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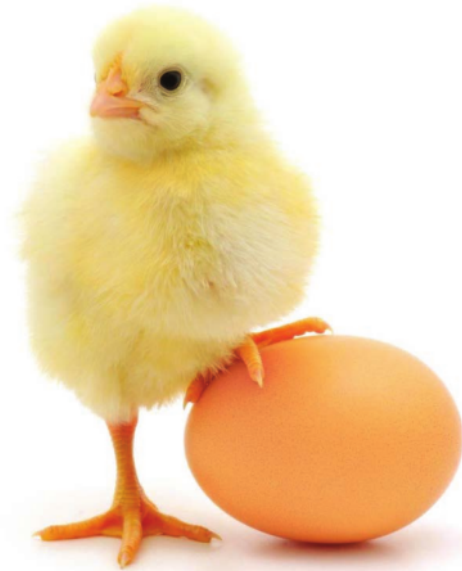
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Research Article

Pituitary-Specific Transcription Factor Gene Polymorphism and its Associations with Body Weight of Super Kampung Chickens Aged Thirteen Weeks

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Abstract

Background and Objective: Pituitary-specific Transcription Factor polymorphism gene of livestock can be used as a basis for selection programs. A study was conducted to categorize Pituitary-Specific Transcription Factor (Pit-1|Tasl) polymorphism genes and their associations with body weight of Super kampung chicken. **Materials and Methods:** Day-old 112 Super Kampung chickens were used in this study. Chicks were kept in an open house with slat floor until the age of 17 weeks. Feed and drinking water were provided *ad libitum*. Blood sample was collected with a syringe (3 mL) from the brachial vein and Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) was used for analysis. **Results:** The results showed that the Pit-1 genes were polymorphic in Super Kampung chickens. There were two alleles, namely C and T, each with a rate of 0.746 and 0.254, respectively and three genotypes, namely CC, CT and TT, each with a frequency of 0.598, 0.295 and 0.107, respectively. Pit-1 gene in the Super kampung chicken population was not in equilibrium. There were correlations between the genotype of fragment genes Pit-1|Tasl exon 6 and the body weight of the Super Kampung chickens at 13 weeks of age. The highest body weight was found in the CC genotype. **Conclusion:** The CC genotype of the Pit-1 gene can be used as a growth marker in Super Kampung chickens.

Key words: Pit1 gene, PCR-RFLP, growth rate, super Kampung chicken, CC genotype

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Super Kampung chickens are a cross-breeds between male Bangkok chicken (local Indonesian chicken) and female layer chicken of Isa Brown strain. These crossbred chickens have been widely bred to meet the demand of raw material for a traditional cuisine. The crossbred chickens have a high phenotypic polymorphism (feather color, skin color, shank color, physical shape and body weight) and are predicted to have high genetic polymorphism as well¹. The high poly²⁹phism provides opportunities for selection of poultry. The Pituitary Specific Transcription Factor-1 (Pit-1) gene is one of the common markers used for select³² of poultries²⁻⁴. Its functions are to bind and reactivate growth hormone (GH), prolactin (Prl)⁷ and thyroid- β -stimulating hormone (TSH- β) promoters^{5,6}. The Pit-1 gene is responsible for the development of the anterior hypophysis of the pituitary gland⁷, induces differentiation of liver progenitor cells for the production of prolactin (Prl)⁸, as well as delaying adrenarache in humans⁹.

Previous scientific experiments found that the Pit-1 polymorphism gene is related to growth, carcass and fatty characters in pigs¹⁰⁻¹³, cattle^{10,14} and broilers^{4,15}. The Pit-1 of the AA genotype of rs80904061 on intron 4 had lower feed intake than those of the AA genotype in pigs¹³. The Pit-1 of the GG genotype of rs13687126 showed higher body weight gain compared to the AG genotype at 49 days. Whilst, at 70 days, birds with the TT genotype of rs13687128 gain more weight with lower feed conversion ratio compared to the CT and CC genotypes.

On the other hand, at 70 days, the Pit-1 of the AA genotype of rs13905622 is able to grow faster and utilize feed more efficiently compared to the AT and TT genotypes. Therefore, it is observed that the Pit-1 gene polymorphism could be used as a foundation in chicken breeding programs⁴. This research was conducted to obtain the Pit-1 gene genotype which can be practiced as growth markers in Super Kampung chickens.

MATERIALS AND METHODS

Animals and treatments: A total of 112 a day old Super Kampung chickens were used in this study. The chickens were kept in an open cage with a slat floor at the Teaching Farm of Animal Science Faculty, Mataram University, Lingsar, West Lombok, Indonesia. The birds have free access to feed and drinking water. Body weight and feed intake were recorded weekly.

Data collection: Blood sample (400 μ L) was collected from the brachial vein using a 3 mL syringe. The blood sampling area was cleaned using 70% alcohol with cotton. The blood sample was put into the Eppendorf tubes that had been filled with 1.5-2.2 mg mL⁻¹ of EDTA (Ethylene Diamine Tetra-acetic Acid) as an anticoagulant. The sample was stored at 4°C.

DNA extraction: DNA was extracted using the Genomic DNA Mini Kit produced by Geneaid. Three hundred microliters (300 μ L) of blood sample were put into a 1.5 mL tube. RBC Lysis Buffer was then added into the tube with 3 \times volume of the samples using inversion method. The mixture was then incubated for 10 min at room temperature. The samples were centrifuged for 5 min at 3000 rpm and then the supernatant was removed. One hundred microliters (100 μ L) of RBC Lysis Buffer were then added. The next step was Cell lysis (stage-1) process and conducted by adding 200 μ L of buffer, followed by shaking and incubation at 60°C for 10 min to clarify the appearance of the sample. During incubation, the tube was turned over every 3 min. Then, 200 μ L of buffer sample was prepared for step 4. In stage 2 (DNA Binding), 200 μ L of absolute ethanol was added and shaken for 10 sec and LD column was placed in a 2 mL collection tube. In the next step, the sample mixture was transferred to GD column and centrifuged at 14000-16000 rpm for 5 min. After that, 2 mL collection tube was removed and then GD column was placed in a new 2 mL collection tube. In stage 3 (washing), 400 μ L of W1 Buffer was added into the GD column and centrifuged at 14000-16000 rpm for 30-60 sec. Next, the GD column was put back into the 2 mL collection tube. Subsequently, 600 μ L of wash buffer was added into the GD column. The column was then centrifuged at 14000-16000 rpm for 30-60 sec. LD column was put back into the 2 mL collection tube. Next, the tube was centrifuged at 14000-16000 rpm for 3 min. At stage 4 (DNA elution), the GD column was transferred to a 1.5 mL tube. One hundred microliters (100 μ L) of the heated elution buffer was added and then centrifuged at 14000-16000 rpm for 30 seconds. DNA samples were stored at -20°C for further use.

PCR amplification: DNA samples obtained from each chicken were used as templates to amplify gene loci using PCR with primer of Forward 5'TGGGAAGAACAGTTTATGGC3' and Reverse 5'CTGGAGTCGTTGAAGTAAGC (483bp)¹⁵. Amplification of DNA was carried out using a total volume of 15 μ L, comprising of 1 μ L of DNA, 4 μ L of 5 \times MyTaq Red Reaction Buffer, 0.3 μ L of primer, 1 μ L of MyTaq HS Red DNA Polymerase and 5.7 μ L of ddH₂O. The mixture of reactant was

put into a 1.5 µL tube to be standardized and then allocated to each tube having DNA samples. The tubes were then put into a PCR machine. Amplification of DNA was done using PCR machine of Applied Biosystem at 95°C for 5 min, 35 rotation for denaturation at 95°C for 10 seconds, annealing at 60°C for 20 sec and elongation at 72°C for 30 sec, then continued with the final elongation stage at 72°C for 5 min in one cycle. PCR products were electrophoresed using 1.5% agarose gel.

RFLP analysis: Product of amplification of the Pit-1 gene in exon 6 was cut using the *TasI* restriction enzyme. Five µl of PCR products was allocated into a 0.5 mL tube filled with 0.9 µL of DW, 0.4 µL of restriction enzymes and buffered 0.7 µL. The mixture was incubated at 37 °C for approximately 16 h.

Electrophoresis of PCR-RFLP products: Electrophoresis of the PCR-RFLP gene product Pit-1 was conducted using 2% agarose gel. Five microliters (5 µL) of PCR-RFLP products were put into gel wells. Five microliters (5 µL) of marker DNA 100 bp was used as a marker. The gel was electrified at 100 V for 30 min. After finishing electrophoresis, agarose gel was visualized using ultraviolet light in the UV Transilluminator machine.

Determination of the Pit-1 genotype: The exon 6 Pit-1 gene fragment genotype was C allele, which had no cutting site indicated by one fragment whose length is the same as the length of PCR product of 483 bp. On the other hand, T allele has the *TasI* restricted enzyme site (↓AATT) and was shown by two fragments of 380 bp and 103 bp respectively (Fig. 1).

Data analysis: Allele frequencies and genotypes were calculated using Nei and Kumar¹⁶ formula. Estimated frequency of heterozygosity observations (Ho) was calculated using Weir¹⁷ formula, while the expected heterozygosity values (H_e) were calculated using the Nei and Kumar¹⁶ formula. Hardy-Weinberg equilibrium was calculated using Chi-Square¹⁸, while the free degree of Hardy-Weinberg

equilibrium test was conducted using the method as described by Allendorf *et al.*¹⁹. The associations between the Pit-1 genotype and body weight were calculated with the help of the GLM procedure of SAS version 9.1²⁰.

RESULTS AND DISCUSSION

Restriction Fragment Length Polymorphism (RFLP) analysis using the *TasI* restriction enzyme against the fragment of Pit-1 exon 6 gene obtained three kinds of fragments (Fig. 2). Fragments that were not truncated by the *TasI* enzyme (483 bp) were TT genotypes, while the truncated fragments (380 and 103 bp) were CC genotypes and combined fragments (heterozygous) (483, 380 and 103 bp) were CT genotypes.

Based on genotype identification, C and T alleles were detected in the Pit-1 gene fragment in exon 6 with frequencies of 0.746 and 0.254, respectively. Homozygous (CC or TT) genotype means that both parents contribute the same gene (allele) and indicated that the chickens received different gene combinations from both parents.

The analysis of the genotype frequency of Pit-1|*TasI* exon 6 genes for Super Kampung chickens are presented in Fig. 3. The genotype proportion of Pit-1 exon 11 genes in all of the studied Super Kampung chicken populations showed that the frequency of CC genotype is the highest compared to those of CT and TT genotypes. This is attributed to the high frequency of C alleles in the entire Super Kampung chicken population. A similar result was obtained for White Recessive Rock and Chinese Xinghua chickens which showed the high CC genotype frequency of Pit-1 genes and low TT genotype frequency¹⁶.

Moreover, the fragments of Pit-1|*TasI* exon 6 gene in Super Kampung chickens were polymorphic and three types of genotype were found in each gene fragment and the allele frequency was more than 0.01^{16,19}. The high frequency of allele C gene Pit-1|*TasI* exon 6 in the Super Kampung chicken population was due to the fact that the chickens were the

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9301 CATTACAGCTGGAGTTAAAATGACCAAGCCTCTTAAACAAAAAAGTATAGCTCTGTGG 9360
9361 TGCACTCTCCTTATTTCAGTAGTATCTGTTACACTTTCTTCTGTCTTCAAAGGCCAAACTTT 9420
9421 TTTTGTTCAGTGATGACAGCACTCAGTTTCCATTCCCTATGGAAGTTAACTGCTCTTTT 9480
9481 GTTCTTGTTTGTGTTTAAATGCAGCTTTATACAATGAAAAAGTTGGCGTGAATGAG 9540
9541 CGGAAGAGGAAGCGCAGAACCAACATAAGGTTATACATATTTATGGAGTTACATGTTATA 9600
9601 AATGCCTTCAGCTATGAAGTTTCATTTGGGGGGTTGAAATCAAAGTGTGTTTTCTGTT 9660
9661 TGTTAATAGTTAATGAAGATAAACTATTCTGAAACATTCAGTGAAAAATAAAAGCACA 9720
    
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Fig. 1: Pit-1|*TasI* exon 6 cut sites in super Kampung chicken population

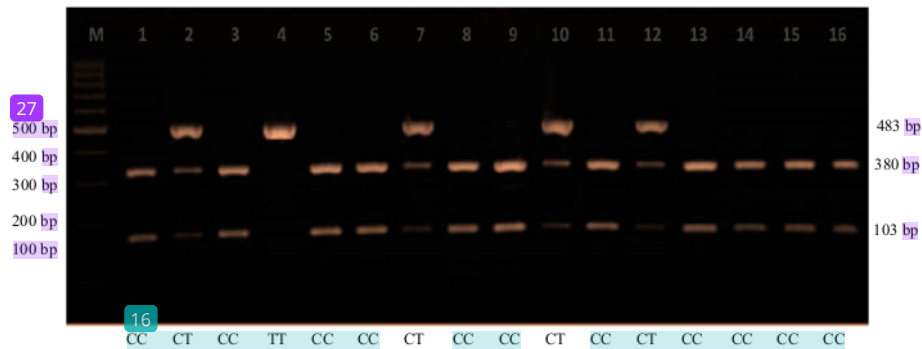


Fig. 2: PCR-RFLP fragment result of Pit-1 exon 6 genes with restriction enzyme in agarose 2%
M = Marker DNA 100 bp. Sample 1-16 = Super Kampung chicken samples

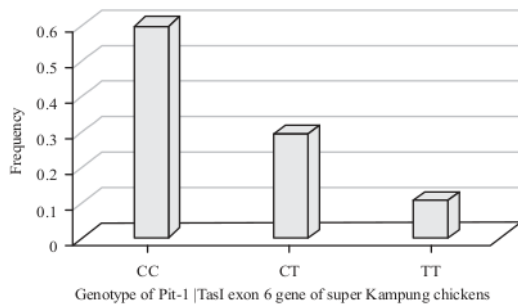


Fig. 3: Genotype frequency of Pit-1|TasI exon 6 gene of Super Kampung chicken

Table 1: Heterozygosity and equilibrium of Hardy-Weinberg Pit-1 gene of Super Kampung chickens

n	Heterozygosity		χ^2
	Ho	He	
112	0.618	0.381	5.852*

n: Samples, Ho: Observed heterozygosity, He: Expected heterozygosity, χ^2 : Chi square (α 5% = 3.841), *: Real (χ^2 count > χ^2 table)

Table 2: Associations between genotype fragment of Pit-1|TasI exon 6 and Body Weight of Super Kampung Chickens at 13 weeks of age

Genotypes	Body weight (g bird ⁻¹)
CC (n = 67)	1217.7 ± 23.4 ^a
CT (n = 33)	1047.1 ± 33.3 ^b
TT (n = 12)	1017.0 ± 55.3 ^b

n: Samples, ^{a,b} means in columns with different letters differ significantly (p < 0.05)

cross-breed of Bangkok chicken (males-Indonesian local chickens) and exotic chickens (laying hen of Isa Brown strains)²¹.

Analysis of the observed heterozygosity values (Ho), expected heterozygosity values (He) and the chi-square test (χ^2) of Pit-1|TasI exon 6 genes for Super Kampung chickens are presented in Table 1. Heterozygosity values were the average percentage of heterozygous loci per individual or the average percentage of heterozygous individuals in the population¹⁶. Genetic polymorphism of a population can be quantitated by using heterozygosity values and can be used to assist selection programs²².

High heterozygosity values indicate high genetic polymorphism within a population and vice versa. In the locus of Pit-1|TasI exon 6 of Super Kampung chicken, it was found that the value of Ho was higher than the value of He. A great dissimilarity between Ho and He values can be implemented as an indicator of genotype difference in the analyzed Super Kampung chicken population²³.

Chi-square test (χ^2) indicate that the genotype frequency of the fragment Pit-1|TasI exon 6 gene of Super Kampung chickens deviates from the Hardy-Weinberg balance. A balanced population is characterized by combining gametes randomly in a great population, leading constant frequencies of genotype and allele from generation to generation¹⁹. In a great population, alteration from one generation to the next generation do not occur when selection, migration, mutation and genetic drift are not found²⁰.

The association analysis between the genotype of the fragment Pit-1|TasI exon 6 gene and the body weight of Super Kampung chickens at 13 weeks of age are presented in Table 2.

Body weight of Super Kampung chickens having CC genotype was heavier than those of having CT and TT genotypes (p > 0.05) (Table 2). This means that there is an association between the polymorphism of the Pit-1 gene and body weight of Super Kampung chickens. The results of this

study enrich the existing knowledge regarding the association between ²³ Pit-1 gene of several livestock and their body weights. Results of the present study are in consonance with Hoseinzadeh *et al.*¹⁴ who ⁷ worked on Holstein cattle, pigs¹³ and chickens¹⁵ and reported that the Pit-1 gene was polymorphic¹⁰ and strongly associated with body weight. Similar result was also reported by Jin *et al.*⁴, who found ³⁵ that the Single-Nucleotide Polymorphism rs13687126 Pit-1 gene is strongly associated with the increased rate of body weight and feed consumption at 70 days. It was further found that individuals with major genotype (GG) at rs13687126 and rs13687128 produced higher body weight compared to heterozygote (GT) and minor genotype (TT) genotypes.

CONCLUSION

¹² The Pit-1 gene in SuperKampung chicken population was polymorphic with C and T alleles and CC, CT and TT genotypes. The highest allele frequency was ¹⁸ C, while the highest genotype frequency was for CC. The Pit-1 gene in SuperKampung chicken populations was not in equilibrium. The Pit-1 gene was strongly associated with the body weight of the Super Kampung chickens. The CC genotype has the highest body weight. Therefore, the CC genotype can be an effective marker for the growth of Super Kampung chickens.

ACKNOWLEDGMENTS

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