic polymorphism at the leptin gene in Iranian Holstein cattle by PCR-RFLP. African Journal Microbiology Research 4(12): 1343-1345. https://doi.org/10.3923/javaa.2010.1420.1422

Syarifulaya N and Sriasih M dan Maskur, 2015. Identifikasi keragaman gen Leptin pada sapi Bali dan kambing Kacang (polymorphism of Leptin gene in Bali cattle and Kacang goat). Jurnal Ilmu dan Teknologi Peternakan Indonesia 1(1): 40-46. https://doi.org/10.29303/jitpi.v1i1.9

Taniguchi Y, Itoh T, Yamada T and Sasaki T, 2002. Genomic structure and promoter analysis of the bovine leptin gene. IUBMB Life 53(2): 131-135. https://doi.org/10.1080/15216540211465

Uemoto YT, Nakano KH and Sato S, 2012. Effects of porcine leptin receptor gene polymorphisms on backfat thickness, fat area ratios by image analysis, and serum leptin concentrations in a Duroc purebred population. Journal of Animal Science 83 (5): 375-385. https://doi.org/10.1111/j.1740-0929.2011.00963

Utomo B, Rimayanti R, Triana IN and Fadholly A, 2021. Melanocortin-4 receptor and leptin as genes for the selection of superior Madrasin cattle. Veterinary World 14(12): 3224-3228. https://doi.org/10.14202/vetworld.2021.3224-3228

Vasconcellos LPMK, Talhari DT, Pareira AP, Coutinho LL and Regitano LCA, 2003. Genetic characterization of aberden angus cattle using moleculer markers. Genetics and Molecular Biology 26(2): 133-137. https://doi.org/10.1590/S1415-47572003000200005

Vignal A, Milan D, Sancristobal M and Eggen A, 2002. A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution 34: 275-305. https://doi.org/10.1186/1297-9686-34-3-275

Viljoen GJ, Nel LH and Crowther JR, 2005. Molecular Diagnostic PCR Handbook. Springer, Dordrecht, Netherland.

Wang L, Raza SHA, Gui L, Li S, Liu X, Yang X, Wang S, Zan L and Zhao C, 2020. Associations between UASMS2 polymorphism in leptin gene and growth, carcass and meat quality traits of cattle: a metaanalysis. Animal Biotechnology 33(2): 279-288. https://doi.org/10.1080/10495398.2020.1805327

Yonatika NO, Widiasih N, Hamidah M, Nurhakim MD, Budiarto H, Bintang DMC, Sani LMI, Lestari DF, Setyaningsing WA, Subhan B and Madduppa H, 2021. Genetic dtructure and phylogenetic relationship of Phyllidella pustulosa species from Seribu Islands, North Sulawesi, Halmahera, and West Papua. IOP Conference Series: Earth Environmental Science 9(44): 12-28. https://doi.org/10.1088/1755-1315/944/1/012028

Yurnalis, 2015. Polimorfisme gen hormon pertumbuhan pada sapi Pesisir Sumatera Barat. Disertasi. Program Pascasarjana, Universitas Andalas, Padang.

Zaituni U, Hendri H and Masrizal M, 2022. Increasing the success of artificial insemination through control of local cattle estrus as a genetic resource. International Journal Health Science 6(S4): 2117–2132. https://doi.org/10.53730/ijhs.v6nS4.6713