Relationship between of Insuline Like growth Factor-2 Gene and Growth Traits in Crosses of Indonesian Local Chicken

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Abstract. Kedu chicken is known as a laying hen, while Sentul and Kampung chicken are known as dual-purpose type chickens. The crossing of Sentul, Kedu and Kampung chicken were produced SKKedu and KeduSK chickens. One of gene that control growth is IGF-2 (Insuline-like Growth Factor 2). This study aims to analyze the diversity of IGF-2 genes and analyze association of genes with growth in SKKedu and KeduSK chickens. The samples used were 23 KeduSK chicken DNA and 17 SKKedu chicken DNA 12 weeks old. Growth measurements consist of body weight, body weight gain, feed consumption and feed conversion ratio. DNA extracted from the blood samples were analysed by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). The diversity of IGF-2 genes was analysed with X2 test. The results showed of IGF-2 gene in SKKedu and KeduSK chickens (CC, TC, dan TT). IGF-2 gene was polymorphic and was in a Hardy-Weinberg equilibrium state. The growth traits of SKKedu and KeduSK chickens based on differences in genotype on IGF-2 genes are almost the same, there were no differences in the growth traits. There was no correlation between IGF-2 genotype of SKKedu and KeduSK chicken with growth traits.

1. Introduction
Indonesia has many kinds of local chicken. Indonesia's local chicken have high adaptation ability and resistant to several disease habitually attacking layer chicken and broiler chicken. Generally, local chicken is raised extensive and semi-extensive as producers of meat and eggs. In 2016, the consumption of Kampung chicken meat is 0.626 kg per capita per year and consumption of Kampung chicken eggs is 3.564 egg per capita per year. This figure was ranked second after the meat consumption of layer chicken reached 5.110 kg per capita per year and consumption eggs at 99.796 eggs per capita per year [1].

Kedu chicken is known as a laying hen, while Sentul and Kampung chicken are known as dual-purpose type chickens. Crossing of Sentul, Kampung, and Kedu chicken produce SKKedu (Sentul, Kampung, Kedu) and KeduSK (Kedu, Sentul, Kampung) chicken. One effort that can be done to increase the productivity of local chickens is through genetic markers-based selection, especially on the nature of growth. One gene that controls growth is IGF-2 (Insuline-like Growth Factor 2). It is known
to affect growth and quality of meat so that the characteristics of the gene of growth in local chickens from the cross can be known. IGF-2 gene is a candidate gene for growth in livestock because it affects development and growth, which regulates somatic growth including muscles, bones, epithelium and fibroblast cells [2]. In an effort to increase the growth of local chickens can be done through selection and crossing. The crossing of local chickens is expected to increase productivity through crossing between clumps to get a high level of heterosis which can further improve genetic quality. Crossing between local chickens and exotic chickens can be a strategy to improve the growth performance of local chickens [3].

This study was conducted to analyze the diversity of IGF-2 genes and determine the relationship or association of genes to the growth characteristics of SKKedu and KeduSK chickens. This research aimed to analyze the diversity of IGF-2 genes and analyzed the relationship of diversity of these genes with growth in SKKedu and KeduSK chickens.

2. Materials and Methods

2.1 Materials
This research used 23 KeduSK chickens DNA and 17 DNA of SKKedu chickens 12 weeks old. The growth properties analyzed were body weight, increase in body weight, feed consumption and feed conversion.

2.2 Methods
The study consisted of blood sampling, DNA extraction, PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism), electrophoresis and data analysis. DNA extraction was carried out based on method of Sambrook [4]. The primers used were primary forward 5'-GCTGGGGACCCAATAGAACC-3' and reverse 5'-TCCCCAGGAGATCACAAATCG-3'.

The stages of PCR consisted of pre-denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation 95°C for 10 seconds, primary amplification 60°C for 20 seconds and extension 72°C for 30 seconds then the ending waspost-extension at 72°C for 5 minutes. PCR is carried out using a thermo cycler machine (Eppendorf® AG 22331). Mix PCR consisted of 0.3 μL primary, 6.2 μL DW (distillation water) and 7.3 μL GoTaq® Green Master Mix (Promega).

The PCR product was cut using the Nla III restriction enzyme with a cutting temperature of 37°C. The restriction mix consisted of 5 μL PCR products, 0.3 μL restriction enzymes, 0.7 μL 1 x buffer and 1 μL DW. The PCR-RFLP results were electrophoresed and visualized using UV transilluminator (AlphaImager® EP).

2.3 Data Analysis
The diversity of IGF-2 genes in SKKedu and KeduSK chickens was analyzed by the X² test. Analysis of the relationship between the diversity of IGF-2 genes and the growth properties of SKKedu and KeduSK chickens at the age of 12 weeks was carried out using the t test.

3. Result and Discussions
The partial fragment of the IGF-2 gene of 40 chickens was successfully amplified and showed 395 bp band. The lengths of PCR products were in good agreements with the reference sequences (GenBank accession number: AH005039.2). This genotyping identified two alleles (C and T) and three genotypes (CC, TC and TT). The T allele was indicated by 256 and 139 bp bands (restricted), while the C allele was suggested by a single 395 bp (unrestricted) (figure 1).
Figure 1. Visualization of the IGF-2/NlaIII gene genotyping in 2% agarose gel (M = marker; CC, TC, TT = genotype).

The frequency of the genotype and frequency of the IGF-2 gene is presented in table 1. The results of the analysis of the diversity of IGF-2 genes and Hardy-Weinberg equilibrium in SKKedu chickens and KeduSK 12 weeks old chicken were performed using the genotype frequency, also shown in table 1.

Table 1. Variation of genotype.

| Chicken  | N  | Genotype Frequency | Allele Frequency | X² |
|----------|----|--------------------|-----------------|----|
|          |    | CC (n)             | TC (n)          | TT (n) | T   | C   |
| KeduSK   | 23 | 0.17 (4)           | 0.61 (14)       | 0.22 (5) | 0.48 | 0.52 | 1.11(ns) |
| SKKedu   | 17 | 0.18 (3)           | 0.65 (11)       | 0.18 (3) | 0.50 | 0.50 | 1.47(ns) |

N: a number of individuals, ns: no significant value at α=0.05

The frequency of T and C alleles of IGF-2 gene in SKKedu chickens were 0.48 and 0.52 respectively, while those in KeduSK chickens were 0.50 and 0.50 respectively. It showed that in both SKKedu and KeduSK chicken species were polymorphic. Alleles are polymorphic if they have an allele frequency of less than 0.99 [5].

The results of Hardy-Weinberg balance analysis of IGF-2 gene with Chi-Square (X²) indicated that the population of SKKedu and KeduSK chickens were in Hardy-Weinberg equilibrium state (P<X²₀₀₀). Hardy-Weinberg’s law describes the frequency of alleles and genotypes in populations experiencing random mating that will remain for generations and there is no factor that cause genetic changes [6].

SKKedu and KeduSK chickens which are cross chickens from several local chickens that have a genetic composition from 3 local chicken species, namely Kampung, Sentul, and Kedu had no effect on the diversity of IGF-2 genes and IGF-2 gene equilibrium in this research.

3.1 Association of IGF-2 Gen Diversity with Chicken Growth SKKedu and KeduSK

Average of body weight, body weight gain, consumption, SKKedu and KeduSK chicken feed conversion based on differences in IGF-2 gene genotypes were shown in Table 2.

Body weight, body weight gain, feed consumption and feed conversion SKKedu and KeduSK chicken based on differences in CC, TC, TT genotypes at the IGF-2 locus were almost the same. However, KeduSK chickens tend growth higher than SKKedu.

Overall, there were no differences in the growth traits of SKKedu and KeduSK chickens from differences in genotype at the IGF-2 locus. There is research on Black Penedesenca chicken and reported that the IGF-2 gene had no effect on chicken performance [7].
Table 2. Average of body weight, body weight gain, consumption, feed conversion 12 week age based on genotype on IGF-2 allele in SKKedu and KeduSK chickens

| Genotype  | SKKedu          | KeduSK          |
|-----------|-----------------|-----------------|
| Body Weight (g) |
| CC        | 1055.8±37.5     | 1153.7±36.5     |
| TC        | 1059.7±30.4     | 1153.7±32.8     |
| TT        | 1012.5±0.00     | 1147.4±34.6     |
| Weight Gain (g) |
| CC        | 111.09±9.68     | 143.72±18.82    |
| TC        | 112.11±7.83     | 143.71±16.43    |
| TT        | 99.91±0.00      | 146.88±17.35    |
| Feed Consumption (g tail⁻¹week⁻¹) |
| CC        | 531.19±16.59    | 568.95±9.27     |
| TC        | 532.93±13.42    | 568.96±8.33     |
| TT        | 512.04±0.00     | 567.35±8.79     |
| Feed Conversion |
| CC        | 5.85±0.30       | 5.04±0.18       |
| TC        | 5.77±0.21       | 5.04±0.16       |
| TT        | 6.19±0.00       | 5.01±0.17       |

*The results of the t test with \( \alpha = 0.05 \) were not significantly different in the variables of body weight, body weight gain, feed consumption, feed conversion.

Table 3. Correlation between genotypes of IGF-2 locus with body weight, weight gain, feed consumption and feed conversion.

| Chicken       | P value | R*  |
|---------------|---------|-----|
| Body Weight   |
| SKKedu        | 0.109   | -0.402 |
| KeduSK        | 0.762   | -0.067 |
| Weight Gain (g) |
| SKKedu        | 0.109   | -0.402 |
| KeduSK        | 0.762   | 0.067  |
| Feed Consumption (g/tail/week) |
| SKKedu        | 0.109   | -0.402 |
| KeduSK        | 0.762   | -0.067 |
| Feed Conversion |
| SKKedu        | 0.098   | 0.414 |
| KeduSK        | 0.762   | -0.067 |
The correlation between genotypes at the IGF-2 locus with body weight, weight gain, feed consumption and feed conversion were shown in table 3. The results showed that there was no relationship between genotypes at the IGF-2 locus and the nature of the observed growth.

There is no correlation between genotype at IGF-2 locus with body weight, weight gain, feed consumption and feed conversion of SKKedu and KeduSK chicken (table 3). This was in line with the results of another research is no association between the diversity of IGF-2 genes and the nature of growth in native chickens [8]. Another factor of four SNP, growth traits, and plasma IGF-2 concentration is no significant associations [7]. No association was found between the IGF-2 gene diversity to slaughter weight and physical properties of the chicken carcass [9]. This showed that the IGF-2 gene cannot be used to characterize the growth traits of SKKedu and KeduSK chickens in this research.

4. Conclusion
IGF-2 gene was polymorphic in SKKedu and KeduSK chicken samples and was in the Hardy-Weinberg equilibrium state. The growth characteristics of SKKedu and KeduSK chickens based on differences in CC, TC, TT genotypes in the IGF-2 gene were almost the same. There was no relationship between genotype at IGF-2 locus and growth traits in SKKedu and KeduSK chickens.

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