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Association of Growth Hormone Gen with KUB Chicken Productivity

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ABSTRACT

This study aims to investigate the diversity of quantitative characteristics and GH genes, along with the association between GH genes and quantitative characteristics. The research material consisted of 96 KUB chickens aged DOC-2 months and corresponding blood samples. The t-test was utilized to determine differences in body weight, weight gain, and body measurements between male and female KUB chickens, and assess the diversity of the GH gene. T^2-Hotelling analysis was employed to compare body measurements between male and female KUB chickens, while principal component analysis was used to identify size and shape characteristics. Male KUB chickens' average body weight, weight gain and body size were significantly ($p<0.05$) higher than females. The analysis of the growth hormone gene exhibited three genotypes: +/+ (0.51), +/- (0.35), and -/- (0.14). Additionally, two alleles were identified: (+) accounting for 0.68% and (-) accounting for 0.32%. The growth hormone gene MspI of KUB chickens demonstrated polymorphism, with X2 count (2.93) < X2 table 0.05 (3.84). The heterozygosity value in the KUB chicken population was 0.43, and the obtained PIC value was 0.38. The genotype +/+ of the growth hormone gene in KUB chickens was significantly higher ($p<0.05$) than genotypes +/- and -/-.In conclusion, male KUB chickens exhibited higher body weight, weight gain, and body measurements compared to female KUB chickens. The chest circumference served as the identifier for body size in both male and female KUB chickens, while the length of the upper body and tibia length distinguished the shape characteristics of male and female KUB chickens, respectively. KUB chickens' growth hormone gene MspI was associated with body weight, weight gain, and body measurements, with the (+/+) genotype being the most favorable.

Keywords: Characterization, Association, Growth Hormone (GH) gene, KUB chicken, MspI enzyme

Introduction

Poultry genetic resources in Indonesia offer a diverse and promising opportunity for animal protein production. Among these resources, local chickens stand out as a potential candidate for development. Local chickens refer to native Indonesian breeds or those imported to Indonesia that have successfully adapted to the local environment (Prawira *et al.*, 2021). These chickens play a significant role in the lives of people and therefore should be conserved and further developed. One local chicken breed with great potential is the KUB chicken, also known as Kampung Unggul Balitnak. According to the Decree of the Minister of Agriculture Number: 274/Kpts/SR.120/2/2014, KUB chickens have been officially recognized as local Indonesian breeds. They are the result of innovative research conducted at the Indonesian Livestock Research Institute in Ciawi-Bogor, involving six generations of selective breeding (Urfa *et al.*, 2017). One of the key advantages of KUB chickens is their rapid growth compared to other native breeds (Mayora *et al.*, 2018). However, their genetic diversity remains relatively high, presenting further selection and improvement opportunities.

Selection is a crucial process in which livestock suitable for development are identified and populations with undesirable traits are eliminated. In the case of local chicken breeds like KUB, selection can be carried out based on the characterization of quantitative traits. These traits, such as body weight, weight gain, and size, have economic value in poultry production (Depison *et al.*, 2022). Nevertheless, assessing the extent to which genetics and environment influence livestock performance through quantitative characteristics can be challenging. Fortunately, advancements in molecular research technologies, especially in the field of genomics, provide opportunities for direct characterization of structural genes. One such gene of interest is the growth hormone (GH) gene, which plays a vital role in regulating body weight, weight gain, and overall body metabolism (Puteri *et al.*, 2019; Mazurowski *et al.*, 2015). By exploring the diversity of GH genes, researchers can utilize molecular markers, such as Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), to gain insights into the genetic variation within KUB chicken populations. Polymerase Chain

Reaction (PCR) is a laboratory technique that enables the amplification of specific DNA sequences. In this method, particular restriction enzymes are employed to detect variations in DNA fragments, which arise due to differences in the location and number of specific restriction enzyme recognition sites. Electrophoresis visualizes these variations as distinct bands (Hidayati *et al*., 2016; Mardiah *et al*., 2021).

Restriction Fragment Length Polymorphism (RFLP), on the other hand, is a method used to analyze DNA sequences by cutting them into fragments using specific restriction enzymes. This technique facilitates the identification of gene polymorphisms (Anggraini *et al.*, 2017).

Materials and Methods

The study utilized a total of 96 KUB chickens and collected blood samples from each chicken. The necessary materials included 70% alcohol and cotton for blood preservation, the Genomic DNA Purification Kit from Promega, isopropanol, 70% ethanol, agarose powder, TBE Buffer solution, distilled water, ethidium bromide (EtBr) staining, loading dye, DNA ladder, forward and reverse primers, Nuclease Free Water, Gotaq Green Mastermix, and MspI restriction enzyme from Thermo Scientific. The equipment used comprised hand gloves, EDTA K3 vascular, tube holder, 3 ml disposable syringe, cool box, stationery, freezer Merk Showcase, oven, autoclave, 200 µL, 1000 µL, 100 µL, and 20 µL micropipettes, Eppendorf pipette tips (yellow, blue, white), Eppendorf microtubes (0.2 mL, 1.5 mL, and 2 mL), microtube rack, centrifuge, Vortec, analytical balance, Erlenmeyer flask, measuring cup, gel doc, power supply for electrophoresis, electrophoretic gel system, gel printer, well comb, mini spin centrifuge, electric heater, PCR thermocycler machine, and water bath.

The research methodology involved both field and laboratory work. The field research utilized an experimental approach with direct observations. KUB chickens' body weight, weight gain, and body measurements were collected from day-old chicks to 2 months of age. The quantitative characteristics assessed included beak length, head length, beak width, head height, head circumference, neck length, neck circumference, wing length, upper body length, lower body length, body height, chest length, chest width, chest circumference, shank length, shank circumference, tibia length, tibia circumference, third finger length, and pubic bone distance. Body weight and weight gain were measured using a digital scale when the chickens reached two months of age. Body length was measured using a digital caliper and tape measure. Each chicken was tagged with a name tag attached to its wing. Blood samples were obtained from the axillary vein of the wing using a syringe, and approximately 1-2 mL of blood was collected into a 3 mL EDTA tube. The samples were temporarily stored in a cool box before being transferred to the freezer for further processing.

According to the protocol provided, the laboratory research involved DNA extraction using the Genomic DNA Purification Kit from Promega. Electrophoresis was conducted using a 1.5% agarose gel stained with ethidium bromide, and the gel was subjected to 100 volts for 60 minutes. The DNA extraction results were visualized using a Gel Doc under UV light. The primers used in the study had a length of 949 bp in Exon 1, with the GenBank access number AY461843. The forward primer was 5'-TGCAAGGAGGGGATATGGAG-3', and the reverse primer was 3'-TTCCCCTAACGTGCTCATGT-5'. PCR amplification was performed using a BIO-RAD PCR machine. The amplification products were visualized by electrophoresis on a 1.5% agarose gel stained with EtBr, applying 100 V for 60 min. The PCR products were then digested with the MspI restriction enzyme (*Arthrobacter luteusI*) at the C↓CGG cut site corresponding to the gene locus. The digestion mixture included 10 µL of PCR amplification product and 10 µL of MspI restriction enzyme, with a total volume of 20 µL. The mixture was incubated in a water bath at 37°C for 3-4 h. Subsequently, electrophoresis was conducted using a 2% agarose gel stained with EtBr, applying 100 V for 120 min to determine the genotypes and allele frequencies. The lengths of the bands were measured and compared using a 100 bp DNA ladder marker and PCR amplification blanks to determine the genotypes of the samples.

T-Test, T^2-Hotelling, and Principal Component Analysis (PCA)

According to the instructions, The T-test determines differences in body weight, weight gain, body size, and genotype differences between male and female KUB chickens (Gaspersz, 2006). Test analysisT2-Hotteling used to analyze between groups. If T^2-Hotelling shows significant results ($P<0.05$), data processing for each livestock group is continued with Principal Component Analysis (PCA) to identify the determinants of KUB chicken size and body shape (Gaspersz, 2006).The determinants of the size and shape of these Indonesian local Chickens were obtained using Principal Component Analysis (PCA). Main Component Analysis aims to explain the structure of variancecovariance (a combination of diverse multivariate data) through a linear combination of certain variables, while in general, it aims to reduce the data and interpret it (Gaspersz, 2006).

Genotype and Allele Frequencies, Hardy-Weinberg Equilibrium, Heterozygosity, and *Polymorphic Information Content (*PIC)

Genotype frequency is the proportion or percentage of a particular genotype in a population, calculated based on the number of genotypes divided by the total sample. The Hardy-Weinberg balance, with the chi-square test (X2) aims to compare the observed data with the hypothesized or expected value. Genetic variability can be seen based on the heterozygosity value and can be calculated using Nei's formula (Nei and Kumar, 2000). Polymorphic Information Content (PIC) is

calculated using the formula by Botstein *et al.* (1980).

Results and Discussion

KUB chicken average body weight and KUB chicken weight gain

The average body weight and weight gain of male and female KUB chickens aged DOC-2 months are presented in Table 1.

Based on Table 1, the average body weight of male KUB chickens at DOC, 1 mon, and 2 mons was 34.96±1.88 g, 385.80±18.64 g, and 775.37±41.83g, respectively. In comparison, female KUB chickens at DOC, 1 mon, and 2 mon had average body weights of 33.57±2.02 g, 371.21±19.46 g, and 716.41±44.37 g. These findings indicate that the KUB chickens in this study had higher average body weights compared to previous research. For instance, previous studies reported the DOC weight of KUB chickens as 34.20±3.09 g, while Sentul chickens weighed 33.85±2.53 g (Putri *et al.*, 2020). Moreover, Depison *et al.* (2022) observed body weights of KUB chickens at DOC, 1 mon, and 2 mon as 33.57 g, 321.14 g, and 699.62 g, respectively. Similarly, Utama *et al.* (2022) reported average body weights of 33.57g, 374.79 g, and 755.39 g for KUB chickens at DOC, 1 mon and 2 mon, respectively. The discrepancies in average body weights among these studies may be attributed to variations in rearing systems and environmental conditions. This finding aligns with the statement by Arianto *et al.* (2019) that the environment plays a significant role in growth. Furthermore, Irmaya *et al.* (2021) suggest that differences in body weight can result from environmental conditions, genetic factors, and management practices.

Regarding weight gain, male KUB chickens exhibited an average gain of 350.75±16.67 g at DOC-1 mon, while female KUB chickens gained 337.64±17.49 g during the same period. At 1-2 mon, male KUB chickens had a weight gain of 389.43±27.19 g, compared to 345.19±25.29 g for female KUB chickens. The body weight gain of KUB chickens in this study exceeded that reported by Depison *et al.* (2022), where KUB chickens at DOC-1 monhad a gain of 287.57±49.35 g, and those aged 1-2 mon had a gain of 389.43±27.19 g for males and 345.19±25.29 g for females. Similarly, Utama *et al.* (2022) found that KUB chickens at DOC-1 mon had a gain of 338.96±22.93 g, while those aged 1-2 mon gained 338.96±22.93 g. These differences may also be influenced by environmental factors and management practices, as indicated by Zurriyati *et al.* (2020), emphasizing the impact of husbandry and environmental conditions on livestock body weight.

The t-test results showed significant differences ($p<0.05$) in body weight at DOC, 1 mon and 2 mon, as well as in body weight gain at DOC-1 mon and 1-2 mon, between male and female KUB chickens. These differences indicate that male KUB chickens have higher average body weights and weight gains compared to female KUB chickens. The disparity in body weight and weight gain between males and females can be attributed to hormone influence, particularly testosterone. Male KUB chickens possess higher levels of testosterone compared to females. Testosterone in roosters stimulates faster growth rates by promoting protein synthesis, which subsequently affects body weight gain (Andini and Purwantini, 2019).

Average body sizes

The results of this study indicated that the average body sizes of KUB chickens aged two months were significantly different ($p<0.05$) higher than female KUB chickens. The results of this study are similar to several other studies. Utama *et al.* (2022) stated that the body size of male KUB chickens is higher than that of females. Super male chickens are taller than super female chickens (Putri *et al.*, 2020); likewise, in Merawang males, it is higher than in female Merawang chickens (Sari *et al.*, 2021). The difference in the average body sizes of male KUB chickens and female KUB is thought to be influenced by the hormone testosterone, which can stimulate increased expenditure of other hormones as growth boosters so that male livestock can be superior to female livestock (Ananda *et al.*, 2020). This condition causes the body size of male KUB chickens to be higher than female KUB chickens. Pagala *et al.* (2018) believe that in male chickens, the growth hormone gene controls a higher production trait than in female chickens.

Analysis of T2 -Hottelling and principal components of body measurements

To assess the vector value of the average body size in KUB male and female chickens, T^2-Hotelling analysis was conducted. This analysis demonstrated that male KUB chickens had significantly larger body sizes than female KUB chickens ($P<0.05$). These differences in body size can be attributed to variations in body frame, as stated by Putra *et al.* (2015), who highlighted the role of genetic factors in determining the body size of livestock. Additionally, Muzani *et al.* (2005) support this notion by suggesting that genetic factors contribute to disparities in livestock body size.

Principal component analysis (PCA) was employed to investigate the discrimination between body size and body shape in chickens. This analytical approach allows for the examination of size and shape equations, and the determination of total diversity (TD) and eigenvalues (λ) in male and female KUB chickens. Refer to Table 3 for the specific details and outcomes of these analyses.

Based on Table 3, the body size values of male and female KUB chickens exhibit a total diversity of 74.8% and 70.1% respectively. These percentages represent the highest proportions of variance among the principal components obtained. The highest eigenvector identified in the body size equation for both male and female KUB

chickens is the chest circumference (CC). This indicates that chest circumference, along with upper body length and tibia length, plays a significant role in determining body size characteristics, as these variables contribute the most to the body size equation. These findings align with Utama *et al.* (2022), who emphasized that chest circumference is an important body measurement for KUB chickens.

In terms of body shape, the similarity of the body shape scores between male and female KUB chickens at two months of age demonstrates a total variance of 7% and 14.5% respectively. These values represent the highest proportions of variance among the principal components obtained for body shape. The highest eigenvector observed in the characteristic equation for body shape in roosters is the upper body length (uBL), while in hens, it is the tibia length (TL). This discrepancy suggests genetic differences between the sexes. According to Putri *et al.* (2020), genetics significantly influence the body shape of chickens, whereas body size is also influenced by environmental factors, regional topography, and husbandry practices. Consequently, the length of the tibia makes a more significant contribution to the body shape equation for KUB chickens.

DNA extraction and growth hormone (GH) gene amplification

The objective of DNA extraction is to obtain DNA of high quality and purity. This study successfully performed DNA extraction on 96 KUB chicken blood samples, resulting in clear and clean DNA bands. The extraction process aims to obtain DNA that is suitable for molecular analysis, as emphasized by Hutami *et al.* (2018). For a visual representation of the DNA extraction process and its results, please refer to Figure 1.

Table 1. Average body weight of DOC up to 2 months of male and female KUB chickens

Age	KUB chicken	
	Male	Female
DOC(g)	34.96±1.88[a]	33.57±2.02[b]
Body weight 1 mon (g)	385.80±18.64[a]	371.21±19.46[b]
Body weight 2 mon (g)	775.37±41.83[a]	716.41±44.37[b]
Weight gain-1 mon (g)	350.75±16.67[a]	337.64±17.49[b]
Weight gain 1-2 mon (g)	389.43±27.19[a]	345.19±25.29[b]

Different superscripts in the same line for each body size of male and female chickens are significantly different (P<0.05).

Table 2. Body sizes of male and female KUB chickens aged 2 months

Body size	Male	Female
Beak length (BL) (mm)	24.39±1.02[a]	19.02±0.90[b]
Head lengt (HL) (mm)	32.54±0.73[a]	26.65±1.72[b]
Beak width (BWd) (mm)	6.45±0.55[a]	5.30±0.36[b]
Head height (HH) (mm)	25.81±0.82[a]	23.20±1.29[b]
Head circumference (HC) (mm)	95.79±1.15[a]	90.20±1.50[b]
Neck length (NL) (mm)	105.21±1.11[a]	99.58±0.85[b]
Neck circumference (NC) (mm)	64.26±1.06[a]	58.89±0.97[b]
Wing length (WL) (mm)	155.84±1.85[a]	145.19±1.69[b]
Upper body length (uBL) (mm)	176.76±1.46[a]	155.79±1.11[b]
Lower body length (lBL) (mm)	226.52±1.66[a]	200.05±0.82[b]
Body height (BH) (mm)	285.34±1.68[a]	270.14±2.47[b]
Bust length (BL) (mm)	92.85±1.68[a]	84.88±2.75[b]
Chest width (CW) (mm)	42.40±1.19[a]	34.81±0.86[b]
Chest circumference (CC) (mm)	264.60±1.42[a]	246.85±1.70[b]
Shank length (SL) (mm)	60.36±1.06[a]	51.63±1.36[b]
Shank circumference (SC) (mm)	37.62±0.99[a]	31.91±0.80[b]
Tibia length (TL) (mm)	85.09±1.11[a]	76.86±1.02[b]
Tibia circumference (TC) (mm)	73.40±1.21[a]	66.51±1.04[b]
Third finger length (tFL) (mm)	47.94±3.03[a]	23.94±1.47[b]
Pubic bone distance (PBD) (mm)	9.93±0.63[a]	8.66±0.49[b]

Different superscripts in the same line for each body size of male and female chickens are significantly different (P<0.05).

Table 3. Equation of body size and body shape with total and eigenvector diversity of male and female KUB chickens

Type			Equality	TD (%)	Λ
Male	Body size	=	0.046 BL + 0.250 HL + 0.254 BWd + 0.242 HH + 0.242 HC + 0.251 NL + 0.249 NC + 0.254 WL + -0.021 uBL + -0.031 lBL + 0.238 BH + 0.251 CL +0.253 CW + 0.256 CC + 0.254 SL + 0.254 SC + 0.041 TL + 0.254 TC + 0.245 tFL + 0.243 PBD	74.8	14.30
	Body shape	=	0.057 PP + 0.008 PK + -0.008 LP + 0.071 TK + 0.071 LiK + -0.029 PL + 0.081 LiL + 0.013 Psa + 0.531 Pta + -0.517 PTB + 0.113 TT + - 0.051 PD + -0.022 LD+ 0.029 LiD + -0.022 PS + 0.048 Lis + -0.624 Pti+ -0.019 LiTi + -0.084 PjK + -0.121 JTP	7.0	1.39
Female	Body size	=	0.057 BL + 0.008 HL + -0.008 BWd + 0.071 HH + 0.071 HC + -0.029 NL + 0.081 NC + 0.013 WL + 0.531 uBL + -0.517 lBL + 0.113 BH + - 0.051 CL + -0.022 CW + 0.029 CC + -0.022 SL + 0.048 SC + -0.624 TL + -0.019 TC + -0.084 tFL + -0.121 PBD	70.1	14.05
	Body size	=	0.240 PP + 0.216 PK + -0.027 LP + -0.048 TK + 0.204 LiK + 0.090 PL + 0.256 LiL + 0.165 Psa + 0.200 Pta + 0.099 PTB + 0.278 TT + 0.226 PD + 0.099 LD + 0.086 LiD + 0.221 PS + 0.388 Lis + 0.418 Pti + 0.410 LiTi + 0.111 PjK + -0.027 JTP	14.5	2.90

BL = Beak length, HL = Head length, BW = Beak width, HH = Head height, HC = Head circumference, NL = Neck length, NC = Neck circumference, WL = Wing length, uBL = Upper body length, lBL = Lower body length, BH = body height, CL = Chest length, CW = Chest width, CC = Chest circumference, SL = Shank length, LC = Shank circumference, TL = Tibia length, TC = Tibia circumference, tFL = Third finger length and PBD = Pubic bone distance.

Figure 1 illustrates the electrophoresis results of KUB chicken DNA extraction, revealing distinct and prominent bands. The clarity and intensity of the bands serve as indicators of successful DNA isolation. A visually appealing appearance with thick bands and low smear intensity indicates a high quality and successful extraction of total DNA (Hutami *et al.*, 2018).

Figure 2 demonstrates the successful amplification of the growth hormone (GH) gene using a suitable annealing temperature of 60°C for 45 seconds. The clear amplification of the GH gene indicates that the annealing temperature used was optimal, not excessively high or low. Annealing temperature is a crucial factor in PCR as it determines the binding of the primers to the DNA template. It is calculated based on the melting temperature (Tm) of each primer, and the correct annealing temperature ensures the production of a robust and accurate PCR product (Mardiah *et al.*, 2021). Therefore, achieving the appropriate annealing temperature is essential. If the temperature is too high, the primers may fail to bind to the DNA, resulting in unsuccessful amplification. Conversely, if the temperature is too low, the primers may bind to unintended regions of the DNA, leading to poor-quality DNA amplification (Hidayati *et al.*, 2016; Rahmadhan *et al.*, 2019).

Genotype and allele frequency

Diversity within the growth hormone (GH) gene in KUB chickens was examined using the MspI restriction enzyme, which targets the C↓CGG cutting site. This analysis successfully revealed three distinct genotypes: (+/+), (+/-), and (-/-). These genotypes were identified based on the presence of four distinct band sizes: 125bp, 148bp, 267bp, and 409bp, representing two alleles denoted as (+) and (-). The occurrence of multiple genotypes in the population signifies the existence of genetic diversity. Such diversity arises from variations in DNA sequences due to repetitions, insertions, deletions, and recombination events among individuals, groups, or the overall population (Nei and Kumar, 2000). Figure 3 illustrates the results of electrophoresis of the KUB chicken Growth Hormone (GH) gene fragments, obtained by digesting the DNA with the MspI restriction enzyme and separating the fragments using a 2% agarose gel.

Figure 3 displays the successful amplification of the growth hormone (GH) gene PCR products using an appropriate annealing temperature of 60°C for 45 s. The amplified GH gene confirms the suitability of the chosen annealing temperature. This finding supports the perspective of Rahmadhan *et al.* (2019), which highlights the critical role of annealing temperature in determining the success of amplification. Selecting an excessively high temperature can hinder primer attachment, leading to failed amplification. Conversely, opting for a temperature that is too low can result in non-specific binding of the primer, yielding DNA with low specificity. Genotype and allele frequencies describe the genetic diversity within a population (Miraj *et al.*, 2022).

Based on Table 4, the results of growth hormone (GH) gene restriction using the MspI enzyme in KUB chickens obtained three genotypes (+/+), (+/-) and (-/-). Genotype frequencies of growth hormone (GH) genes in KUB chickens were +/+ (0.51), +/- (0.35), and -/- (0.14). The value of the allele frequency (+) of KUB chicken was 0.68%, and allele (-) was 0.32%. The results of this study indicate that the growth hormone gene in KUB chickens is polymorphic. This result follows the opinion of Gurning *et al.* (2018) and Sihombing *et al.* (2019), which state that a locus is declared polymorphic if the number of alleles in the population is more than one. A locus is said to be polymorphic if it has variations in alleles in a population, whereas if it does not, it is said to be monomorphic (Azizah *et al.*, 2015).

Hardy-Weinberg balance (H-W)

Based on the data presented in Table 4, it can be observed that the X2 count value (2.93) is lower than the X2 table value at a significance level of 0.05 (3.84). This finding indicates that there is no significant difference within the KUB chicken

Figure 1. Electrophoresis of DNA extraction results.

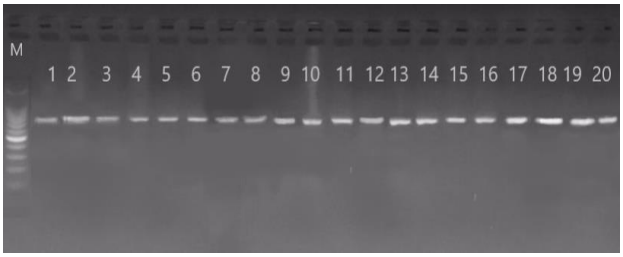

Figure 2. Electrophoresis results of GH gene PCR products with a lenght of 949 bp using 1000 bp DNA Ladder.

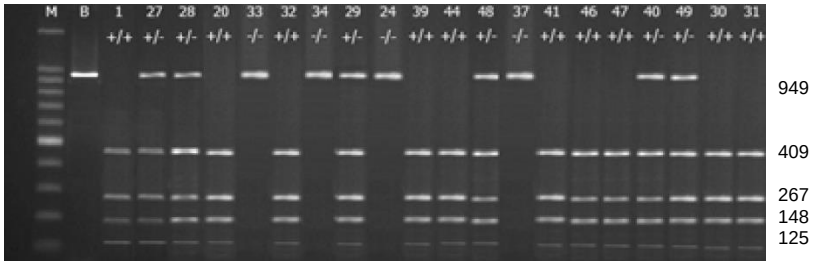

Figure 3. Results of electrophoresis PCR-RFLP GH|MspI (M = Marker, B = Blank Fragmen amplifikasi gen growth hormone).

Table 4. Genotype, allele frequencies, Hardy-Weinberg balance test (HW), heterozygosity value (h) and PIC (polymorphic information content) values

Locus	N	Genotypes	Genotypes frequency	Allele frequency	X^2	H_0	H_e	PIC
		+/+	0.51	68%				0.38
KUB|GH|*Msp1*	96	+/-	0.35		2.93	0.32	0.50	
		-/-	0.14	32%				

population (P>0.05). Consequently, it can be inferred that the KUB chicken population adheres to the Hardy-Weinberg equilibrium, which implies that mating occurs randomly. The Hardy-Weinberg law states that a population can achieve equilibrium if the frequencies of alleles and genotypes remain constant across generations (Gurning *et al.*, 2018). In this context, the chi-square value serves as an indicator of balance between the observed and expected values. In this case, the chi-square value is not significant at the 5% (or 0.05) level, further supporting the notion of a balanced state (Akramullah *et al.*, 2020).

Heterozygosity (h)

According to the data presented in Table 4, the observed heterozygosity value (Ho) for KUB chickens was determined to be 0.32, while the expected heterozygosity value (He) was calculated as 0.50. This finding suggests that the diversity within the KUB chicken population is classified as moderate, indicating relatively distant genetic relationships among individuals. These results align with the findings of Wang *et al.* (2015), who proposed that a population with an observed value smaller than the expected value indicates a relatively distant kinship relationship.

By considering the heterozygosity values, we can infer that the KUB chicken population exhibits a moderate level of genetic diversity, suggesting variations within the population. This information provides insights into the genetic landscape of KUB chickens and highlights the importance of maintaining and managing their genetic diversity for future breeding programs.

Polymorphic information content (PIC)

The level of informativeness of a marker or identifier can be determined by assessing its Polymorphic Information Content (PIC) value, which falls into three categories: low (≤ 0.25), medium (0.25 < PIC < 0.5), and high (≥ 0.5) (Hartati and Soewandi, 2022). In the case of the growth hormone (GH) gene in KUB chickens, the PIC value was found to be 0.38 based on the study's results. This categorizes the GH gene as medium in terms of informativeness. The PIC value of 0.38 indicates that the growth hormone gene in KUB chickens carries a moderate level of genetic diversity and serves as a reasonably informative identifier for the GH gene fragment (Msp1). This finding aligns with the findings of Terryana *et al.* (2017), where a high PIC value corresponds to a marker or character with substantial genetic diversity.

By considering the medium PIC value of the growth hormone gene in KUB chickens, researchers can utilize this marker to gain insights into the genetic makeup and variations within the KUB chicken population. This information is valuable for breeding programs, conservation efforts, and further studies aimed at understanding the genetic diversity and traits of KUB chickens.

Association of growth hormone (GH) genes with quantitative KUB chickens

Average body weight at 2 mon old, body weight gain at 1-2 mon old, and CL (chest length), CC (chest circumference), SL (shank length) and WL (wing length) at 2 mon old Growth Hormone gene in various genotypes are presented in Table 5.

Based on the data presented in Table 5, it is evident that KUB chickens with the +/+ genotype exhibited higher average body weight at two months old, body weight gain during the 1-2 monperiod, and body measurements at two months old compared to the +/- and -/- genotypes. This finding highlights the potential of KUB chickens with the +/+ genotype as informative genetic markers and valuable seed sources for future selection programs. These results are consistent with a study conducted by Batubara *et al.* (2016), which reported that cattle with the (-/-) genotype had higher body weight and body measurements compared to the (+/+) and (+/-) genotypes.

Based on the data presented in Table 5, it is evident that KUB chickens with the +/+ genotype exhibited higher average body weight at two months old, body weight gain during the 1-2 monperiod, and body measurements at two months

Table 5. Average body weight at 2 mon old, weight gain at 1-2 mon old, CC, uBL, and TL at 2 mon old KUB chicken growth hormone gene in various genotypes

Description (g)	Genotipe		
	+/+	+/-	- /-
Body weight 2 mon			
Male	808.22 ± 32.62[a]	746.74± 9.40[b]	727.57± 4.53[c]
Female	753.30 ± 20.47[a]	689.51 ± 17.46[b]	645.05 ± 10.22[c]
Combined	781.32 ± 38.76[a]	718.12 ± 32.16[b]	689.48 ± 43.44[c]
Body weight gain 1-2 mon			
Male	410.71 ± 20.69[a]	369.36 ± 6.62[b]	363.08± 0.58[c]
Female	366.65 ± 11.68[a]	328.64 ± 9.32[b]	306.21 ± 3.66[c]
Combined	389.18 ± 27.83[a]	349.01 ± 21.14[b]	336.83 ± 29.60[c]
Body size			
Chest circumference (CC)	257.15 ± 8.92[a]	254.99 ± 9.4[b]	254.26 ± 9.32[c]
Upper body length (uBL)	167.34 ± 10.61[a]	165.86 ± 10.89[b]	164.96 ± 10.43[c]
Tibia length	81.82 ± 4.20[a]	80.51 ± 4.32[b]	79.61 ± 4.10[c]

Different lowercase letters on the same row are significantly different (P < 0.05).

old compared to the +/- and -/- genotypes. This finding highlights the potential of KUB chickens with the +/+ genotype as informative genetic markers and valuable seed sources for future selection programs. These results are consistent with a study conducted by Batubara (2016), which reported that cattle with the (-/-) genotype had higher body weight and body measurements compared to the (+/+) and (+/-) genotypes.

Furthermore, the mean difference test (t-test) analysis demonstrated significant differences (P<0.05) in the average weight, weight gain, CC, uBL and TL of the GH gene between the +/+ genotype and the +/- genotype, as well as between the +/- genotype and the -/- genotype. This indicates that the GH gene of KUB chickens with the +/+ genotype has a stronger association with body weight, weight gain, and body measurements compared to the other genotypes.

These findings suggest that the high production of the GH gene with the +/+ genotype leads to increased body weight, weight gain, and body measurements compared to the +/- and -/- genotypes. Therefore, it can be concluded that the GH|MspI gene is closely associated with the body weight, weight gain, and body measurements of male and female KUB chickens, with the +/+ genotype being the most favorable. This aligns with the assertion made by Pagala *et al.* (2018) that the Growth Hormone (GH) gene influences growth and metabolism through interactions with specific receptors on the target cell surface, making it a promising basis for selection in the development of KUB chickens.

Conclusions

Based on the results and discussion, the following conclusions can be drawn: 1) Male KUB chickens exhibit higher body weight, weight gain, and body measurements compared to female KUB chickens, indicating sexual dimorphism in these traits. 2) Chest circumference serves as a reliable indicator of body size for both male and female KUB chickens. Conversely, upper body length and tibia length are crucial in determining the overall shape of male and female KUB chickens. 3) The growth hormone gene (MspI) in KUB chickens displays polymorphism, indicating the presence of genetic variation within the population. 4) KUB chickens' growth hormone gene (MspI) is associated with body weight, weight gain, and body measurements.

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