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Research Article

Sequence Analysis and Polymerase Chain Reaction of Growth Hormone Gen Exon 5 in Pesisir and Friesian Holstein Cattle Crosses

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Abstract | The growth and development of livestock were significantly influenced by genes, particularly, growth hormone (GH). The gene holds potential as a candidate in the Marker Assisted Selection program, which involves using genetic marker technology to enhance livestock selection for desirable growth traits. One approach to improving the performance of Pesisir with Holstein Friesian cattle crosses involves identifying growth hormone variations through analysis of the exon 5 gene. This study aimed to identify the characteristics of qualitative and quantitative traits and growth hormone patterns in crossbreeds between Pesisir and Friesian Holstein (FH) cattle. A total of 60 crossbreeds of Pesisir and FH cattle were used. The method employed involved a molecular analysis of growth hormone in the exon 5 gene using Polymerase Chain Reaction (PCR), alongside a qualitative analysis of the characteristics exhibited by the FH and Pesisir cattle crosses. The identification results regarding the diversity of the GH gene found 14 point mutations, including six transversions, namely, C>G 1343 bp, T>G 1376 bp, C>A 1438 bp, G>C 1570 bp, A>C 1720 bp, and G>A 1744 bp. Additionally, seven transitional mutations were detected, including G>A 1359 bp, T>C 1409 bp A>G 1454 bp, G>A 1485 bp, G>A 1506 bp, G>A 1538 bp, C>T 1659 bp, and one insertion C 1838 pb. This study found two alleles and three genotypes with a general heterozygosity value where H0>He. The growth hormone gene from the Pesisir and FH cattle crosses were polymorphic, with imbalanced genotype frequencies, and the body size of males was longer than that of females.

Keywords | Growth Hormone, Pesisir Cattle, Holstein Friesian Cattle, Gene Exon 5

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INTRODUCTION

The population and productivity of cattle in Indonesia are facing a decline, specifically in terms of growth. To address this issue and harness livestock genetic resources, focused efforts are essential. One of the viable efforts is genetic quality improvement, which has the opportunity potential to spur increased productivity and boost the cattle population. According to Afriani et al. (2019), factors capable of boosting cattle productivity and population include increasing genetic quality, improving rearing management, and limiting production. Genetic quality improvement can be achieved through selection and crossing (Widyas et al., 2022). The cross between Pesisir and FH cattle is expected to improve the genetic quality of livestock.

One factor influencing the change in the growth rate percentage is Growth Hormone, which is needed in tissue,

fat metabolism, reproduction, and general body development (Beauchemin et al., 2006). A complex system of somatropin axis controls growth in cattle. Genes involved in this axis, particularly GH play a crucial role in postnatal growth, specifically bone and muscle development, mediated by insulin-like growth factor 1 (IGF-1) (Sellier, 2000). The bovine GH gene, located on the 19th chromosome (Tatsuda et al., 2008), exhibits polymorphisms (Dario et al., 2005). A substitution of leucine (L) with Valine (V) at position 127 in exon 5 has been identified, through Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) using glycolate oxidase 1 (Go1) restriction enzymes (Hradecka et al., 2008).

At the DNA level, livestock selection can be done by assessing certain genes' diversity. One way to achieve this is to find the cattle growth hormone gene, a national treasure that should be preserved and utilized to increase livestock production. Advances in molecular markers (DNA Markers) have facilitated identifying genetic changes resulting from crosses and their relationship with quantitative and qualitative traits in livestock (Singh et al., 2014). In addition, molecular markers can also differentiate between breeds, specifically to preserve and maintain the purity of certain breeds. The growth and development of livestock are influenced by GH (Hu et al., 2021). This gene holds potential as a candidate in the Marker Assisted Selection (MAS) program, which employs genetic marker technology to enhance growth and carcass traits in cattle (Pintaka, 2018). Enhancing the genetic attributes of breeds to yield livestock with improved phenotypes and genotypes is significantly impacted by the inherent presence of genes (Talenti et al., 2022). The GH gene is needed for tissue growth, fat metabolism, regulation of reproduction, lactation, and general body growth (Beauchemin et al., 2006). It has also been linked to growth characteristics in beef cattle (Hale et al., 2000). In addition, the gene plays a role as the main regulator of growth after birth and metabolic processes associated with growth rate, body composition, health, milk production, and aging through adjustments with other genes (Ge et al., 2003). The GH gene is related to live weight and body weight gain in beef cattle, encoding growth and development hormones (Pierzchale et al., 2004; Schneider et al., 2013). Consequently, the gene has become the most intensive object of study in ruminants linking GH mutations with production traits (Zhao et al., 2004; Misrianti et al., 2012). The GH gene was located on chromosome 19 and has a long sequence size nucleotides of 1869 base pairs (bp) consisting of five exons separated by four introns (Amiri et al., 2018). Several polymorphic regions have been reported in different regions of the gene (Sedykh et al., 2021). Therefore, this study aimed to identify qualitative and quantitative characteristics of the GH gene in crosses of Pesisir and FH cattle by sequencing analysis of the Exon 5 gene.

MATERIALS AND METHODS

Blood sampling was conducted based on the following criteria, live cattle in Koto XI Tarusan, Bayang, and Pesisir Selatan Regency, Indonesia, have various coat colors, aged between 1-6 months, male or female. A total of 60 samples were taken as blood from crosses of Pesisir and FH cattle. The blood was collected through the jugular vein up to 5 ml and placed into the EDTA tube. One of the photos of a Pesisir and FH cattle crosses is shown in Figure 1.

Figure 1: Pesisir and Friesian Holstein Cattle Crosses

DNA extraction

DNA extraction was performed using blood samples and extracted with genomic G-Spin™ Total DNA Extraction Kit from iNtRON Biotechnology.

Primer of Growth Hormone Gene

The amplification of the GH exon 5 gene and the end region was done with the Eppendorf Thermal Cycler machine using the Polymerase Chain Reaction (PCR) method with a base length of 824 bp. The primers used to amplify the GH gene segment in the exon five region and the tip region were the forward primer 5'-GCAGTGGAGGATGATGGTG-3' and the reverse primer 5'-AGGAAAGGACAGTGGGAGTG-3'. Primer serves to recognize the sequence of base pairs to be amplified before sequencing.

PCR Product Sequencing and Cut site detection

PCR product sequencing represent the last step to determine the nucleotide sequence of the fragment resulting from amplification using the PCR technique.

Analyzing qualitative and quantitative characteristics

Qualitative traits in livestock were analyzed by observing the color of the fur, eye circles, muzzle, wattles, and horns. Body measurements (quantitative characteristics) were carried out with the cow standing upright on a flat plane (the "parallelogram" position). The characteristics measured included body weight, head length and width, body length, chest circumference, and shoulder height.

Data analysis

The GH gene was identified after PCR, followed by sequencing. Meanwhile, the individual characteristics of crosses between Pesisir and FH cattle were assessed with qualitative and quantitative measurements. The results obtained were then presented descriptively (Alhamda, 2016). The Hardy-Weinberg equilibrium was tested with a chi-square test to determine whether the genotype and allele frequencies of the GH gene were in balance (Nafiu et al., 2020).

RESULTS AND DISCUSSION

Amplification of the GH Gene Segment by the PCR Method from Crosses of Pesisir Cattle and Holstein Frisien (FH) Cattle

Amplification of the GH gene segment was carried out using primer pairs Forward: 5'- GCA GTG GAG GAT GAT GGT G-3' and Reverse 5'- AGG AAA GGA CAG TGGGAG TG-3' with a fragment length of 824 bp. Among the 60 DNA samples comprising 37 from male crossbreed cattle of Pesisir and FH, as well as 23 from a crossbreed of Pesisir and FH, electrophoresis on agarose 1% showed successful amplification of PCR products. The amplification results were presented below in Figure 2.

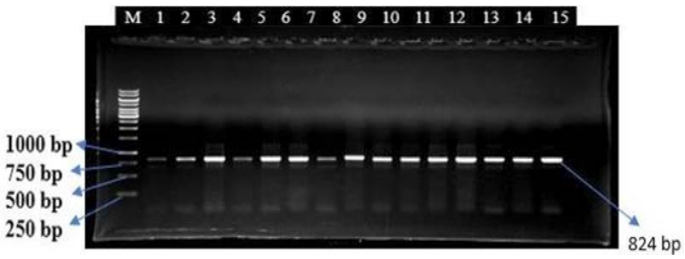

Figure 2: Results of amplification of the GH gene segment using the PCR method

Figure 2 shows that the primers used succeeded in amplifying the exon 5 GH gene with a fragment length of 824 bp. A successful amplification is indicated by the presence of a single band of DNA or fragment expected within a gel well. A primer is non-specific when it produces an amplification product of more than one fragment or when the length does not match the predicted size during primer design. Faint and spread-out bands signify broken intermolecular bonds, while well-defined bands indicate high-concentration, intact DNA (Doublet et al., 2019). The successful outcome of the electrophoresis amplification process is determined by the presence of a singular DNA band within a gel block; however, it is noteworthy that not all samples exhibit uniform DNA band density (Afriani et al., 2022).

According to Viljoen et al. (2005), the success of DNA amplification depends on the interaction of PCR components in the right concentration. Common PCR optimization steps include primer annealing temperature, as well as Mg^{2+}, primer, and target DNA concentrations. Sorber et al. (2017) stated that the smear formed during electrophoresis stemmed from residual washing solutions. Zhang et al. (2012) added that such smears could indicate the presence of contaminants other than isolated DNA. The accuracy of the PCR conditions relies on the precision of the reaction mixture and temperature conditions in each cycle (Rahayu et al., 2006).

Gene Identification of Growth Hormone

Gene identification of Growth Hormone was conducted by sequencing using 1st *Base* services in Singapore, sent through Genetika Science Indonesia. A total of 60 DNA samples from the crossbreeding of Pesisir and FH cattle were submitted for analysis. Sequencing results in electropherogram data were then analyzed using the MEGA 11 application for sequencing of nucleotide bases. The reference sequence used was Gene Bank NC_037346.

Based on the alignment analysis results, the electropherogram data obtained demonstrated mutations. An overview of mutation events is shown in Table 1. Table 1 shows that 14 variations were found in the exon 5 region in the Pesisir and FH cattle cross samples. This diversity originated from SNPs, involving sequences, recombination, and insertion. A mutation is an event that potentially causes changes in genetic material with inherited properties. This phenomenon is due to changes in the number and types of nitrogenous bases, as well as shifts in the location of the sequence along the nucleotide chain. Based on the alignment analysis results, several point mutations appeared in the sequence of the growth hormone gene from crossbreeding of Pesisir and FH cattle as shown in Figure 3-5.

Based on the analysis results, 13 point mutations consisting of 7 transitions, 6 transversions, and 1 insertion point were discovered. Transition-type mutations occur when a pyrimidine base consisting of thymine (T) or cytosine (C) replaces another pyrimidine base, or when a purine base comprised of adenine (A) or guanine (G) substitutes

Table 1: Mutations in Growth Hormone Genes

No	Mutation	Mutation Position (pb)	Mutation Type
1	C>G	1343	Transverse
2	G>A	1359	Transition
3	T>G	1376	Transverse
4	T>C	1409	Transition
5	C>A	1438	Transverse
6	A>G	1454	Transition
7	G>A	1485	Transition
8	G>A	1506	Transition
9	G>A	1538	Transition
10	G>C	1570	Transverse
11	C>T	1659	Transition
12	A>C	1720	Transverse
13	G>C	1744	Transverse
14	Ins A	1838	Insertion

Note: pb = base length

another purine within double-stranded DNA. Each base pairs specifically with its counterpart on the complementary strand; Adenine (A) + Thymine (T) and Cytosine (C) + Guanine (G). Meanwhile, transversion mutations occur when a purine base replaces a pyrimidine base or vice versa. Muladno (2010) stated that nucleotide diversity does not depend on the DNA strand length and the sample size. This provides an opportunity for even a small sample size to offer valuable information and a comprehensive understanding of the broader population.

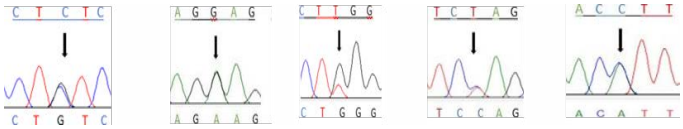

Figure 3: Gene mutation 1438 bp.

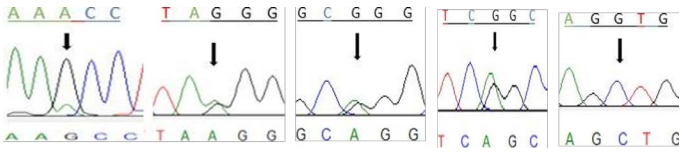

Figure 4: Mutation gene C1570 bp.

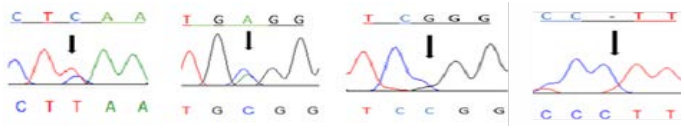

Figure 5: Mutation gene Ins C 1838 pb

Genotype and Allele Frequency

The genotype and allele frequencies obtained from the analysis were presented in Table 2. Table 2 shows that the diversity of the GH gene is polymorphic. Genetic diversity among subpopulations can be identified by examining similarities and differences in allele frequencies (Li et al., 2000). Allele frequency denotes the relative occurrence of an allele within a population (Nei and Kumar, 2000). Genotype and allele frequencies are influenced by selection, gene mutation, the merging of two populations with different gene frequencies, crossing over, outcrossing, and genetic drift (Rahmatullah et al., 2016).

Heterozygosity

Heterozygosity is one of the parameters used to measure the level of genetic diversity based on allele frequencies at each locus (Anggraeni et al., 2009). Genetic diversity, specifically expressed as expected heterozygosity (H), stands as a prevalent metric employed in evaluating genetic variability within populations, as commonly observed in statistical analyses of genetic variations. (Harris and DeGiorgino, 2017). The heterozygosity analysis results of Pesisir and FH cattle crosses are shown in Table 3.

Table 3 presents the heterozygosity analysis data for Pesisir and FH cattle crosses. The results showed that the average value of H0>He indicates a higher presence of individuals with heterozygous genotypes. This prevalence of heterozygous genotype suggests a potential for outbreeding. Ablondi et al. (2021) Alterations in nucleotide bases, commonly referred to as mutations, serve as a prevalent foundation for detecting and characterizing genetic diversity. Polymorphism is a variation of the genetic structure within a population (Kasprzak-Filipek et al., 2019).

Hardy-Weinberg balance

Hardy-Weinberg gene frequencies within a population remain constant over time, and the total number of alleles among individuals is also fixed across generations. The GH gene balance was analyzed using the chi-square test and the results are shown in Table 4.

Based on Hardy-Weiberg balance analysis data, seven mutations were significantly different, and six were not, presumably due to the outbreeding cattle population. According to Vasconcellos et al. (2003), several events, such as the accumulation of genotypes, division, mutations, selection, migration, and endogamy, can cause an imbalance in the population. The Hardy-Weinberg law states that allele and genotype frequencies in a population will remain constant unless influenced by certain factors. This condition will only occur when no forces or certain factors change the ratio of alleles and loci. According to the law of balance, a genotype is composed of pairs of alleles from the male and the parent (Nafiu et al., 2020).

Table 2: Genotype and Allele Frequency Analysis Data

No	Position (bp)	N	Genotype Frequency			Allele Frequency	
1	1343	60	TT	TC	CC	C	G
			(0,48)	(0,52)	(0)	(0,74)	(0,26)
2	1359	60	GG	GA	AA	G	A
			(0,5)	(0,5)	(0,5)	(0,75)	(0,25)
3	1376	60	TT	TG	GG	T	G
			(0,07)	(0,93)	(0)	(0,54)	(0,46)
4	1409	60	TT	TC	CC	T	C
			(0,5)	(0,5)	(0)	(0,75)	(0,25)
5	1438	60	CC	THAT	AA	C	A
			(0,97)	(0,03)	(0)	(0,98)	(0,02)
6	1454	60	AA	AG	GG	A	G
			(0,93)	(0,07)	(0)	(0,96)	(0,04)
7	1485	60	GG	GA	AA	G	A
			(0,43)	(0,57)	(0)	(0,71)	(0,29)
8	1506	60	GG	GA	AA	G	A
			(0,95)	(0,05)	(0)	(0,975)	(0,025)
9	1538	60	GG	GA	AA	G	A
			(0,97)	(0,3)	(0)	(0,98)	(0,02)
10	1570	60	GG	GC	CC	G	C
			(0,1)	(0)	(0,9)	(0,1)	(0,9)
11	1659	60	CC	CT	TT	C	A
			(0,68)	(0,32)	(0)	(0,84)	(0,16)
12	1720	60	AA	AC	CC	A	C
			(0,3)	(0,7)	(0)	(0,65)	(0,35)
13	1744	60	GG	AC	CG	G	C
			(0,3)	(0,7)	(0)	(0,99)	(0,01)
14	1838	60	-	-	-	-	-

Note: pb = base length, N = Number of Samples

Table 3: Heterozygosity analysis data of Pesisir and Frisian Holstein cattle crosses

No	Position (pb)	Heterozygosity	
		H0	He
1	1343	0,516	0,28
2	1359	0,5	0,375
3	1376	0,93	0,49
4	1409	0,5	0,375
5	1438	0,03	0,039
6	1454	0,07	0,076
7	1485	0,57	0,411
8	1506	0,05	0,048
9	1538	0,03	0,029
10	1570	0	0,18
11	1659	0,32	0,268
12	1720	0,7	0,455
13	1744	0,02	0,0198

Information: H0 = Observation, He = Expectation

Table 4: Hardy-Weinberg Balance Analysis Data

No	Mutation Position (pb)	X2 count	X2 tab (0,05)	Information
1	1343	7,22	5,991	Real Different
2	1359	7,67	5,991	Real Different
3	1376	46,12	5,991	Real Different
4	1409	7,67	5,991	Real Different
5	1438	0,079	5,991	No Real Difference
6	1454	0,185	5,991	No Real Difference
7	1485	9,13	5,991	Real Different
8	1506	0,039	5,991	No Real Difference
9	1538	0,079	5,991	No Real Difference
10	1570	60	5,991	Real Different
11	1659	0,577	5,991	No Real Difference
12	1720	17,39	5,991	Real Different
13	1744	0,079	5,991	No Real Difference

Description: $X^2h<X^2t(0.05)$ = Not Significantly Different, $X^2h>X^2t(0.05)$ = Significantly Different

CONCLUSION

In conclusion, fourteen-point mutations were identified in the GH gene, including six transversions, namely, C>G 1343 bp, T>G 1376 bp, C>A 1438 bp, G>C 1570 bp, A>C 1720 bp, G>A 1744 bp. Moreover, seven transitional mutations were identified including G>A 1359 bp, T>C 1409 bp A>G 1454 bp, G>A 1485 bp, G>A 1506 bp, G>A 1538 bp, C>T 1659 bp, and one insertion C 1838 bp. These mutations collectively showed two alleles and three genotypes with general heterozygosity values where H0>He. The GH gene from Pesisir and FH cattle crosses exhibited polymorphism, characterized by imbalanced genotype frequencies.

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NOVELTY STATEMENT

Exploring the intricate genetic landscape, our research delves into the sequence analysis and polymerase chain reaction of the growth hormone gene exon 5 in crossbred Pesisir and Friesian Holstein cattle, unraveling novel insights into the molecular intricacies that shape the phenotypic traits of these unique hybrids. This study could be used as a reference or basic information for further research to complete the molecular genetics framework for improving the genetic quality of Pesisir cattle and other livestock species.

CONFLICT OF INTEREST

All authors confirm there is no conflict of interest in this publication.

AUTHOR CONTRIBUTION

All authors equally share duties and responsibilities.

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