

## Polymorphism of Sikumbang Jonti ducks Growth Hormone (GH) Gene using PCR-RFLP Methods

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**ABSTRACT:** This research aimed to identify polymorphism of the GH gene in Sikumbang Jonti ducks using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method as a candidate for genetic marker selection. This research used 123 blood samples of male and female Sikumbang Jonti ducks. The ducks were eight weeks old and kept intensively at the UPT Fakultas Peternakan Universitas Andalas. Blood samples of Sikumbang Jonti ducks were taken through the brachial vein as much as  $\pm 2$  mL. The Genomic DNA Purification Kit (Promega) protocol isolated DNA from blood samples. A pair of primers F amplified the total DNA: 5'-GGA CAG CCT GAG GAA AGA GT-3' and R: 5'-GTG GAA GGT GGG GAG ACT TC-3', which produced a fragment region of the GH gene exon 3 with a cut region of part intron two and part intron 3 with a primary length of 833 bp. The amplified product was restricted by the enzyme *TasI* which recognizes the cutting site ( $\downarrow$ AATT). Based on the restriction results, two (2) genotypes were obtained, namely homozygous (-/-) as many as four (4) and homozygous (+/+) as many as 119. The analysis of restriction products included the allele frequency, namely the allele (+) of 0.98 and the allele (-) of 0.02, and genotypes frequency of homozygous (-/-) of 0.03 and homozygous genotypes (+/+) of 0.97. The diversity of the GH gene is in the Hardy-Weinberg equilibrium. Based on the research results, it can be concluded that there is diversity in the GH gene of the Sikumbang Jonti duck, and it is in the Hardy-Weinberg equilibrium, so it can be used as a candidate for the genetic marker of ducks.

**Keywords:** Diversity, Germplasm, Local Duck, Payakumbuh

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### INTRODUCTION

Indonesia is a country that has great potential for the development of livestock businesses, especially poultry farming (Fajri et al., 2022). It can be seen in the investment value of poultry farming in Indonesia which is the highest compared to other livestock sectors (DirjenPKH, 2020). One type of poultry that has the potential to be developed in Indonesia is duck livestock. Duck has the potential to be developed; there are still large market opportunities, and the high value of a community towards duck meat, especially in the form of processed meat products duck is *itik lado hijau*, especially in Sumatera Barat. Saputri (2017) stated that people like processed duck products in the form of *itik*

*lado hijau*, even many consumers who buy these products are tourists who are interested in trying so that the processed duck product can become a character or attraction for tourists in Sumatera Barat.

The development of processed ducks in Sumatera Barat raises several significant obstacles, one of which is the difficulty of obtaining superior quality and available ducks. It is feared that this obstacle will cause the local duck population in Sumatera Barat, which has unique genetic characteristics and appearance, to become extinct. This can be accomplished by using a molecular genetic identifier in livestock breeding, a program selection that can be performed more accurately and efficiently.

The molecular technique is expected to determine ducks' genetic diversity so that they can be selected for superior and quality local duck genes. Molecular techniques for identifying gene diversity can be done with the RFLP (Restriction Fragment Length Polymorphism) method. The restriction fragment pattern is analyzed when DNA is cut by the polymerase enzyme (Al-Samarai et al. 2015). According to Yurnalis et al. (2017), the growth hormone (GH) gene can be used as a genetic marker in duck selection efforts to improve duck performance (meat) and meet the needs of animal protein in Indonesia.

Sumatra Barat has several types of local ducks, including the Sikumbang Jonti duck. Sikumbang Jonti duck is a Sumatera Barat germplasm originating from Payakumbuh District, in the Kenagarian Koto Baru, Payobasuang Sub-District. The Sikumbang Jonti duck has the advantage of being resistant to heat stress (Subekti et al., 2019). Based on some of the explanations above, it is necessary to conduct research to identify gene diversity using the RFLP method on GH genes to search for superior genetic marker candidates for the Sikumbang Jonti duck.

## MATERIALS AND METHODS

### Materials

The research material used in this study was 123 blood samples of Sikumbang Jonti ducks divided into 81 males and 42 females. The ducks were eight weeks old and kept intensively at the UPT Fakultas Peternakan, Universitas Andalas.

### Methods

The duck blood sample was taken  $\pm 2$  mL through the brachial vein (part of the duck wing) using a disposable syringe; then, the blood sample was inserted into the EDTA vacutainer tube and stored at  $-20^{\circ}\text{C}$ . DNA isolation was carried out from blood samples using Promega's genomic DNA purification

kit. DNA isolated was amplified by primer pair F: 5' GGA CAG CCT GAG GAA AGA GT-3' and R: 5'-CCA GGA TCC GTC CCT TCC TCC AC -3', which resulted in fragments on the region around exon 3 GH genes, that was the region of part of intron two and part of intron 3 with a primary length of 833 bp.

Invitro amplification was carried out with a PCR machine (Eppendorf Mastercycler gradient) with a pre-denaturation program at a temperature of  $95^{\circ}\text{C}$  for 30 seconds, annealing at a temperature of  $59^{\circ}\text{C}$  for 30 seconds, and an extension of  $72^{\circ}\text{C}$  for 1 minute and, a final extension of  $72^{\circ}\text{C}$  for 5 minutes with 34 cycles. PCR amplification reagent using Master Mix (Promega®) with the following composition:

- Sample DNA as much as two  $\mu\text{l}$ ,
- Master Mix as much as 15  $\mu\text{l}$ ,
- The primer mix of F and R as much as three  $\mu\text{l}$ ,
- Nuclease Free Water as much as ten  $\mu\text{l}$ .

Results of GH gene amplification were electrophoresed in agarose 1.5% with ethidium bromide staining and observed using a UV transilluminator. The restriction of the GH gene is carried out by restricting the GH gene amplification product with the enzyme *TasI* (Promega®); the procedure is as follows:

- Take the PCR product as much as 20  $\mu\text{l}$ ,
- Add enzyme *TasI* as much as 10  $\mu\text{l}$ ,
- Insert the tube containing the PCR product mixture and enzymes into the water bath incubator at a temperature of  $65^{\circ}\text{C}$  for 4 hours.

The restriction results for *TasI* were visualized by electrophoresis of restriction products on 2% agarose gel with ethidium bromide and DNA benchtop (Marker) and run using an electrophoresis machine (ThermoSCIENTIFIC®) at a voltage of 100 volts for 2 hours. The electrophoretic product was observed with the trans-illuminator UV

beam host. Based on observations of the results of electrophoresis with the UV Transilluminator, an image of the cutting band will be obtained with three possible genotypes:

- The homozygote is not truncated (-/-) if only one band is sized along with the amplification fragment (833 bp).
- The homozygote is truncated (+/+) if two or more bands are out of position/below the amplification fragment size (833 bp).
- The heterozygous (+/-), if two or more bands with one band are at the position/size of the amplified fragment and the other band is below the position of the amplified fragment.

### Data Analysis

The genotype diversity of each individual can be determined from the DNA strands of the genes found. Each sample was compared by size (marker) that is the same and the calculated frequency allele. The allele frequency can be calculated using the formula (Nei and Kumar, 2000):

$$\chi_{i=1} = \frac{2n_{ii} + \sum_{j \neq i} n_{ij}}{2N}$$

While the genotype frequency is calculated by the formula (Nei and Kumar, 2000):

$$\chi_{ii} = \frac{\sum_{i=1}^n n_i}{2N}$$

Note:

- $\chi_i$  = Frequency of the i allele;
- $\chi_{ii}$  = Frequency of genotype ii;
- $n_{ii}$  = number of samples with genotype ii;
- $n_{ij}$  = number of samples with genotype ij;
- N = Number of samples

The test for hardy-weinberg equilibrium was carried out to determine whether the allele frequency and genotype

frequency of the GH-*TasI* gene in the reared duck population was still at a balance of  $p^2+2pq+q^2$  which was tested by chi-square ( $\chi^2$ ) according to Nei and Kumar (2000) with the following formula:

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

Note:

$\chi^2$  = chi-square

O = number of observations of the I genotype

E = expected number of the i genotype

## RESULTS AND DISCUSSION

### Polymorphism of GH Gene

Growth hormone gene amplification using PCR (Eppendorf Martercyler Gradient) machine at an annealing temperature of 59 °C visualized by agarose electrophoresis 1.5%. The polymer used in this study successfully amplified the 833 bp exon region of the GH gene (Figure 1). Based on Figure 1, the electrophoresis results are explicitly applied. This is because there is only one band with a length of 833 bp corresponding to the amplification fragment's length in each well. According to Sarbaini et al. (2017), gene amplification can be stated explicitly if there is only one DNA band in each well at the time of electrophoresis.

Results of PCR amplification of gene fragments GH products | *TasI* gel electrophoresis on agarose 2% gain of two (2) pattern ribbon-cutting results (Figure 2), namely: the first is a pattern of four (4) ribbon clipped with ribbon size 448 bp, 327 bp, 121 bp, and 100 bp; and the second is a pattern of one (1) band outside the content post/above the amplification fragment size (833 bp), then it is called (-/-) with a band size of 833 bp. The length of the band can be determined based on the *TasI* enzyme, which recognizes the AATT cutting site. The occurrence of some

bands in the electrophoresis results because a sequence bases AATT the DNA fragments were observed, so severed by enzyme *TasI* and formed a ribbon with the size of 448 bp, 327 bp, 121 bp, and 100 bp. Determination of the number and size fragments are truncated in the skewer with a marker used and used as a marker calculation tape length DNA is analyzed. The part of the DNA subjected to this restriction enzyme's cutting action is called the identification sequence. This enzyme only hit one (1) cutting site: AATT. Saputra et al. (2020) stated that restriction enzymes cut DNA fragments at specific base sequences.

The results in this study show the same results as the research of Yurnalis et al. (2017) and Nova et al. (2016), who found diversity in the GH gene using the PCR-RFLP method in Sikumbang Jonti ducks. However, the research of Sarbaini et al. (2017), who used the PCR-RFLP method on the exon one region GH gene, showed monomorphism results in Sikumbang Jonti local ducks. The GH gene in Sikumbang Jonti ducks has a lot of polymorphic compared to other Sumatera Barat local ducks, such as the Bayang ducks, so the use of GH gene diversity in Sikumbang Jonti ducks as candidates for genetic markers for selection in forming superior duck seeds is very supportive.

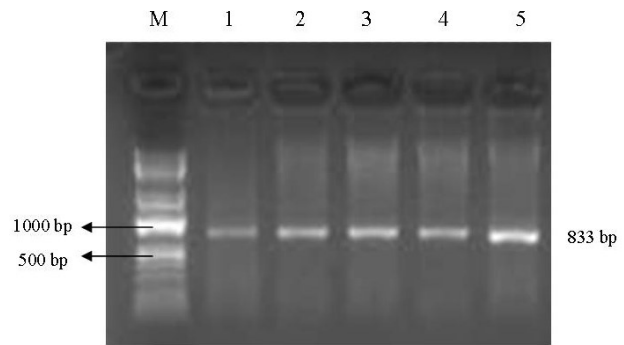


Figure 1. Amplification results of GH Gene in Sikumbang Jonti ducks; M=500 bp ladder standard size

Masti et al. (2021) found variations in the GHR gene of Sikumbang Jonti ducks. This shows that in addition to the GH gene's polymorphism, the GH receptor gene can also be used as a candidate for genetic marker selection for Sikumbang Jonti ducks.

### Genotype Frequency and Allele Frequency

Based on the results of genotypic observations in the individual samples presented in Figure 2, the genotype and allele frequencies are obtained as presented in Table 1. Based on Table 1, it can be known that out of 123 samples obtained, genotype homozygotes (+/+) of 119 samples or 0.97%, genotype homozygous (-/-) as much as four samples or 0.03%, and Heterozygous genotypes (+/-) were 0 samples or 0.00%.

Table 1. Frequency of Genotype and Allele GH Gene (*TasI*) Sikumbang Jonti, duck

| Population            | N   | Genotype (n) |       |       | Genotype Frequency (%) |       |       | Allele Frequency (%) |      |
|-----------------------|-----|--------------|-------|-------|------------------------|-------|-------|----------------------|------|
|                       |     | (+/+)        | (+/-) | (-/-) | (+/+)                  | (+/-) | (-/-) | (+)                  | (-)  |
| Sikumbang Jonti ducks | 123 | 119          | 0     | 4     | 0,97                   | 0,00  | 0,03  | 0,98                 | 0,02 |

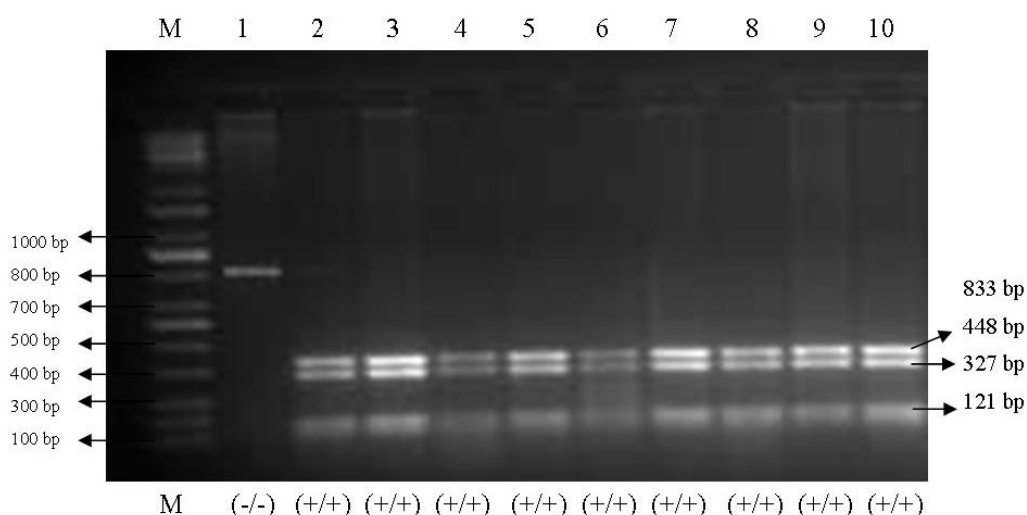


Figure 2. Restriction results of GH Gene in Sikumbang Jonti ducks; M=100 bp ladder standard size

From the three genotype e frequencies obtained, the highest frequency is found in the homozygous (+/+) genotype. This shows that mutations are rare in the GH-*TasI* gene for the exon three regions because the population of Sikumbang Jonti ducks were found to have a predominantly genotype (+ / +) than genotype (- / -) or (+/-). This is the same as the research of Yurnalis et al. (2017) and Nova et al. (2016) which states that the diversity in the GH gene has the same pattern, namely that there is one homozygous genotype that is very dominant or has the highest frequency compared to other heterozygous or homozygous genotypes.

The analysis of the frequency of allele gene exon 3 of the GH area using *TasI* restriction enzymes in Sikumbang Jonti ducks shows that diversity in the GH gene of Sikumbang Jonti ducks is polymorphism. It is

because the right value of the frequency of an allele (+) and (-) smaller than equal to 99%. This is in line with the opinion of Nei and Kumar (2000), which state that a gene is said to be polymorphic if the two allele frequency values of an observed population are less than or equal to 99%.

### Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium determines whether the observed data deviates from the expected. This can be done by using the chi-square test, which compares the calculated results with the chi-square test. From the calculation results obtained genotype frequencies of two (2) homozygous type genotype (+/+)/(p<sup>2</sup>) and homozygous (-/-)/(q<sup>2</sup>), whereas genotype heterozygous (+/-)/(2pq) were not found.

Table 3. Chi-Square ( $\chi^2$ ) of GH Gene (*TasI*) Sikumbang Jonti duck

| Population            | N   | Frequency Observation |      |      | Frequency Expectation |      |      | Chi-Square ( $\chi^2$ ) |
|-----------------------|-----|-----------------------|------|------|-----------------------|------|------|-------------------------|
|                       |     | +/+                   | +/-  | -/-  | +/+                   | +/-  | -/-  |                         |
| Sikumbang Jonti ducks | 123 | 0,97                  | 0,00 | 0,03 | 0,94                  | 0,06 | 0,00 | ns                      |

The chi-square results show that the diversity of the GH gene is in the Hardy-Weinberg equilibrium. This shows that the duck population in the study was enormous; there was no selection, migration, mutation, or genetic drift. Noor (2008) stated that a large enough population would not change from one generation to another if there is no selection, migration, mutation, founder, and genetic drift.

## CONCLUSION

Based on the results of the study, it can be concluded that there is diversity in the GH gene of Sikumbang Janti ducks, and it is in the Hardy-Weinberg equilibrium so that it can be used as a candidate for the genetic marker of ducks.

## REFERENCES

- Al-Samarai, F. R., and A. A. Al-Kazaz. 2015. Molecular Markers: An Introduction and Applications. *European Journal of Molecular Biotechnology* 9(3):118-130.
- Dirjen PKH [Direktorat Jenderal Peternakan dan Kesehatan Hewan]. 2020. *Statistik Peternakan dan Kesehatan Hewan 2020*. Jakarta (ID): Direktorat Jenderal Peternakan dan Kesehatan Hewan.
- Fajri, F., F. Maulana, A. A. B. Persada, D. Sandri, B. P. Febrina, W. M. Lestari, A. L. R. Hutabarat, and M. Zein. 2022. Potential of Ration Based on Local Raw Materials as A Substitute of Commercial Ration for Crude Protein, Crude Fat, and Crude Fiber. *Agrotropica: Journal of Agricultural Sciences* 5(2): 109-115.
- Masti, H, Yurnalis, T. D. Nova, and T. Rafian. 2021. Keragaman Gen Growth Hormone Receptor (GHR) Ekson 10 pada itik Sikumbang Janti. *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan* 9(1): 15-19.
- Nei, M., and S. Kumar. 2000. *Molecular Evolution and Phylogenetics*. New York (United States): Oxford University Press, Inc.
- Noor, R. R. 2008. *Genetika Ternak*. Penebar Swadaya, Jakarta (ID).
- Nova, T., Y. Yurnalis, A. K. Sari. 2016. Keragaman Genik Gen Hormon Pertumbuhan (GH|MboII) pada itik Sikumbang Janti Menggunakan Penciri PCR-RFLP. *Jurnal Peternakan Indonesia* 18(1): 44-52.
- Saputra, E. A., M. F. Ulum, and J. Jakaria. 2020. Association of SNP g.643G>A of MYF5 gene polymorphism with body weight and body measurements in Bali cattle. *J. Indonesian Trop. Anim. Agric.* 45(1):1-6.
- Saputri, W. 2017. *Preferensi Konsumen Terhadap Itik Lado Hijau Di Kota Bukittinggi*. Skripsi. Universitas Andalas, Padang (ID).
- Sarbaini, S., Y. Yurnalis, H. Hendri, R. Dahnil. 2018. Analisis Keragaman Exon-1 Gen Hormon Pertumbuhan pada Itik Lokal (Bayang) Sumatera Barat Menggunakan Metoda PCR-RFLP. *Jurnal Peternakan Indonesia* 20 (2): 124-129.
- Subekti, K., D. D. Solihin, R. Afnan, A. Gunawan, dan C. Sumantri. 2019. "Polymorphism of Duck HSP70 Gene and mRNA Expression under Heat Stress Conditions." *International Journal of Poultry Science* 18(12):591-97.
- Yurnalis, Y., H. Husmaini, and S. Sabrina. 2017. Polymorphisms of Growth Hormone Gene Exon 1 and Their Associations with Body Weight in Pitalah dan Kumbang Janti Ducks. *Int. J. Poult. Sci.*16:203-208.