Wed, 28 Sep 2022, 07:02 AM

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We have received the reports from our reviewers on your manuscript, "Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle", which you submitted to Journal of Animal Health and Production with MH20220804060845-R1.

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Table 1:

Write title as: ‘Mutation detection in FSHR gene of Pesisir Cattle’

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Table 2: Better to add some identity for first two rows.

Table 3:

Write title as: ‘Genotype frequency and allele frequency of FSHR gene.’

Replace comma (,) with point (.) for all values, (e.g., 0,1 should be 0.1).

Table 4:

Write heading as: ‘Chi-square test results for Hardy-Weinberg equilibrium of FSHR gene of Pesisir cattle.’

Write ‘No’ as ‘Serial No.’

Replace all non English terms (e.g., Posisi Mutasi) with English terms.

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Define asterisk (**) in table footnote.

Cite table 4 in the text (lines 150-157).

Figure 1:

Write title as: PCR product amplification of the exon 9 of the FSHR gene in Pesisir cattle (M = Marker).

Other than Figure 1 (PCR product amplification…), merge all figures (label them as A, B, C, D and E) with the following headings:

Figure 2. Transition A→G at position 8 (A), transition T→C at position 213 (B), Transition G →A at position +252 (C), transversion A→T at position +49 (D), and transversion A→C at position +232 (E).

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Journal of Animal Health and Production - Submission Proof

Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle

Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle

Journal of Animal Health and Production - Submission Proof

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Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (*FSHR*) Gene in Pesisir Cattle

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Abstract

This study aimed to resequencing of 962 bp of exon 9 and intron 9 of follicle stimulating hormone receptor (*FSHR*) gene in Pesisir cattle to identify single nucleotide polymorphisms (SNP). The samples used in this study were blood samples (n= 70) collected from Pesisir cattle (aged 2-5 years). DNA was isolated and the targeted region was amplified using the polymerase chain reaction (PCR) technique. Then the amplified products were sequenced. The results showed that the population of Pesisir cattle used in this study was polymorphic. There were 5 polymorphisms in the exon 9 and intron 9

regions, which were located at the 18 A>G, 213 T>C, +49 A>T, +232 A>C, and +252 G>A. There were 3 transition type mutations (at positions 8, 213 and +252) and 2 transversion type mutations (at positions +49 and +232). Based on the result of this study, it can be stated that the genotype frequency of Pesisir cattle population was not in the Hardy-Weinberg equilibrium.

Key words: SNP, *FSHR* gene, Pesisir cattle, DNA isolation, single nucleotide polymorphism

INTRODUCTION

Pesisir cattle is one of the Indonesian cattle from West Sumatra, which has a smaller body size compared to other local cattle breeds. The adult body weight of Pesisir cattle is ranging from 200 – 400 kg, and they are mainly raised on rural farms with poor reproduction management (Afriani *et al.*, 2022). Pesisir cattle have a significant contribution to the meat demands of the people of West Sumatra. As a local breed, Pesisir cattle have several desirable characteristics, such as their ability to survive in harsh environmental conditions with high reproductive efficiency (Sarbaini, 2004). The potential genetic resources of local Indonesian cattle are very diverse and have not been used optimally. The diverse genetic resources of a breed may make a population more resistant to live and higher adaptability to environmental changes (Frankham et al., 2002). Because of the growing cattle population in Indonesia, it is advisable to explore the genetic resources of local breeds.

The livestock productivity can be improved up to the genetical potential using the molecular selection tools. The implementation of selection at the molecular level for the local genetic resources has not been widely used. This utilization can be carried out as an effort to obtain superior local Indonesian cattle that have high productivity and reproduction capabilities (Nasution, 2013). Talenti et al. (2022) stated that improving the genetic quality of breeds to produce the livestock with better phenotypes and genotypes, is strongly influenced by the presence of genes in it. One of the important genes that influencing reproduction in cattle is the follicle stimulating hormone (*FSH*) gene. The FSH stimulate the gonads in females, however it can not work directly into the cell without the help of its receptor i.e., follicle stimulating hormone receptor (*FSHR)*. The *FSHR* works directly into cells and help in increasing the production of ovum cells in females as it is a transmembrane receptor that interacts with follicle-stimulating hormone. The *FSHR* gene is required to carry the *FSH* gene to the target tissue so that it can be translated into protein to become the FSH hormone. The *FSHR* gene found on chromosome 11 consisting of 10 exons and 9 introns with length of 194885 (NCBI GenBank access code NC-037338). One of the functions of *FSHR* is to support the activation of specific receptors in stimulating follicular growth in the ovaries (Fan and Hendrickson, 2005).

Detecting genetic variation based on nucleotides can be done using the SNP (single nucleotide polymorphism) technique via a PCR (polymerase chain reaction) product. The SNP technique has the advantage that it is easy to apply and read the

data. SNP is a marker in genomic variation between individuals so that it can be used to detect alleles that carry important traits in an individual (Mmeka et al. 2013). This study was conducted with aim to identify the SNPs in the *FSHR* gene (exon 9 and intron 9). The target DNA that was amplified in this study was the *FSHR* gene fragment located at exon 9 with a length of 185 bp.

MATERIALS AND METHODS

This study used the blood samples that were collected from female Pesisir cattle. A total of 70 Pesisir cows (aged 2-5 years) were used to collect samples from a cattle breeding center i.e., BPTUHPT Padang Mengatas, Payakumbuh, West Sumatra, Indonesia.

DNA Isolation and *FSHR* Gene Amplification

DNA isolation from blood samples was carried out using a commercial extraction kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, USA). The isolated DNA was then visualized using 1% agarose gel and stored at -20ºC until used.

Gene amplification was carried out using the PCR machine with a pair of primers F: 5'-TTA GGC CCT GTG ACT GTG AG-3' and R: 5'-CTG GCA AAG AGG GAA CAA GAG-3' (Yurnalis, 2019). The PCR reactions were prepared with Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Chicago, USA) using a predenaturation program at 95ºC for 5 minutes, denaturation at 95ºC for 45 seconds, annealing at 58ºC for 45 seconds, initial extension at 72ºC for 1 minute and final extension at 72ºC for 5 minute. PCR was performed with 35 cycles. The results of the amplification of the *FSHR* gene were seen by electrophoresis using 1% agarose gel and Ethidium Bromide liquid; and were visualized under a UV transmulator. Gene amplification was said to be successful if the agarose gel showed bands formed in each well-containing DNA samples of PCR products. The target DNA was determined by comparing the position of the band formed with the position of the DNA ladder band. The results of the electrophoresis were documented using a digital camera.

Sequencing

The PCR product from the *FSHR* exon 9 gene was sequenced using the services of a commercial company (1st BASE Singapore).

Data analysis

All data were saved in Excel Spread Sheets. Genotypic and allelic frequencies were determined using the formula of Nei and Kumar (2000).

1. Genotype frequency

$$\chi_i \frac{\sum n_i}{N}$$

where,

x_i = observed genotype

N = total number of individual samples

n_i = total number of individual samples with genotype i

2. Allele frequency

$$\chi_i = \frac{(2n_{ii} + \sum_{j \neq i} n_i)}{2n}$$

Where,

x_i = allele frequency i

n_{ii} = number of individuals for the genotype ii

n_{ij} = number of individuals for the genotype ij

n = number of samples

3. Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium was tested with chi-square test, where χ^2 is the chi-square test, O_{ij} is the number of genotypic observations and E_{ij} is the expected number of genotypes

$$\chi^2 = \sum\sum \frac{(O_{ij} - E_{ij})}{E_{ij}}$$

RESULTS AND DISCUSSION

FSHR

In current investigation, amplification of the *FSHR* gene fragment was carried out in Pesisir cattle that showed the position of the *FSHR* gene at exon 10 with a PCR product of 962 bp long. The amplification results of the study were shown in Figure 1.

The amplification process during electrophoresis can be declared successful if in one gel block only one DNA band is visible, but not all samples showed the same density of DNA band. This was due to differences in the concentration of extracted DNA in the template used for PCR as well as differences in the concentration of DNA that was successfully amplified (multiplied) (Sambrook and Russell, 2001). Zulkarnaim et al. (2010) reported the amplification of GHR|AluI gene fragment performed on Bali, Limousin, Simmental, and Pesisir cattle and showed the position of the GHR gene at exon 10 with a 298 bp PCR product.

FSHR Gene Diversity and Mutation Detection

All the PCR products samples (n=70) were sequenced using the services of 1st BASE Singapore and analyzed using the SeqManTM version 4.00 DNASTAR program (DNASTAR Inc., Madison, Wisconsin, USA). The reference sequence used in this study was the *FSHR* Exon 9 gene sequence which was obtained from NCBI Genbank No. NC-037338. The results of the sequencing showed that there is diversity in the exon 9 and some intron 9 regions (Table 1).

Five SNPs were detected, two were found in exon 9, and three in intron 9. Two types of mutations were obtained; transition and transversion. The computer-generated sequencing electropherogram showed the mutation of the exon 9, as shown in Figure 2-4.

According to Asaf et al. (2014) SNPs in the coding region can directly affect the protein, SNPs in the intron region can affect splicing, while SNPs in the promoter may affect gene expression. Nevertheless, both intronic and exonic mutations can affect performance (Helal et al., 2021; Mi et al., 2021; Yadav et al., 2021). Although introns are not transcribed into proteins, many research results showed different functions of introns, one of the most important functions is increasing protein abundance. This indicates that although the intron region is not involved in protein synthesis, variations in introns can affect the translation process. The effect of introns was first observed in Simian Vacuolating viruses which produce undetectable protein products upon deletion of their intron sequences (Gruss et al., 1979; Hamer et al., 1979).

In this study, 5 point mutations were found in exon 9 and intron 9, namely 3 points of transition mutation and 2 points of transversion mutations. The detected transition mutations wereA>G at position 8, T>C at position 213 and G>A at position +252. Meanwhile, two points of transversion mutations were deteced, namely A>T at the +49 position and A>C at the +232 position. Windelspect (2007) stated that mutations occur due to the substitution of pyrimidine base (thymine and cytosine) with another pyrimidine base or between one purine base (adenine and guanine) and another purine base. Mutations or changes in nucleotide bases are widely used as the basis for identifying genetic diversity (Ablondi et al. 2021).

Based on the results of the sequencing, there were 30 haplotypes in exon 9 and intron 9 in the *FSHR* gene of Pesisir cattle. The main differences between individuals and diversity in the population of Pesisir cattle were shown in Table 2. The results showed that there were differences in haplotypes indicating individual differences. According to Akbar *et al.* (2014), different haplotype types in each individual can be used as a reference in individual identification. The more diverse types of haplotype composites in a population, the higher the level of genetic diversity (Baksir et al., 2022).

Genotype Frequency and Allele Frequency

Based on the number and types of genotypes in the exon 9 and intron 9 *FSHR* genes in Pesisir cattle, the genotype and allele frequencies were shown in Table 3.

Table 3 shows that there are 5 positions of SNP, namely 8 A – G, 213 C – T, +49 A – T, +232 A – C and +252 G – A with 3 pairs of genotypic frequencies each and having the highest allele frequency of 0.9. The results of this study illustrate that the genotype frequency distribution in this study is polymorphic (various) because the value is less than 0.99 or more than 1%. As stated by Nei and Kumar (2000) that the locus of an allele is said to be polymorphic (various) if the frequency is equal to or less than 0.99 or more than 1%. If the opposite happens then it is monomorphic (uniform). Polymorphic properties are very important to analyze because it is one of the requirements for a gene to be used as a genetic marker (Hartl and Clark, 1997).

Hardy-Weinberg Equilibrium

The results of the chi-square test (χ^2) on the genotype frequency of the *FSHR* gene showed a difference between the ratio of observations and the ratio of expectations or a state of Hardy-Weinberg imbalance that occurs in the Pesisir cattle population ($P>0.05$). This might be due to the small population of Pesisir cattle which restricted the random mating in the Pesisir cattle population. In accordance with Falconer and Mackay (1983) who stated many factors can affect the Hardy-Weinberg imbalance, including mutations, gene flow, migration, selection, genetic drift, and the absence of random mating. Vasconcellos et al. (2003) also suggested that some factors such as accumulation of genotypes, divided population, mutation, selection, migration and mating in the same group/population (endogamy) can cause imbalances in the population.

CONCLUSION

The results of current study on Pesisir cattle declared that in the exon 9 and intron 9 regions of the *FSHR* gene, there were 5 polymorphic SNPs. The Chi-square test shows that the allele frequency and genotype frequency in the population of Pesisir cattle are not in Hardy-Weinberg equilibrium (χ^2 h> χ^2 t).

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CONFLICT OF INTEREST

All authors state that there is no conflict of interests.

NOVELTY STATEMENT

This study on the *FSHR* gene of Pesisir cattle to identify SNPs could be used a reference or basic information for further research to complete the molecular genetics framework for the improvement of the genetic quality of Pesisir cattle as well as other livestock species.

AUTHORS CONTRIBUTION

Tinda Afriani (TA), Yurnalis (Y) and Jaswandi (J) conducted research, Mangku Mundana (MM), Adisti Rastosari (AR) and Anna Farhana (AF) collected data, Y wrote the manuscript, Endang Purwati (EP) and J revised the manuscript, and TA helps in editing of the manuscript.

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Table 1. Mutation detection FSHR gene of Pesisir Cattle

No	Mutation	Position	Mutation Type
1	A → G	8 (exon 9)	Transition
2	T → C	213 (exon 9)	Transition
3	A → T	+49 (intron 9)	Transversion
4	A → C	+232 (intron 9)	Transversion
5	G → A	+252 (intron 9)	Transition

232 **Table 2.** The result of haplotype in exon 9 and intron 9 of FSHR gene in Pesisir Cattle (aligned with GenBank reference).

233

Haplotype	Control Area Sequence Number				
	8	213	+49	+232	+252
	A	T	A	A	G
1	AG	.	.	AC	.
2	.	.	.	AC	.
3	AG	.	.	CC	.
4	.	.	.	.	.
5	.	.	.	AC	.
6	.	.	.	CC	GA
7	.	.	.	AC	.
8	AG	.	.	AC	.
9	AG	.	.	CC	.
10	GG	CC	TT	CC	.
11	AG	.	.	AC	.
12	AG	.	.	CC	GA
13	.	.	.	AC	GA
14	.	.	.	CC	AA
15	.	.	.	.	.
16	AG	TC	.	AC	.
17	AG	.	.	CC	GA
18	AG	.	.	CC	.
19	AG	TC	.	CC	GA
20	GG	CC	TT	CC	.
21	.	.	.	AC	GA
22	.	TC	.	AC	.
23	AG	.	.	AC	.

234

235

236 **Table 3.** The result of genotype frequency and allele frequency FSHR gene.

SNP Position	N	Genotype Frequency			Allele Frequency	
8	70	GG	AG	AA	G	A
A – G		0,1	0,46	0,45	0,33	0,67
213	70	TT	TC	CC	T	C
T – C		0,83	0,07	0,1	0,86	0,14
+49	70	AA	AT	TT	AC	T
A – T		0,86	0	0,14	0,86	0,14
+232	70	CC	AC	AA	C	A
A – C		0,36	0,47	0,17	0, 60	0,40
+252	70	GG	GA	AA	G	A
G – A		0,82	0,17	0,01	0,9	0,1

237 Description: SNP = *Single Nucleotide Polymorphism*

238 N = Number of samples

239

240 **Table 4.** The result of chi-square test on Hardy-Weinberg equilibrium of FSHR gene in Pesisir cattle.

241

No	Posisi Mutasi	χ^2 hitung	χ^2 tabel		Keterangan
			0,05	0,01	
1	18 A → G	187,56	5,991	9,210	**
2	213 T → C	62,46	5,991	9,210	**
3	+49 A → T	72,53	5,991	9,210	**
4	+232 A → C	146,93	5,991	9,210	**

5	+252 G → A	104,59	5,991	9,210	**

Description: $x^2_h > x^2_t$ (0,05) = significantly different

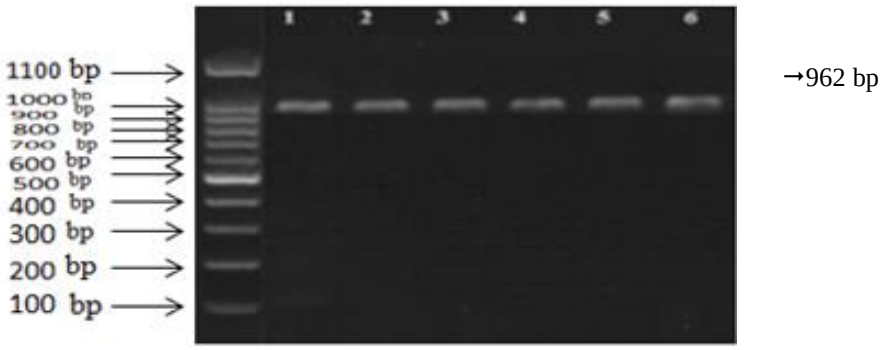

Figure 1. PCR product amplification of the FSHR exon 9 gene in Pesisir cattle (M = Marker)

Figure 1. Transition A→G at position 8, transition T→C at position 213

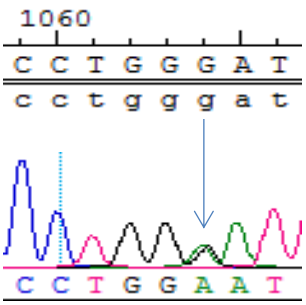

Figure 2. Transition G →A at position +252

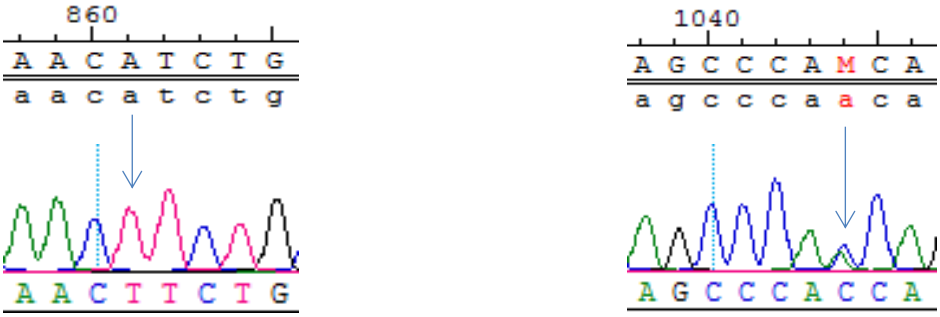

Figure 3. Transversion A→T at position +49, tranversion A→C at position +232

Revision Being Processed

S.no	Manuscript ID	Journal Name	Manuscript Title	Submitting Author	Date Submitted	Status
1	MH202208040608 45-R1	Journal of Animal Health and Production	Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle	Dr Tinda Afriani	08 Sep 2022	Awaiting Reviewer Invitation

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Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle

Identification of Single Nucleotide Polymorphism (SNP) In Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle

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Journal	Journal of Animal Health and Production
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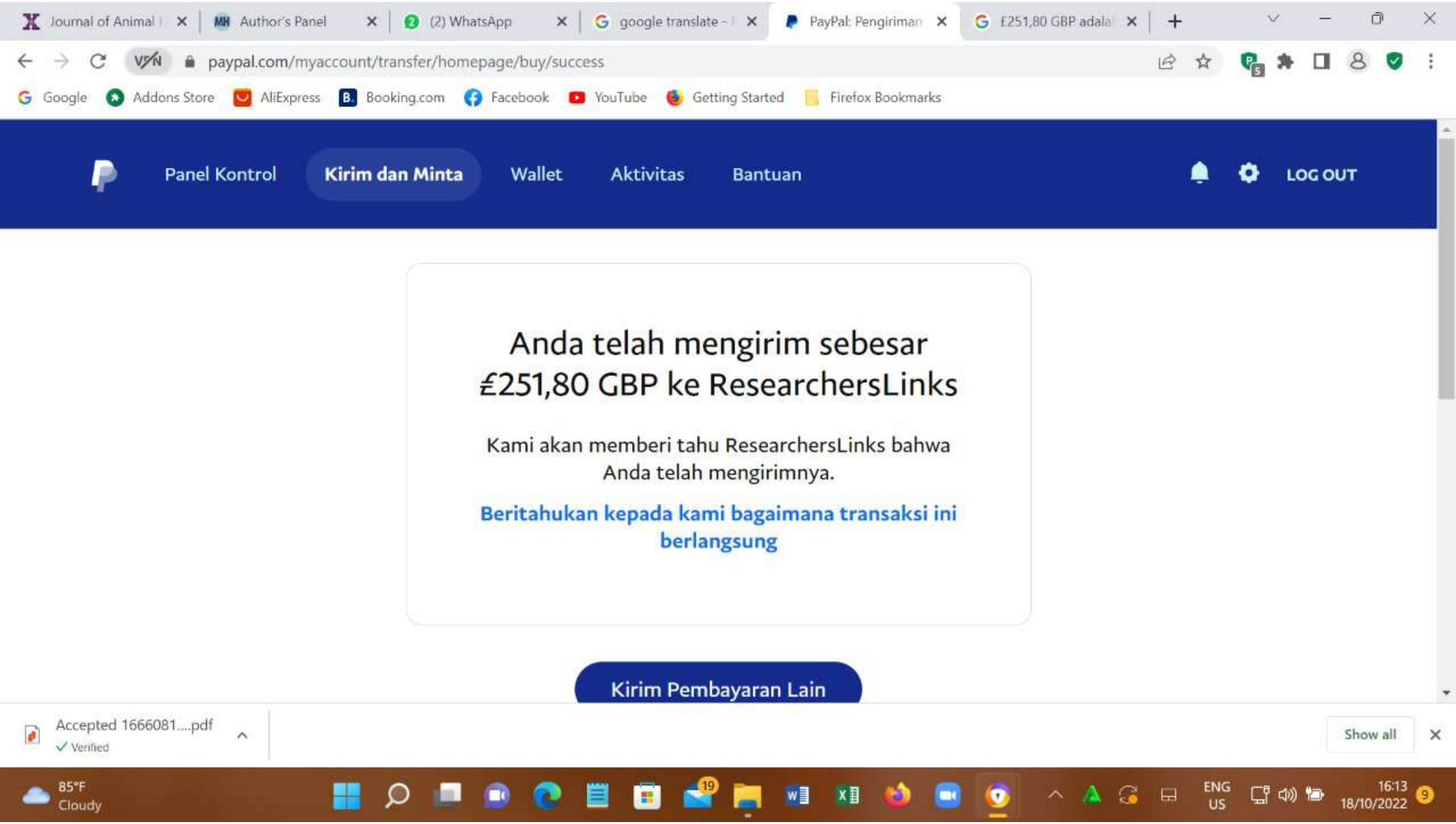
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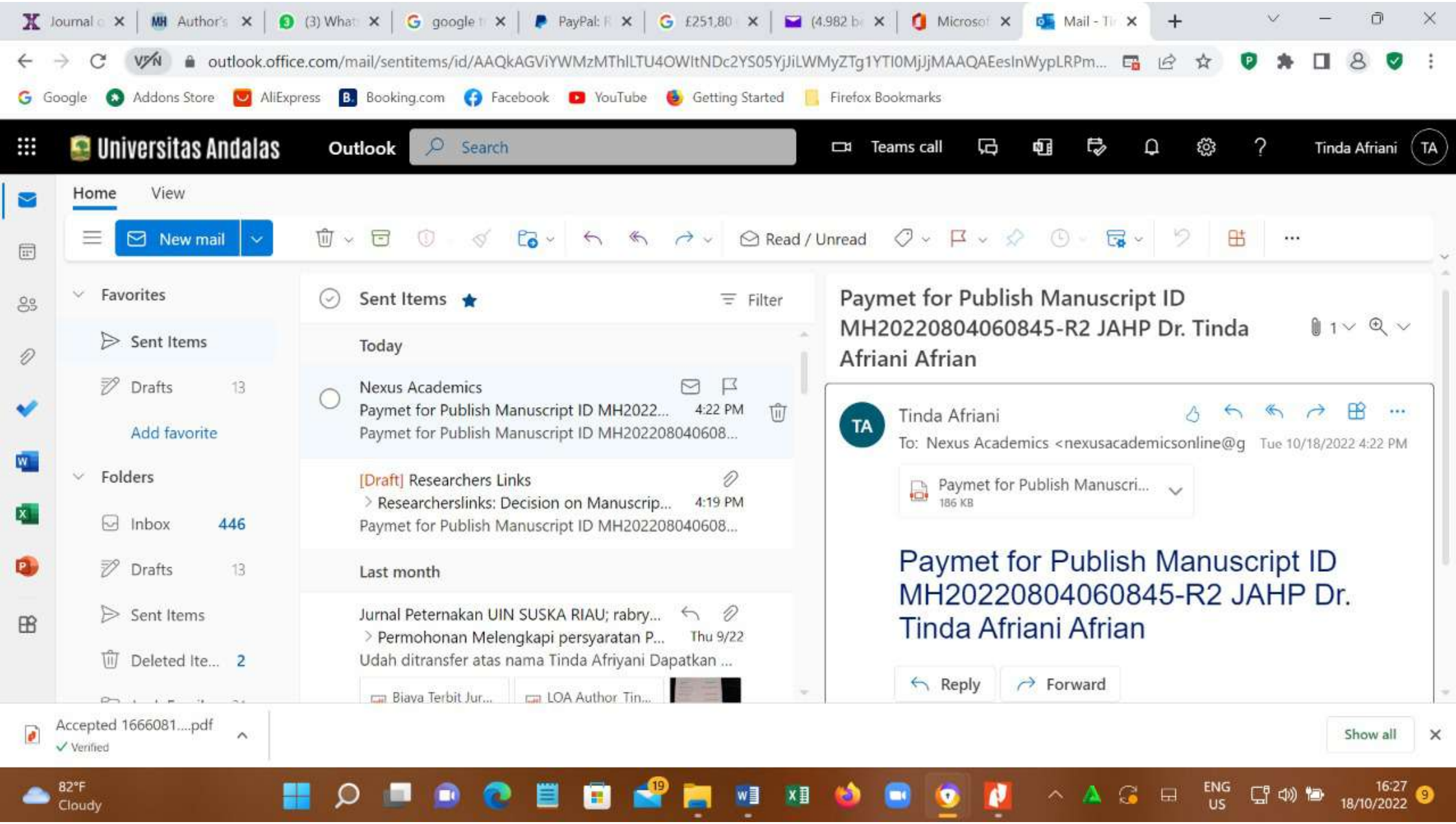
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According to Asaf et al. (2014) SNPs in the coding region can directly affect the protein, SNPs in the intron region can affect splicing, while SNPs in the promoter may affect gene expression. Nevertheless, both intronic and exonic mutations can affect performance (Helal et al., 2021; Mi et al., 2021; Yadav et al., 2021). Although introns are not transcribed into proteins, many research results showed different functions of introns, one of the most important functions is increasing protein abundance. This indicates that although the intron region is not involved in protein synthesis, variations in introns can affect the translation process. The effect of introns was first observed in Simian Vacuolating viruses which produce undetectable protein products upon deletion of their intron sequences (Gruss et al., 1979; Hamer et al., 1979).

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As shown in Table 4, the results of the chi-square test (χ^2) on the genotype frequency of the *FSHR* gene showed a difference between the ratio of observations and the ratio of expectations or a state of Hardy-Weinberg imbalance that occurs in the Pesisir cattle population (P>0.05). This might be due to the small population of Pesisir cattle which restricted the random mating in the Pesisir cattle population. In accordance with Falconer and Mackay (1983) who stated many factors can affect the Hardy-Weinberg imbalance, including mutations, gene flow, migration, selection, genetic drift, and the absence of random mating. Vasconcellos et al. (2003) also suggested that some factors such as accumulation of genotypes, divided population, mutation, selection, migration and mating in the same group/population (endogamy) can cause imbalances in the population.

CONCLUSION

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Identification of Single Nucleotide Polymorphism (SNP) in Exon 9 and Intron 9 of Follicle Stimulating Hormone Receptor (FSHR) Gene in Pesisir Cattle

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Faculty of Animal Science Andalas University Padang West Sumatra Indonesia.

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Keywords | SNP, *FSHR* gene, Pesisir cattle, DNA isolation, single nucleotide polymorphism

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***Correspondence** | Tinda Afriani, Faculty of Animal Science Andalas University Padang West Sumatra Indonesia; **Email**: tindaafriani@ansci.unand.ac.id
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INTRODUCTION

Pesisir cattle is one of the Indonesian cattle from West Sumatra, which has a smaller body size compared to other local cattle breeds. The adult body weight of Pesisir cattle is ranging from 200 – 400 kg, and they are mainly raised on rural farms with poor reproduction management (Afriani et al., 2022). Pesisir cattle have a significant contribution to the meat demands of the people of West Sumatra. As a local breed, Pesisir cattle have several desirable characteristics, such as their ability to survive in harsh environmental conditions with high reproductive efficiency (Sarbaini, 2004). The potential genetic resources of local Indonesian cattle are very diverse and have not been used optimally. The diverse genetic resources of a breed may make a population more resistant to live and higher adaptability to environmental changes (Frankham et al., 2002). Because of the growing cattle population in Indonesia, it is advisable to explore the genetic resources of local breeds.

The livestock productivity can be improved up to the genetical potential using the molecular selection tools. The implementation of selection at the molecular level for the local genetic resources has not been widely used. This utilization can be carried out as an effort to obtain superior local Indonesian cattle that have high productivity and reproduction capabilities (Nasution, 2013). Talenti et al. (2022) stated that improving the genetic quality of breeds to produce the livestock with better phenotypes and genotypes, is strongly influenced by the presence of genes in it. One of the important genes that influencing reproduction in cattle is the follicle stimulating hormone (*FSH*) gene.

The FSH stimulate the gonads in females, however it can not work directly into the cell without the help of its receptor i.e., follicle stimulating hormone receptor (*FSHR)*. The *FSHR* works directly into cells and help in increasing the production of ovum cells in females as it is a transmembrane receptor that interacts with follicle-stimulating hormone. The *FSHR* gene is required to carry the *FSH* gene to the target tissue so that it can be translated into protein to become the FSH hormone. The *FSHR* gene found on chromosome 11 consisting of 10 exons and 9 introns with length of 194885 (NCBI GenBank access code NC-037338). One of the functions of *FSHR* is to support the activation of specific receptors in stimulating follicular growth in the ovaries (Fan and Hendrickson, 2005).

Detecting genetic variation based on nucleotides can be done using the SNP (single nucleotide polymorphism) technique via a PCR (polymerase chain reaction) product. The SNP technique has the advantage that it is easy to apply and read the data. SNP is a marker in genomic variation between individuals so that it can be used to detect alleles that carry important traits in an individual (Mmeka et al., 2013). This study was conducted with aim to identify the SNPs in the *FSHR* gene (exon 9 and intron 9). The target DNA that was amplified in this study was the *FSHR* gene fragment located at exon 9 with a length of 185 bp.

MATERIALS AND METHODS

This study used the blood samples that were collected from female Pesisir cattle. A total of 70 Pesisir cows (aged 2-5 years) were used to collect samples from a cattle breeding center i.e., BPTUHPT Padang Mengatas, Payakumbuh, West Sumatra, Indonesia.

DNA Isolation and *FSHR* Gene Amplification

DNA isolation from blood samples was carried out using a commercial extraction kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, USA). The isolated DNA was then visualized using 1% agarose gel and stored at -20°C until used.

Gene amplification was carried out using the PCR machine with a pair of primers F: 5'-TTA GGC CCT GTG ACT GTG AG-3' and R: 5'-CTG GCA AAG AGG GAA CAA GAG-3' (Yurnalis, 2019). The PCR reactions were prepared with Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Chicago, USA) using a predenaturation program at 95°C for 5 minutes, denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, initial extension at 72°C for 1 minute and final extension at 72°C for 5 minute. PCR was performed with 35 cycles. The results of the amplification of the *FSHR* gene were seen by electrophoresis using 1% agarose gel and Ethidium Bromide liquid; and were visualized under a UV transmulator. Gene amplification was said to be successful if the agarose gel showed bands formed in each well-containing DNA samples of PCR products. The target DNA was determined by comparing the position of the band formed with the position of the DNA ladder band. The results of the electrophoresis were documented using a digital camera.

Sequencing

The PCR product from the *FSHR* exon 9 gene was sequenced using the services of a commercial company (1st BASE Singapore).

Data analysis

All data were saved in Excel Spread Sheets. Genotypic and allelic frequencies were determined using the formula of Nei and Kumar (2000).

Genotype frequency

where,

x_i = observed genotype

N = total number of individual samples

n_i = total number of individual samples with genotype i

Allele frequency

Where,

x_i = allele frequency i

n_{ii} = number of individuals for the genotype ii

n_{ij} = number of individuals for the genotype ij

n = number of samples

Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium was tested with chi-square test, where χ^2 is the chi-square test, O_{ij} is the number of genotypic observations and E_{ij} is the expected number of genotypes

RESULTS AND DISCUSSION

FSHR

In current investigation, amplification of the *FSHR* gene fragment was carried out in Pesisir cattle that showed the position of the *FSHR* gene at exon 10 with a PCR product of 962 bp long. The amplification results of the study were shown in Figure 1.

The amplification process during electrophoresis can be declared successful if in one gel block only one DNA band is visible, but not all samples showed the same density of DNA band. This was due to differences in the concentration of extracted DNA in the template used for PCR as well as differences in the concentration of DNA that was successfully amplified (multiplied) (Sambrook and Russell,

Table 1: Mutation detection in FSHR gene of Pesisir Cattle

Serial No.	Mutation	Position	Mutation Type
1	A → G	8 (exon 9)	Transition
2	T → C	213 (exon 9)	Transition
3	A → T	+49 (intron 9)	Transversion
4	A → C	+232 (intron 9)	Transversion
5	G → A	+252 (intron 9)	Transition

Table 2: The result of haplotype in exon 9 and intron 9 of FSHR gene in Pesisir Cattle (aligned with GenBank reference).

	Control Area Sequence Number				
Position	8	213	+49	+232	+252
Haplotype	A	T	A	A	G
1	AG	.	.	AC	.
2	.	.	.	AC	.
3	AG	.	.	CC	.
4	.	.	.	.	.
5	.	.	.	AC	.
6	.	.	.	CC	GA
7	.	.	.	AC	.
8	AG	.	.	AC	.
9	AG	.	.	CC	.
10	GG	CC	TT	CC	.
11	AG	.	.	AC	.
12	AG	.	.	CC	GA
13	.	.	.	AC	GA
14	.	.	.	CC	AA
15	.	.	.	.	.
16	AG	TC	.	AC	.
17	AG	.	.	CC	GA
18	AG	.	.	CC	.
19	AG	TC	.	CC	GA
20	GG	CC	TT	CC	.
21	.	.	.	AC	GA
22	.	TC	.	AC	.
23	AG	.	.	AC	.

Table 3: Genotype frequency and allele frequency of FSHR gene.

SNP Position	N	Genotype Frequency			Allele Frequency	
8	70	GG	AG	AA	G	A
A – G		0.1	0.46	0.45	0.33	0.67
213	70	TT	TC	CC	T	C
T – C		0.83	0.07	0.1	0.86	0.14
+49	70	AA	AT	TT	AC	T
A – T		0.86	0	0.14	0.86	0.14
+232	70	CC	AC	AA	C	A
A – C		0.36	0.47	0.17	0.60	0.40

+252	70	GG	GA	AA	G	A
G – A		0.82	0.17	0.01	0.9	0.1

Description: SNP = *Single Nucleotide Polymorphism*
N = Number of samples

Table 4: Chi-square test result for Hardy-Weinberg equilibrium of FSHR gene of Pesisir cattle

Serial No.	Mutation Position	χ^2 value	χ^2 level		Description
			0.05	0.01	
1	18	187.56	5.991	9.210	**
	A → G				
2	213	62.46	5.991	9.210	**
	T → C				
3	+49	72.53	5.991	9.210	**
	A → T				
4	+232	146.93	5.991	9.210	**
	A → C				
5	+252	104.59	5.991	9.210	**
	G → A				

Description: $x^2_h > x^2_t$ (0.05) = (**) significantly different

2001). Zulkarnaim et al. (2010) reported the amplification of GHR|AluI gene fragment performed on Bali, Limousin, Simmental, and Pesisir cattle and showed the position of the GHR gene at exon 10 with a 298 bp PCR product.

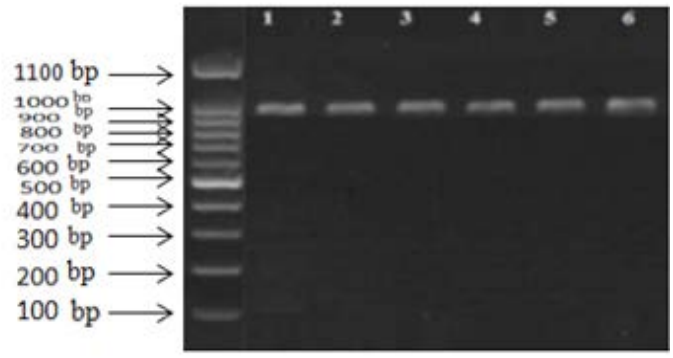

Figure 1: PCR product amplification of the exon 9 of the FSHR gene in Pesisir cattle.

FSHR Gene Diversity and Mutation Detection

All the PCR products samples (n=70) were sequenced using the services of 1st BASE Singapore and analyzed using the SeqManTM version 4.00 DNASTAR program (DNASTAR Inc., Madison, Wisconsin, USA). The reference sequence used in this study was the *FSHR* Exon 9 gene sequence which was obtained from NCBI Genbank No. NC-037338. The results of the sequencing showed that there is diversity in the exon 9 and some intron 9 regions (Table 1).

Five SNPs were detected, two were found in exon 9, and three in intron 9. Two types of mutations were obtained; transition and transversion. The computer-generated sequencing electropherogram showed the mutation of the exon 9, as shown in Figure 2.

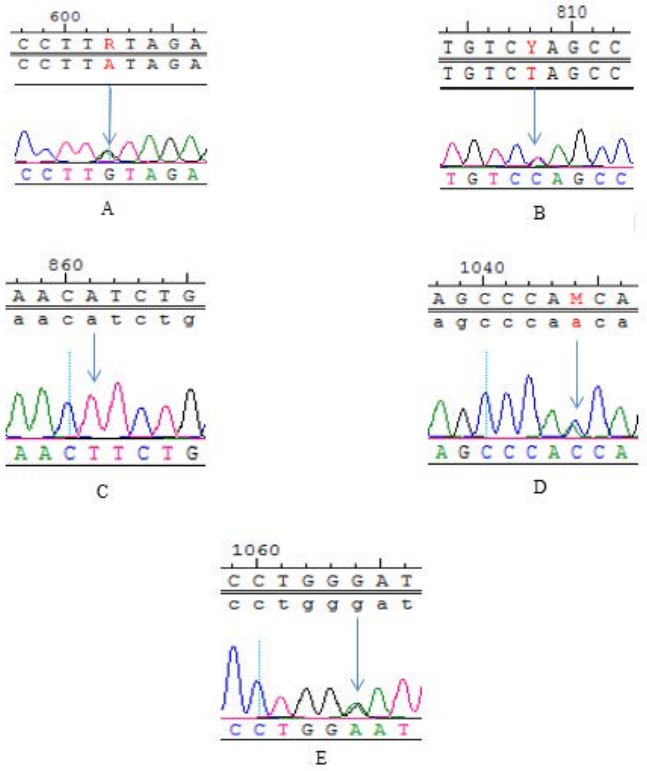

Figure 2: Transition A→G at position 8 (A), transition T→C at position 213 (B), Transversion A→T at position +49 (C), tranversion A→C at position +232 (D), Transition G →A at position +252 (E)

According to Asaf et al. (2014) SNPs in the coding region can directly affect the protein, SNPs in the intron region can affect splicing, while SNPs in the promoter may affect gene expression. Nevertheless, both intronic and exonic mutations can affect performance (Helal et al., 2021; Mi et al., 2021; Yadav et al., 2021). Although introns are not transcribed into proteins, many research results showed different functions of introns, one of the most important functions is increasing protein abundance. This indicates that although the intron region is not involved in protein synthesis, variations in introns can affect the translation process. The effect of introns was first observed in Simian Vacuolating viruses which produce undetectable protein products upon deletion of their intron sequences (Gruss et al., 1979; Hamer et al., 1979).

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Based on the results of the sequencing, there were 30 haplotypes in exon 9 and intron 9 in the *FSHR* gene of Pesisir cattle. The main differences between individuals and diversity in the population of Pesisir cattle were shown in Table 2. The results showed that there were differences in haplotypes indicating individual differences. According to Akbar et al. (2014), different haplotype types in each individual can be used as a reference in individual identification. The more diverse types of haplotype composites in a population, the higher the level of genetic diversity (Baksir et al., 2022).

Genotype Frequency and Allele Frequency

Based on the number and types of genotypes in the exon 9 and intron 9 *FSHR* genes in Pesisir cattle, the genotype and allele frequencies were shown in Table 3.

Table 3 shows that there are 5 positions of SNP, namely 8 A – G, 213 C – T, +49 A – T, +232 A – C and +252 G – A with 3 pairs of genotypic frequencies each and having the highest allele frequency of 0.9. The results of this study illustrate that the genotype frequency distribution in this study is polymorphic (various) because the value is less than 0.99 or more than 1%. As stated by Nei and Kumar (2000) that the locus of an allele is said to be polymorphic (various) if the frequency is equal to or less than 0.99 or more than 1%. If the opposite happens then it is monomorphic (uniform). Polymorphic properties are very important to analyze because it is one of the requirements for a gene to be used as a genetic marker (Hartl and Clark, 1997).

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As shown in Table 4, the results of the chi-square test (χ^2) on the genotype frequency of the *FSHR* gene showed a difference between the ratio of observations and the ratio of expectations or a state of Hardy-Weinberg imbalance that occurs in the Pesisir cattle population (P>0.05). This might be due to the small population of Pesisir cattle which restricted the random mating in the Pesisir cattle population. In accordance with Falconer and Mackay (1983) who stated many factors can affect the Hardy-Weinberg imbalance, including mutations, gene flow, migration, selection, genetic drift, and the absence of random mating. Vasconcellos et al. (2003) also suggested that some factors such as accumulation of genotypes, divided population, mutation, selection, migration and mating in the same group/population (endogamy) can cause imbalances in the population.

CONCLUSION

The results of current study on Pesisir cattle declared that in the exon 9 and intron 9 regions of the *FSHR* gene, there were 5 polymorphic SNPs. The Chi-square test shows that the allele frequency and genotype frequency in the population of Pesisir cattle are not in Hardy-Weinberg equilibrium (χ^2 h> χ^2 t).

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

All authors state that there is no conflict of interests.

NOVELTY STATEMENT

This study on the *FSHR* gene of Pesisir cattle to identify SNPs could be used a reference or basic information for further research to complete the molecular genetics framework for the improvement of the genetic quality of Pesisir cattle as well as other livestock species.

AUTHORS CONTRIBUTION

Tinda Afriani (TA), Yurnalis (Y) and Jaswandi (J) conducted research, Mangku Mundana (MM), Adisti Rastosari (AR) and Anna Farhana (AF) collected data, Y wrote the manuscript, Endang Purwati (EP) and J revised the manuscript, and TA helps in editing of the manuscript.

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