Original Article

Association of growth hormone gene polymorphism with body weight body weight Kampung chicken

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Abstrak

Tujuan: Mengetahui polimorfisme gen *Growth Hormone* (GH) dan hubungan polimorfisme gen *Growth Hormone* (GH) dengan bobot badan ayam Kampung.
Metode: Metode penelitian adalah eksperimen menggunkan 50 sampel darah ayam Kampung umur 3 bulan. Metode meliputi: ektraksi DNA, amplifikasi PCR, karakterisasi dan identifikasi PCR-RFLP dengan enzim MspI. Data dikoleksi yaitu: frekuensi genotype dan alel. Lokus bersifat polimorfik, keseimbangan populasi pada keragaman gen GH MspI dihubungkan dengan bobot badan umur 3 bulan dianalisis menggunakan uji beda rata-rata.
Hasil: Gen GH MspI ayam Kampung bersifat polimorfik, gen GH ayam Kampung berada pada ketidakseimbangan populasi, gen GH MspI ayam Kampung mempunyai keragaman sangat informatik dan signifikan polimorfik pada genotype A2A2 (P<0.05) dan berasosiasi dengan sifat bobot badan ayam Kampung umur 3 bulan.
Kesimpulan: Gen GH MspI ayam Kampung memiliki hubungan dengan bobot badan umur 3 bulan, bersifat polimorfik dan dapat dijadikan acuan dalam melakukan seleksi untuk meningkatkan mutu genetik.

Kata Kunci: Ayam kampung; Gen GH *(Growth Hormone)*; Polimorfisme

Abstract

Objective: The study was aimed to obtain growth hormone gene polymorphism and association growth hormone gene polymorphism with body weight in Kampung chicken.
Methods: The research method was an experiment using 50 blood samples from 3 month old Kampung chickens. Methods of DNA extraction, PCR amplification, characterization and identification using PCR-RFLP with MspI enzyme were used in this study. Data of genotype and allele frequencies were collected. The polymorphic locus, equilibrium population on the diversity of the gene GH MspI association with body weight at three months of age was analyzed by using the mean difference test.
Results: Gene GH MspI of Kampung chicken was polymorphic, the gene GH of Kampung chicken was in an imbalance population, gene GH MspI of Kampung chicken had a very informative diversity and significant polymorphic on the genotype A2A2 (P<0.05) and associated with bodyweight Kampung chickens aged three months.
Conclusions: Gene GH MspI of Indonesian chicken has an association with body weight aged three months, polymorphic, and can be used as a reference selection to improve genetic quality.

Keywords: *Growth hormone gene*; Kampung chicken; Polymorphism

INTRODUCTION

Indonesia is a country with the potential to develop genetic resources. One of the genetic resources that have the potential to be developed is a local chicken. Local chickens or Indonesian native chickens are non-breed chickens that have the advantage of being easy to adapt to the environment, not susceptible to disease, and having a relatively high selling price. One of the local chickens raised by Indonesian is Kampung chicken.

Kampung chicken is a source of genetic wealth for local livestock in Indonesia. Kampung chicken has the advantages of easy maintenance, relatively low costs and high resistance to disease [1]. Kampung chicken has relatively low productivity and relatively high diversity. This high degree of diversity provides opportunities for selection. Compared with purebred chickens, kampung chickens have weaknesses such as low productivity and high diversity in qualitative characteristics, so that genetic quality improvements can make through selection [2];[3];[4]. Selection is an activity to select livestock that is considered suitable to be developed while livestock that is not potential needs to remove. Selection can do either through livestock performance or traits that are visible from the outside or genetically. Selection based on livestock performance such as body weight, weight gain, body measurement takes longer, and the accuracy in this selection is still low. Advances in the molecular field can identify genetic diversity in native chickens through genes that have economic value, such as growth. One of these genes regulating growth is the Growth Hormone (GH) gene.

The Growth Hormone (GH) gene is a gene that controls growth and plays a role in the body's metabolism. Identification of GH gene polymorphisms is essential to obtain initial information about the properties of genes that have economic value [5]. The genetic diversity of GH genes can be related to body weight in Kampung chickens; the relationship of this diversity looks at the variety of GH genes that are influenced by genetics itself or from the environment. The high genetic variation of native chickens indicates the potential to improve genetic quality in native chickens [6].

The diversity of growth hormone genes is known by determining alleles and genotypes in each individual through the PCR-RFLP approach using restriction enzymes [7]. One of the methods of identifying the growth hormone (GH) gene characterization we can use is the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) marker.

PCR is a technology that can multiply a sample of DNA fragments contained in a complex of genomic macromolecules from various sources (animals, plants, bacteria, and viruses) into 2n times enzymatically [8]. Restriction Fragment Length Polymorphism (RFLP) is a widely used technique to detect variations at the DNA level. RFLP detection is carried out based on the possibility of differences in the length of the target DNA fragment produced after the cutting process with a restriction enzyme [9].

Until now, not widely known and there is still a lack of data related to the association of the gene Growth Hormone (GH) with body weight of Kampung chickens. Therefore, information needs that can use as a reference in the selection and increasing in productivity of Kampung chickens in the future. Based on the description above, research has been carried out on the Association of Growth Hormone Gene Polymorphisms with Body Weight of Kampung Chickens.

MATERIALS AND METHODS

Materials

This research was conducted at the Animal Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University, from April 5 – June 5, 2021. Amount 0f 50 blood samples of the age 3- month Kampung chickens collected (Figure 1). The system of raising native chickens in colony cages with continuous feeding and drinking (ad libitum). The size of the cage used is 4x3x1.8 m, equipped with a feed, drinking and lighting system. The feed is used from PT. Japfa Comfeed Indonesia Tbk. BR1 for ages 0-1 months with a composition of min.3000 Kcal/kg energy, protein 21.0-23.0%, fat min. 5%,

Figure 1. Kampung Chickens

fiber max. 5%, calcium 0.8-1.1%, phosphorus min. 0.50%. BR2 for 2-3 months of age with an energy composition of min. 3100 Kcal/kg, protein 19.0-20.0%, fat min. 5%, fiber max. 5%, calcium 0.8-1.1%, phosphorus min. 0.45%.

Methods
Blood collection

The method used in this study was a blood draw Kampung chicken, DNA extraction, PCR amplification and PCR-RFLP. Blood draw Kampung chicken was conducted using 3 months old Kampung Chicken were obtained by taking blood using a syringe in the axillary vein of the wing. 1-2 ml of blood was taken and then put into a 3 ml tube and mixed with EDTA powder to prevent clotting. Blood was stored in a freezer at a temperature below -20oC before further processing.

DNA extraction

DNA extraction was conducted using Genomic DNA Purification Kit protocol from Promega. The results of DNA extraction were then electrophoresed using 1.5% agarose stained with Ethidium Bromide, then the electrophoresis machine was run with a voltage of 200 volts for 1 hour. Furthermore, the results of DNA extraction were observed using UV light on the Gel Documentation

system (Biometra-German) which was further documented. A pair of primer for GH gene shown in Table 1. Below:

PCR amplification

Amplification PCR was conducted with Thermoocycler from BIO-RAD. The composition is as follows 3 μl Forward and Reverse primers, 2 μl genomic DNA, 10 Nucleuse free water/DDW (double distillation water), and 15 μl Gotaq Green Mastermix from Promega which was inserted into a microtube tube PCR 0.2 ml for total mixture is 30 μl, visualization of PCR products was observed using a UV light and documented with Gel Documentation (Biometra-Germany). The amplification process was carried out according to the PCR steps as in Table 2.

PCR-RFLP was carried out by incubation using a waterbath with the following composition: 10 l of the PCR product and 10 l of the enzyme *restriction MspI* (C|CGG) at 37°C for 4 hours. Visualization of PCR-RFLP was observed using a UV light and documented with Gel Documentation (Biometra-Germany).

Data Analysis
Allele frequency

Table 1. Length and location of the gene GH and primers used for PCR Amplification

Locus	Length (bp)	Location	Primary Sequence	Gene Bank
GHD01a	950	Exon 1	F:5'TGC AAG GAG GGG ATA TGG AG³' R: 5'TT CCC CTA ACG TGC TCA TGT3'	AY461843

Table 2. PCR analysis optimization temperature

Stage	Temperature	Duration	Cycle
Predenaturation	95°C	5 minutes	1x
Denaturation.	95°C	45 seconds	
Annealing	60°C	45 seconds	35x
Extension	72°C	1 minute	
Final Extension	72°C	5 minutes.	1x

Genotype frequency was calculated by calculation as follow:

$$F_1 = \frac{\Sigma X_i}{N}$$

Note:

xi = observed genotype
N = Total Population

The allele frequency of the Growth Hormone (GH) gene is the proportion of a particular allele in a population compared to all alleles occupying the same locus, obtained from the PCR-RFLP characterization analysis analyzed using the formula [10]:

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

Note:

xi = frequency of the i-th allele,
nii = number of individuals of genotype ii,
nij = number of individuals with genotype ij,
N = total number of samples.

Hard-weinberg equilibrium

The Hardy-Weinberg (HW) balance with the chi-square test (X2) aims to compare the data from our observations (observed) with the hypothesized or expected values (expected). Hardy-Weinberg balance (HW) with chi-square test (X2) according to [11] as follows:

$$X^2 = \frac{(\text{obs} - \text{exp})^2}{\text{exp}}$$

Note:

2 = test *Chi-square*,
Obs = the number of observations of the i-th genotype
Exp = expected number of genotype i

Polymorphic Information Content

Polymorphic Information Content (PIC) was calculated using the formula [12].

$$\text{PIC} = 1 - \sum_{i=1}^{n} p_1^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Note:

PIC = Polymorphic Information Contents
Pi = frequency of the I allele,
N = number of alleles

T test

Differences in the mean body weight of native chickens against the genotype of gene GH *MspI* fragments were analyzed using the t-test [13].

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{\sum (X_{J1} - \overline{X}_1)^2}{n_1(n_1 - 1)} + \frac{\sum (X_{J2} - \overline{X}_2)^2}{n_2(n_2 - 1)}}}$$

Note:

t = value of t count
X_1 = sample mean in the first group,
X_2 = sample mean in the second group,
X_{J1} = the value of the J-observation in the first group
X_{J2} = the value of the J-observation in the second group
n_1 = number of samples in the first group, and
n_2 = number of samples in the second group.

RESULTS

DNA extraction and amplification of growth hormone gene

DNA extraction results from a total of 50 samples of Kampung chicken blood using the Genomic DNA Purification Kit from Promega, The collected DNA were presented in Figure 2.

Based on Figure 2 above, DNA bands produced contained smears at the bottom of the tape. Success in DNA extraction can see from the presence or absence of smears that
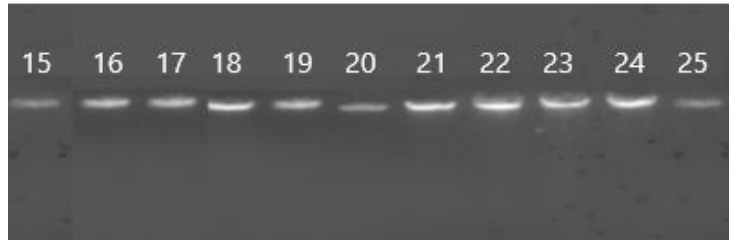
Figure 2. Electrophoresis Results of Genomic DNA Purification Kit. DNA Extraction

Figure 3. Sequences gen GH (Growth Hormone) Kampung chicken enzyme MspI

appear from the results of DNA bands; smears see if other elements participate in the DNA extraction process.

Sequences for The GH gene Kampung chicken are presented in Figure 3. The GH gene for Kampung chicken has a length of 950 bp located in exon 1 because it has 3 cut points enzyme MspI (C|CGG).

The amplification of the PCR product of the Growth Hormone gene Kampung chicken is presented in Figure 4 dan 5. The results of The amplification PCR show that the length of the PCR product is 950 bp, and Marker is an indicator of the length of the PCR product that is sized as expected.

Genotype and allele frequency

The diversity of the Growth Hormone (GH) gene in Kampung chicken is known from the identification of genotypes through PCR-RFLP markers using the MspI (C*CGG) cutting enzyme with a GH primer length of 950 bp for Kampung chickens. There are three

truncated points, namely 148 bp, 267 bp, and 409 bp from 4, namely 148 bp, 125 bp, 267 bp and 409 bp.

The results of cutting the Growth Hormone (GH) gene of Kampung chicken using the MspI restriction enzyme are presented in Figure 6.

Based on Figure 6 , it can see that the results of cutting with the MspI enzyme contained several genotypes of A1A2, A2A2, A3A3, A1A1, A1A3 and three alleles of A1, A2, and A3. The A1A2 genotype was cut into three bands with the intersection points at 950 bp, 409 bp, and 148 bp. The A2A2 genotype was cut into two bands with the intersection points at 409 bp and 148 bp, and the A3A3 genotype was cut into three bands with the cut points located at 409 bp, 267 bp and 148 bp, the A1A1 genotype was cut into two bands with the cut points located at 950 bp and 148 bp. In comparison, the A1A3 genotype was truncated into four bands with the cut points located at 950 bp, 409 bp, 267 bp. and 148 bp. The amount
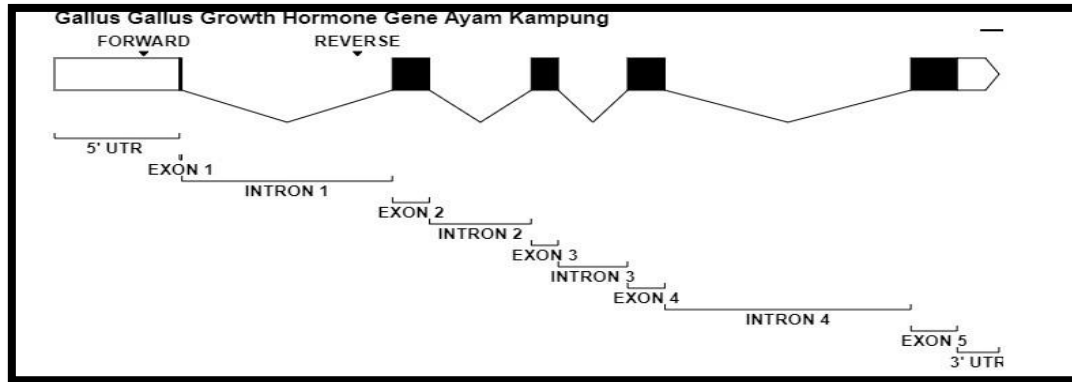
Figure 4. Gene structure of GH (Growth Hormone) Kampung chicken Genbank: No.AY461843

Figure 5. Results of electrophoresis Amplification of the PCR product of the Growth hormone gene for Kampung chicken

Figure 6. The results of restriction electrophoresis of the Growth Hormone (GH) gene using the MspI cutting enzyme

of 50 samples in this study showed genotypes of A1A2 (3), A2A2 (9), A3A3 (15), A1A1 (13), and A1A3 based on analysis.

The result of genotipe frequency and allele frequency of the GH (Growth Hormone) gene of Kampung chicken is shown in Table 3.

Based on Table 3. it can be explained that there are four kinds of genotypes arising from the GH (Growth Hormone) gene in native chickens (A1A2, A2A2, A3A3, A1, A1 and A1A3) and allele frequencies (A1, A2, and A3). Genotype A1A2 has a frequency value of 0.06; A2A2 is 0.18; A3A3 is 0.30; A1A1 is 0.26 and A1A3 is 0.20. In this study, genotype A3A3 had the highest genotype frequency value of 0.30 and A1A2 was the lowest genotype frequency with value of 0.06. Allele A1 had an allele frequency value of 0.39; A2 had a value of 0.21, and A3 had a value of 0.40.

Equilibrium hardy-weinberg and polymorphic informative content (PIC)

Based on Table 3, the results of the Chi-square test of the GH (Growth Hormone) gene in Kampung chicken were significantly different, P<0.01. with a Chi-square value of 38.36, it can say that the gene (Growth Hormone) in Kampung chicken describes an imbalance state of the population. It means there is a balance between genotype and expected value. Based on Table 3, the GH gene fragment had a PIC value of 0.64 in a very informative (high) position as a genetic marker in Kampung chickens. The genotype of the GH gene in Kampung chickens is highly polymorphic (diverse).

Relationship between gene polymorphism GH (Growth Hormone) with body weight kampung chicken

Table 4 shows that the average body weight (BB) in Kampung chickens aged three months GH gene when analyzed with PCR-RFLP/ MspI, resulted in genotypes A1A2 A2A2, A3A3, A1A1, A1A3. The analysis of the average difference test showed that the body weight (BB) of Kampung chickens was significantly different (P<0.05) between each genotype.

DISCUSSION

DNA extraction and amplification gene growth hormone

The results of DNA extraction electrophoresis resulted in the results of DNA bands that were not thick. The resulting DNA bands would affect the quality of DNA. DNA quality depends on the lysis process in breaking down the cell nucleus and the concentration of DNA rehydration given. [7] The results of the extraction of DNA which are visible, are shown by the DNA purification process that occurs ideally to obtain pure DNA; pure DNA produced can be seen with clear and clean DNA bands.

The results of the PCR amplification product bands were visible, and can be influenced by the quality and the concentration of DNA from the extraction results, primers, temperature at each PCR stage. The success of the resulting band means the ability to amplify the primer to produce the accuracy of the DNA band. Annealing temperature used in the amplification of PCR products is 60°C for 45 seconds, and 35 cycles, the selection of annealing temperature is very decisive for the success of amplification when the primer will attach to the target DNA and PCR reagents. [14] The success of the amplification depends on selection of the right annealing temperature because the primer attaches to the DNA strand when suitable to t requires an optimal temperature. If the selected temperature is too high, the amplification process will fail, and the primer will not

Table 3. Genotype frequency, allele frequency, Hardy-Weinberg Equilibrium**,** and PIC (Polymorphic Information Content) value

Line-Locus	N	Genotype	Genotype Frequency	Allele Frequency	X^2 count	PIC value
Kampung chicken	50	A1A2	0.06	A1=0.39		
GH|MspI		A2A2	0.18	A2=0.21	38.36*	0.64
		A3A3	0.30	A3=0.40		
		A1A1	0.26			
		A1A3	0.20			

Table 4. Average body weight (BB) on the GH gene fragment MspI Kampung chicken

Genotype				
A1A2	A2A2	A3A3	A1A1	A1A3
s1038.0±58.1[a]	1144.0±93.1[b]	1093.2±77.7[c]	1114.8±62.3[d]	1071,2±66,1[e]

Different letters superscript in the same column for each genotype means are significantly different (P<0.05)

attach to the DNA. If the temperature is too low, it will cause the primer to stick to the other side, and the DNA formed has low specificity. [15] Bangkok chicken DNA was successfully extracted, indicated by the overall band obtained that was clearly visible, quite clear, not thick or thin, indicating that the DNA concentration was appropriate. Bangkok chicken myostatin gene PCR product was successfully amplified with annealing temperature of 60°C for 45 seconds.

Genotype and allele frequency, hardy-weinberg equilibrium, (PIC)

The variety of genotypes obtained by more than two alleles that appeared, namely A1, A2, and A3 at a locus, means that the locus has multiple alleles. Multiple alleles arise as a result of gene mutations at that locus. [16] If there are more than two kinds of alleles at a locus, then that the locus has a series of alleles and is called multiple alleles. Multiple alleles have more than two alleles, although no diploid organism has more than two kinds of alleles for each factor. The emergence of these multiple alleles might occur due to gene mutation events

Gene mutations can cause changes in nucleotide bases resulting in changes in the composition of amino acids; mutations occur because of changes in the genetic material of genes or chromosomes in a cell. Mutations in genes can give rise to other alleles. [17] The genotype results in a mutation at the 3934th site, which causes a change in the base from guanine (G) to adenine (A), resulting in a change in amino acids, from glutamic acid (GAA) to lysine (AAA). [18] Mutations occur at the DNA level due to changes in DNA bases (A=ademin, T=thymine, G=guanine, S=cytosine) in the form of substitution, deletion, insertion, and inversion.

The analysis of the study based on the allele frequency of the gene GH (*Growth Hormone*) in Kampung chickens can be said to be polymorphic (various) with the allele frequency value of the four alleles not being more than 0.99 or more than 99%. [10] When the allele is less than 99% of a population, it can say that the allele is polymorphic (various).

[19] that when the chi-square value shows a match between the observed value

and the expected value, the Chi-square value is not significant at the 5% or 0.05 level.

The imbalance in the population of Kampung chickens in this study was because by selected the Kampung chicken rearing system in a cage with each cage has a ratio of one male and six females and the absence of free-range chickens so that there is no random mating between other native chickens. The genotypes that appear were very diverse and had more than two alleles so that there was a mutation in the gene. [7] Population imbalance can be suspected because of the selection, the absence of random mating, the uncontrolled mating system, giving rise to selection opportunities, narrowing the population (*bottleneck effect*), and migration. [20] The balance of genotypes against the population is significant if there is no selection, mutation, migration, and *genetic drift*. *Genetic drift* is a change in genotype frequency resulting from random fluctuations in the probability of mating patterns, sampling errors, and sudden changes in frequency due to environmental factors. On the contrary, when there is an imbalance in the frequency of genotypes and alleles to the population, it can be caused by the accumulation of genotypes, divided populations, mutations, selection, migration, and marriage in the same group (endogamy).

[12] When the PIC value 0.50 it indicates a very informative locus, the PIC value $0.25 < PIC < 0.50$ indicates a reasonably informative locus, and the PIC value 0.25 indicates a low informative locus. [21] The PIC value is an ideal index to measure allele fragment polymorphism [22] The PIC value can be used as a level of determination of genetic information and for purposes of determining the presence of polymorphic alleles, meaning that it has the same function as the heterozygosity value.

Relationship between gene polymorphism GH (Growth Hormone) with body weight kampung chicken

[23] While the BB genotype's average body weight and weight gain had a higher average value than the AA genotype. The B allele positively affected body weight and weight gain in Indonesian Kampung chickens until the age of 4 months. A significant difference ($P<0.05$) occurred in the bodyweight of

chickens aged four months, and a significant difference (P<0.05) occurred in the weight gain of chickens with a period of 2 to 4 months. [24] The GH gene in Arian broiler chickens can affects somebody's composition of Arian broilers, seen from the significant GH gene (P<0.05) associated with weight body weight and weight percentage of the lower thigh at six weeks of age.

The GH gene in native chickens is very informative, meaning that it is very diverse. It can use as a reference in selecting and increasing the productivity of native chickens. [24] when a genotype can be said to be polymorphic (diverse), it is possible to use it on essential traits that consider economically valuable and can help in the selection program. The GH (Growth Hormone) gene in Kampung chicken is polymorphic so that it can use as a gene for markers of growth characteristics in making selection. However, it is necessary to do further research to see the point of mutation contained in the GH gene of this Kampung chicken so that more information about the GH gene is obtained.

CONCLUSION

Based on the study results, it is possible to conclude that the GH (Growth Hormone) gene locus of Kampung chicken is polymorphic, but there has been a mutation in the GH gene, which can see from the emergence of more than two alleles. Hardy-Weinberg Equilibrium describes an imbalance state of the population. The population of Kampung chickens has a very informative diversity value. It has a polymorphic association to the A2A2 genotype of the GH gene of Kampung chickens with a body weight of 3 months.

CONFLICT OF INTEREST

The author declares that he has no conflict of interests.

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