Identification of Quantitative Characteristic and Association Between ACTA-1 Gene and Body Weight in Local Chicken

A S Andini¹, Ismoyowati² and D Purwantini²

¹Postgraduate Master Program of Animal Science, Jenderal Soedirman University, Purwokerto, Indonesia
²Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, Indonesia

E-mail: anissriandini997@gmail.com

Abstract. This study aims to identify the quantitative characteristics of local chickens and examine the presence of polymorphisms based on the nucleotide sequences of ACTA-1 genes. The material used is a local chicken consisting of 25 Pelung and 25 Native chickens. The quantitative data uses t test. Identification ACTA-1 gene polymorphism is carried out by PCR method and Sequencing of PCR product. The quantitative characters, of male Pelung and Native chickens significantly different, involving the length of tarsometatarsus, tarsometatarsus circumference, comb height and body weight. Meanwhile, female Pelung and Native chickens show significant differences in femur length, tibia length, tarsometatarsus length, tarsometatarsus circumference, third finger length, wing length, comb height and body weight. The sequencing result indicates the presence of SNPs (Single Nucleotide Polymorphism) among them c.584 T > G, c.585 T> A, and c.657 T> C. Furthermore, in the base c.657 T> C the heterozygosity value of 0.18. Based on correlation value at c. 585 T>A shows that AA genotype has a significant effect on body weight (P<0.05). Therefore, the ACTA-1 gene is an important marker, which can be used to improve the economic characteristics found in local chickens.

Keywords: ACTA-1 gene, local chicken, polymorphism, quantitative traits, SNP

1. Introduction

Local chickens spread in Indonesia have different characteristics, including native chicken, Pelung chicken, Bekisar chicken, Ketawa chicken, and many others. The advantages of native chicken compared to other chickens are simple, easy maintenance and relatively low maintenance costs. Native chicken has body weight which is classified into two parts, namely light and medium type. Light type local chicken is a chicken that has a body weight of 1.5 kg as adults (above the age of 24 weeks), while medium type chicken has a body weight of 2.5 kg as adults [1].

Many Pelung Chicken is one of the genetic sources that have an advantage in the sound of a melodious chef, as well as a relatively large body size. The Pelung and Sentul chickens are intensively maintenance at the age of 20 weeks have a body weight of 2.20 kg and 1.60 kg, while the extensive maintenance of each chicken has a body weight of 1.60 kg and 1.10 kg. Observations regarding qualitative and quantitative characteristics are necessary to do. One of the most important quantitative...
characteristics is weight weighing. Body weight greatly affects the economic value of the livestock [2]. There are 12 Native Thai chickens types, which weight from 1 to 1.5 kg at 2.7 months [3].

The gene approach through the analysis of SNP (Single Nucleotide Polymorphisms) has been successfully performed to identify some DNA markers. The dominant SNP is inherited and can be used to study genetic diversity. The discovery of SNPs found in genes that affect the composition of fatty acids and gene expression is an important way of knowing the characteristics of a gene [4].

Some genes or more can affect body weight, one of which is the ACTA-1 gene (actin, alpha1, skeletal muscle). Actin is a major component of the microfilament system in eukaryotic cells, which plays an important role in the supra molecular regulation of other proteins [5]. The genetic relationship between the ACTA-1 gene and the inner organ has a significant influence. The cytoskeleton of the actin found in all living beings is a very important trait of a cell. Furthermore, the filament actin controls the overall shape of the cell and its ability to meet other cell substrates [6]. Skeletal muscle is an important source for livestock because it is influenced by the amount and muscle fiber. Embryo muscle fibers determine the amount of meat produced when a chicken becomes an adult [7]. The aim of the study is to identify quantitative characteristics, genetic diversity based on the ACTA-1 gene as well as to review the ACTA-1 gene relationship with the body weight in local chickens.

2. Methodology

Animals and Sampling
The material used is 50 local chickens, consisting of 25 Pelung chickens and 25 Native chickens. Quantitatively identified data that includes body weight, length of the femur, tibia length, tibia circumference, third finger length, wing length, height and chest length. Each livestock is taken by 1 ml of blood.

Primer design
The primary used is referring to the previous journal according to [8] i.e. primary forward-5’ ACTGGGACGAGATGGAGAAG3’ and reverse 5’TCCAGAGCCACATAGCACAG3.

Amplification DNA
The pre-denaturation stage is performed for 5 minutes at the temperature of 95°C, then the denaturation is done for 1 minute at the temperature of 95°C, and followed by annealing for 1 minute at a temperature of 62°C. Next stage is elongation where PCR reaction will be terminated, thermocycler conditions are maintained at the temperature of 72°C for 1 minute. The final stage of PCR is post-elongation, this stage is the reaction stage to enhance the DNA extension and performed for 7 minutes at a temperature of 72°C. The PCR reaction is repeated 35 cycles to achieve maximum results. The reaction of PCR (PCR product) is then electrophoresis on the 1.5% agarose gel and visualized using Ultra Violet rays [9].

Sequencing DNA
Sequencing method consists of several phases, those are sample preparation, cycle sequencing, purifying, and DNA sequencing. The results of readings by the sequencer machine are indicated by an electroforegram that is shaped like an up and down curve with different colors. The blue color indicates C base, red color indicates base T, black color indicates the base G, the green color of A wave, and purple or light blue indicates N (error).

Data analysis
The results of quantitative characteristic measurements are analyzed using the t test [10] with the formula

\[ t_{test} = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(N_1 - 1)Sd_1^2 + (N_2 - 1)Sd_2^2}{N_1 + N_2 - 2} \frac{N_1 + N_2}{\sqrt{N_1 * N_2}}}} \]

\( \bar{Y}_1 \) = Average phenotypic characteristics of Native chickens
\( \bar{Y}_1 \) = Average phenotypic characteristics of Pelung chickens
The sequencing results are processed using the Bioedit and Clustal Omega programs, after which the genes, genotypes and values of heterozygosity were searched [11, 12].

Formula:

\[ F_{An} = \frac{\sum_{\text{gen}} \text{ACTA1} A}{\sum_{\text{gen}} \text{ACTA1} A + \sum_{\text{gen}} \text{ACTA1} n} \]

\[ F_{An} \quad \text{= gene frequency } A \text{ on the loci to } n \]

Genetic variety is calculated using the individual formula heterozygosity (h) and the intensity of heterozygosity (H̅) [13, 14] is that:

\[ h = 1 - \sum q_i^2 \]

\[ H̅ = \frac{\sum n}{r} \]

3. Result and Discussion

The quantitative characteristics are observed in this study include femur length, tibia length, tarsometatarsus length, tarsometatarsus circumference, third finger length, wings length, comb height, sternum length, and body weight. Data retrieval is done in Cianjur area. Locally researched chickens include Pelung chicken and Native. The average morphometric in the local male chicken is found in Table 1.

| No | Morphometric         | Pelung chicken       | Native chicken        |
|----|----------------------|----------------------|-----------------------|
| 1  | Femur length (mm)    | 119.87 ± 11.90\(^b\) | 138.25 ± 12.72\(^a\) |
| 2  | Tibia length (mm)    | 162.31 ± 20.25\(^a\) | 159.00 ± 15.77\(^b\) |
| 3  | Tarsometatarsus length (mm) | 118.12 ± 7.15\(^a\) | 99.25 ± 10.44\(^b\) |
| 4  | Tarsometatarsus circumference (mm) | 64.94 ± 8.57\(^a\) | 52.00 ± 5.30\(^b\) |
| 5  | Third finger length (mm) | 77.84 ± 8.67\(^a\) | 71.00 ± 3.13\(^b\) |
| 6  | Wings length (mm)    | 197.46 ± 10.69\(^a\) | 187.50 ± 10.77\(^b\) |
| 7  | Comb height (mm)     | 69.21 ± 3.75\(^a\) | 32.5 ± 21.39\(^b\) |
| 8  | Sternum length (mm)  | 212.22 ± 22.06\(^a\) | 183.75 ± 24.43\(^b\) |
| 9  | Body weight (g)      | 3836.19 ± 518.03\(^a\) | 2258.75 ± 463.32\(^b\) |

Different superscripts between chicken types showed significant differences (P<0.05)

The result shows that the Pelung male has a relatively high body size than the Native male. The production of an individual or livestock is influenced by the genetic and environmental factors given. Differences in body size can be caused by hormones contained therein. Males have a testosterone hormone that causes male growth to be faster than females. Androgen hormones and testosterone can affect the rate of bone growth. Testosterone in males affects the metabolism of protein synthesis, resulting in increased body weight, making meat tissues larger as well as relatively high rates of bone growth [15]. The difference between the male and female body size is also found. Female body weight is lighter compared to the weight of the rooster. This can be due to the growth difference between a male and female in addition to sexual dimorphism [16].

The femur length of Native chicken is relatively larger than that of Pelung chicken. The femur length in Pelung male has a length of 119.87 ± 11.90 cm while in Native male it is 138.25 ± 12.72 cm. This difference in femur height can be caused by environmental factors and genetics. Bone size is relatively greater than body weight in local African chickens, this is due to crossing with other chicken [17]. The average morphometric of local female chickens are found in the Table 2.

According to the Table 2, indicating the body size in the female Pelung has a relatively higher value compared to the female chicken. This may be due to genetic and environmental factors. Genetic and environmental factors have a close relationship in expressing its genetic capacity. The larger the size of the body is produced, the bigger the size of the body is observed. Poultry growth expressions are regulated by two hormones namely growth hormone and triiodotironin (T3). The Growth hormone is synthesized directly by Somatotroph in the caudal lobe in the anterior pituitary [18].
Table 2. The average morphometric of local female chickens

| No | Morphometric                   | Pelung Chicken | Native Chicken |
|----|--------------------------------|----------------|----------------|
| 1  | Femur length (mm)              | 112 ± 6.51<sup>a</sup> | 91.41 ± 6.99<sup>b</sup> |
| 2  | Tibia length (mm)              | 135 ± 6.30<sup>a</sup> | 103.65 ± 12.01<sup>b</sup> |
| 3  | Tarsometatarsus length (mm)     | 96.5 ± 4.17<sup>a</sup> | 66.58 ± 6.01<sup>b</sup> |
| 4  | Tarsometatarsus circumference (mm) | 54 ± 3.71<sup>a</sup> | 38.10 ± 2.47<sup>b</sup> |
| 5  | Third Finger length (mm)        | 60 ± 1.72<sup>a</sup> | 49.87 ± 2.00<sup>b</sup> |
| 6  | Wings length (mm)              | 173.5 ± 6.83<sup>a</sup> | 148.45 ± 16.60<sup>b</sup> |
| 7  | Comb height (mm)               | 29.5 ± 4.37<sup>a</sup> | 16.06 ± 8.56<sup>b</sup> |
| 8  | Sternum length (mm)            | 165.5 ± 56.22<sup>a</sup> | 140.80 ± 7.33<sup>b</sup> |
| 9  | Body weight (g)                | 2360 ± 67.21<sup>a</sup> | 1332.22 ± 107.45<sup>b</sup> |

Different superscripts between chicken types showed significant differences (P<0.05)

Genetic diversity based on locus Gen ACTA-1. The results of sequencing show polymorphisms due to the mutation of thymine into guanine (584 T > G), mutation of thymine to adenine (585 T > A) as well as changes from thymine to Cytosine (657 T > C). Polymorphism in the ACTA-1 gene can be seen in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Polymorphism of ACTA-1 gene shown in SNPs (c. 584 T>G, c. 585 T>A, and c. 657 T>C). A= adenine, G=guanine, C= cytosine, T=thymine.

The basis for conducting the selection is calculated using Genotype frequency, gene frequency, and heterozygosity. In each base, different results are obtained. Calculation results on base pair 584 T > G have a Genotype frequency value of TG = 0.5, TT=0.25 and GG = 0.20, as well as the frequency values of the genes obtained, are T = 0.525, C = 0.5 and G = 0.475, with heterozigosity value is 0.498. The next point is known on the base 585 T > A has a Genotype frequency i.e. TA = 0.50, TT = 0.25 and AA = 0.25 and has a frequency value of genes i.e. T = 0.5, and A = 0.5 with heterozigosity value is 0.50. The frequency of genotype on a base 657 T > C produces two genotype of which are TT = 0.8 and TC = 0.2 with a frequency value of T = 0.9 and C= 0.10, with heterozigosity is 0.18. The highest genotypic frequency was obtained in TT observed in 306 chickens, besides that TT had a 10 times TMA increase compared to other genotypes. Alleles are categorized as polymorphism when the allele frequency does not exceed 0.99 [19]. The presence of polymorphism is important because it can be used in obtaining important economic value properties and can assist in selection programs based on genetic polymorphism [20].

Association Between ACTA-1 Gene with Body Weight on Local Chicken. The result of correlation value shows that the AA genotype has a significant on body weight (P>0.05), while the TA and TT
genotypes do not have a significant effect on body weight (Table 3). The polymorphic (marker) found in the ACTA-1 gene has a real relationship with body weight. Polymorphic which has a real influence on body weight or other traits can be used as a selection tool [20]. The average body weight is base pair point c.585 T>A show in Table 3.

**Table 3.** The average body weight is based pair point c. 585 T>A

| No | Genotype | Body Weight (gram) | Correlation |
|----|----------|--------------------|-------------|
| 1. | AA       | 2930.972 ± 859.84  |             |
| 2. | TA       | 2184.999 ± 992.34  | 0.46        |
| 3. | TT       | 2072.072 ± 466.69  |             |

Different letters show a significant effect (P>0.05)

Based on the results obtained c.585 T>A shows the highest correlation value that is 0.46, c. 584 T>G shows a correlation of 0.40 and c.657 T>C shows a correlation value of 0.09. In c.585 T>A has high heterozygosity value among the other base pairs, therefore c.585 T>A it is used as a reference to determine the relationship between body weight with ACTA-1 gene base on its genotype. The average body weight in BB genotypes are relatively higher compared to genotype AA, this indicates that the B allele has a positives effect on body weight [19]. Association between the ACTA-1 gene and body weight has a significant effect because of the high genetic correlation is that the frequency of G alleles could increase body weight. Therefore, the ACTA-1 gene is a very important marker, which can be used to improve the economic characteristic found in local chickens[8].

4. **Conclusion**

The results show that there are significant differences between quantitative characteristics in chicken Pelung chicken with Native chicken. The sequencing results indicate that there are three SNP (Single Nucleotide Polymorphism) identified in the study of C. 584 T > G, c. 585 T > A, and C. 657 T > C. Based on the research on ACTA-1 gene has a significant effect on body weight (P<0,05).

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