Diversity of the Monoamine Oxidase A Gene in Beef Cattle

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ABSTRAK

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Monomamine Oxidase A (MAOA) merupakan gen yang turut mengontrol sifat-sifat agresif. Gen MAOA berperan dalam mengkodekan enzim monoamine oksidase A yang berperan dalam katabolisme neurotransmiter, termasuk dopamin, norepinefrin, dan serotonin. Penelitian ini bertujuan untuk mengidentifikasi keragaman gen SNP MAOA pada sapi potong dengan metode sekuensing. Penelitian ini menggunakan 127 sampel DNA sapi untuk identifikasi keragaman, antara lain sapi Bali, Limousin, Wagyu, PO, Madura, dan Wagyu-Bali (F1). Polimorfisme gen MAOA yang terletak pada promotor dan ekson 1 dianalisis menggunakan metode sekuensing. Frekuensi genotipe, frekuensi alel, nilai heterozigositas, dan keseimbangan Hardy-Weinberg dihitung menggunakan program PopGen32. Hasil penelitian menunjukkan bahwa gen MAOA yang terletak di daerah promotor memiliki enam SNP, salah satunya adalah SNP g.385G>A, sedangkan gen MAOA yang terletak di ekson 1 bersifat monomorfik. Metode PCR-RFLP digunakan untuk menyelidiki polimorfisme gen SNP g.385G>A MAOA menggunakan enzim restriksi RSaI. Gen MAOA terdeteksi pada 3 genotipe yaitu GG, GA, dan AA. SNP g.385G>A bersifat polimorfik pada sapi Bali, PO, Madura, dan silangan Wagyu-Bali (F1), sedangkan monomorfik pada sapi Limosin dan Wagyu. Penelitian lebih lanjut diperlukan untuk mengeksplorasi implikasi fungsional SNP g.385G>A dan hubungannya pada perilaku agresif sapi.

Kata Kunci: Sapi Pedaging, Keragaman Genetik, Gen MAOA, Single Nucleotide Polymorphism

ABSTRACT

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Monoamine Oxidase A (MAOA) is a gene that controls aggressive traits. The MAOA gene plays a role in encoding the monoamine oxidase A enzyme, which plays a role in the catabolism of neurotransmitters, including dopamine, norepinephrine, and serotonin. This study aims to identify the diversity of the MAOA SNP gene in beef cattle using sequencing methods. This research used 127 cattle DNA samples to identify diversity, including Bali, Limousin, Wagyu, PO, Madura, and Wagyu-Bali (F1) cattle. MAOA gene polymorphisms in the promoter and exon 1 were analyzed using sequencing methods. Genotype frequencies, allele frequencies, heterozygosity values, and Hardy-Weinberg balance were calculated using the PopGen32 program. The results showed that the MAOA gene in the promoter region has six SNPs, one of which is SNP g.385G>A, while the MAOA gene in exon 1 is monomorphic. The PCR-RFLP method was used to investigate the SNP g.385G>A MAOA gene polymorphism using the RSaI restriction enzyme. The MAOA gene was detected in 3 genotypes: GG, GA, and AA. SNP g.385G>A is polymorphic in Bali, PO, Madura, and Wagyu-Bali cross (F1) cattle while monomorphic in Limousin and Wagyu cattle. Further studies are necessary to explore the functional implications of SNP g.385G>A and their relationship to aggressive behaviors in cattle.

Key Words: Beef Cattle, Genetic Diversity, MAOA Gene, Single Nucleotide Polymorphism

INTRODUCTION

Indonesia has diverse genetic resources for beef cattle, from local to introduced cattle. Bali cattle, one of the local cattle of Indonesia, are domesticated forms of wild Banteng (*Bos javanicus*), and they are noted for their adaptability to harsh environments (Anwar et al. 2017). However, they also exhibit behavioral traits such as defensive aggressiveness, especially when exposed to stressful conditions like transportation, unfamiliar environments, or improper handling. Bali cattle, in particular, exhibit a higher level of aggressiveness than introduced breeds, especially when restrained or threatened, making their management more challenging (Cooke 2014). This aggressiveness, combined with their hardiness, has driven interest in further research to balance productivity with behavior traits that impact cattle management practices.

The utilization of genetic resources in beef cattle aims to increase productivity, and proper management practices are essential, including effective livestock handling (Amiano et al. 2020). Cattle farming in

Indonesia often follows traditional methods, but modern practices emphasize improving productivity through better breeding, feeding, and handling techniques (Adinata et al., 2023). However, managing problematic livestock, especially those exhibiting aggressive behaviors, can significantly affect productivity. Aggressive behavior in cattle poses a risk to farmworkers and house workers, as it can lead to injury during routine activities like feeding, milking, or health checks (Lindahl et al. 2016). Many farms in Indonesia still rely on manual labor, and this risk has become more pronounced (Laya et al., 2024). Aggressiveness, such as headbutting or kicking, is especially dangerous when dealing with large animals like beef cattle (Titterington et al., 2022). This behavior disrupts management and reduces productivity by increasing stress levels in cattle and the handlers (Eusebi et al., 2018). In Indonesia, addressing these behavioral traits through selective breeding is a crucial strategy to reduce the risks associated with aggressive farming.

Several genes control aggressive traits in cattle, and the monoamine oxidase A (MAOA) gene is one of the key regulators, which are chemicals that transmit signals between nerve cells in the brain (Eusebi et al. 2021). Located on the X chromosome, the MAOA gene consists of 15 exons and 14 introns (Edgnülü et al. 2014). MAOA gene encodes the enzyme monoamine oxidase A, which is responsible for the catabolism of neurotransmitters like dopamine, epinephrine, norepinephrine, and serotonin (V and Husain RS 2017). These neurotransmitters regulate mood, emotions, stress response, and physical movements (Ferreira et al. 2015). After these chemicals send signals between neurons, the MAOA enzyme helps break them into inactive components. This process prevents an excessive buildup of neurotransmitters in the synapse, the gap between neurons. Some individuals carry a variant of the MAOA gene known as MAOA-L (low activity). This variant leads to a less effective enzyme, which slows the breakdown of neurotransmitters. As a result, neurotransmitters like serotonin and dopamine remain in higher concentrations in the brain, which can affect emotional regulation (Sacco et al. 2017). The MAOA enzyme is essential in brain areas such as the amygdala, which regulates emotions like fear and anger, and the prefrontal cortex**,** which is responsible for impulse control and decision-making. When MAOA activity is low, the brain's ability to regulate emotional responses is reduced, potentially leading to more aggressive or uncontrolled behavior (Yen et al., 2021). Mutations or disruptions in the MAOA gene can lead to imbalances in neurotransmitter regulation, which increases the likelihood of aggressive behavior in cattle (van Rhijn et al., 2022). This aggressiveness not only endangers farmworkers but also disrupts the overall management of the herd, causing delays in handling, reduced efficiency, and a higher risk of accidents (Peden et al. 2019).

Biomolecular technology can detect MAOA gene diversity more accurately. One biomolecular technique that can identify the diversity of the MAOA gene is Polymerase Chain Reaction - Restriction Fragment length polymorphism (PCR-RFLP) using restriction enzymes. RFLP can detect high levels of polymorphism and has been widely used to identify genes that produce essential traits (Khasanah et al. 2016). Exploration of MAOA gene diversity has been reported in several types of livestock, such as Lidia and Mexican Spanis cattle, which are known as aggressive breeds of cattle for agility and sport (Eusebi et al. 2020), local Indonesian sheep (Handiwirawan 2012), and Yorkshire (Chen et al. 2019), as well as in domesticated types of cattle such as Angus and Simmental cattle (Lühken et al. 2010). Based on the research results above, information on the MAOA gene has been carried out intensively in various livestock worldwide. However, studies of the MAOA gene in Indonesian beef cattle have never been conducted. Therefore, it is necessary to conduct research to identify and analyze the diversity of MAOA genes in beef cattle in Indonesia.

MATERIALS AND METHODS

Sample collection

The DNA samples used in this research were 127 cattle DNA samples from several representative beef cattle in Indonesia, that are 76 samples of native Indonesian cattle, namely Bali cattle, from BPTU-HPT Denpasar, Bali, and BPTP Kupang, NTT, and local cattle consisting of 15 PO cattle samples from UPTD Ciamis, West Java. The introduced cattle consisted of 6 samples of Wagyu cattle from BET Cipelang Bogor, West Java, and 9 Limousin cattle from BPTU-HPT Padang Mangatas, West Sumatra. Hybridized cattle consisted of 12 samples of Madura cattle from VBC Sapudi Island, Madura, East Java, and 8 Wagyu-Bali cross (F1) cattle from UPTD Kupang, West Nusa Tenggara. The DNA samples are from the collection of the Animal Molecular Genetics Laboratory, Faculty of Animal Husbandry, IPB.

DNA extraction and amplification

Extraction of cattle blood samples uses a DNA kit to obtain DNA samples and follows the procedure of the DNA kit used, namely Geneaid's Genomic DNA Mini Kit. The primers were designed from MAOA gene sequence data on the Ensembl web (www.ensembl.org) with accession number ENSBTAG00000016206. Primers were designed using Primer 3 (https://primer3.ut.ee/) and validated with Primer Stats (www.bioinformatics.org). The primer sequences that have been designed in the promoter fragment and exon 1

are the forward primers 5'-TAC ACA CCA CCT TGC ACT CA-3' and the reverse 5'-AGT GGA CTC TTG TGT GGA CA-3' with a length of 548 bp. The forward primer sequences 5'-TGT CCA CAC AAG AGT CCA CT-3' and reverse 5'-TCC ACA CTG ACC TGA GAT GC-3' were used to amplify a 341 bp long target sequence located at exon one position. 0.5-2 µL of DNA was put into the PCR tube, then 14 µL of premix solution was added, which consisted of 0.2 µL of forward primer and 0.2 µL of reverse primer, 6.1 µL of DW, and 7.5 µL of Red Master Mix. The mixture was incubated in a thermal cycler for the amplification process. The initial stage of the amplification process is the predenaturation stage at a temperature of 95ºC for 1 minute. The second stage consists of 35 cycles, each consisting of a denaturation process at 95ºC for 15 seconds, primary annealing at 57ºC for 15 seconds, and extension at 72ºC for 10 seconds. The final stage is primary elongation at 72ºC for 5 minutes. PCR product results were visualized using 1.5% agarose gel and 1% fluorosafe staining at 100 volts for 35 minutes.

Sequencing and PCR-RFLP

The PCR product results of the promoter fragment and exon 1 of the MAOA gene were sequenced using analysis services, namely the Macrogen Company, Seoul, South Korea. The PCR product of the promoter fragment and exon 1 of the MAOA gene was prepared in 20 µL per sample using forward primers for analysis. The genotype determination of the obtained single nucleotide polymorphisms (SNPs) was carried out using the PCR-RFLP technique using the RSaI restriction enzyme, which was incubated at 37 °C for 4 hours. The determination of cutting enzymes was carried out using the Nebcutter program (www.labtools.us/nebcutter-v2-0/). RFLP results were visualized using 2% agarose gel and 1.5% fluorosafe staining at 100 volts for 35 minutes. The marker used was 2 µL of 100 bp marker. The results of DNA electrophoresis in the form of bands are visible with the help of a UV transilluminator light.

Data analysis

The diversity of the MAOA gene in beef cattle samples was analyzed using the allele frequency, genotype frequency, heterozygosity value, and Hardy-Weinberg balance approach using the PopGen 32 application. Allele and genotype frequencies are calculated as follows:

$$X_i = \frac{\left(2n_{ii} + \sum_{j \neq 1} n_{ij}\right)}{(2N)}$$

$$X_{ii} = \frac{n_{ii}}{N}$$

Where X_i is the frequency of the allele, X_{ii} is the frequency of the genotype, n_{ii} is the number of individuals with genotype ii, n_{ij} is the number of individuals with genotype ij, and N is the total sample.

Estimating observed heterozygosity (Ho) and expected heterozygosity (He) is carried out to determine genetic diversity and estimate the balance of alleles in a population. The estimated heterozygosity value was calculated as follows:

$$H_o = \sum_{i \neq j}^{N} \frac{n_{ij}}{N}$$

$$H_e = 1 - \sum_{i=i}^{q} X_i^2$$

where Ho is the observed heterozygosity (population), He is the expected heterozygosity, n_{ij} is the number of heterozygous individuals, N is the number of individual observations, X_i is the allele frequency, and q is the number of alleles. The HWE was analyzed using the Chi-square test as follows:

$$X^2 = \sum \frac{(O - E)^2}{E}$$

X^2 is the Chi-square, O is the observed value, and E is the expected value.

RESULTS AND DISCUSSION

DNA amplification and SNP identification

The MAOA gene was successfully amplified in the promoter and exon 1 fragments with PCR product lengths of 548 bp and 341 bp, respectively. The results of MAOA gene amplification are presented in Figure 1.

The bands showing the appropriate size in gel electrophoresis after amplification using the PCR method indicate the success of the amplification reaction. Several factors can influence the success of amplification in the PCR method, namely the quantity and quality of the initial DNA used (Putra et al. 2021). The amount of DNA that is too low can produce less bright amplicon bands, while poor or degraded DNA quality can hinder the amplification process. PCR conditions such as annealing temperature, primer concentration, and number of amplification cycles also play an important role. Non-optimal settings of these parameters can result in low amplification efficiency (Hashim and Al-Shuhaib 2019).

Identification of the SNPs of the MAOA gene in the promoter resulted in the discovery of six new single nucleotide polymorphisms (SNPs) that differentiate Bali cattle from other cattle breeds, which can be used to characterize aggressive characteristics in Bali cattle. No SNP was found in the MAOA gene in the exon 1 fragment. The results of the six SNPs obtained are shown in Table 2.

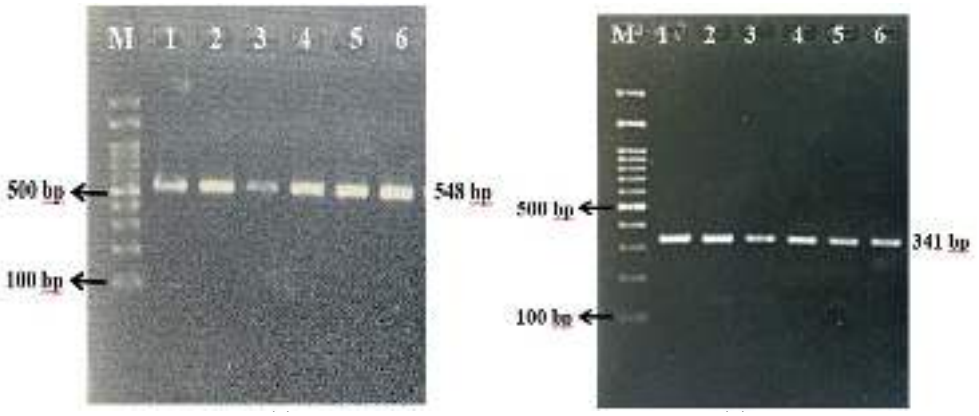

Figure 1. Electrophoresis visualization of PCR amplification results of the MAOA gene sequence promoter fragment (a) and exon 1 (b) on a 1.5% agarose gel; M: 100 bp marker; 1-3: Bali cattle samples, 4-6: Limousin cattle samples

Table 1. SNP identification on the MAOA gene promoter in Indonesian Bali cattle

SNP	Mutation	Restriction Enzymes
g.98A>C	Transversion	-
g.145G>T	Transversion	*Cac*8I
g.216A>C	Transversion	*Mse*I
g.340T>G	Transversion	-
g.349A>C	Transversion	-
g.385G>A	Transition	*Rsa*I

SNP= single nucleotide polymorphism

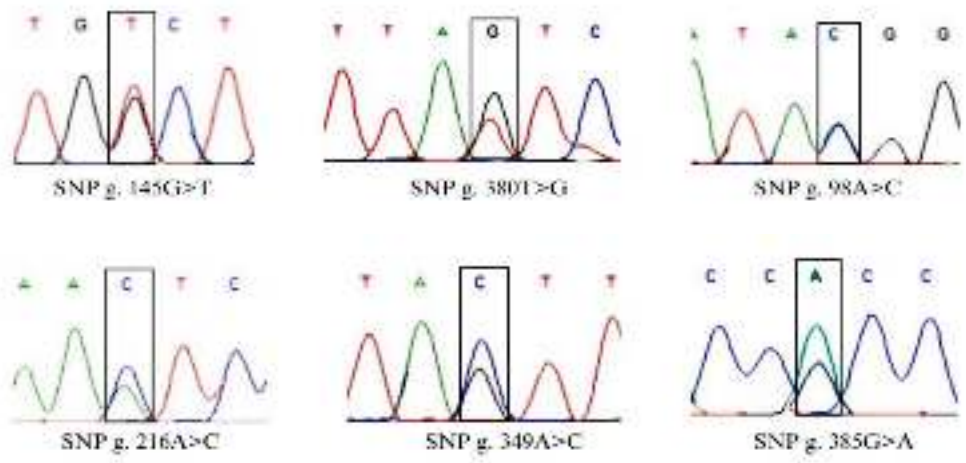

Figure 2. Partial sequencing maps and SNP identification promoter region MAOA gene in Bali cattle

SNP g.385G>A is a transition mutation, which is a replacement of a purine base with another purine base (G>A) or a replacement of a pyrimidine base with another pyrimidine base (C>T), while g.98A>C, g.145G> T, g.216A>C, g.340T>G, g.349A>C are transversion mutations, which is a replacement of a purine base with a pyrimidine base or vice versa (Setyani et al. 2021). Nucleotide changes in the promoter region do not change the amino acids.

Genetic diversity of the MAOA gene

RFLP was performed with a cutting enzyme after detecting an MAOA gene mutation in the promoter fragment. Based on the results of the six SNPs obtained, SNP g.385G>A was then analyzed using the PCR-RFLP technique, namely using the *RSa*I (GT|AC) enzyme (Figure 3). The PCR-RFLP results obtained three genotypes: GG, GA, and AA. The AA genotype has one

Figure 3. PCR-RFLP visualization of SNP g.385G>A MAOA gene promoter fragment on a 2.0% | *Rsa*I (5'-GT | AC-3') agarose gel. M: 100 bp marker; SNP g.385G>A: GG, AG, and AA

Table 2. Diversity of SNP g.385G>A MAOA gene in several breeds of beef cattle

Cattle	N	Genotype Frequency			Allele Frequency		Ho	He	χ^2 Chi-Square
		GG	GA	AA	G	A			
Bali	76	0.00	0.12	0.88	0.06	0.94	0.12	0.11	ns
Limosin	9	1.00	0.00	0.00	1.00	0.00	0.00	0.00	*
Wagyu	6	1.00	0.00	0.00	1.00	0.00	0.00	0.00	*
PO	15	0.40	0.60	0.00	0.70	0.30	0.60	0.44	ns
Madura	13	0.85	0.15	0.00	0.92	0.08	0.15	0.14	ns
WagyuxBali (F1)	8	0.00	0.75	0.25	0.38	0.63	0.75	0.50	ns

N= number of samples; Ho= observed heterozygosity; he= expected heterozygosity; χ^2 table (0,05:1) = 3,84; *= unexplained; ns = not siginificant (χ2 count < χ2 table)

band with a length of 548 bp, the GG genotype has two bands with a length of 358 bp and 190 bp, and the GA genotype has three bands with a length of 548 bp, 358 bp, and 190 bp. The diversity of SNP g.385G>A MAOA gene using the PCR-RFLP technique in several beef cattle breeds is presented in Table 2.

The findings from this research indicate that the single nucleotide polymorphism (SNP) g.385G>A in the MAOA gene, specifically in the promoter region, shows variation across different cattle breeds. The genotype associated with aggressive traits is often linked to polymorphic conditions in this region of the MAOA gene. In Bali cattle, the A allele frequency is significantly higher, with an actual frequency of 0.94, suggesting that this allele may contribute to the manifestation of aggressive behaviors. In contrast, the G allele is more common in other breeds, such as Limousin and Wagyu, where the MAOA gene is monomorphic, implying that these breeds may exhibit fewer aggressive tendencies due to the dominance of the G allele. The MAOA gene encodes the enzyme monoamine oxidase A, which plays a crucial role in neurotransmitter regulation, including serotonin, dopamine, and norepinephrine. These neurotransmitters affect mood regulation, emotional responses, and stress handling. Mutations or variations in this gene, particularly in the promoter region, can lead to abnormal levels of neurotransmitters, which may manifest in increased aggression (Ziegler and Domschke 2018). The higher frequency of the A allele in Bali cattle suggests that this variation in the MAOA gene could predispose these animals to more aggressive behaviors compared to monomorphic breeds for the G allele. Regarding breed-specific aggressive traits, Bali cattle, which exhibit a polymorphic condition for the SNP g.385G>A with a high A allele frequency, are likely more prone to aggression, which aligns with observations of Bali cattle's defensive aggressiveness in response tohandling stressful situations (Sari et al., 2021). In contrast, Limousin and Wagyu cattle, which are monomorphic for the G allele, are less likely to display such aggressive behaviors, likely due to selective breeding that has reduced genetic diversity in this region.

These findings highlight the importance of understanding genetic diversity when considering cattle management and breeding programs aimed at balancing productivity and behavior.

Heterozygosity values measure genetic diversity in populations, which can contribute to selection programs (Putri et al. 2021). The number of samples and alleles and their frequency influence the heterozygosity value. The heterozygosity value of SNP g.385G>A shows a Ho value that is higher than the He value in Bali, Wagyu, PO, Madura cattle, and Wagyu-Bali crosses (F1), which indicates high genetic diversity of the MAOA gene in Bali, Wagyu, PO cattle. , Madurese and Wagyu-Bali crosses (F1), while the Limousin and Wagyu cattle showed values that did not vary (0.00). The heterozygosity value ranges from 0 to 1. If the heterozygosity value is close to 0, then the heterozygosity value is low, whereas if it is close to 1, then the heterozygosity value is said to be high (Li et al., 2013). High observed heterozygosity indicates that the observed population has a high level of genetic diversity (Karimah et al., 2021). The research results showed that the Ho value of Bali cattle and Madura cattle tended to be in the low category, while the Ho value of PO cattle and Wagyu-Bali cross (F1) cattle tended to be in the high category. The adaptability obtained from natural selection in tropical areas causes limited genetic diversity in Bali and Madura cattle. In order to maintain good performance, Bali cattle need a suitable living environment that includes nutrition and climate. Significant differences between Ho and He values can indicate a genotypic imbalance in the analyzed population (Dairoh et al., 2022).

The balance of alleles in a population is seen based on the chi-square value calculated based on the difference in observed and expected genotype frequencies (Pratiwi et al. 2016). The population is in equilibrium if the calculated chi-square value is smaller than the table chi-square value (Ismail et al., 2020). The results of the analysis showed that Bali, PO, Madura, and Wagyu-Bali cross (F1) cattle were in Hardy-Weinberg equilibrium (equilibrium, P>0.05). The Hardy-Weinberg equilibrium value of Limousin and Wagyu cattle cannot be analyzed because they are monomorphic. In the equilibrium of a large population, allele frequencies will be stable from generation to generation, and there will be no influencing factors such as selection, migration, mutation, or genetic drift (Jakaria et al., 2023). A population is in genetic balance if the genotype frequencies (p2, 2pq, q2) and allele frequencies (p and q) are constant from generation to generation because the combination of gametes occurs randomly in a large population. Although polymorphisms in SNP g.385G>A have the potential to be candidate genetic markers, this study requires direct observation or measurement of aggression traits in the sampled cattle breeds. Therefore, further studies are needed to investigate the functional significance of SNP g.385G>A and their possible association with behavioral traits in cattle.

CONCLUSION

The results of this study found six specific SNPs, namely SNP g.98A>C, SNP g.145G>T, SNP g.216A>C, SNP g.340T>G, SNP g.349A>C, and SNP g.385G>A in the promoter region of the MAOA gene. In SNP g.385G>A, three genotypes were found using the PCR-RFLP method using the RSaI restriction enzyme, namely genotypes GG, GA, and AA. SNP g.385G>A is polymorphic in Bali, PO, Madura, and Wagyu-Bali cross (F1) cattle while monomorphic in Limousin and Wagyu cattle. Further studies are necessary to explore the functional implications of SNP G.385G>A and their relationship to aggressive behaviors in cattle.

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