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Original Article

Morphometric characterization and effect of growth hormone (GH) gene polymorphism on growth traits of Kerinci duck (*Anas platyrhynchos*)

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Abstrak

Tujuan: Untuk mengetahui asosiasi keragaman gen GH terhadap sifat pertumbuhan dan mengetahui karakteristik morfometri itik Kerinci.
Metode: Metode penelitian adalah eksperimen menggunakan 96 sampel darah itik Kerinci. Metode meliputi pengambilan data karakteristik kuantitatif (bobot badan, pertambahan bobot badan, ukuran-ukuran tubuh), sampel darah itik Kerinci, kegiatan ekstraksi DNA, amplifikasi PCR dan restriksi dengan enzim *AluI*. Analisis data meliputi uji-t, *T²-Hotelling*, analisis komponen utama, frekuensi genotip, alel, keseimbangan hardy-weinberg, heterozigositas, dan PIC.
Hasil: Karakteristik kuantitatif itik Kerinci jantan berbeda nyata (P<0,05) lebih tinggi dibandingkan itik Kerinci betina. Hasil analisis Gen GH|AluI itik Kerinci bersifat polimorfik. Populasi itik Kerinci berada dalam kesetimbangan Hardy-Weinberg (P>0,05). Nilai Keragaman itik Kerinci Ho<He. Karakteristik kuantitatif gen GH itik Kerinci bergenotip +/+ berbeda nyata (P<0,05) lebih tinggi dibanding genotip +/- dan -/-.
Kesimpulan: Karakteristik kuantitatif itik Kerinci jantan lebih tinggi dibandingkan itik Kerinci betina. Penciri ukuran tubuh itik Kerinci adalah panjang tulang dada, panjang shank, dan lingkar shank, dan penciri bentuk tubuh adalah panjang sayap. Gen GH|AluI itik Kerinci bersifat polimorfik dan memiliki asosiasi dengan karakteristik kuantitatif dengan genotipe terbaik yaitu genotipe +/+.

Kata Kunci: Asosiasi; Gen *growth hormone*; Itik Kerinci; Karakterisasi

Abstract

Objective: To determine the association of GH gene diversity with growth traits and to obtain the morphometric characteristics in Kerinci ducks.
Methods: The research method was an experiment using 96 blood samples of Kerinci duck blood. The methods included data collection on quantitative characteristics (body weight, body weight gain, body measurements), blood samples from Kerinci ducks, DNA extraction activities, PCR amplification and restriction with AluI enzyme. Data analysis included t-test, T2-Hotelling, principal component analysis, genotype frequency, allele, Hardy-Weinberg balance, heterozygosity, and PIC.
Results: The quantitative characteristics of male Kerinci ducks were significantly different (P<0.05) higher than female Kerinci ducks. Analysis of the Kerinci duck GH|AluI gene was polymorphic. The population of Kerinci ducks was in Hardy-Weinberg equilibrium (P>0.05). Diversity Value of Kerinci

Duck Ho<He. Quantitative characteristics of GH gene of Kerinci duck genotype +/+ were significantly different (P<0.05) higher than genotype +/- and -/-.
Conclusions: The quantitative characteristics of male Kerinci ducks were higher than female Kerinci ducks. Characteristics of the body size of Kerinci duck were the length of sternum, length of shank, and circumference of shank, and identifier of body shape was length of wings. The Kerinci duck GH|AluI gene was polymorphic and has associations with quantitative characteristics, with the best genotype being the +/+ genotype.

Keywords: Association; Growth Hormone gene; Kerinci duck; Characterization

INTRODUCTION

Indonesia has a diversity of poultry, one of the potential sources of animal food to be developed. The local duck is one of the poultry that plays a role in providing animal food. Local ducks come from certain areas that usually have names according to regions and varied phenotypic characteristics. For example, one of the local ducks in Indonesia is the Kerinci duck that originated from Kerinci Regency, Jambi Province.

The Kerinci duck is one of the germplasms that Minister of Agriculture degree No. 2834/ Kpts/LB.430/8/2012 the has determined, so it needs to be preserved. Unfortunately, not much research has been done on Kerinci ducks, even though obtaining basic data on genetic diversity is important. One of the efforts to obtain basic data needs to be characterized. Characterization, in general, can be done based on the performance of ducks quantitatively, such as body measure ments, bodyweight, and body weght gain. However, quantitative characterization of its performance is difficult to determine how many genetic or environmental influences are, so it is necessary to carry out molecular characterization. Molecular characterization plays an important role in characterizing genetic diversity more efficiently and in a shorter time. Characterization of genetic diversity through genes that have economic value such as growth can be carried out in-depth analysis of structural genes that play an important role in the growth of Kerinci ducks. One of the determining genes in controlling growth traits is the Growth Hormone (GH) gene. For example, the GH gene is a determining gene in controlling growth traits. Characterizing genetic diversity via genes which have monetary fee, such as boom, may be executed via an in-intensity evaluation of structural genes that play a function within the growth of Kerinci ducks. One of the determining genes in controlling growth traits is the GH gene.

The GH gene is a gene who plays a role in the body's metabolism with controls increase [1]. Efforts to determine the variety of GH genes can be carried out with molecular markers of the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Polymerase Chain Reaction (PCR) is a technique that aims to multiply DNA copies resulting from enzymatic amplification by target DNA sequences [2]. Restriction Fragment Length Polymorphism (RFLP) is a method that plays a role in detecting variations at the DNA level. The advantages of the PCR-RFLP technique are that it is simple, fast, and inexpensive [3]. Until now, the diversity of GH genes related to GH gene expression on production traits such as body weight, weight gain, and size is not widely known. If these two methods, namely the molecular method and their performance, are related, then they can be used as the basis for selection. GHR, GHRL, and IGF1 genes that have been used as candidate genes in finding linkages between genotypes and phenotypes in livestock [4]. GH gene sequence in duck ducks have a length of 5218 base pairs (PB) consists of 5 exons and 4 similar introns in different mammal species. GH genes are very polymorphic in various livestock. Many polymorphisms have been identified in GH duck genes, for example in Pitalah ducks and Kumbang Janti duck [5]. Peking and Mulard ducks [6], Tsaiya ducks, Muscovy ducks [7]. A previous study [5] found a very significant relationship between GH exon 1 gene and duck body weight at 5-8 weeks. Thus, also in the study Mazurowski et al [6] found a significant relationship between the diversity of GH exon 2 genes and the weight of Mulard ducks.

Based on the description, as well as the lack of information related to the GH gene in Kerinci

ducks, a study was conducted on "Morphometric characterization and effect of growth hormone (GH) gene polymorphism on growth traits of Kerinci duck (*Anas platyrhynchos*)".

MATERIALS AND METHODS

Materials

This research was carried out in two stages: research in the area and laboratory. Area research became finished in Koto Majidin Village, Kerinci Regency, Jambi Province. Research inside the laboratory became achieved in the Animal Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University. The study starts on November 20, 2021, until January 20, 2022. The materials used were 96 Kerinci ducks and 96 Kerinci duck blood samples consisting of 41 males and 55 females. The materials used are 70% alcohol, cotton, isopropanol, 70% ethannol, agarose powder, TBE Buffer, equates, microtube eppendorf,microtube rack, centrifuge, vortec, analytical balance, gel doc, electrophoretic gel system, PCR machine, waterbath, well comb, mini spin sentrifuge, power supply electrophoresis, autoclave, and microtube PCR

Methods

The methods used in this study were Kerinci duck blood sampling, DNA extraction, PCR amplification and PCR-RFLP. Blood collection for Kerinci ducks was carried out using Kerinci ducks aged 3 months. Blood sampling is carried out with attention to animal welfare and its implementation was carried out by a veterinarian in accordance with standard procedures [8]. It was obtained by taking blood using a syringe in the wing axillary vein. At least 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 -20^{o}C before further processing. Kerinci ducks were reared from the age of DOD to the age of 3 months intensively with a cage size of 4 x 8 m. The feed used was Japfa Comfeed production with BR1 Energy Composition (kcal/kg): 4,100, Protein (%): 21, Fat (%): 3-7, Calcium (%): 0.9-1.1, Phosphorus (%): 0.6-0.9, and BR2 Energy (kcal/kg): 4,100, Protein (%):19, Fat (%): 3-8, Calcium (%): 0.9-1.1, Phosphorus (%): 0.6-0.9. (Br 1 for 0-4 weeks and Br 2 for 4-12 weeks), using the ND vaccine on DOD, and the drugs used are vitamin C, tetra-chlor.

This research became finished in two stages: in the field and laboratory. The first stage became achieved in the field, which included taking quantitative data, and blood collection for Kerinci ducks. Body weight data collection was obtained from the weighing results. The body measure ments were taken as many as 18 parameters according to the instructions of [9]. Kerinci duck blood samples were obtained by taking blood using a syringe in the axillary vein of the wing. The laboratory's second stage was carried out, including DNA extraction, PCR amplification, and restriction using the AluI enzyme. DNA extraction from duck blood was carried out using the Genomic DNA Purification Kit Protocol from Promega. Then the DNA extraction results were amplified using a primer with a product length of 879 bp which was expected to amplify the growth hormone gene. The primer used was 879 bp in length in Exon 1 with no access to GenBank AB158760.2., and this primer was designed using the Primer 3 Plus program. Primer Forward $^{5'}$GGG AAA CCA CCT CTT TTG CT$^{3'}$ and Reverse $^{5'}$CAG GGA CAG TGA CTC AAC CA$^{3'}$. The process of cutting the amplification results using the PCR-RFLP method using the AluI restriction enzyme (AG↓CT) cutting on 850 bp and 912 bp.

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 Figure 1.

Amplification PCR was conducted with Thermoocycler from BIO-RAD. The composition was 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 was 30 µl, visualization of PCR products was observed using a UV light and documented with Gel Documentation (Biometra Germany). The amplification process was carried

Segment Position	Length (bp)	Primer Name	Sequence (5' 3')	Annealing Temperate
272-1150	879	GHD1-F GHD1-R	5'-GGGAAACCACCTCTTTTGCT-3' 5'-CAGGGACAGTGACTCAACCA-3'	60°C

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241  ccacccagct ctatccccac taacgataaa tgggaaacca cctcttttgc tttatgtcag
301  aggtgtccca gtctggcttg tcagccctgt taactgtggg ccagaccctg cctggagcag
361  gcaggaaaat taggagcact ttctatctat gcggggaaat tccaccatgt aaaagcactg
421  atctgatttg gggtggctct tccatgatga taaaaccgtt atttgcaata aacagcagaa
481  tatggagaaa tcattcagtg ctaatttcat ccctaggcaa acatcctccc caacctttcc
541  atctatgtat aaatgactac aattaggtag caccattgcg aacacgtgtg catttatgca
601  tggagaagat atagagaggt tgttgtgatc atgaacacat atatacattt taaacagacc
661  ccctactata taaggggtgt ctcaacagtt gccattacca gcctagatga aaggaagaaa
721  cattcacttt caagcaacat ctgagcaact ctccaggcag aaatggctcc aggtactctt
781  ctttatttca gtttacgagg attgccaatg cggctacagg cagcattgtg tccaaagaag
841  ggcaataaag ctggtgaagg gtctagagaa caagtcttat taggagcagc cgtgggcact
901  ggggttgttt agctggaga aaaggtggct caggagagac cttaccacac cctacaatta
961  ccttaaagga ggctgtagca aggtggggat caggctcttc tcccaggtac taagtggtaa
1021 gatgagggga aatggcctca agttgtgcca ggggaggttt aggttggata ttagaagaag
1081 tttctttact gaaagggttg tgtggcactg gaataggctg cccagggaag tggttgagtc
1141 actgtccctg gaggtcatca tgaaacatgt agatgtagaa gttagtagta tgttttcatg
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GH FWD

EXON 1

ALU 1

GH REV

Figure 1. GH gene sequences in ducks accessed at GenBank No. access: AB158760.2

out according to the PCR steps in 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 AluI (AG↓CT). 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

Differences between quantitative characteristics and differences between male and female Kerinci duck genotypes were analyzed by t-test based on [10] instructions.

$$t = \frac{X_1 - X_2}{\sqrt{\frac{\sum(X_{J1} - X_1)^2}{n_1(n_1 - 1)} + \frac{\sum(X_{J2} - X_2)^2}{n_2(n_2 - 1)}}}$$

Notation:

t = value of t be count

X_1 = pattern imply in the first organization,

X_2 = pattern mean in the second institution,

X_{J1} = the cost of the J- remark within the first institution

X_{J2} = the value of the J- statement in the X_{J2} second organization

N_1 = wide variety of samples in the first N_1 organization, and

N_2 = variety of samples within the second N_2 organization

T²-hotteling

Vector cost of the average sizes of body of male and female Kerinci ducks were analyzed using the T²-Hotelling test [10].

$$T^2 = \frac{n_1 n_2}{n_1 + n_2}(X_1 - X_2)S_{G^{-1}}(X_1 - X_2)$$

Next:

$$F = \frac{n_1 + n_{2-p-1}}{(n_1 + n_2 - 2)p}T^2$$

can be allotted F with levels of freedom $V_1 = p$ and $V_2 = N_1 + N_2 - p - 1$

Statment:

T^2 = belief of T²-Hotelling statistic

F = calculated belief for T2-Hotelling

n_1 = the variety of observation facts inside n_1 the first institution of duck

n_2 = the range of observation records inside the 2d institution of duck

X_1 = vector suggest value of a random X_1 variable inside the first institution of X_1 duck

X2 = vector represents a belief of a random variable in the 2nd organization of duck

SG_{-1} = the inverse of the composite diversification matrix (the inverse of the SG matrix)

P = variety of measuring variables.

Two organizations are declared identical if T².

$$T \leq \frac{(n_1 + n_2 - 2)p}{n_1 + n_2 - p - 1}F\alpha: v_1, v_2. \quad \text{and}$$

Stated different if T^2

$$T \geq \frac{(n_1 + n_2 - 2)p}{n_1 + n_2 - p - 1} F\alpha: v_1, v_2.$$

If the T^2-Hoteling take a look at showed full-size consequences ($P<0.05$), then the facts processing for every group of Kerinci duck became persisted with the Principal Component Analysis (PCA).

Principal component analysis

Principal Component Analysis (PCA) to decide the size of body and shape of body of the Kerinci duck based [10].

$$Y_j = \mathbf{a}1jX1+\mathbf{a}2jX2+\mathbf{a}3jX3+\ldots\ldots+\mathbf{a}19Jx$$

Note:
Yj = j-th main issue (j = 1, 2; 1 = size, 2 = form)
$X_{1,2,3...}$ = variable to at least one,2,3….18
$a_{ij,2j,3j,..}$ = i-th variable eigenvector (1,2,three,….18) and j-th main aspect

Genotype and allele frequency

The genotype frequency is projected primarily based on the number of positive genotypes divided by using the full pattern:

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

Note:
x_i = found genotype
N = overall sample

The allele frequencies of GH genes received from the PCR-RFLP characterization evaluation have been analyzed the use of [11] method:

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

Note:
X_i = frequency of the i-th allele,
N_{ii} = variety of duck of genotype ii,
N_{ij} = wide variety of duck of genotype ij,
N = general range of samples.

Hardy-weinberg equilibrium

Hardy-Weinberg equilibrium with chi-square test (X^2) according [10] as follows:

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

Note:
x^2 = Chi-square test,
Obs = quantity of observation of the i-th genotype
Exp = predicted wide variety of genotype i

Heterozygotes

Expected heterozygosity (He) was calculated using the formula [11].

$$\hat{h} = 2n\,(1 - \Sigma xi^2)/(2n - 1)$$

Note:
xi = allele frequency of –i locus
n = number of samples
$\hat{h}$ = locus heterozygosity

Polymorphic information content

Polymorphic Information Content (PIC) is calculated based on [10]:

$$PIC = 1 - \sum p_1^2$$

Note:
PIC = Polymorphic Information Content,
P_i = frequency of the i allele.

RESULT

Quantitative characteristics of Kerinci duck

The average body weight at the age of 2 and 3 months and the body weight gain at the period of 2-3 months for male and female Kerinci ducks are served on Table 1.

The t-test analysis results show that the average body weight at the age of 2 and 3 months and the increase in body weight at the age of 2-3 months for male Kerinci ducks were significantly different ($P<0.05$) higher than that of female Kerinci ducks.

Average body sizes of the Kerinci duck

The average sizes of body male and female Kerinci ducks aged 3 months include, length of beak (BeL), width of beak (BeW), length of head (HeL), height of head (HeH), length of neck (NeL), length of back (BaL), sternum length (StL), length of wing (WiL), length of femur (FeL), length of tibia (TiL),

Table 1. Average body weight at the age of 2 and 3 months and body weight gain at the period of 2-3 months for male and female Kerinci ducks.

Age (g)	Male	Female
Body weight at 2 months	1250.45±35.92[a]	1040.95±42.36[b]
Body weight at 3 months	1673.91±47.25[a]	1441.18±41.94[b]
Body weight gain at 2-3 months	423.46±53.19[a]	400.23±37.15[b]

Different lowercase superscripts on the same line were significantly different (P<0.05).

length of shank (SaL), circumference of shank (SaC), length of third finger (TFiL), chest circumference (CeC), body length (BoL), head circumference (HeC), neck circumference (NeC), and tibial circumference (TiC). The t-test analysis results show that the average sizes of body male Kerinci ducks were significantly different (P <0.05) higher than that of female Kerinci ducks.

***T²-hotelling* analysis and principal components analysis of body sizes in Kerinci ducks**

The T2-Hotelling analysis results in this study show that the sizes of body male Kerinci ducks were significantly larger (P<0.01) compare to female Kerinci ducks.

The equations size of body, shape of body, total diversity, and eigenvectors of male and female Kerinci ducks aged 3 months are served on Table 2.

Table 2. shows that the equation for body size values of male and female Kerinci ducks has 63.8% and 73.6%, respectively of total diversity. The highest eigenvectors in the body size equation in male and female Kerinci ducks were the sternum length (StL), shank length (SaL), and shank circumference (SaC). The equation for shape of body score of male and female Kerinci ducks had a total diversity of 7.3% and 7.3%, respectively. The highest eigenvectors in the shape of body equation in male and female Kerinci ducks is wing length (WiL).

DNA extraction and PCR amplification of Kerinci duck growth hormone genes

DNA extraction was successfully carried out on 96 Kerinci duck blood samples. The results of the DNA extraction electrophoresis

Table 2. Equalization of size of body and shape of body with total diversity and eigenvectors of male and female Kerinci ducks.

Kerinci ducks			Equation	TD (%)	Λ
Male	Size of body	=	0.11 BeL + 0.255 BeW + 0.23 HeL + 0.252 HeH + 0.246 NeL + 0.267 BaL+ 0.279 StL + 0.07 WiL + 0.256 FeL + 0.262 TiL + 0.278 SaL + 0.273 SaC + 0.158 TFiL + 0.263 CeC + 0.265 BoL + 0.21 HeC + 0.263 NeC + 0.168 TiC.	63.8	11.5
	Shape of body	=	0.314 BeL + 0.206 BeW + -0.035 HeL + -0.213HeH + 0.031 NeL + -0.058 BaL + 0.035 StL + 0.728 WiL + -0.134 FeL + -0.01 TiL + 0.022 SaL + -0.088 SaC + 0.398 TFiL + -0.043 CeC + -0.052 BoL + -0.277 HeC + -0.064 NeC + 0.085 TiC.	7.3	1.3
Female	Size of body	=	0.26 BeL + 0.173 BeW + 0.165 HeL + 0.2 HeH + 0.212 NeL + 0.225 BaL+ 0.262 StL + 0.122 WiL + 0.241 FeL + 0.258 TiL + 0.262 SaL + 0.262 SaC + 0.259 TFiL + 0.251 CeC + 0.25 BoL + 0.256 HeC + 0.261 NeC + 0.261 TiC.	73.6	13.2
	Shape of body	=	0.267 BeL + 0.062 BeW + 0.244 HeL + 0.142HeH + 0.213 NeL + 0.37 BaL + 0.236 StL + 0.464 WiL + 0.111 FeL + -0.09 TiL + -0.182 SaL + -0.217 SaC + -0.255 TFiL + -0.32 CeC + 0.023 BoL + -0.263 HeC + -0.217 NeC + -0.142 TiC.	7.3	1.3

Description: length of beak (BeL), width of beak (BeW), length of head (HeL), height of head (HeH), length of neck (NeL), length of back (BaL), sternum length (StL), length of wing (WiL), length of femur (FeL), length of tibia (TiL), length of shank (SaL), circumference of shank (SaC), length of third finger (TFiL), chest circumference (CeC), body length (BoL), head circumference (HeC), neck circumference (NeC), and tibial circumference (TiC).

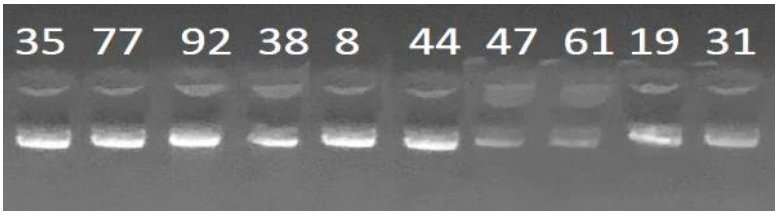

Figure 2. Results of DNA extraction electrophoresis

numbers 35, 77, 92, 38, 8, 44, 47, 61, 19, 31 = individual sample.

in Figure 2. show that the DNA bands obtained are clear and not too thick, meaning that the concentration of DNA obtained is relatively high. Some DNA samples whose concentration level is still low can be seen from the thin bands, but some other DNA samples have bands that are not too thick so that DNA can be used for the next step.

The GH gene PCR product fragment was amplified using a pair of primers Forward: 5'GGG AAA CCA CCT CTT TTG CT3' and Reverse 3'CAG GGA CAG TGA CTC AAC CA5'. This primer was designed using a primer 3 plus located on Exon 1 with length of product is 879 bp. (Figure 3) indicates that amplified primary length of the GH gene is 879 base pairs (bp).

Genotype and allele frequency

The diversity of growth hormone genes in Kerinci duck was identified using AluI cutting enzyme with AG↓CT cutting sites at 579 bp, 238 bp, and 62 bp. This restriction resulted in three genotypes, +/+, +/-, and -/- (Figure 4), and two alleles, + and -.

Based on Table 3, the genotype frequencies in the GH AluI gene in Kerinci ducks are +/+ (42%), +/- (38%), and -/- (20%), with allele frequencies (+) of 61% and (-) by 39%. The growth hormone gene of Kerinci duck in this study is polymorphic.

Hardy-weinberg (HW) equilibrium

Based on Table 3, X^2_{count} (3.48) < X^2_{table} 0.05 (3.84), this condition indicates that the Kerinci duck population is not significantly different at the 0.05 level; thus, the Kerinci duck population can be expressed in equilibrium Hardy Weinberg's.

Heterozygosity

The genetic diversity of the Kerinci duck GH|AluI gene was obtained based on the heterozygosity value in Table 3. below, which shows that the value of H0<He. The observed heterozygosity value (H0) was 0.38, and the expected heterozygosity (He) was 0.48. This value indicates that the diversity of Kerinci ducks was classified as moderate with relatively distant genetic relationships.

Polymorphic information content (PIC)

Based on Table 3. below, the PIC value in the Kerinci duck GH|AluI gene was 0.42. Therefore, the PIC value in the GH|AluI gene was in the moderate category, which means that the primer was quite informative as a marker for the GH|AluI gene fragment.

Association of growth hormone genes with quantitative characteristics in Kerinci ducks.

The average body weight at 3 months of age, body weight gain at 2-3 months, StL, SL,

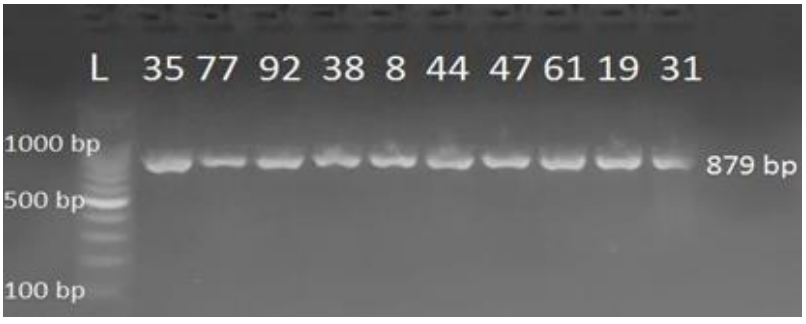

Figure 3. PCR results from the GH gene.

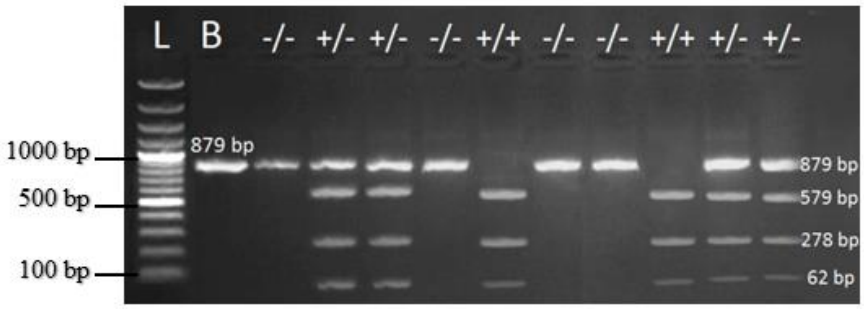

Figure 4. Results of GH|AluI PCR-RFLP electrophoresis.

L = Ladder, B = Blank fragment of GH gene amplification

SC, and WL at 3 months of age, and the Kerinci duck GH gene for various genotypes are served on Table 4.

Table 7. shows the average weight of body at 3 months, body weight gain at the age of 2-3 months and the body measurements at the age of 3 months. The male and female Kerinci ducks genotype +/+ were higher than the genotypes +/- and -/-. The t-test analysis results showed that the mean of quantitative characteristics, against the Kerinci duck GH gene using PCR-RFLP genotype +/+ was significantly different (P<0.05) higher than genotype +/- as well as genotype +/- significantly different (P<0.05) higher than genotype -/-.

DISCUSSION

The average weight of body male and female Kerinci ducks aged 2 and 3 months in this study was higher than in several other studies on the body weight of ducks. For example, the average weight of body male Alabio ducks aged 2 and 3 months, respectively, was 1053.58±59.39 g and 1347.92±97.53 g [12], female Leizhou Black ducks in China aged 2 and 3 months, respectively, was 781.73±36.43 g and 110.33±36.29 g [13]. The outcomes of this observe are not a lot exclusive from the research of [14], which stated that the average weight of body male and female Kerinci ducks aged 2 months, respectively, was 1283.60 ±70.61 g and 1137.72±41.09 g. This condition shows that the weight of body male and female Kerinci ducks aged 2 and 3 months is quite good compared to several other studies.

The average weight gain of body male and female Kerinci ducks aged 2-3 months from this study was higher than several other studies who stated that the average weight gain of body Leizhou Black ducks in China male and female aged 2-3 months was 345.69 g and 318.60 g [13], male and female Cihateup-Alabio (CA) crossed ducks aged 2-3 months was 374.12 g and 261.33 g [15]. This condition indicates that the weight gain of body male and female Kerinci ducks aged 2-3 months is better than in several other studies.

The average weight of body at the age of 2 and 3 months and the weight gain of body at the period of 2-3 months for male Kerinci ducks from this study were higher than female Kerinci ducks. This difference is thought to be due to the presence of androgen hormones resulting in the rapid growth of male Kerinci ducks. This is the opinion [16], who state that androgen hormones cause the rapid growth of male ducks.

Based on the *T²-Hotelling* analysis, the average body sizes of male Kerinci ducks aged 3 months from this study were higher than female Kerinci ducks. This difference was due to the body skeleton of male Kerinci ducks being larger than female

Table 3. Genotype frequency, allele, Hardy-Weinberg (HW) equilibrium, heterozygosity and PIC (Polymorphic Information Content) value.

Line-Locus	N	Genotype	Genotype frequency	Allele frequency	X^2 count	H_0	H_e	PIC value
Kerinci		+/+	0.42	0. 61				
duck	96	+/-	0.38		3.48^{ns}	0.38	0.48	0.42
GH|*AluI*		-/-	0.20	0. 39				

Description: ns= non significant.

Table 4. For various genotypes, average quantitative characteristics of the Kerinci duck GH gene.

Description (g)	Genotype		
	+/+	+/-	-/-
Body weight at 3 months			
Male	1719.40±24.67[a]	1660.65±16.45[b]	1562.74±6.96[c]
Female	1488.38±26.79[a]	1434.94±8.57[b]	1354.26±15.08[c]
Combined	1580.79±117.45[a]	1526.44±113.00[b]	1463.98±107.53[c]
Body weight gain at 2-3 months			
Male	436.01±11.08[a]	406.67±5.48[b]	340.96±7.04[c]
Female	434.75±7.08[a]	402.27±7.65[b]	338.88±8.66[c]
Combined	435.25±8.78[a]	404.06±7.11[b]	339.97±7.70[c]
Body measurement at 3 months			
StL	140.36±1.48[a]	135.35±1.85[b]	131.07±0.65[c]
SaL	51.77±0.75[a]	47.98±0.56[b]	45.25±1.02[c]
SaC	39.59±0.63[a]	37.66±0.51[b]	34.12±1.58[c]
WiL	239.07±0.70[a]	234.58±1.85[b]	231.02±0.55[c]

Superscripts of different letters on the same line are significantly different (P<0.05).

Kerinci ducks. This is the opinion [17-19], who states that an animal's size of body is influenced by the size of the animal's body frame. The longer the skeleton is in normal size, the more muscle attached to it, resulting in a larger carcass piece [20].

The sternum length (StL), the shank length (SaL), and the shank circumference (SaC) are the characteristics of the body size of male and female Kerinci ducks because they have the biggest contribution to the size similarity. The results of this study are the same as the research [21] who states that the size of body characteristics of poultry are shank circumference and shank length. Therefore, the results of this study indicate that StL, SaL, and SaC may be used as fabric for attention inside the choice of Kerinci ducks. Wing length can be used as a selection parameter to improve the shape of body score of Kerinci ducks.

The results of DNA extraction showed that there were thick and thin bands indicating that the concentration of DNA produced was high and low. This is to the statement of several researchers who stated that high concentrations of DNA produced thick and bright bands, while a low concentration of DNA produced a thin and opaque band [22,23]. DNA extraction is said to be successful if there is no smear from the DNA bands [24]. This study's primary length of the GH gene amplified was 879 base pairs (bp). The outcomes of this observe are not same from those of [23], who stated that the amplified primary length of the GH gene was 801 bp. This is due to the use of different primers. [25] stated that very short primers could make it trouble to read the band after cutting the use of enzymes.

This study's GH|AluI gene produced three genotypes, +/+, +/-, and -/-, with two alleles + and – with cutting position at 850 bp and 912 bp. Mutation occurs around the cutting position. The outcomes of this observe are not a lot exclusive from the research of [23], who stated that diversity of GH genes in exon 1, which was cut with the MboII enzyme, showed that the GH gene of Sikumbang Janti ducks was polymorphic characterized by the appearance of genotypes (+/+), (+/-), and (-/-) and two alleles + and -. Sutopo *et al.* [26] stated that the population of Demak and Pekalongan ducks found two alleles on the GH gene, (+) and (-), which formed homozygous characters (+/+) and (-/-) and heterozygous (+/) characters. This condition indicates that the genotypes of Kerinci ducks vary. A population can be polymorphic; one of the alleles is less than 99% [11,27].

This study's population of Kerinci ducks was in equilibrium with Hardy Weinberg's Law. This condition indicates that the GH gene in Kerinci ducks is in a state of equilibrium so that it can be stated that the mating occurs randomly. The outcomes of this observe are not a lot exclusive from the other studies, who noted that the observed population of Pekin ducks and Muscovy ducks at the GH/BsmFI intron 2 locus indicated that the population was in Hardy-Weinberg equilibrium [6], in the population of Sikumbang Janti ducks GH|MboII exon 1 shows

a balance of Hardy Weinberg's Law [23]. Akramullah *et al.* [28] stated that if the chi-square value shows equilibrium between the observed and expected values, the chi-square value is insignificant at the 5% or 0.05 level.

Molee *et al.* [29] stated that the body weights and body sizes of poultry with genotype AA were higher than that of AB and BB. The GH gene of Kerinci ducks with genotype +/+ had better associations with quantitative charac teristics than other genotypes. The outcomes of this observe are not a lot exclusive from [23], which states that the variety of the GH BsmFI gene in Pekin ducks in Poland is associated with body weight and body weight gain. This condition shows that the high production of genotype +/+ in GH gene can increase the quantitative characteristics faster than genotype +/- and -/-.

The higher GH gene production, the faster the growth, while the lower GH gene production, the more stunted growth. Edward *et al.* [30] stated that GH is a hormone involved in many processes as diverse as growth, adiposity, glucose homeostasis, and reproduction. In this condition, it was stated that the GH|AluI gene was associated with quantitative characteristics of male and female Kerinci ducks, with the best genotype being the +/+ genotype. The results of this study can be used as a basis for selection in the framework of the Kerinci duck breeding program in the future. The next stage will try to develop Kerinci ducks with the genotype (+/+), and sequencing analysis.

CONCLUSION

Based on the results of discussion that quantitative characteristics of male Kerinci ducks are higher than female Kerinci ducks. Characteristics of size of body of the Kerinci duck are the sternum length, the shank length, and the circumference of shank, and the identifier of shape of body is the wings length. In addition, the Kerinci duck GH|AluI gene is polymorphic. Therefore, the Kerinci duck GH|AluI gene is associated with quantitative characteristics with the best genotype, the +/+ genotype.

CONFLICT OF INTEREST

The author declares that we have no conflict of interest.

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