Genetic Diversity Analysis of Exon-2 Growth Hormone (GH) Gene in Crossbred Chicken (Sentul X Arab) using PCR-RFLP Technique

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Abstract. The research aims to analyze the genetic diversity of the GH gene using the PCR-RFLP technique. Fifty blood samples were collected from crossed Sentul and Arab Chickens aged three months. DNA was extracted from whole blood samples, and its quantity was evaluated by bio-drop technique. Extracted DNA was amplified for gene GH using a PCR machine. The PCR product was restricted by MspI (C↓CGG) and Hae-III (GG↓CC) enzymes for identifying the fragment length diversity. The variables observed in this study were DNA quantitative analysis, the genotype, and alleles of the obtained genotype. The result shows the extracted DNA is of good quality because it has values >1.8 and <2.0, which means there's no contamination in the DNA. GH gene was amplified with primers of 519 bp and showed that a DNA band matched the target in the specified primer design. Analysis of Genetic Diversity shows the monomorphic GH gene in this research. The genotype obtained at the GH-HaeIII locus is AA, and at the GH-MspI locus is TT. Both enzymes weren't restricted to these amplification design sites.

1 Introduction

Poultry farming is one of the contributors to meeting national animal protein needs, which every year experiences an increasing rate along with increasing demand for poultry meat and eggs. The poultry in demand for meat and eggs is local chicken. Local chickens are included in genetic resources that have lived and developed since immemorial and have been adapted for a long time in Indonesia [1, 2]. Local chickens that are currently popular are Sentul chickens and Arabian chickens. Sentul and Arabian chickens need attention because their potential is enormous enough to meet the demand for meat and eggs [3, 4]. However, both Sentul chickens and Arabian chickens have not been utilized optimally because their productivity still needs to improve. As is known, the Sentul chicken is a dual-purpose type capable of producing 140 eggs/year. Meanwhile, Arabian chickens are laying-type chickens, producing 190-250 eggs annually [5]. Meanwhile, Arabic chicken meat has not been utilized because it has a color that does not suit people's interests.

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Efforts can be made to overcome this problem by carrying out cross-breeding, which aims to increase livestock productivity, both production and reproductive characteristics. With crosses, it is hoped that they will be able to pass on the advantages of each family to their offspring [6, 7]. Crossing is one of the factors that causes genetic diversity in chickens [8]. The existence of high genetic diversity can be used as an effort to form new varieties. With the development of the field of molecular genetics, it is possible to determine the level of genetic diversity at the DNA level, which can determine the genetic potential of an individual [9, 10]. Genetic diversity analysis can be carried out on a gene in livestock, one of which is the growth hormone gene. The growth hormone gene is included in the genes that influence chickens' production performance and physiology [11].

One of the techniques that can be used to analyze genetic diversity is the PCR RFLP technique. The PCR technique is an in vitro DNA amplification technique that can duplicate large amounts of DNA relatively quickly. PCR-RFLP is a method capable of analyzing mutations in DNA sequences with the help of restriction endonuclease enzymes [12]. This enzyme has a role in obtaining the DNA sequence by cutting the DNA genome into fragments by cutting the phosphodiester bonds in the DNA sequence.

2 Materials and Methods

2.1 Study Site & Animal Sampling

Chicken blood samples were taken at the Faculty of Animal Science, Jambi University, followed by laboratory research from July to September 2023 at the Animal Biotechnology Laboratory, Faculty of Animal Science, Brawijaya University, to analyze the genetic diversity of the GH gene in livestock using PCR RFLP. The material used was blood samples from 50 Sentul x Arabian chickens aged 3 months.

2.2 DNA extraction

The initial procedure carried out in this study was taking blood samples from the axillary vein using a 3 ml syringe. Then, the blood sample is put into an EDTA tube and stored in a cool box filled with ice gel and ice packs. Next, the blood samples were taken to the Laboratory of the Faculty of Animal Science, Brawijaya University. The first procedure in laboratory research is to carry out DNA extraction.

DNA extraction using Genomic DNA mini kit from Geneaid. DNA extraction is the initial procedure in research on molecular technology, which has several stages, namely lysis, DNA binding, DNA washing, and DNA eluting according to the instructions from the Genomic DNA Kit [12]. Next, a quantitative DNA test was carried out using Biodrop DNA Spectroscopy, which aims to check the purity of the DNA that has been extracted. Quantitative DNA analysis is needed to determine the contamination and purity of DNA [13].

2.3 DNA Amplification and Restriction Analysis

The next procedure is to prepare primers based on the GH gene sequence accessed in Genbank with no. AY461843. These primers include forward primer (5'-GTAATGCAGCCACTTCTC-3') and reverse primer (5'GGCATCAAGCTAATGAGG-3'), which are composed of exon 2 of the growth hormone gene. After preparing the primers, the GH gene is amplified using the PCR technique by preparing a composition: a pair of primers, Green master mix, and nuclease-free water. The processes that occur in the PCR technique are pre denaturation, denaturation, annealing, extension, and final extension [14].
Visualization of PCR products with a mixture of agarose, TBE 0.5x, and Florosafe DNA using the electrophoresis method.

PCR products that have been electrophoresed will then be subjected to diversity analysis via RFLP (Restriction fragment length polymorphism) analysis. Cutting is carried out using the enzymes MspI and HaeIII. Genetic diversity was visualized using an electrophoresis machine, agarose, TBE 0.5x, Florosafe DNA, and Loading Blue Dye. After going through the electrophoresis process, the product is observed via a Gel Doc machine.

3 Results and Discussion

3.1 DNA Quantitative Analysis

DNA extraction is the initial stage in molecular identification to obtain extraction results in genomic DNA. DNA extraction aims to obtain DNA with high purity without being mixed with other cell components such as carbohydrates and proteins. DNA used in the molecular analysis must not be contaminated with other products [15]. DNA extraction consists of several stages, namely lysis or destruction of the membrane of the cell wall [6], DNA binding to bind DNA released from the cell, DNA washing, and DNA eluting. The method that can be used to determine the purity of the extracted DNA is to use a nanodrop spectrophotometer. Nanodrop is an effective technique for viewing DNA quality quickly [6]. It has the principle that ultraviolet radiation can be absorbed by nucleotides and proteins in solution due to the presence of purine and pyrimidine bases [12]. The quality of DNA needs to be considered if it is to be used for further analysis, such as PCR [16, 17]. Some contaminants that can be identified using UV light spectroscopy are proteins, polysaccharides, and RNA [13].

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<tbody>
<tr>
<td>Standard Value</td>
<td>&gt;100 ng/µL</td>
<td>1.8 − 2.0</td>
<td>2.0-2.2</td>
<td>1.8 − 2.0</td>
</tr>
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</table>

Table 1 presents the average results of the Biodrop spectrophotometer. The results show that the extracted DNA is of good quality because it has values >1.8 and <2.0. According to [18], Biodrop spectrophotometer quantity results with values below 1.8 indicate contamination from protein and phenol solutions, while values above 2.0 indicate RNA contamination. The principle of the spectrophotometer is to calculate the difference in UV light where the double band of DNA can absorb UV light at 260 nm. In comparison, contaminants in the form of protein and phenol will absorb light with a wavelength of 280 nm. DNA purity can be measured by the ratio of absorbance to wavelengths of 260 nm and 280 nm [19].

3.2 Growth Hormone Gene Amplification

GH gene amplification was done using the PCR technique using a BioRad T100 Thermal Cycler PCR machine. The proper primers and PCR components must support the continuity of the gene amplification process. Then, it is necessary to optimize the annealing temperature as one of the determinants of the success of the amplification process. The annealing stage is essential because attaching the primer to the DNA strand requires optimal temperature. If the temperature is not optimal, the amplification process will fail because the primer will not
bind to the DNA strand [20, 21, 22, 23]. For this reason, several temperature optimizations were carried out to obtain the most suitable temperature for the PCR product. Table 2 presents temperature optimization for PCR products in this study.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Stage</th>
<th>Temperature (°C)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x</td>
<td>Pre Denaturation</td>
<td>95</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>35 x</td>
<td>Denaturation</td>
<td>95</td>
<td>10 Seconds</td>
</tr>
<tr>
<td>35 x</td>
<td>Annealing</td>
<td>58</td>
<td>20 Seconds</td>
</tr>
<tr>
<td>35 x</td>
<td>Extension</td>
<td>72</td>
<td>30 Seconds</td>
</tr>
<tr>
<td>1 x</td>
<td>Final Extension</td>
<td>72</td>
<td>5 Minutes</td>
</tr>
</tbody>
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Qualitative testing was carried out to determine PCR product amplification results. This test is carried out using an electrophoresis machine, which is then viewed using a Gel DOC machine. When using an electrophoresis machine, you need to pay attention to the voltage used because it will affect the speed of DNA migration in the agarose gel. After all, DNA is a molecule with a negative charge [12]. The voltage used is 100 Volts. Confirmation of the PCR product can be seen in Figure 1. The research results show that a DNA band matches the target in the specified primer design. The band formed is a single band located at position 519 bp in exon 2 of the growth hormone gene. These results show that the amplification carried out was appropriate, and there was no contamination from other materials. Amplification of appropriate genes indicates appropriate temperature optimization [24, 25, 26].

![Fig. 1. PCR Product by Agarose Gel Electrophoresis.](image)

### 3.3 Analysis of Genetic Diversity

Analysis of the genetic diversity of the GH (growth hormone) gene is known using the RFLP technique. RFLP is an analysis technique for cutting nucleotide sequences at specific locations with the help of restriction endonuclease enzymes. This enzyme can cut DNA at the recognition point. Then, diversity can be seen through the bands formed during electrophoresis [12]. Restriction enzymes are found in bacteria, and researchers have used restriction enzymes and proven to have substantial value in biotechnological applications [27]. Analysis using the RFLP technique has the advantage that the analysis process is simple and faster [28].

The restriction enzymes used in this research are the Hae-III and the MspI. Type II restriction enzymes include the Hae-III enzyme and the MspI enzyme. This enzyme recognizes and cuts at the point (5'-GGCC-3'), while the MspI enzyme cuts at the point (5'-CCGG-3'). The Restriction Endonuclease enzyme will cut DNA at the phosphodiester bond and recognize 4 – 8 nucleotide sequences [29]. The genetic diversity analysis of the GH gene that has been carried out is presented in Figure 2 and Figure 3.
Genetic diversity with growth hormone candidate genes was chosen to classify the genetic diversity of Sentul x Arabian chickens. Based on the results of observations, it shows that both the HaeIII enzyme and the MspI enzyme only have one allele, namely the A allele and the T allele. The genotype obtained at the GH-HaeIII locus is AA, and at the GH-MspI locus is TT. The results of this study show that the GH gene is monomorphic. Figure 2 and Figure 3 show that there is no genetic diversity, whether cutting uses the Hae-III enzyme or the MspI enzyme. The absence of polymorphism in the livestock studied could be due to limited sample size, the presence of null alleles, or the selected loci having low effectiveness [30]. The results of this observation are similar to the observations of [26], who stated that the Bangkok chickens observed were monomorphic because only 1 allele and 1 genotype were found.

4 Conclusion

DNA Quantitative Analysis shows that there is no contamination from other ingredients. Genetic diversity analysis was conducted on exon 2 of the growth hormone gene using HaeIII and MspI restriction enzymes, showing the monomorphic GH gene in this research. Both enzymes weren't restricted to these amplification sites.

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