

## **Phenotypic Performance and The Characterization of Growth Hormone (GH|AluI) in Bangkok Chicken Breed**

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**ABSTRACT:** This study aimed to determine Bangkok chicken's phenotypic characteristics and growth hormone genes using the PCR-RFLP method. The samples used were 50 Bangkok chickens. Research in the field is taking phenotypic traits data. Research in the laboratory is carried out by DNA isolation, amplification, and restriction. This study showed that DOC weight, body weight, and bodyweight gain of DOC-1 month, 1-2 months, 2-3 months of Bangkok chickens were significantly different ( $P < 0.05$ ) higher than female Bangkok chickens. The results of the molecular analysis of DNA isolation were precise, then the amplification of the GH gene fragment with a product length of 997 bp and restriction obtained a ++ genotype. Allele frequencies and genotypes obtained in the Bangkok chicken GH|AluI genes are monomorphic. Conclusion: the phenotypic characteristics of the average body weight, the body weight gain of male Bangkok chicken was higher than that of female. Allele frequencies and genotypes of Bangkok chicken are monomorphic.

**Keywords:** Bangkok Breed, Phenotypic, PCR-RFLP, Growth Hormone

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## INTRODUCTION

Local chickens have the potential to be developed in order to meet protein needs of animal origin (Utama *et al.*, 2022). Local chicken is one of the livestock that has contributed to fulfilling the protein needs of animal origin for the people of Indonesia. The need for chicken meat every year has increased because the price is affordable for all people (Umam *et al.*, 2014).- Local chicken has several advantages, including resistance to the diseases, easily adapted to the various environmental conditions, easily maintained, delicious and savory taste, and high selling price (Nuraini *et al.*, 2018). However, local chicken breeds have disadvantages such as low productivity.

Bangkok chicken is one of the local chicken domestications of four wild species in Indonesia (FAO, 2008).-Bangkok breed has a diverse product characteristic due to the long adaptation in Indonesia. Bangkok chickens as superior breeds, both as laying hens and broilers, as well as fighting cocks (Sitanggang *et al.*, 2016). The characteristics diversity of Bangkok breed can further be exploited as the basis for selection to increase productivity and for conservation purpose. The body weight of Bangkok chickens aged DOC, 1, 2, and 3 months at the same age was higher than that of KUB, Sentul, Kampung and Merawang chickens. (Depison *et al.*, 2022)

Selection to increase productivity needs character information with economic value including body weight, body weight gain, and body measurements. Characterization is an activity to determine livestock performance which can be used for selection considerations. However, this conventional selection has drawbacks, such as taking a long time to get the result. Recently, molecular characterization showed an important role with a quick and effective process to characterize genetic diversity.

Characterization of genetic diversity related to production traits that have economic value, such as growth, can be carried out by in-depth analysis of structural

genes or other parts that play an important role in livestock growth. One of the genes that are important and determinant in controlling growth traits is the Growth Hormone (GH) gene. One of the characterization and identification of GH genes can be done by using the molecular identifier of the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The restriction enzyme used to analyze DNA results from the PCR-RFLP method is AluI. The restriction enzyme AluI was also used to determine the association of GH gene diversity with growth traits and to determine the morphometric characteristics of Kerinci ducks (Salsabila *et al.*, 2022).

PCR is a technique to replicate DNA segments. This process will produce a large number of copies from a small sample. PCR technique is more widely used due to the simplicity and high success rate of obtained DNA sequence amplification. PCR-RFLP is a technique that can detect codominant traits or distinguish between homozygous and heterozygous. PCR-RFLP has been widely used to obtain genetic population descriptions and accelerate the characterization of high economic value traits such as growth. This study aims to obtain information about performance diversity and Growth Hormone (GH) genes in free-range chickens so that later it can be used as a reference in selecting and increasing the productivity of native chickens in the future.

## MATERIALS AND METHODS

We analyzed 50 samples of Bangkok chicken blood and body weight data. Each chicken has a name tag on its wing. Every month the body weight and weight gain of male and female Bangkok chickens are measured. Data recorded includes DOC weight up to 3 months and weight gain for DOC-1 month, 1-2 months and 2-3 months using digital scales. The formula for body weight gain (g) is body weight II-body weight I. Laboratory materials including 70% alcohol, DNA Purification Extraction Kit (Promega - USA), agarose powder, TBE

Buffer solution, distilled water, ethidium bromide (EtBr) staining, loading dye, DNA ladder, primer, nucleus free water, Gotaq Green Mastermix, and Thermoscientific brand *AluI* restriction enzyme were used in this study. We used several equipments in this study including hand glove, centrifuge, 1000 µl, 200 µl, 100 µl, 20 µl micropipette, Eppendorf tip pipette, microtube rack, tube axygen 0.2 ml, 1.5 ml, and 2 ml, vortex, analytical balance, Erlenmeyer, measuring cup, gel doc, electrophoresis power supply, electrophoretic gel system, gel printer, well comb, electric heater, water bath, freezer, mini spin centrifuge, and PCR machine.

This genetic characterization consists of several stages, including DNA extraction, DNA qualification, and PCR-RFLP. We used genomic DNA Purification Kit protocol from Promega for the DNA extraction. At the same time, DNA qualification was carried out by electrophoresis using 1.5% agarose gel stained with ethidium bromide (EtBr), with a voltage of 200 volts for 60 minutes. Gel doc visualized the electrophoresis results and showed the thickness of the DNA band subjectively. Gene candidate amplification using primer pairs was shown in Table 1. below:

**Table 1.** Length, location and sequence of primary pairs

| Locus | Length (bp) | Location | Sequence                                                                                    | Gen Bank |
|-------|-------------|----------|---------------------------------------------------------------------------------------------|----------|
| GHD02 | 996         | Exon 2   | F: 5'GTT CCC AGT CCT CAC CCA C <sup>3</sup><br>R: 5'CAG TGC TGT GTT TAC CCA GG <sup>3</sup> | AY461843 |

Amplification was conducted using Gotaq PCR mix with a total volume of 20 l consist of 3 l Forward and Reverse primers, 2 l genomic DNA, 10 l Nuclease free water/DDW (double distillation water), and 15 µl PCR mix inserted into a microtube tube PCR of 0.2 ml. The amplification process was carried out according to the PCR steps in Table 2.

The amplification results were prepared by electrophoresis using 1.5% agarose gel with ethidium bromide (EtBr) staining 200 volts for 60 minutes, then we used UV light from gel doc to visualize the

result. The amplified band was cut with the *AluI* restriction enzyme from Thermoscientific brand according to the gene locus with the following composition of 10 l diluted enzyme with 10 l of PCR product DNA.

Electrophoresis was using 2% agarose gel stained with ethidium bromide (EtBr) and the electrophoresis took 120 minutes at 100 volts and then visualized with the help of UV light using a gel doc. DNA Ladder determined the genotype identification of each sample based on the size and pattern of 100 bp.

**Table 2.** Optimal temperature, time and cycle of PCR stages

| Stages                        | Growth Hormone Gene |                            |       |
|-------------------------------|---------------------|----------------------------|-------|
|                               | Temperature (°C)    | Time (hour:minutes:second) | Cycle |
| Pre – denaturation            | 95                  | 00:05:00                   | 1x    |
| Denaturation                  | 95                  | 00:00:45                   |       |
| Annealing                     | 60                  | 00:00:45                   | 35x   |
| Extention                     | 72                  | 00:01:00                   |       |
| Final extention               | 72                  | 00:05:00                   | 1x    |
| Enzym restriction <i>AluI</i> | 37                  | 04:00:00                   |       |

## Data Analysis

### Genotype and allele frequency

Genotype frequency is the proportion or percentage of a particular genotype in a

population, calculated based on the number of genotypes divided by the number of samples.

$$F = \frac{\sum X_i}{N}$$

*Description :*

F = Genotype frequency

$x_i$  = Observed genotypes

N = Total population

The allele frequency is the proportion of a particular allele in a population compared to all alleles occupying the same

locus from the PCR-RFLP characterization analysis. We analyzed allele frequency using formula from Nei and Kumar (2000):

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

*Description :*

$x_i$  = The allele frequency of the samples,

$n_{ii}$  = Sample genotype ii,

$n_{ij}$  = Sample genotype ij,

N = Total sample.

**t – test**

We performed t-test to show the difference in average body weight and

weight gain of Bangkok chickens breed with the following formula from Mendenhall (1987).

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sum (X_{j1} - \bar{X}_1)^2}{n_1(n_1-1)} + \frac{\sum (X_{j2} - \bar{X}_2)^2}{n_2(n_2-1)}}}$$

*Description :*

t = t - value

$\bar{X}_1$  = sample mean in the first group,

$\bar{X}_2$  = sample mean in the second group,

$X_{j1}$  = the value of the j-th observation in the first group,

$X_{j2}$  = the value of the j-th observation in the second group,

$n_1$  = number of samples in the first group, and

$n_2$  = number of samples in the second group.

## RESULT AND DISCUSSION

### Average body weight of male and female Bangkok chickens

The average body weight of male and female Bangkok chickens aged DOC (*Day old chicken*), one month, two months, and three months are presented in table 3.

Table 3 showed the average body weight for male and female chickens at one month, two months, and three months. Overall, bodyweight of male Bangkok

chickens from DOC - 3 months was significantly different ( $P < 0.05$ ) higher than female. The difference in body weight between male and female chickens is due to the differences in growth hormones, growth hormone in male chickens controls higher production characteristics than female chickens (Pagala *et al.*, 2019).

This research is compared with Puteri *et al.* (2020), which states that the body weight of local chickens starting from the

age of DOC-3 months in the KUB chicken is 34.2 g, 235.64 g, 713.15 g and 1108,42 g, in the Sentul chicken is 33.85 g, 217.06 g, 632.88 g and 1021.44 g, meanwhile in Arab chicken is 33.45 g, 210.5 g, 591.2 g and 874,57 g. The average body weight gain

from this study was higher than that from Depison et al. (2022) which stated that the body weight gain for DOC-3 months old KUB chickens was 1.1116.63g Sentul chickens 1077.58g, Kampung chickens 1052.48g and Merawang chickens 961.95g.

**Table 3.** Average body weight of male and female Bangkok chickens

| Age     | Sex                        |                            |
|---------|----------------------------|----------------------------|
|         | Male                       | Female                     |
| DOC     | 39.92±2.52 <sup>a</sup>    | 37.102±1.85 <sup>b</sup>   |
| 1 Month | 414.22±38.24 <sup>a</sup>  | 363.7±25.36 <sup>b</sup>   |
| 2 Month | 856.14±68.69 <sup>a</sup>  | 770.58±20.26 <sup>b</sup>  |
| 3 Month | 1369.41±92.69 <sup>a</sup> | 1212.03±38.27 <sup>b</sup> |

Note: Different superscripts in the same line for each sex of chickens are significantly different ( $P < 0.05$ )

### Bodyweight Gain of male and female Bangkok chickens

Bodyweight gain of male and female Bangkok breed for DOC to one month, one month to two months, and two month to three months are presented in Table 4.

Table 4. shows that the average body weight gain of male Bangkok chickens for DOC-1 month, 1-2 months, and 2-3 months were 374.30±36.16g, 441.91±36.98g, 513.27±53.00g, respectively.

The average body weight gain of female Bangkok chickens starting from the age of DOC-1 month, 1-2 months, and 2-3 months were 326.60±24.49g, 406.88±27.39g, 441.45±40.27g. This research is different from Rahayu *et al.*, (2021), which states that the body weight gain of Bangkok chickens starting from the age of DOC-3 months is 334.21 g, 415.57 g and 456.88 g. This difference is presumably due to genetic differences in the chickens used. Arianto *et al.* (2019) state that several factors influence livestock growth, including genetic and environmental factors. These results indicate that this study is better than previous studies, namely Abdu *et al.* (2021) on sentul chickens, Prawira *et al.* (2021) on free-range chicken, Sari *et al.* (2021) on Merawang chickens and Utama *et al.* (2022) on KUB chickens.

The average body weight gain of male Bangkok chickens starting from the age of

DOC - 3 months was higher and significantly different ( $P < 0.05$ ) than female Bangkok chickens. The different hormone profile between male and female Bangkok chickens leads to the difference in the growth performance between male and female. Rahayu *et al.* (2014) showed that testosterone in roosters can stimulate an increase in growth hormone secretion.

The results of the average difference test (t-test) showed that the body weight gain at the age of 2-3 months was significantly more ( $P < 0.05$ ) higher than the body weight gain at the age of DOC-1 month and 1-2 months as well as weight gain. 1-2 months old body weight was significantly different ( $P < 0.05$ ) higher than the weight gain of DOC-1 months of age for both males and females. These results indicate that the highest average body weight gain is achieved at 2-3 months for both males and females.

This difference is thought to be due to genetic differences and external factors such as feed that affect Bangkok chicken body weight gain. Following the opinion of Sari *et al.* (2021), which states that differences in body weight gain occur due to genetic factors, maintenance management, and feed. Sitanggang *et al.* (2016) Genetic and environmental factors have a close relationship to express individual genetic capacity perfectly.

**Table 4.** Bodyweight gain of male and female Bangkok breed

| Sex    | DOC-1 Month                | 1-2 Month                  | 2-3 Month                  |
|--------|----------------------------|----------------------------|----------------------------|
| Male   | 374.30±36.16 <sup>cA</sup> | 441.91±36.98 <sup>bA</sup> | 513.27±53.00 <sup>aA</sup> |
| Female | 326.60±24.49 <sup>cB</sup> | 406.88±27.39 <sup>bB</sup> | 441.45±40.27 <sup>aB</sup> |

Note: Different lowercase superscripts in the same line for each month of chickens are significantly different ( $P < 0.05$ ); different superscripts of capital letters in the same column for each month of chickens are significantly different ( $P < 0.05$ )

### DNA Extraction

The results of DNA extraction of blood samples using the DNA Purification Kit protocol from Promega were then electrophoresed using 1.5% agarose and visualized with UV light. Figure 1 showed

the results of DNA Extraction. Sophian (2021) states that DNA lysis must be processed to carry out molecular analysis to remove the genetic material. We can do this step with the chemical and enzymatic processes.



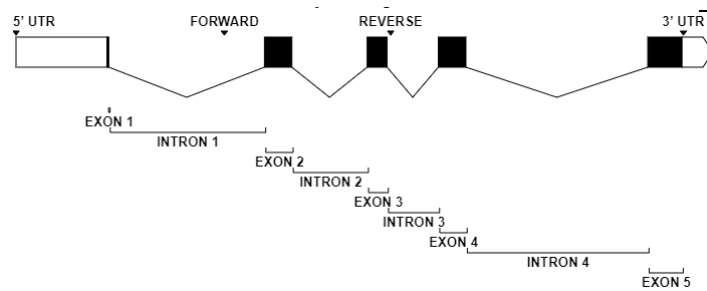
**Figure 1.** Electrophoresis of total DNA extracted from DNA using the DNA Purification Kit protocol from Promega

Figure 1. shows that the results of the electrophoresis of Bangkok chicken DNA isolation are precise. The DNA extraction stage was carried out correctly and according to the recommended work procedure based on the DNA Purification Kit protocol from Promega. Arianti and Sister (2018) DNA isolation is a series of processes to separate DNA from other cell components. DNA isolation is the most important initial stage in molecular research. The success in isolating DNA is determined by the suitability between the results of DNA extraction and the amount of DNA solution added to the effects of band electrophoresis, which is visible, which indicates if there is DNA. Hidayati *et al.* (2016) state that the thick and bright bands

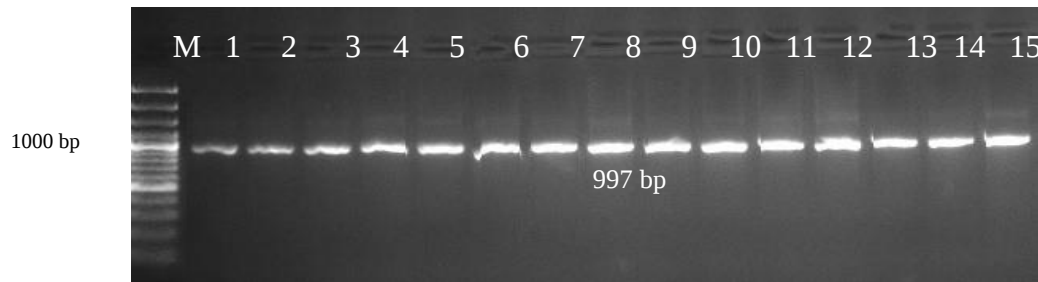
qualitatively indicate a high concentration of DNA isolation results, while the thin bands indicate a small concentration of DNA produced. Nova *et al.* (2016) state that DNA purification is a process to separate DNA from cell lysates (proteins, carbohydrates, lipids) and other contaminants. Gene Structure of Bangkok Chicken Growth Hormone (GH) Genbank: AY461843 in Figure 2.

### Amplification of Growth Hormone (GH) Gene Fragment in Bangkok Chicken

The product of amplification of the Growth Hormone (GH) gene fragment obtained has a length of 997 bp. The amplification results were electrophoresis using 1.5% agarose visualized under UV light on gel doc presented in Figure 3.



**Figure 2.** Gene Structure of Bangkok Chicken Growth Hormone (GH) Genbank: AY461843



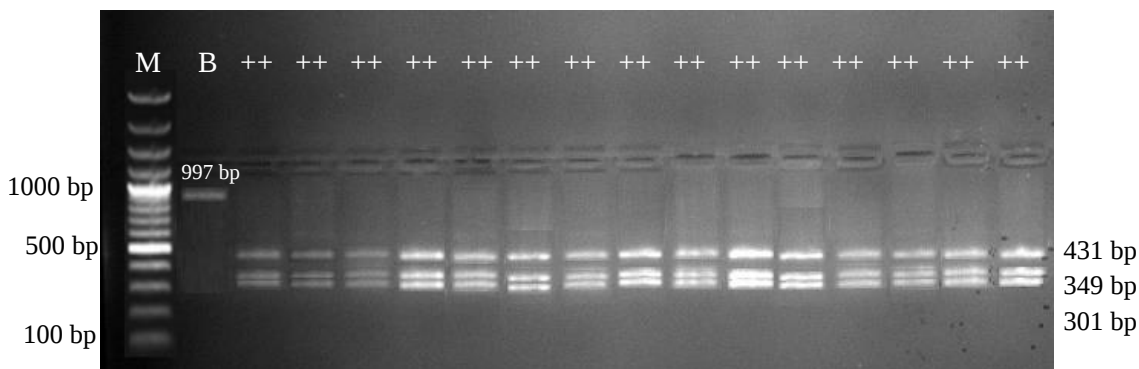
**Figure 3.** Electrophoresis of Growth Hormone (GH) PCR products

Figure 3 shows that the Growth Hormone (GH) gene in Bangkok chicken was amplified with a product length of 997 bp. GH gene amplification was successful at an annealing temperature of 60°C for 45 seconds. It was successful for amplification in Bangkok chicken genes with the myostatin gene (Rahmat *et al.*, 2022). The choice of temperature in the PCR process is very important because the temperature is the most critical factor in determining the success of a PCR, especially the annealing temperature (Wijaya *et al.*, 2018). The annealing temperature also depends on the

base composition, length, and primer concentration. According to Hidayati *et al.* (2016), the annealing temperature is the temperature at which the primer will attach to the DNA template. The melting temperature ( $T_m$ ) can be calculate by the annealing temperature based on each primer. The search for optimal conditions of the annealing temperature is critical.

#### Growth Hormone Gene Restriction Results

Bangkok chicken growth hormone gene identified using *AluI* cutting enzyme with AG↓CT cutting site are in figure 4.



**Figure 4.** Visualization of GH/*AluI*. RFLP results

**Table 5.** Genotype frequency and Allele frequency of Bangkok chicken

| Gene    | n  | Allele | Genotype | Genotype frequency | Allele frequency |
|---------|----|--------|----------|--------------------|------------------|
| GH AluI | 50 | +      | ++ = 50  | ++ = 100 %         | +                |
|         |    | = 50   | + - = 0  | + - = 0 %          | = 100 %          |
|         |    | - = 0  | -- = 0   | -- = 0 %           | - = 0 %          |

The characterization of the Bangkok chicken growth hormone gene using the AluI cutting enzyme obtained five cut points of 301 bp, 130 bp, 69 bp, 80 bp, and 349 bp. Based on Figure 6. The GH|AluI fragment cutting results resulted in three strips of bands, namely 431 bp, 349 bp, and 301 bp. The number of bands that should be obtained is six bands, namely 301 bp, 130 bp, 69 bp, 80 bp, 349 bp, and 68 bp. It happens because some of the intersection points are too small or too short. Spetiawan *et al.* (2016) The use of restriction enzymes is expected to produce a DNA restriction pattern (cutting) to determine the differences between the individuals used. If there is a cutting site, the restriction enzyme will cut the DNA at a known site so that the DNA sequence separates into DNA bands.

Table 5 shows the genotype frequency of the GH|Alu1 gene in Bangkok chickens. There is only one genotype and one allele, ++ and +. The allele frequency of + allele is one. Nei (1987) states that an allele is monomorphic if it has an allele frequency equal to or less than 0.99 (99%). The results of this study have similarities with research by Sumantri *et al.* (2010), which states that the GH|AluI locus is monomorphic. The number of samples is relatively small, and the samples only come from one population, so that diversity is not found (Hidayati *et al.*, 2016). Agung *et al.* (2017) state that low genetic diversity can occur in a livestock group due to the selection process and the lack of new male introductions in a population.

## CONCLUSION

The conclusion in this study is the phenotypic traits of the average body weight, and the body weight gain of male Bangkok chickens is higher than females.

Allele frequencies and genotypes of Bangkok chicken are monomorphic.

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