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Association of Growth Hormone Genes with Performance of Crossbreeds of Sentul Chicken and Arab Chicken Using PCR-RFLP

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ABSTRACT. This study evaluated the body weight, weight gain, body measurements, and genetic diversity of the Growth Hormone (GH) gene in male and female Sentul-Arab (SeA) crossbred chickens. It also explored the association between the GH gene and these traits. The research involved 30 male and 30 female SeA chickens reared from day-old chicks (DOC) to four months of age. The experimental method used direct observation to collect data, including body weight, weight gain, body measurements, and blood samples. Data were analyzed using t-tests, the Hotelling's T^2 test, and Principal Component Analysis (PCA). Molecular analyses assessed genotype and allele frequencies, Hardy-Weinberg equilibrium, heterozygosity levels, and Polymorphic Information Content (PIC). The results indicated that male SeA chickens had significantly higher ($P<0.05$) body weight, weight gain, and body measurements compared to females. GH gene analysis revealed polymorphism, with genotype frequencies of +/+ (0.43), +/- (0.35), and -/- (0.22), consistent with Hardy-Weinberg equilibrium. The heterozygosity levels indicated moderate genetic diversity, while the PIC value fell within the low category. In conclusion, male SeA chickens exhibited superior body weight, weight gain, and body measurements compared to females, with chest circumference and shank length being critical indicators of body size. The polymorphic GH|TaqI gene was associated with body weight, weight gain, and body measurements, with the +/+ genotype showing the most favorable effects.

Keywords: Arab chicken, growth hormone, PCR-RFLP, Sentul chicken

INTRODUCTION

Indonesia is a tropical nation rich in diverse flora and fauna, with local chickens being one of the most common examples of this biodiversity. In Indonesia, these local chickens are primarily raised for meat and egg production. The benefits of keeping local chickens include straightforward maintenance, minimal land requirements, adaptability to various environmental conditions, disease resistance, and relatively high market prices. Among the different breeds of local chickens, Sentul, and Arab chickens stand out as up-and-coming promising development candidates.

Sentul chickens are a native breed from Ciamis Regency in West Java Province, cultivated for generations primarily as meat producers (Ihsanudin *et al*., 2022). These local meat-type Sentul chickens have significant breeding potential due to their ability to yield relatively high meat production.

Arab chickens (*Gallus turcicus*) are a native breed from Belgium that has successfully adapted to the Indonesian environment. These chickens are primarily known for their egg-laying capabilities and possess considerable breeding potential (Alwi *et al*., 2019). While Sentul chickens achieve better weight than Arab chickens, their egg production is lower.

One method to enhance livestock performance is crossbreeding Sentul chickens with Arab chickens. Crossbreeding involves the mating of individuals from different lines or strains to achieve a blend of characteristics from both parent breeds. The resulting crossbreed is anticipated to exhibit a higher average performance than the average performance of either parent. Body weight, weight gain, and measurements can indicate livestock's high and low performance. However, these factors are influenced by the interaction between genetics and the environment, making it necessary to determine the extent of their genetic and environmental impact. Advancements in molecular technology enable us to assess structural genes directly. One gene that is significant for growth is the Growth Hormone (GH) gene.

GH is a growth hormone that influences the growth and metabolism of livestock through interactions at the cell surface (Pagala *et al*., 2018). It plays a crucial role in maintaining glucose availability by promoting the absorption of amino acids into proteins, enhancing fatty acid oxidation and their release from adipose tissue, and counteracting insulin's effects on increasing glucose levels (Fassah *et al*., 2023). The diversity of the GH gene is associated with body weight, and this relationship can be observed in the variations of the GH gene, which are affected by both genetic factors and environmental conditions (Rahmat *et al*., 2022). The polymerase chain reaction-

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restriction fragment length polymorphism (PCR-RFLP) marker is one method used to identify genetic diversity in GH.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) marker is one technique used to assess genetic diversity in GH. Polymerase chain reaction (PCR) is an in vitro technique for amplifying DNA. This method employs thermostable polymerase enzymes to amplify DNA molecules in a laboratory setting (Nugroho *et al*., 2021). Restriction fragment length polymorphism (RFLP) is a technique for detecting DNA sequences by observing variations in DNA fragments produced by specific restriction enzymes, which indicate gene polymorphism (Anggraini *et al*., 2017). The PCR-RFLP method offers advantages such as lower costs and shorter processing times, allowing for real-time results compared to other techniques (Guan *et al*., 2018).

Based on the provided details and the limited knowledge about the diversity of growth hormone genes derived from crossing Sentul and Arab chickens, we propose conducting research titled "Investigating the Association Between Growth Hormone Genes and the Performance of Crossbred Sentul and Arab Chickens Utilizing PCR-RFLP."

MATERIALS AND METHODS

Place and Time

This research was carried out in two phases. The first phase involved the maintenance of chickens resulting from the crossbreeding of Sentul and Arab chickens (SeA), conducted in RT 03, RW 01, Mendalo Indah Village, Jaluko District, Muaro Jambi Regency, from February 11, 2023, to August 1, 2023. The second phase took place at the Basic and Integrated Laboratory (UPT-LDT) of Jambi University, running from January 21, 2024, to February 21, 2024.

Material

All the animal procedures for animal experimentation in this study were approved by the Ethical Clearance Committee of the Faculty of Animal Science, Jambi University, Indonesia (No ID approval: Ref.01/UN21.7/ECC/2024). The materials used in this study were 2 male Sentul chickens and 10 female Arab chickens taken randomly from 5 male Sentul chickens and 25 female Arab chickens. The Sentul chickens and Arab chickens were crossed, resulting in 30 male and 30 female crosses of Sentul chickens and Arab chickens (SeA). SeA chickens were raised from DOC age to 4 months, then 60 blood samples were taken.

In the field, the equipment included cages, 3 kg feeders for baby chicks, 5 kg hanging feeders, 2 litre drinking containers for chickens, commercial feed, vaccines, medications, calipers, digital scales, and measuring tapes. The feeds used were BR1 and BR2 from PT. Japfa Comfeed Indonesia Tbk, with relatively different nutritional content in protein (Table 1).

Table 1. Composition of BR1 and BR2 feed

Nutrient content	BR1	BR2
Energy (Kcal/kg)	4100	4100
Protein %	21	19
Fat %	3-7	3-8
Calcium %	0.9-1.1	0.9-1.1
Phosphorus %	0.6-0.9	0.6-0.9

In the laboratory, we utilized a range of precision equipment, including; gloves, EDTA K3 vials, tube holders, 3 ml disposable syringes, cool boxes, stationery, freezers, ovens, autoclaves, micropipettes (200 µl, 1000 µl, 100 µl, and 20 µl), Eppendorf pipette tips (yellow, blue, and white), Eppendorf microtubes (0.2 ml, 1.5 ml, and 2 ml), microtube racks, centrifuges, Vortex mixers, analytical balances, Erlenmeyer flasks, measuring cups, gel documentation systems, electrophoresis power supplies, electrophoretic gel systems, gel printers, well combs, mini spin centrifuges, electric heaters, PCR thermocyclers, and water bath.

Method

Chickens Raising

The method employed in this study was experimental, involving direct observation. SeA chickens are kept in colony cages measuring 3 m x 2 m, as many as 4 units, each cage contains 15 SeA chickens. The cage rests on the ground, lined with wood shavings (litter). The cage is located in Mendalo Indah Village, located at 103º31'39.0"-103º32'48.3 GT and 1º36'39.2"-1º37'25.6" LS, and is located in the western part of the capital city of Muaro Jambi Regency. SeA chickens are fed in the morning and evening, while drinking water is given ad libitum.

The data collected included the body weight of day-old chicks (DOC) at 1, 2, 3, and 4 months, weight gains from DOC to 1 month, from 1 to 2 months, from 2 to 3 months, and from 3 to 4 months, as well as the body size of SeA chickens at 4 months of age and blood samples from the SeA chickens. The body measurements of SeA chickens were measured using digital scales, calipers, and measuring tape. Blood samples were obtained

using a syringe from the axillary vein of the wing, with each sample ranging from 1 to 2 ml. The collected blood was placed in a 3 mL tube and mixed with EDTA powder to prevent clotting. The samples were initially stored in a cool box and then transferred to a freezer below -20 °C for further processing.

Identification of GH Gene Diversity with PCR-RFLP

The second stage of the study was conducted in the laboratory and involved DNA extraction, PCR amplification, and restriction using the Taq1 enzyme. DNA extraction followed the protocol of the Genomic DNA Purification Kit from Promega. The extracted DNA was then amplified using a pair of primers designed to produce an estimated product of 656 bp in intron 4, with accession number AY461843.1. The forward primer sequence was 5' ACC-TAT-ATT-CCG-GAG-GAC-CAG 3', and the reverse primer sequence was 3' GAA GAA-CAC-TCT-CCA-GCT-GCT 5'. Amplification of the GH gene was performed using PCR techniques on a conventional PCR machine. The reaction mixture consisted of 3 µl of each primer (forward and reverse), 2 µl of genomic DNA sample, 10 µl of nuclease-free water, and 15 µl of GoTaq Green Master Mix (Taq Polymerase), resulting in a total volume of 30 µl. The mixture was briefly centrifuged before being placed into the PCR machine at the optimal temperature for amplification. The amplification process was carried out under PCR conditions starting from the predenaturation stage at a temperature of 95 °C for 1 minute followed by 35 cycles of denaturation at a temperature of 95 °C for 30 seconds, then annealing at a temperature of 60 °C for 45 seconds then extension at a temperature of 72 °C for 60 seconds and final extension using a temperature of 72 °C for 5 minutes. The amplification results were analyzed by electrophoresis, where 3 µl of the PCR products were loaded onto a 1.5% agarose gel stained with FluoroSafe and run at 100 volts for 35 minutes. The PCR products underwent incubation for 4 hours in a thermocycler before being digested with the Taq1 restriction enzyme at the TCG↓A cutting site. The final results of the GH gene restriction were then subjected to electrophoresis on a 2.5% agarose gel stained with FluoroSafe and visualized under a UV transilluminator. Genotype identification was based on the resulting DNA fragments, which were compared to a 100 bp marker.

Data Analysis

Differences in body weight, weight gain, body size measurements, and variations between male and female SeA chicken genotypes were analyzed using the t-test according to Mendenhall's guidelines (1987). The average body size values for male and female SeA chickens were assessed using the T^2-Hotelling test. If the T^2-Hotelling test indicated a significant difference ($P<0.05$), Principal Component Analysis (PCA) was performed to identify the factors influencing body size and shape in SeA chickens. The association of GH gene polymorphism with the performance of SeA chickens, including average body weight at 4 months of age, weight gain at 3-4 months of age, body size, and body shape, were analyzed using a T-test. Molecular data analysis involves calculating:

1. Genotype Allele Frequency

Genotype frequency is the proportion or percentage of a particular genotype in a population, calculated based on the number of a genotype divided by the total sample:

$$F1 = \frac{\Sigma Xi}{N}$$

Description:
Xi = observed genotype
N = Total Population

The allele frequency of the Growth Hormone gene is the proportion of a particular allele in a population compared to all alleles occupying the same locus, obtained from PCR-RFLP trait analysis, analyzed using the Nei and Kumar formula.

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

Description:
X_i = Frequency of allele i,
n_{ii} = Number of individuals with genotype ii,
n_{ij} = Number of individuals with genotype ij,
N = Total number of samples.

2. The Hardy-Weinberg (H-W) Equilibrium

Hardy-Weinberg (H-W) equilibrium with chi-square test (X2) aims to compare observed data with the expected value of hypothesis one. Hardy-Weinberg (H-W) equilibrium with chi-square test (X2) is as follows:

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

Description:
X^2 = chi-square test
Obs = number of observations of the i-th genotype
Exp = number of expected genotypes of the i-th

3. Heterozygosity

Genetic diversity (heterozygosity) is calculated based on the Allele Frequency at each DNA Locus using Nei's formula.

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

Description:
X_i = frequency of allele of the i-th locus
N = number of samples
h = heterozygosity of the locus

4. Polymorphic Information Content (PIC)

Polymorphic Information Content (PIC) is used as a standard for assessing genetic markers based on DNA genetic bands from PCR amplification.

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

Description:
PIC = Polymorphic Information Content,
Pi = frequency of alleles I,
N = number of alleles

RESULTS AND DISCUSSION

Average Body Weight and Body Weight Gain of SeA Chickens

The results of the average difference test (t-test) indicated that the average body weight of male SeA chickens at DOC and at 1, 2, 3, and 4 months was significantly higher (P<0.05) than that of females (Table 2). The average body weight of SeA chickens in this study was lower than the findings reported by Abdu *et al*. (2021) which indicated that the body weights of male Sentul chickens at day-old (DOC) and at 1, 2, 3, and 4 months were 35.98±3.07 g, 400.63±44.89 g, 781.63±50.92 g, 1281.49±50.92 g, and 1501.22±152.64 g, respectively. For female Sentul chickens, the body weights at DOC and 1, 2, 3, and 4 months were reported as 28.74±2.38 g, 341.48±24.34 g, 687.32±66.20 g, 1163.92±51.73 g, and 1369.22±102.61 g. The results of this study were also lower than those found by (Depison *et al*., 2022), which stated that the average body weights of male and female Sentul chickens at DOC and ages of 1, 2, 3, and 4 months were 29.44 g, 326.22 g, 671.38 g, 1107.02 g, and 1374.37 g respectively. Generally, the body weight of male higher than female. The difference in body weight between male and female SeA chickens is believed to be influenced by the testosterone hormone produced by the testes. Testosterone acts as an androgen steroid and is a growth regulator in male livestock (Prawira *et al*., 2021). The increased secretion of steroids in males is attributed to heightened testosterone production from the testes, resulting in a faster growth rate for males than females (Mardiah *et al*., 2021). Furthermore, this discrepancy in body weight is believed to be influenced by genetic factors in SeA chickens, which inherit traits equally from male and female parents; thus, this study demonstrates a heterosis effect. According to Dika et al. (2024), heterosis refers to the inheritance of advantageous traits from parents, and body weight being one of the heterosis traits that significantly impacts livestock productivity.

Table 2. Average body weight (BW) of male and female SeA chickens aged DOC–4 months

Parameter (g)	Male	Female
BW DOC	31.12±1.52[a]	27.41±1.58[b]
BW 1 month	190.93±27.77[a]	171.13±17.13[b]
BW 2 month	617.88±52.40[a]	570.37±55.48[b]
BW 3 month	1007.52±63.18[a]	945.89±65.65[b]
BW 4 month	1172.21±73.45[a]	1097.25±81.82[b]

Note: The lowercase superscripts on the same row indicated there is the significantly different (P<0.05).

The weight gain of SeA chickens from DOC to 1 month is relatively low, but it increases from 1 to 2 months. However, weight gain decreases between 2 to 3 months and 3 to 4 months (Table 3). This pattern is believed to occur because the 1 to 2-month age range corresponds to the meat growth phase, while by 2 to 3 months and 3 to 4 months, the bone growth process may have reached its maximum capacity.

Table 3. Body weight gain (BWG) of male and female SeA chickens aged DOC–4 months

Parameter (g)	Male	Female
BWG DOC-1 month	159.81±26.85[a]	143.72±15.92[b]
BWG 1-2 month	426.94±35.50[a]	399.24±43.32[b]
BWG 2-3 month	389.64±14.88[a]	375.52±13.25[b]
BWG 3-4 month	164.69±16.81[a]	151.36±23.54[b]

Note: The lowercase superscripts on the same row indicated there is the significantly different (P<0.05).

The findings of this study were lower than those reported by Irmaya *et al*. (2021), which indicated that the average weight gain of Sentul chickens at DOC to 1 month, from 1 to 2 months, from 2 to 3 months, and from 3 to 4 months was 184.31 g, 414.72 g, 388.57 g, and 165.92 g, respectively. Additionally, the average weight gain of SeA chickens in this study was also less than the results found by Depison *et al*. (2020), which stated that the average weight gain of Sentul chickens at DOC to 1 month, from 1 to 2 months, and from 2 to 3 months was 183.21±42.91 g, 415.82±95.84 g, and 388.57±100.39 g. This discrepancy is believed to

stem from genetic differences and variations in the research environment. According to Depison *et al*. (2020), livestock growth is influenced by multiple factors, including genetics, environmental conditions, and management practices. According to Gultom *et al*. (2021), increased body weight gain is linked to elevated growth hormone levels at 21 and 35 days of age. This growth phase necessitates a diet with high nutritional quality, and this period of growth must be supported by providing quality feed for optimal development (Lukmanudin *et al*., 2018). The results of the average difference test (t-test) indicated that the weight gain of male SeA chickens from DOC to 1 month, from 1 to 2 months, from 2 to 3 months, and from 3 to 4 months was significantly higher (P<0.05) than that of females. This difference is thought to be influenced by gender, as male chickens tend to gain more weight than females due to variations in growth hormone levels (Prawira *et al*., 2021). The faster growth observed in male livestock is attributed to androgen hormones, which promote muscle protein synthesis (Hamdani *et al*., 2017). The disparity in weight gain between males and females can be attributed to hormonal influence, particularly testosterone. Testosterone in roosters stimulates faster growth rates by promoting protein synthesis, which subsequently affects body weight gain (Andini *et al*., 2019).

Average Body Sizes of Sentul Chicken and Arab Chicken

The average body size of male SeA chickens was significantly greater (P<0.05) than that of female SeA chickens. The body size of SeA chickens, both male and female, has large values, namely height, chest circumference, wing length, while the body parts with small sizes are beak width and pubis width (Table 4). The average body size of SeA chickens in this study are lower than the results of the study by by Irmaya *et al*. (2021), which indicated that the average measurements for various body sizes of 4-month-old Sentul chickens were as follows: head height 41.11±0.99 mm, chest width 61.41±3.16 mm, chest length 126.78±5.39 mm, tibia length 126.49±5.39 mm, shank length 83.54±6.11 mm, neck circumference 95.10±5.43 mm, neck length 140.54±5.23 mm, upper body length 212.76±6.97 mm, tibia circumference 107.80±8.28 mm, shank circumference 48.56±2.68 mm, and body height 293.17±11.17 mm. The differences in results are likely due to variations in environmental conditions and the specific strains of chickens being crossed. According to Depison *et al*. (2020), variations in livestock body sizes can be attributed to several factors, including chicken strains, gender, and environmental influences.

Table 4. Body sizes of male and female SeA chickens aged 4 months

Body Size (mm)	Male	Female
Beak length	20.84±1.32[a]	19.7±1.08[b]
Beak width	11.42±1.00[a]	10.62±0.97[b]
Head height	31.83±1.79[a]	27.56±3.12[b]
Head length	46.4±3.05[a]	43.80±2.59[b]
Chest width	46.73±2.49[a]	41.71±3.54[b]
Chest length	114.13±9.61[a]	106.15±8.23[b]
Tibia length	115.86±5.47[a]	108.55±5.71[b]
Shank length	78.98±4.22[a]	71.83±4.00[b]
Third toe length	70.07±4.09[a]	59.55±6.09[b]
Pubis width	12.70±0.37[a]	12.38±0.41[b]
Head circumference	113.58±4.90[a]	110.08±3.68[b]
Neck circumference	73.54±4.94[a]	68.00±4.52[b]
Neck length	124.18±8.51[a]	115.05±5.13[b]
Upper body length	168.72±5.72[a]	160.22±9.23[b]
Wing length	214,79±8,05[a]	207.53±7.97[b]
Chest circumference	227.47±12.49[a]	208.51±8.53[b]
Lower body length	193.79±7.71[a]	188.43±6.06[b]
Tibia circumference	92.52±5.39[a]	87.66±5.67[b]
Shank circumference	32.76±4.02[a]	28.13±3.85[b]
Body height	265.24±10.01[a]	255.44±10.43[b]

Note: The lowercase superscripts on the same row indicated there is the significantly different (P<0.05) for each body size of male and female chickens.

The results of this study indicated that the average body size of male SeA chickens was significantly greater (P<0.05) than that of female SeA chickens. These findings align with those of Sari and Wiyanto (2021), who reported that the average body size of 4-month-old male Merawang chickens was also significantly higher (P<0.05) than that of females. This difference in body size suggests that male chickens exhibit more significant frame size growth than females. Sahrani *et al*. (2022), noted that larger body sizes in livestock correspond to increased body weight. Additionally, Lukmanudin *et al*. (2018) mentioned that diversity in livestock can arise from genetic variation, which include additive genes, predominant genes, and epistatic genes that may influence.

T^2-Hotelling Analysis and Principal Components of Body Sizes of Sentul Chicken and Arab Chicken (SeA Chicken)

The T^2-Hotelling analysis was conducted simultaneously to compare the body sizes of male and female SeA chickens. Principal Component Analysis (PCA) was employed to identify the

characteristics of body shape and size (Sari and Wiyanto, 2021). The results of the T^2-Hotelling analysis in this study indicated that the body sizes of male SeA chickens were significantly different ($P<0.01$) from those of female SeA chickens.

The similarity of body size measurements for male and female SeA chickens showing a total diversity of 78% and 80%, with eigenvalues of 15.72 and 16.01 (Table 5). This percentage represents the most significant proportion of diversity among the primary components that influence body size. The chest circumference (CC) is the highest eigenvector in the body size equation for male and female SeA chickens. CC is a crucial characteristic of body size, as it contributes significantly to the similarity in body size and should be considered when selecting SeA chickens.

Table 5. Similarities in body size and body shape with total diversity and eigenvectors of male and female SeA chickens

Gender	Equation	TD (%)	Λ
Male	Body Size = 0.23 BL+0.241 BW+0.239 TK+0.243 HL+0.233 CW+0.234 CL+0.199 TL+0.203 SL+ 0.241 TFL+0.239 PW+0.135 HC+0.245 NC+0.213 NL+0.205 UBL+0.199 UWL+0.246 CC+ 0.175 LBL+0.241 LiTi+0.238 SC+0.239 BH	78	15.72
	Body Shape =0.154 BL+0.128 BW+0.172 TK+0.155 HL+0.211 CW+0.173 CL–0.357 TL+0.258 SL+ 0.157 TFL+0.048 PW–0.519 HC+0.038 NC–0.189 NL–0.316 UBL+0.161 UWL+0.098 CC– 0.323 LBL+0.13 LiTi+0.184 SC–0.122 BH	13	2.59
Female	Body Size = 0.24 BL+0.24 BW+0.231 TK+0.242 HL+0.227 CW+0.218 CL+0.215 TL+0.234 SL+ 0.24 TFL+0.238 PW+0.091 HC+0.235 NC+0.203 NL+0.211 UBL+0.239 UWL+0.245 CC+ 0.166 LBL + 0.234 LiTi+0.241 SC+0.227 BH	80	16.01
	Body Shape = 0.151 BL+0.142 BW+0.139 TK+0.129 HL+0.138 CW–0.154 CL–0.302 TL+0.155 SL+ 0.129 TFL+0.116 PW–0.614 HC+0.148 NC–0.359 NL–0.199 UBL–0.171 UWL–0.025 CC– 0.304 LBL+ 0.137 LiTi+0.153 SC+0.013 BH	10	2.18

Description: BL= beak length, BW= beak width, TK= head height, HL= head length, CW= chest width, CL= chest length, TL= tibia length, SL= shank length, TFL= third finger length, PW= pubis width, HC= head circumference, NC= neck circumference, NL= neck length, UBL= upper body length, UWL= upper wing length, CC= chest circumference, LBL= lower body length, LiTi= tibia circumference, SC= shank circumference, BH= body height

These findings align with those of Wahyuni *et al.* (2022), which indicated that chest circumference is also the highest eigenvector in the body size equations for Sentul and Kampung chickens. The observed similarity in body size can be attributed to the relatively uniform environmental conditions at the research location. Puteri *et al*. (2020), state that environmental factors or topography significantly influence body size similarity.

The similarity in body shape between male and female SeA chickens at 4 months of age shows total diversity values of 13% and 10%, respectively, with eigenvalues of 2.59 and 2.18. These values represent the highest proportion of diversity among the primary components analyzed. The shank length (SL) emerged as the highest eigenvector in assessing the body shape similarity for male and female SeA chickens, indicating that SL significantly contributes to the similarity in body shape. These findings contrast with those of Wati *et al.* (2023), who reported that in male Kampung chickens, the similarity in body shape is determined by back length, while in female Kampung chickens, it is defined by chest length. The observed differences in body shape similarity can be attributed to genetic variations and chicken strains. According to Irmaya *et al*. (2021), genetic factors can lead to differences in body size and shape in livestock.

Genotype and Allele Frequency

The GH gene was successfully amplified by PCR at a size of 676 bp (Figure 1). The diversity of the SeA chicken GH gene was analyzed using the Taq1 restriction enzyme, which recognizes the TCG↓A cutting site. The restriction analysis revealed three genotypes: +/+, +/-, and -/-. The results showed one band at 676 bp, two at 288 bp and 388 bp, and three at 676 bp, 388 bp, and 288 bp (Figure 2). Specific restriction enzymes can provide insights into the diversity of a DNA fragment, as indicated by variations in band positions and the number of bands produced due to the action of particular restriction enzymes (Pratama *et al*., 2023).

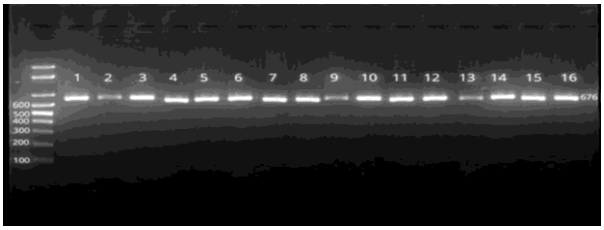

Figure 1. PCR amplification results of GH genes in Sea Chicken

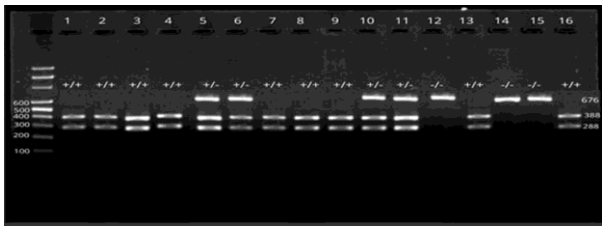

Figure 2. The PCR-RFLP results of the GH Gene Polymorphism in SeA chicken

The genotype frequencies of the GH gene in SeA chickens, specifically the +/+, +/, and -/- genotypes, which are 0.43, 0.35, and 0.22, respectively. The frequency of the +/+ genotype is higher than that of the +/- and -/- genotypes, with allele frequencies of + (61%) and - (39%) (Table 6). These findings suggest that the GH gene in SeA chickens is polymorphic. According to Tinda *et al.* (2022), an allele is considered polymorphic if its frequency is less than 0.95. A population can be classified as polymorphic if it contains more than one allele at a given locus and if at least one of the alleles has a frequency of less than 99% (Allendorf, 2017).

Table 6. Genotype frequency and allele frequency of GH Gene in SeA chickens

Strain locus	N	Genotype	Genotype frequency	Alel	Allele frequency
SeA Chickens GH|Taq1	60	+/+	0.43	+	61%
		+/-	0.35		
		-/-	0.22	-	39%

Hardy-Weinberg Equilibrium (HWE)

The Chi-Square test analysis showed that X^2 value (3.44) is smaller than the critical X^2 value of 0.05 (3.84) (Table 7). This result indicated that the SeA chickens are in Hardy-Weinberg equilibrium ($P<0.05$). Our findings are consistent with those of Pratama *et al.* (2023), who reported that Sentul chickens at the GH/Msp1 locus also exhibit Hardy-Weinberg equilibrium, demonstrating that genotype and allele frequencies remain stable across generations.

Table 7. Hardy-Weinberg Equilibrium (HWE) test analysis

Strain	N		Genotype			Allele Frequency		X^2 Test
			(+/+)	(+/-)	(-/-)	(+)	(-)	
SeA Chickens	60	O	26	21	13	0.61	0.39	3.44
		E	22.33	28.55	9.13			

Description: O= observation, E= experiment

The HWE was used to see the balance of genotypes in the samples we observed. Allendorf (2017) noted that a population is in equilibrium if the calculated X^2 value is lower than the critical X^2 value. The equilibrium of a livestock population can be affected by an irregular mating system, allowing male livestock to mate with the same female repeatedly (Al-Sobri *et al.*, 2022). Suppose there is a discrepancy in the frequencies of genotypes and alleles within a population. In that case, it may result from factors such as genotype accumulation, population subdivision, mutation, selection, migration, or inbreeding (Rahmat *et al.*, 2022).

Heterozygosity

Table 8 presents the estimation of heterozygosity values for SeA chickens, including the observed heterozygosity (H_o) and expected heterozygosity (H_e) for the GH|Taq1 gene. The heterozygosity values for the GH|Taq1 gene in SeA chickens are $H_o = 0.35$ and $H_e = 0.48$. The observed heterozygosity (H_o) is lower than the expected heterozygosity (H_e), with H_o (0.35) < H_e (0.48). This lower observed heterozygosity compared to the expected value indicates a relatively moderate level of diversity in the GH gene among SeA chickens. Heterozygosity is a measure of genetic diversity within a population; a higher H_o value signifies greater diversity, while a lower H_o value indicates reduced diversity (Saifullah *et al.*, 2023). Factors such as sample size, number of alleles, and allele frequencies can also influence heterozygosity (Pratama *et al.*, 2023).

Table 8. Heterozygosity value of SeA chickens

Strain	N	PCR-RFLP identifier	Heterozygosity	
			H_O	H_E
SeA Chickens	60	*Growth Hormon|Taq1*	0.35	0.48

Polymorphic Information Content (PIC)

Genetic diversity can be assessed using heterozygosity and polymorphic information content (PIC) values. The PIC value evaluates the level of genetic information and the presence of polymorphic alleles. Heterozygosity is directly proportional to the PIC value, essentially a corrected measure of heterozygosity.

Tabel 9. Polymorphic Information Content (PIC)

Strain	N	PIC	Class
SeA Chickens	60	0.42	Middle

The PIC value is utilized as a standard for evaluating genetic markers based on PCR-amplified DNA bands and is categorized into three classes: PIC > 0.5 is considered very informative, 0.25 < PIC < 0.5 is moderate, and PIC < 0.25 is low (Al-Sobri *et al.*, 2022). The PIC serves as

a parameter indicating the informativeness of a marker. According to Table 9, the PIC value for the GH gene in SeA chickens is 0.42, placing it in the medium category. It suggests the primer is informative as a GH|Taq1 gene fragment marker. These findings are consistent with those of Alfano *et al*. (2023), who reported a PIC value of 0.38 for the GH gene in KUB chickens. Terryana *et al*. (2017) noted that a high PIC value reflects a marker or trait with significant genetic diversity.

Association of Growth Hormone Gene with Performance of Sentul Chicken and Arab Chicken Crossbreeding Results (SeA Chicken)

The association of GH gene polymorphism with SeA chicken performance was carried out to determine the variation of the GH gene genotypes that have the highest influence on chicken growth traits. The chicken performances, including average body weight at 4 months of age, weight gain at 3-4 months of age, body size, and body shape characteristics of the GH gene, were grouped based on the GH genotype. The association of the GH gene polymorphism with chicken performance can be seen in Table 10.

Table 10 indicates that the average body weight at 4 months of age, body weight gain from 3 to 4 months, chest circumference (CC), and shank length (SL) at 4 months are greater in SeA chickens with the (+/+) genotype compared to those with the (+/-) and (-/-) genotypes. It suggests that the analysis of average differences in the SeA chicken GH gene, using PCR-RFLP, shows that the (+/+) genotype is significantly higher (P<0.05) than the (+/-) genotype and the (+/-) genotype is also significantly higher (P<0.05) than the (-/-) genotype. It implies that the SeA chicken GH gene with the (+/+) genotype is more strongly associated with body weight, weight gain, and body measurements than those with the (+/-) and (-/-) genotypes. These findings are consistent with those of Alfano et al. (2023), who reported that KUB chickens with the (+/+) genotype exhibit higher average body weight, weight gain, and body size compared to those with the (+/-) and (-/-) genotypes. Additionally, the GH gene with the (+/+) genotype demonstrates superior quantitative traits compared to GH genes with other genotypes (Salsabila et al., 2022). The physiological effects of growth hormone are initiated by binding to the transmembrane GH receptor and activating JAK2, an intracellular protein tyrosine kinase linked to the receptor (2). This sets off a sequence of protein phosphorylation cascades that activate multiple transcription factors, such as AP-1 and Stats1, 3, 5a, and 5b and ultimately result in growth performance (Rotwein and Chia 2010).

Table 10. The association of GH gene polymorphism with the average body weight at 4 months old, body weight gain at 3-4 months old, and body size at 4 months old of SeA chicken

Description	Genotype		
	+/+	+/-	-/-
Body Weight at 4 Months			
Male	1229.71±34.74^{a}	1165.44±24.04^{b}	1065.92±41.56^{c}
Female	1177.07±31.34^{a}	1077.45±25.50^{b}	977.23±41.54^{c}
Combined	1205.41±42.14^{a}	1115.16±50.79^{b}	1024.99±60.83^{c}
Body Weight Gain at 3-4 months			
Male	181.32±24.57^{a}	162.07±16.47^{b}	147.10±5.85^{c}
Female	171.59±11.81^{a}	144.12±20.85^{b}	125.36±5.72^{c}
Combined	176.83±19.99^{a}	151.81±20.75^{b}	137.06±12.56^{c}
Body Size			
Chest circumference	227.16±12.73^{a}	213.71±11.61^{b}	206.56±9.13^{c}
Shank length	78.72±4.59^{a}	73.64±5.38^{b}	71.61±2.91^{c}

Note: The lowercase superscripts on the same row indicated there is the significantly different (P<0.05).

CONCLUSION

Based on the research results, it can be concluded that male SeA chickens exhibit higher body performance than females. The body size characteristics of male and female SeA chickens include chest circumference and shank length, critical shape determinants. The Growth Hormone|Taq1 gene polymorphism in SeA chickens is polymorphic and had an association with body performances, including body weight, weight gain, and body measurements. The genotype +/+ of the GH gene polymorphism had a higher value of the body performances of SeA chicken. The GH gene could be a potential gene marker for selecting SeA chicken with the best performance.

CONFLICT OF INTEREST

The authors declared that all the material presented in this manuscript has no conflict of interest regarding any financial issues.

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