

Genetic Diversity of the Prolactin Gene in Three Indonesian Ducks

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ABSTRACT

The Prolactin gene is a candidate gene associated with egg production due to its crucial role in the production and reproduction of poultry. This study aimed to identify the polymorphisms of the prolactin gene in Indonesian local duck breeds. For that purpose, three duck breeds, namely Bayang (n= 25), Turi (n= 26), and Magelang (n= 14), were used for genotyping of the prolactin gene using polymerase chain reaction (PCR) amplification and direct sequencing method. The primers used were primer forward: 5'- TGCAAACCATAAAAGAAAAGA-3' and reverse: 5'-CAATGAAAAGTGGCAAAGCAA-3'. Two single nucleotide polymorphisms (SNPs) in intron 4 of the prolactin gene were detected: C5796A and T5817C. The frequencies of the 5796C (0.85) and 5817T (0.85) alleles were highest in the total population. For the C5796A locus, the CC genotype had the highest frequency (0.69), followed by CA (0.31), without AA genotype. For the T5817C, the TT genotype had the highest frequency (0.69), followed by TC (0.31), without CC genotype. The genotype frequency distributions in all breeds at every locus were in Hardy-Weinberg equilibrium ($P > 0.05$). Future studies could further expand the effect of the SNPs in the prolactin gene on economically essential traits, especially egg production in ducks.

Keywords: Prolactin gene; Genetic; Polymorphism; Indonesian; Ducks

1. INTRODUCTION

Local duck is one of Indonesia's germplasm, and most of them are raised as egg producers [1]. The population of local ducks in Indonesia currently reaches 58.2 million heads. In Indonesia, the egg production rate has increased from 2019 by 328.686 tons to 332.907 tons in 2020 [2]. The increased number of duck egg production indicated that the community and the food industry's use and consumption of local duck eggs have increased. Ducks contribute 13.4% to the total poultry egg production in Indonesia. It shows that ducks play an essential role as part of protein sources. Hence, it is necessary to increase duck' egg production.

The production and quality of duck eggs are strongly influenced by the maintenance system carried out by breeders. Besides, genetic factors are one of the factors that affect egg production and egg quality. Nowadays, genetic selection using molecular markers of a gene has been widely used [3]. One candidate gene that affects egg production and poultry reproduction is the prolactin

gene (PRL) [4]. The PRL gene is synthesized mainly in the anterior pituitary in all vertebrates [5]. The PRL gene is one of the growth hormone gene families that control variations in egg production by reducing egg biosynthesis during the brooding period [6]. This gene consists of 5 exons and 4 introns, encoding 229 amino acids [4].

Several studies have been focused on the association of PRL gene polymorphisms and economical traits. Previously, studies on the PRL gene had been widely carried out in Chinese duck, Khaki Campbell Ducks, and Italian duck [5,7,8]. In addition, the study of the PRL gene was also conducted in several breeds of Indonesian local duck, i.e., Bayang, Tegal, Magelang ducks [9,10]. Li et al. [11] reported mutation T1326C in the intron 1 region had a positive association with egg weight in Gayou duck. Wang et al. [5] found SNP C-5961T polymorphism at exon 5 was significantly associated with egg production and egg weight in local ducks in China. Chang et al. [12] found six SNPs in

Brown Tsaiya ducks (T233C, T295CG, G309T, C381A, G3941T, A 3957C) located in the non-coding region, and all those SNPs were associated with egg weight at 40 weeks of age and fertility rate except for SNP T295C. However, the analyses of the prolactin gene in Indonesian local duck breeds (Bayang, Turi, and Magelang) were not much reported. Therefore, the objective of this study was to investigate the polymorphisms of the prolactin gene in three local Indonesian duck breeds.

2. MATERIAL AND METHODS

2.1. Material

The material used in this study was blood samples of female Indonesian local duck breeds. A total 65 individuals consist of three duck population, namely Bayang (n = 25), Turi (n = 26), Magelang (n = 14) ducks.

2.2. Methods

Finding of polymorphisms in the prolactin gene was conducted in three steps: DNA extraction, amplification, and direct-sequencing. The BioEdit program ver. 7 was used to analyze the sequencing's result and genotyping. The genetic diversity's parameters were calculated by PopGen program ver. 3.2.

2.2.1. DNA Extraction

The DNA was extracted following the standard protocols of gSYNCTM DNA extraction Kit (Geneaid, Taiwan).

2.2.2. Amplification

Four hundred bp PCR products were amplified in Thermo Cycler following Damayanti et al. [13] procedure. Twenty-five microlitre of mixture reaction comprising 9.5 μ L double-distilled water (DDW), 12.5 μ L MyTaq HS Red Mix (Bioline, UK), 0.5 μ L of each primer (forward: 5'-TTCGTTTGGGGCAAGTCAAG-3' and reverse: 5'-GGAAACGCTCACCAACATGT-3'), and 2 μ L of DNA. The amplification conditions were started with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s, and the final extension at 72 °C for 10 min. The PCR products were then visualized in 2%

electrophoresis gel stained with ethidium bromide (EtBr).

2.2.3. DNA Sequencing

Using an automated DNA sequencer, the 20 μ L PCR products were sent to UGM's Central Laboratory for one-direction (forward) sequencing. The sequencing results were then analyzed using Clustal Omega and BioEdit ver 7.0 program to identify the prolactin gene's polymorphism and genotyping each sample. Manual detection of the electropherogram was used to confirm the SNP. Double peaks or different nucleotide peaks detected were detected as the SNP.

2.2.4. Data Analysis

Based on the genotype from each SNP C5796A and T5817C, the genotype and allele frequencies, and χ^2 test for Hardy-Weinberg equilibrium were calculated following Antonius et al. [14] instructions using PopGen ver 3.2.

3. RESULT AND DISCUSSIONS

3.1. Results

Two SNPs, namely SNP C5796A and T5817C found using the sample's sequence alignment (Fig 1). Both SNPs were located in the fourth intron of the prolactin gene. Based on the electropherogram, the CC and CA genotypes (SNP C5796A) and the TT and TC genotypes (SNP T5817C) showed clear peaks (Fig 2 and 3). The result of genetic diversity analysis (allele and genotype frequencies, and chi-square test value) of SNPs C5796A and T5817C in Bayang, Turi, Magelang, and total populations are shown in Table 1. The frequencies of the 5796C (0.85) and 5817T (0.85) alleles were highest in the total population. For the C5796A locus, the CC genotype had the highest frequency (0.69), followed by CA (0.31), without AA genotype. For the T5817C, the TT genotype had the highest frequency (0.69), followed by TC (0.31), without CC genotype. The genotype frequency distributions in all breeds at every locus were all in Hardy-Weinberg equilibrium ($P > 0.05$) in all the breeds.



Figure 1 SNP identification by sample's sequence alignment in Clustal Omega program

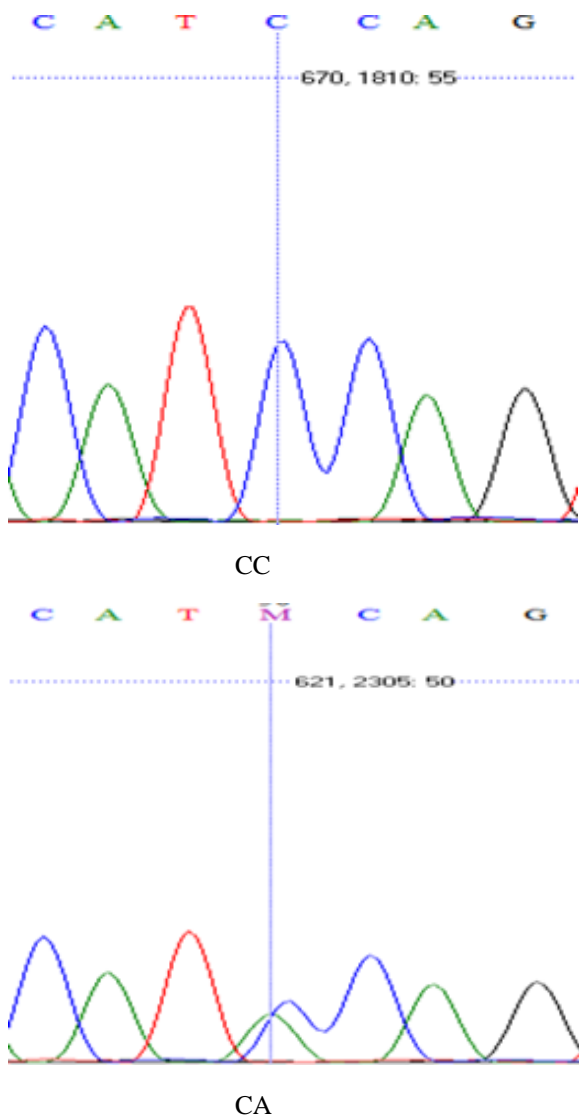


Figure 2 Visualization of CC and CA genotypes of SNP C5796A in the electropherogram.

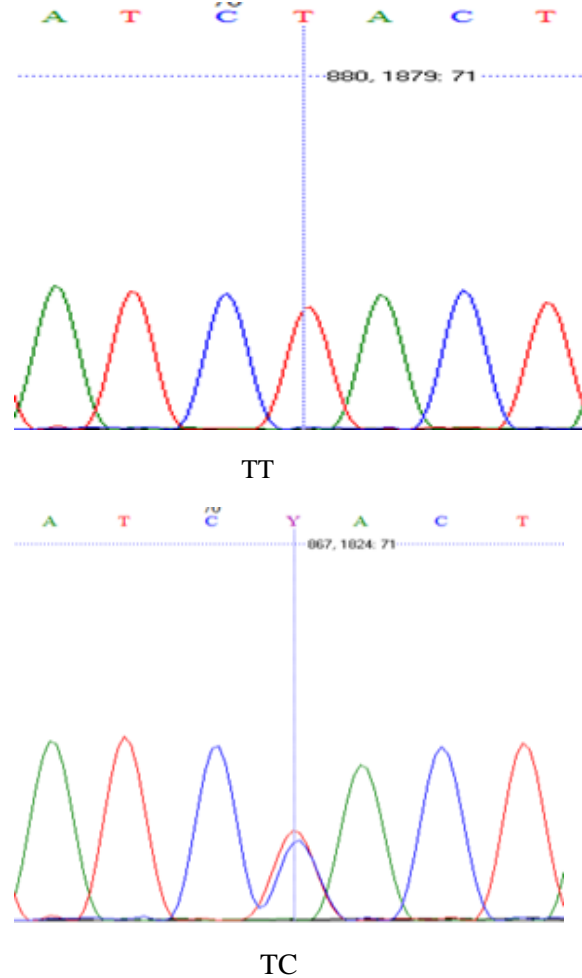


Figure 3 Visualization of TT and TC genotypes of SNP T5817C in the electropherogram.

3.2. Discussions

In Indonesia, duck's egg contributed to fulfilling protein needs in any level of society. Egg production depends on reproduction performance which is categorized as a low-heritable trait. Therefore, studying a candidate gene for reproduction is a vital to understanding the mechanisms to improve egg-laying performance. The prolactin gene is one of the genes associated with egg production in ducks. Two SNPs (C5796A and T5817C) were found in the intron 4 of prolactin gene in the studied Indonesian local duck breeds (Bayang, Turi, and Magelang). Some SNPs in coding and non-coding region within the prolactin gene were reported in the previous study. The SNP T-1326C and A-412G in intron 1 were reported significantly associated with egg production and egg weight [15]. In 5'-proximal, also a non-coding region, three SNPs (A-410G, G-268A, and T-226A) have a positive association with egg production in geese [16]. These results indicated that even though the mutations were in the non-coding region, they could still affect the phenotype. Chorev et al. [17] stated that intron is part of the gene

that does not code the amino acids. However, the intron is involved in each step of mRNA processing, such as transcription initiator, transcription terminator, time delays in a transcribed intron, transcription regulation, alternative splicing, and RNA stabilizer. SNPs in non-coding regions affect gene expression by affecting regulatory elements, and some intronic SNPs activate cryptic splice sites, leading to alternative splicing [18]. Thus, further study is needed to explore the functions of these non-coding SNPs.

The CC genotype had the highest frequency (0.69) in this study, followed by CA (0.31) for the C5796A locus. The TT genotype had the highest frequency (0.69), followed by TC (0.31) for the T5817C locus. The 5796-AA and 5817-CC genotypes were absent in all studied samples. The 5796C (0.82 – 0.86) and 5817T

(0.82 – 0.86) alleles had the highest frequencies than 5796A (0.14 – 0.18) and 5817C (0.14 – 0.18) in Bayang, Turi, and Magelang ducks. This result was in line with Wang et al. [5] that found C and T alleles were dominant in the F1 stock of China native ducks. Yurnalis et al. [9] reported in different SNP, PRL|DraI mutation, the C allele (0.80) was higher than the T allele (0.20) in female Bayang ducks. In an enormous population, a polymorphic site is considered an SNP if it has less than 99 percent allele frequency or less than 95 percent in the small population [19]. According to the χ^2 test value, the sample population (Bayang, Turi, and Magelang) was in agreement with Hardy-Weinberg's equilibrium ($\chi^2 < 5.59$). The genetic diversity based on SNPs within-population will be constant between generations as long as there is no mutation, migration, selection, and controlled mating.

Table 1. Allele and genotype frequencies and chi-square test's value of SNP C-5796A and T-5817C in Bayang, Turi, and Magelang ducks.

Breed	SNP	Genotype Frequency		Allele Frequency		χ^2
Bayang	C-5796A	AA	0.00	A	0.14	0.56
		CC	0.72	C	0.86	
		AC	0.28			
	T-5817C	CC	0.00	C	0.14	0.56
		TT	0.72	T	0.86	
		CT	0.28			
Turi	C-5796A	AA	0.00	A	0.15	0.74
		CC	0.69	C	0.85	
		AC	0.31			
	T-5817C	CC	0.00	C	0.15	0.74
		TT	0.69	T	0.85	
		CT	0.31			
Magelang	C-5796A	AA	0.00	A	0.18	0.51
		CC	0.64	C	0.82	
		AC	0.36			
	T-5817C	CC	0.00	C	0.18	0.51
		TT	0.64	T	0.82	
		CT	0.36			
Total population	C-5796A	AA	0.00	A	0.15	2.03
		CC	0.69	C	0.85	
		AC	0.31			
	T-5817C	CC	0.00	C	0.15	2.03
		TT	0.69	T	0.85	
		CT	0.31			

4. CONCLUSION

In conclusion, two new SNPs of prolactin gene were detected in Indonesian local duck breeds. The SNP C5796A and T5817C were located in intron 4 region. The 5796C and 5817T alleles frequency was higher than the 5796A and 5817C alleles. The 5796-CC and 5817-TT genotypes has the highest genotype frequency. The distribution of allele and genotype frequencies were in Hardy-Weinberg equilibrium in all tested population.

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