



Polymorphism Association of *Pituitary Positive Transcription Factor-1* Gene with Body Weight Traits in BC₁ Hybrid Chicken (*Gallus gallus gallus* Linnaeus, 1758) from Cross Breeding between Female F₁ Broiler and Male Pelung

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ABSTRACT

Pituitary Positive Transcription Factor-1 gene is closely related to chicken growth and productivity. This research was conducted to detect Single Nucleotide Polymorphism in the exon 6 Pituitary Positive Transcription Factor-1 gene and its association with the bodyweight growth in the first backcross hybrid chicken. Procedures of the research included crossbreeding female first filial broiler chicken with male Pelung chicken to obtain first backcross hybrid chicken, Day Old chick hatched were maintained during 49 days, the bodyweight on the Day-Old chick measured every seven days, DNA was isolated by Chelex 5% method, Pituitary Positive Transcription Factor-1 gene was amplified by PCR, DNA band was visualized utilizing electrophoresis, and the PCR product was sequenced using Sanger method. The DNA sequence was aligned using Clustal omega software to gain Single Nucleotide Polymorphism. The Single Nucleotide Polymorphism was analyzed using the Pearson correlation test between chicken body weights of 49-days-old chickens with the polymorphism points. The conclusion indicated that the bodyweight of the first backcross hybrid chicken was higher than the Pelung chicken but lower than the first filial broiler chicken. Single Nucleotide Polymorphism was not found on the exon 6 Pituitary Positive Transcription Factor-1 gene in the first backcross hybrid chicken.

Keywords: Growth, Hybrid chickens, PIT-1 gene, SNP

INTRODUCTION

Indonesian native chickens or known as '*ayam buras*' (non-broiler chickens) are very popular by Indonesians, especially in rural areas. Indonesian native chickens are classified into four functional groups such as meat and egg producer, singing chicken, used in traditional ceremonies, fancy, and fighting cock (Hidayat and Asmarasari, 2015). According to (Zein and Sulandari, 2009) a genetic molecular study, informed that all domesticated chicken populations came from one ancestor (monophyletic), namely red jungle fowl (*Gallus gallus*) originated from Southeast Asia. Indonesian local chickens were developed through a process of domestication and known as native chickens. Native chickens were the result of a cross between jungle fowl *Gallus bankiva* and *Gallus varius* scattered in the territory of Indonesia, especially in Java and Nusa Tenggara. Local chickens or often known as '*ayam kampung*' have superiority in the quality of their meat and egg, but this superiority is not followed by good productivity capability in meat and egg (Zein and Sulandari, 2009).

The productivity of local chickens is relatively low, as an implication of the extensive system of maintenance. Indonesian local chickens must be maintained optimally to support the small-scale poultry industry so that it becomes a solution to fulfill the increasing demand for domestic food consumption (Daryono et al., 2010). The efforts to improve the productivity of local chickens include selection and crossbreeding programs. According to Cheng (2010), selective breeding is aimed to produce a superior chicken breed with adjusted phenotype quality according to human needs. The targeted selection program will provide a high economic mean in the use of local chickens, namely by improving the quality of local chickens through the crossing and selective breeding programs of specific characters. Other basic information such as specific characteristics, origin, performance, and productivity of local chickens are needed to optimize the utilization of local chickens in Indonesia. This information is expected to make Indonesian local chickens better known, developed, and preserved, so that they can be used sustainably (Sulandari et al., 2007). Therefore, we need research that can study genetic diversities and identify genes responsible for the growth of hybrid chickens.

With the progress of molecular genetics, the selection program can be carried out earlier through analysis at the DNA level. Pituitary Positive Transcription Factor-1 (PIT-1) gene is the one gene that is closely related to chicken growth and productivity (Miyai et al., 2005). As stated by Jiang et al. (2004) exon 6 in PIT-1 gene has a significant

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relationship to improving the growth of chicken weight. The purpose of this study was to obtain hybrid chickens that inherited superior characteristics from both broodstocks with good growth characteristics resembling broiler chickens and good phenotypic characters, body resistance, good quality of meat and eggs resembling local chickens, and genetic quality improvement through molecular selection. Therefore, this study also analyzed the relationship between exon 6 polymorphisms of the PIT-1 gene with hybrid chicken body weight.

MATERIALS AND METHODS

Ethical approval

The procedure in this research has been conducted following the guidelines of the ethical committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Chicken cross breeding

In this study, the first backcross (BC₁) hybrid chickens were used as a result of crossing between female F₁ broiler chicken and male Pelung chicken. The Day Old Chick (DOC) was maintained for seven weeks with lighting 24 hours using 10 watts light bulb, air temperature $\pm 30^{\circ}\text{C}$ and 40-50% humidity, feed by BR I (protein 21,00-23,00% and energy 3000 kcal/kg) made by PT. Japfa Comfeed Indonesia Public Listed Company (Plc) *ad libitum*. 12 broods were consisting of 7 males and 5 females. Furthermore, DOC was raised intensively for 7 weeks in special cages to minimize outside influences that can interfere with health, facilitating growth monitoring, and facilitating chickens' feeding. The DOC body weight measurement every 7 days was aimed to observe DOC growth during the observation period for 7 weeks. Quantitative character measurements and qualitative character observations were carried out on the last day of observation on the 49th day.

DNA isolation

DNA isolation with Chelex 5% method with the modified concentration of Chelex according to the optimization phase. A total of 10 μl of chicken blood was put into a 1.5 mL tube, added with 1 ml of Tris-EDTA (TE) buffer. Then put into a 1.5 mL microcentrifuge tube, centrifuged at a speed of 13,000rpm for 3min. The supernatant was transferred to the new eppendorf tube, then the pellet was added with 200 μl of 5 percent Chelex solution, 18 μl of dithiothreitol (DTT) 0.05 M, 2 μl of proteinase K, then mix various samples rapidly 30s with vortex and incubated at 56°C for 2 h, and vortex in every 15 min. Then incubated at 100°C for 8 min, and centrifuged at 13,000 rpm for 3 min. The supernatant was transferred to a 1.5 ml microcentrifuge tube, and stored at -20°C (Butler, 2009).

DNA amplification

The amplification of Pituitary Positive Transcription Factor-1 (PIT-1) gene was carried out by PCR, with the reaction composition of Bioline PCR kit as much as 12.5 μl , 5'-GGCACTTTGGAGAACAAAGC-3' forward primer as much as 1.25 μl , 5'-CTCGTGGTGCTCCTTGATAA-3' reverse primer as much as 1.25 μl , 5 μl of DNA samples, and 5 μl of ddH₂O so that the total volume was 25 μl . The specific primer used was MR5 (for exon 6 with access code AJ236855) from *Gallus gallus* (Nie et al., 2008). The used PCR program was 95°C initial denaturation for 5 min, followed by 35 denaturation cycles at 95°C for 15s, annealing at 60°C for 60 s, and extension at 72°C for 60 s, extra extension at 72°C for 10 min (Van As et al., 2000).

Agarose preparation

Agarose was weighed according to agar concentration (genome =1%) (PCR yield 1.8-2%). Next, it was put in a beaker glass and added with Tris-borate-EDTA (TBE) according to the chamber volume. Then it was put in the oven, heat until it dissolves (clear). A mold was set and installed with the comb. The agar was added with 2-3 μl of flourosave, then poured into the mold. The agarose was left to solidify.

Electrophoresis

Electrophorator was prepared. Agar was inserted into the electrophorator Mupid-exUTM. Tris-borate-EDTA/TBE (immersion) was added until the agar was sinked. The sample was inserted into the well. Electrophorator was closed, turn on, time was set (20-30 min =100 volts, 1h =50 volts) then visualized under UV light by AnalytikJenaTM gel imaging system and documented with GelDocTM Documentation System.

Sequencing with Sanger method

The PCR product was sequenced by the Sanger sequencing method (Sanger et al., 1977) in first Base Company, Selangor, Malaysia.

Data analysis

The correlation between chicken weights was analyzed using SPSS 16.0 one-way ANOVA program statistical test and post hoc LSD method to assess the significance between chicken strains. The data of DNA sequencing were assembled using the Gene Studio program, multiple sequences were alignment using Clustal Omega software, and Pearson correlation test between chicken body weight with Single Nucleotide Polymorphism (SNP, Arnedo et al., 2007).

RESULTS AND DISCUSSION

Chicken growth

In this study, crossings between female F₁ broiler chickens and male Pelung chickens were carried out and resulted in the first backcross or BC₁ hybrid chickens. The comparison of the weight of BC₁ hybrid chicken, Pelung, broiler, and F₁ broiler chicken for 7 weeks is presented in figure 1.

The average weight of chickens from the lowest to the highest starting from Pelung chicken, BC₁ hybrid chicken, F₁ broiler chicken, and broiler chicken. The average body weight of BC₁ hybrid chickens (161.44 gr) for the seven weeks showed lower results compared to the average weight of F₁ broiler chickens (648.14 g) (Roosdianto, 2010), and broiler chickens (1409.57 gr) (Suryaman, 2010), but higher than the average weight of Pelung chickens (112.38 gr). This was based on the inherited character of the broodstocks, the BC₁ hybrid chickens carried the character of broiler chickens which was rapid growth, and thus BC₁ hybrid chickens had a higher weight than Pelung chickens. The growth and development of chickens were influenced by certain factors, including intrinsic factors such as genetics and sex, and extrinsic factors such as the process of chicken breeding, environmental factors, and types of feed (Oktafiantari, 2016).

The significance of the chicken types to the chicken weights for 7 weeks is shown in table 1. The BC₁ hybrid chicken has a higher growth rate compared to Pelung chicken but has a lower growth rate than broiler chicken and F₁ chicken. Table 1, a significant difference is obtained because the significance value of 0.00 is less than the standard deviation of 0.05. Thus, it means that the types of chicken affect chicken weight. The BC₁ hybrid chicken growth is between the Pelung and broiler Chicken growth lines because BC₁ hybrid chickens have both bloodlines. Therefore, it is important to further investigate the causes of these differences in chicken growth, by assessing the polymorphism of the exon 6 PIT-1 gene which has been recognized as one of the genetic markers for chicken growth.

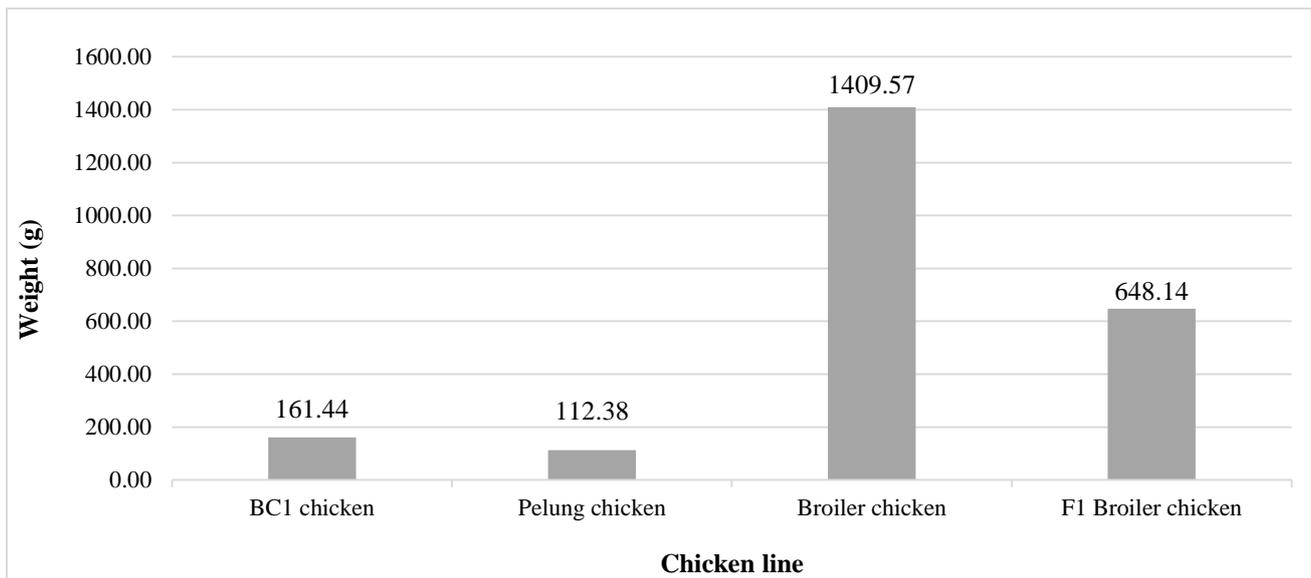


Figure 1. Comparison of mean body weights of BC₁, Pelung, Broiler and F₁ Broiler chickens for seven weeks

Table 1. The differences of chicken weights in BC₁, Pelung, Broiler and F₁ Broiler chickens during seven weeks

Chicken line	Age (week)						
	1	2	3	4	5	6	7
Hybrid BC ₁	48.67± 6.18 ^b	65.33± 9.64 ^b	95.17± 24.37 ^b	122± 4306 ^b	156.4± 43.79 ^b	230.92± 45.49 ^b	419.08± 100.6 ^b
Broiler	194.0± 0.00 ^d	461.00±0.00 ^d	842.00±0.00 ^d	1309±0.00 ^d	1817± 0.00 ^d	2347± 0.00 ^d	2897± 0.00 ^d
Pelung	32.33±2.52 ^a	44.33±11.93 ^a	57.33±17.21 ^a	84.00±27.22 ^a	124.3±30.10 ^a	185.67±42.19 ^a	258.67±54.09 ^a
F ₁ broiler	94.30±0.00 ^c	230.00±0.00 ^c	387.00±0.00 ^c	583.70±0.00 ^c	833± 0.00 ^c	1100.3±0.00 ^c	1308.70±0.00 ^c

Pituitary positive transcription factor-1 gene polymorphism

The molecular selection was performed as a way to improve genetic quality. This study was aimed to detect the presence of PIT-1 gene polymorphism on the weight growth of hybrid chickens. The Pituitary Positive Transcription Factor-1 (PIT-1, POU1F1, or GHF1) gene in chickens was located on chromosome 1 with a length of 14 kb as a genetic marker that had been used to aid in the early selections based on the relationship between markers and the expected quantitative traits (Yamada et al., 1993). The PIT-1 gene was one of the genes that were closely related to the growth and productivity of chickens because the PIT-1 gene controlled the expressions of the coding genes for growth hormone and prolactin hormone (Miyai et al., 2005). Therefore, it could be expressed that the PIT-1 gene was a gene candidate that had the prospect of being used as a genetic marker in the local chicken selection program. Electrophoresis was carried out to determine the results of DNA fragments amplification by PCR. The results of electrophoresis can be seen in figure 2 and figure 3.

Based on exon 6 PIT-1 gene visualization, the 12 BC₁ samples had a nucleotide length of 180 bp (Figure 2a). In the PCR result of the exon 6 PIT-1 gene (Figure 2b), the samples 1-4 are broiler chickens that have nucleotide length 180 bp and the samples 5-8 are Pelung chickens also have nucleotide length 180 bp. DNA amplification results showed good fragments that were shown by the appearance of thick and clear DNA bands, then from the amplification results by PCR, a sequencing process was carried out to determine the nucleotide sequences of the genes. The alignment of the exon 6 PIT-1 gene is shown in table 2.

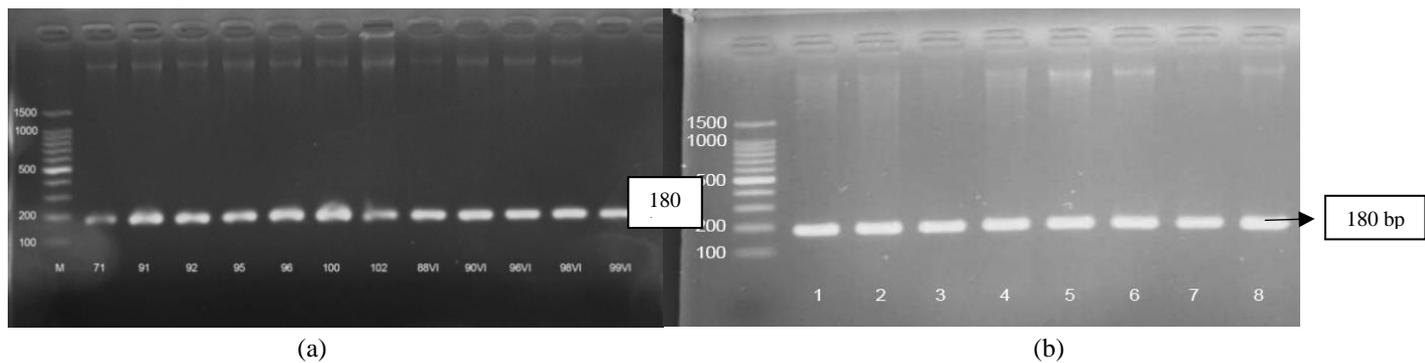


Figure 2. The results of exon 6 Pituitary Positive Transcription Factor-1 gene (180 bp) amplification by PCR (a) hybrid BC₁ chickens (b) 1, 2, 3, 4: Broiler chickens; 5, 6, 7,8: Pelung chickens

Table 2. The single nucleotide polymorphism of exon 6 pituitary positive transcription factor-1 gene

Sample No.	PIT-1 gene polymorphism		Haplotype	Chicken weight on day 49 (g)
	Exon 6			
	Substitution	Substitution		
AJ236855	A	T	Reference	-
Hybrid BC ₁ 1	A	T	Reference	680.00
Hybrid BC ₁ 2	A	T	Reference	490.00
Hybrid BC ₁ 3	A	T	Reference	493.00
Hybrid BC ₁ 4	A	T	Reference	471.00
Hybrid BC ₁ 5	A	T	Reference	369.00
Hybrid BC ₁ 6	A	T	Reference	414.00
Hybrid BC ₁ 7	A	T	Reference	352.00
Hybrid BC ₁ 8	A	T	Reference	348.00
Hybrid BC ₁ 9	A	T	Reference	368.00
Hybrid BC ₁ 10	A	T	Reference	338.00
Hybrid BC ₁ 11	A	T	Reference	349.00
Hybrid BC ₁ 12	A	T	Reference	357.00
Broiler 1	G	A	1	-
Broiler 2	G	A	1	-
Broiler 3	G	A	1	-
Broiler 4	G	A	1	-
Pelung 1	G	A	1	321.00
Pelung 2	G	A	1	224.00
Pelung 3	G	A	1	231.00

Notes: A: Adenine; G: Guanine; T: Thymine

Based on table 2, the exon 6 PIT-1 gene in BC₁ hybrid chickens has the same nucleotide structure as the reference (AJ236855), so that these nucleotide sequences do not make new haplotypes. But Pelung chicken and broiler chicken have 2 SNP located in the coding region. The SNPs consist of 2 substitution points including Adenine to Guanine and Thymine to Adenine. Pelung chicken and broiler chicken form the same nucleotide sequence so that from the 2 SNP it will form 1 same haplotype. Table 3 is the results of the Pearson correlation test used to determine the correlation between the weight of chickens and the points of polymorphism. The results of the study showed that the two points of polymorphism were A928G substitution and T929 substitution. The substitution A928G consisted of GG genotype (mutant phenotype) in Pelung chicken with a 49th day and average weight of 258.7 g, and the AA genotype (wild type phenotype) in BC₁ hybrid chicken with weight average 419.08 g. For the second polymorphism point, T929A consisted of genotype TT (mutant phenotype) in Pelung chicken with an average weight of 49 days to 258.67 g and AA genotype (wild type phenotype) in hybrid chicken BC₁ has weight chicken average 419.08 g. Genotype frequency at A928G substitution point and T929A substitution point have the same value which is 0.5. The correlation coefficient at both points was -0.588. The A928G substitution point and T929A substitution point had a significance value smaller than 0.05, which was 0.021. Based on table 3 it can be described the mutant phenotype at both points affecting the decrease in chicken weight. So that it can be concluded that the relationship between the point of polymorphism and chicken weight was a significantly negative medium correlation. Whereas in a previous study conducted by Jiang et al. (2004) that on MR5 or exon 6 PIT-1 gene there were SNP associated significantly with the phenotypic characters of chickens' growth. A deletion occurred in C nucleotide which caused a change in the amino acid arrangement after the point of mutation, which was caused by a frameshift mutation. As a result of the frameshift mutation, the protein structure changed which was resulted in an error of protein function, or a decrease in protein formation.

Table 3. The correlation test results of Pituitary Positive Transcription Factor-1 gene polymorphism on the chicken mean weight on the 49th day

Polymorphism	A928G Substitution		T929A Substitution	
	GG (mutant)	AA (wild type)	AA (mutant)	TT (wild type)
Genotype frequency	0.5	0.5	0.5	0.5
Mean chicken weight on day 49 (g)	258.67	419.08	258.67	419.08
Correlation coefficient (r)	-0.588		-0.588	
Significant level	0.021 (P < 0.05)		0.021 (P < 0.05)	
Conclusion	Significant with moderate negative correlation		Significant with moderate negative correlation	

CONCLUSION

The conclusion showed that first backcross hybrid chickens resulting from a cross between female F₁ broiler chicken and male Pelung chicken body weight was lower with the average weight at the 7th week was 419,08 g compared to F₁ broiler chickens, but higher than the Pelung chickens. There was not exon 6 Pituitary Positive Transcription Factor-1 gene polymorphism found in first backcross hybrid chickens resulting from a cross between female F₁ broiler chickens and male Pelung chickens.

DECLARATIONS

Author`s contributions

D. Retnosari designed the plan of study, collected data and samples, contributed to analyses, and wrote the manuscript. R.Kilatsih and I.S. Maulidi checked the final form of the manuscript. Trijoko revised the research article and facilitating the experimental work. B.S. Daryono helped in designing the plan of study, facilitating the experimental work, providing the experimental tools, revising the research article.

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Competing interests

The authors have not declared any conflict of interest.

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