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Polymorphism Analysis of TGF-β2 Gene and Its Association with Body Weight and Body Size Measurements of Tolaki Chicken

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ABSTRACT

This study aims to identify the transforming growth factor-beta2 (TGF-β2) gene then associate it with body weight and body size measurements in Tolaki chicken. Tolaki chicken used in this study amounted to 60 hens, the results of hatching in the Lab. Halu Oleo University Poultry Unit, Kendari. Maintenance from 6 weeks to 12 weeks. Observed data include; body weight, back length, chest circumference, shank length, tibia length, femur length, and wing length, were measured every week during the study. The diversity of the TGF-β2 gene was identified using the PCR-RFLP method. The genotype frequency values of TT (39), TC (18), and CC (3), respectively 0.65, 0.30, and 0.05. The value of frequency of T allele and C allele are 0.80 and 0.20 of the total population, respectively. The results of the chi-square TGF-β2 gene are in Hardy-Weinberg balance. Statistical results showed no significant difference (P> 0.05) between the TT, TC and CC genotypes in the TGF-β2 gene with the weight parameters and body measurements of the Tolaki chicken.

Keywords : Tolaki Chicken, TGF-B2 Gene, Body Weight, Body Size Measurements

I. INTRODUCTION

Tolaki chicken is one of Indonesia's local chickens, which is spread especially in the Southeast Sulawesi region and is generally raised by the Tolaki tribal community (Badaruddin et al. 2013 and Aku and Pagala, 2010). Tolaki chicken appearance physically has a relatively smaller and leaner posture compared to native chicken. Specifically male chickens that have a body position upright and form a slope of 30-40° when viewed from the back of the body from the base of the tail to the tip of the neck. Aside from being a producer of meat and eggs, the Tolaki chicken is also often used as a saver chicken by the snacker or during traditional ceremonies (Aku and Pagala, 2010).

Generally, the growth of Tolaki chicken is relatively slow, so that it takes quite a long time to reach the ready slaughter weight. One effort that can be taken to overcome this is through genetic improvement. Improvement of genetic traits, which include qualitative and quantitative traits is very much needed in conducting breeding programs. The existence of genetic improvement is expected to increase the productivity of Tolaki chicken in the future.

The nature of growth is influenced by environmental factors (feed, management, climate) and gene control. There are many genes that control the nature of growth, including growth hormone (GH), growth hormone receptor (GHR), growth hormone-releasing hormone (GHRH), insulin-like growth factor-1 (IGF-1), Pit-1, bone morphogenetic protein 15 (BMP-15), and transforming growth factor-beta (TGF-β) (NCBI 2019). The TGF-β superfamily consists of four

different isoforms, namely TGF-β1, TGF-β2, TGF-β3, and TGF-β4 (Huminiecki et al. 2009). Transforming Growth Factor-beta2 (TGF-β2) is one of the genes that can be used as a candidate marker of the nature of production (growth) (Muhsinin *et al.* 2017).

The use of the TGF-β2 gene as a marker is expected to give rise to good proxies. To produce superior seeds, which are characterized by body weight and greater body size. Body size is a quantitative trait that can be used as a requirement in finding livestock germs. The greater the order in normal size is expected, also attach more and more muscles to produce larger carcass pieces. Based on this background it is necessary to research "Association of TGF-β2 gene polymorphisms on body weight and body measurements in 6-12 weeks old Tolaki chicken".

II. METHODS AND MATERIAL

Research Material

Tolaki chicken used in this study amounted to 60 female chickens. Maintenance from 6 weeks to 12 weeks in individual cages in the Lab. Halu Oleo University Poultry Unit, Kendari. Tolaki chicken is obtained by hatching. The hatching eggs are obtained from Tolaki chicken broodstock taken in the Konawe Selatan District community, Southeast Sulawesi Province. Before being put into a cage, first, weigh the weight and give the leg number. Bodyweight, back length, chest circumference, shank length, tibia length, femur length, and wing length were measured every week during the study. Laboratory analysis is carried out in the Lab. Molecular Genetics in Animal Science, Department of Animal Production and Technology, IPB University.

DNA extraction

At the end of the second week, blood is taken from the brachial vein in the wing area. This blood draw is used as a sample for DNA extraction. DNA extraction uses the phenol-chloroform method (Sambrook and Russel, 2001).

Amplification of Polymerase Chain Reaction (PCR) TGF-β2 gene

Amplification of the TGF-β2 gene fragment was carried out by the Polymerase Chain Reaction (PCR) Amplification method. Primary use, according to Muhsinin et al. (2017), at the SNP mutation point (640) T>C with a PCR product length of 284 bp. Primary reaction uses forward (5'GGTTCAGTGCAAGGCATTTC3') and reserve primer (R:5'CTTCTGTCAAGTGCAGTGAG3').

DNA amplification with a total volume of 15 µL, consisting of 1 µL DNA, 6.2 µL DW, 0.30 µL primer, 7.5 µL 2 x GMM, and 0.50 µL MgCl2. The reactant mixture was put into a 1.5 mL tube to be homogenized, then distributed to each tube containing a DNA sample and then put into a PCR machine. DNA amplification took place in the System 9700 PCR machine with a predenaturation temperature of 95 °C for 5 minutes, a cycle for the stages of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 20 seconds and elongation at 72 °C for 30 seconds, then with the final elongation stage at 72 ° C for 5 minutes in one cycle. PCR products were electrophoresed using 1.5% agarose gel.

Analysis of Restriction Fragment Lenght Polymorphism (RFLP) TGFβ2 gene

The determination of the TGF-β2 gene genotype in Tolaki chickens will use the RFLP method. Amplification products from the TGF-β2 gene were cut using restriction enzymes. The restriction enzyme used in the exon 1 TGF-β2 gene is RsaI, 5 µL of PCR product was transferred to a 0.5 mL tube added 1 µL DW, 0.3 µL of RsaI restriction enzyme and 0.7 µL of Tango buffer. The mixture was incubated at 37 ° C for approximately 16 hours (overnight).

Data Analysis

Allele and TGF-β2 gene frequency

The genotype obtained from PCR-RFLP, then calculated the allele frequency, genotype frequency, Hardy-Weinberg equilibrium value, using the Popgene32 application program (Yeh et al., 1999).

TGF-β2 gene association with body weights and body size measurements

The process of weighing and measuring body measurements for Tolaki chickens, according to Koch (1973) is as follows:

a. Bodyweight is obtained by weighing using a scale in kg.
b. Back length is obtained by measuring the length of the bone from the border of the spine with the cervical vertebrae to the tip of the coccyx using calipers (cm).
c. Chest circumference is obtained by measuring the circumference of the chest using a measuring tape (cm).
d. The length of the shank is obtained by measuring the length of the tarsometatarsus bone (shank) using calipers (cm).
e. The length of the tibia is obtained by measuring the length of the tibia bone from the patella to the tip of the tibia using calipers (cm).
f. The length of the femur is obtained by measuring the length of the femur bone, using calipers (cm).
g. The length of the wing length is obtained by measuring the length of the humerus bone, the radius of the ulna, and the metacarpus to phalanges using calipers (cm).

The association of TGF-β2 gene diversity with body weights of Tolaki Chicken body measurements was performed using analysis of variance with the GLM (General Linear Model) procedure (SAS Inst. Inc., Cary, NC). Significant differences between general averages and genotypes were performed by Duncan's test. Significant differences are indicated by the value of $P < 0.05$. The model used is as follows:

$Yijk = \mu + \alpha i + \varepsilon ijk$

Information:

Yijk = Observation value
μ = Common midpoint
αi = Effect of genotype
εijk = Effect of trial error

III. RESULTS AND DISCUSSION

Amplification of the TGF-β2 Gene

Amplification of the TGF-β2 gene fragment in Tolaki chickens using the System 9700 PCR machine was successfully carried out under annealing temperature of 51 oC for 20 seconds and obtained a PCR product with a length of 284 bp. This is consistent with the research of Li et al. (2003): Tang et al. 2010): Muhsinin et al. (2017). The results of the TGF-β2 gene implication in exon one are presented in Figure 3. The RFLP results using the RsaI enzyme in the TGF-β2 gene fragment successfully identified two alleles, namely the T and C alleles (Figure 1). The two alleles produce three genotypes namely TT, TC and CC (Figure 2). Allele T has one band which is 284. Allele C has two bands, which are 184 bp and 100 bp. The combination of T and C alleles has three bands namely 284 bp, 184 bp, and 100 bp.

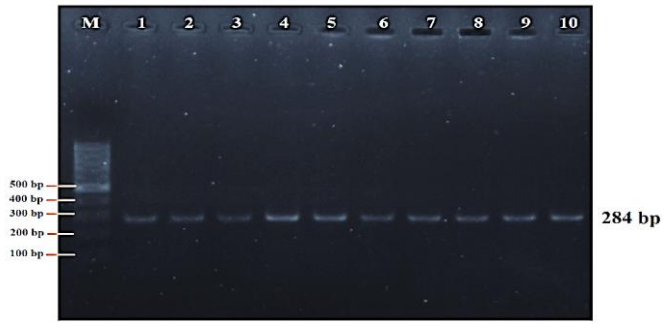

Figure 1. Visualization of the results of the amplification of TGF-β2 gene fragments in Tolaki chickens using 1.5% agarose gel. (M = 100 bp DNA marker; 1-10 = chicken Tolaki DNA sample)

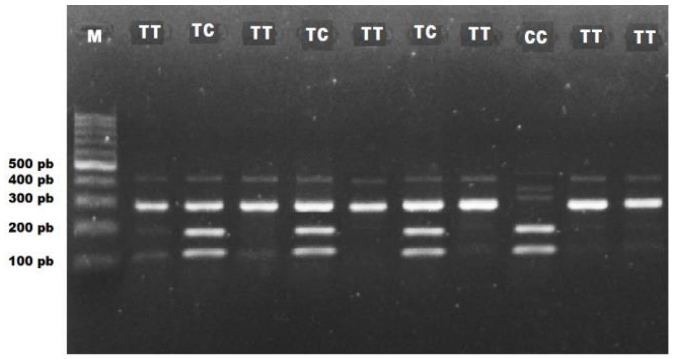

Figure 2. Visualization of PCR-RFLP results of TGF-β2 gene fragments using 2% agarose gel (M = 100 bp DNA marker; 1-10 = Tolaki chicken DNA sample)

Allele Frequency and Genotype TGF-β2 Gene

The diversity of TGF-β2 genes in Tolaki is polymorphic (Table 1). The genotype frequency values of TT (39), TC (18), and CC (3) were 0.65, 0.30, and 0.05, respectively. The value of frequency of T allele and C allele are 0.80 and 0.20 of the total population, respectively. The results of the chi-square test ($\chi 2$) TGF-β2 gene are in Hardy-Weinberg equilibrium {$\chi 2$ count (3.29) <$\chi 2$ table (3.84)}.

Table 1. Genotype frequencies, allele frequencies, and Hardy-Weinberg balance in TGF-β2 gene fragments for Tolaki chickens

Population	Locus	N	Genotype Frequency		Allele Frequency		*Chi-square* ($\chi 2$)
Tolaki Chicken	*RsaI*	60	TT (39)	0.65	T	0.80	3.29
			TC (18)	0.30	C	0.20	
			CC(3)	0.05			

Note: N (total population), $\chi 2$ tables (0.05) = 3.84 for db 1

Association of TGF-β2 Genes with Body Weight and Body Size Measurements

Statistical tests showed no significant difference (P> 0.05) between the TT, TC, and CC genotypes in the TGF-β2 gene with the parameters of body weight and body measurements of the Tolaki chicken (Table 2). This result is in line with the findings of Li et al. (2003) in local Chinese chickens aged 2-8 weeks. However, it is different from the findings of Tang et al. (2010) found this SNP association with local Chinese chicken body weight at 11 and 17 weeks of age, and Niarami et al. (2013) TGF-β2 gene was significantly (P <0.01) related to the nature of body composition (body weight, final body weight, carcass percentage, and breast percentage) of broiler chickens. Tang et al. (2010) reported that TT genotypes had higher body weight compared to CC genotypes at 11 and 17 weeks of age. From the various results of these studies, the effect of the TGF-β2 gene in chickens produced variable results.

Associated with the results of research obtained on the use of TGF-β2 gene markers as MAS (marker-assisted selection) or GAS (assisted selection genotype), which shows that genotype has no relationship with body weight and body sizes of Tolaki chicken aged 6- 12 weeks. This is likely influenced by the number of individuals with CC type in the statistical analysis is relatively small (CC = 3), so it needs a larger number of individuals or samples. Also, the TGF-β2 gene is a group of genes that control the resistance characteristics of chickens (Tohidi et al. 2012). The TGF-β2, TGF-β3 and TGF-β4 genes, besides belonging to the GH gene group, also belong to the cytokine gene group (Susan 2011). Cytokines are a type of protein that is involved in cell communication as a mediator to enhance the immune response (Wibawan 2013). So that good environmental conditions (feed, temperature, health) in this study resulted in production performance that is not much different.

Table 2. Association of the TGF-β2 gene on body weight and body size measurements of the tolaki chicken.

Parameter	Week	TT (n=39)	TC (n=18)	CC (n=3)	Signifikan
Body Weight (g)	6	229,87±30,14	233,06±26,02	297,33±15,31	ns
	9	388,87±57,23	384,88±37,86	411,67± 6,66	ns
	12	525,79±65,91	528,83±49,42	535,67±20,01	ns
Back Length (cm)	6	9,97±1,68	9,94±0,87	10,67±0,58	ns
	9	11,77±0,84	11,82±0,81	11,67±0,58	ns
	12	14,92±0,77	14,89±0,68	14,67±0,58	ns
Chest circumference	6	14,62±0,71	14,72±0,67	15,33±0,58	ns
(cm)	9	15,47±0,95	15,59±0,80	16,67±0,58	ns
	12	19,98±1,96	19,27±2,62	19,67±0,58	ns
Shank Length (cm)	6	3,77±0,43	3,89±0,32	4,17±0,76	ns
	9	5,74±0,54	5,77±0,57	6,17±0,29	ns
	12	6,38±0,49	6,47±0,50	6,67±0,58	ns
Tibia Length (cm)	6	5,71±0,51	5,75±0,60	5,77±0,68	ns
	9	8,49±0,81	8,66±0,45	8,67±0,29	ns
	12	9,66±0,45	9,51±0,57	9,33±0,58	ns
Femur Length (cm)	6	4,36±0,49	4,61±0,50	5,17±0,29	ns
	9	7,33±0,52	7,29±0,36	7,33±0,58	ns
	12	8,29±0,37	8,42±0,43	8,33±0,58	ns
Wing Length (cm)	6	10,56±0,74	10,67±0,69	10,67±0,58	ns
	9	18,45±1,22	18,97±0,87	19,17±0,76	ns
	12	16,59±1,02	16,50±0,51	17,00±1,00	ns

Note: n = number of individuals; ns = not significant (P> 0.05)

IV.CONCLUSION

The TGF-β2 gene in Tolaki chicken is polymorphic and is in Hardy-Weinberg balance. Association of TGF-β2 gene diversity did not show any difference (P> 0.05) on body weight and body size measurements of tolaki chicken.

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