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Association of Prolactin Gene with Egg Production in PMp Ducks

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ABSTRACT: A new strain of duck, namely PMp duck as broiler type, has been developed recently in Indonesia. PMp duck was the result of crossbreeding between Pekin and white Mojosari ducks and followed by *inter-se* mating for 4 generations. A selection program based on the production of eggs was conducted on this PMp duck to prepare it as female parental line that would be crossed with male Muscovies to produce mule ducks. Accuracy of selection can be improved if genes controlling the egg production are known. Although in exon 5 and 3' flanking region of prolactin gene has been known to play a role in egg production and egg weight of Peking duck, in PMp ducks have not yet been carried out. The study aim was to investigate the relationship between PRL genotypes of single nucleotide polymorphism (SNP) and egg production of PMp ducks. Primer pairs of exon 5 of the prolactin gene were designed based on the duck genomic sequence database. Polymorphisms were detected by the direct sequencing technique. Two SNPs in the 3' flanking region of prolactin gene were identified, namely T6049- and T6052A. The result of the association analysis between SNPs and egg production showed no significant effect (P> 0.05). It can be concluded that there were SNPs in 3' flanking region of prolactin gene on PMp duck, but they cannot be used as marker genes for egg production, because all genotypes showed the same level of production. Further investigations on more duck populations with large sample sizes are needed to confirm this finding.

Keywords: Prolactin gene, SNP, PMp duck, egg production

INTRODUCTION

A new strain of duck, namely PMp duck as broiler type, has been developed recently in Indonesia. PMp duck is the result of crossbreeding between Pekin and white Mojosari ducks and followed by inter-se mating for four generations. Pekin duck is a commercial duck from China that specialized as broiler ducks. In two months, body weight of Pekin duck can reach 2-3 kg. Mojosari duck is one of the Indonesian native local duck from East Java. In six months, body weight of Mojosari is only 1.7 kg. A genetic approach to cross Pekin and Mojosari ducks for producing crossbred is one of the methods to improve meat performance of Indonesian local duck. Duck PMp has a body weight of about 2-2.2 kg at the age of 10 weeks. However, its egg production is still relatively low with a coefficient of variation of egg production relatively is high, so the selection is required to increase egg production.

A selection program based on the production of eggs is conducted on this PMp duck to prepare it as a female parental line that would be crossed with male Muscovies to produce mule ducks. The accuracy of selection can be improved if genes controlling the egg production are known. Chang *et al.* (2012) reported that genetic markers linked to loci influencing economically important traits could be used to enhance the speed and effectiveness of progress in animal breeding. Once an association between DNA polymorphism and a trait is found, the DNA polymorphism can be considered a candidate genetic marker for marker-assisted selection (MAS) programs. One of the

candidate genes that control of importance traits economically in poultry is the prolactin gene. This prediction is based on the function of the hormone prolactin that was crucial in poultry namely to encourage of hatching behavior and regulate of follicular development (Susanti *et al.,* 2012). On chickens, research on prolactin genes that linked to traits production had been widely reported.

Unlike in chickens, research on ducks prolactin gene has not been done yet, especially at the local ducks Indonesia. However, overall prolactin gene of duck has been sequenced as shown in Figure 1.

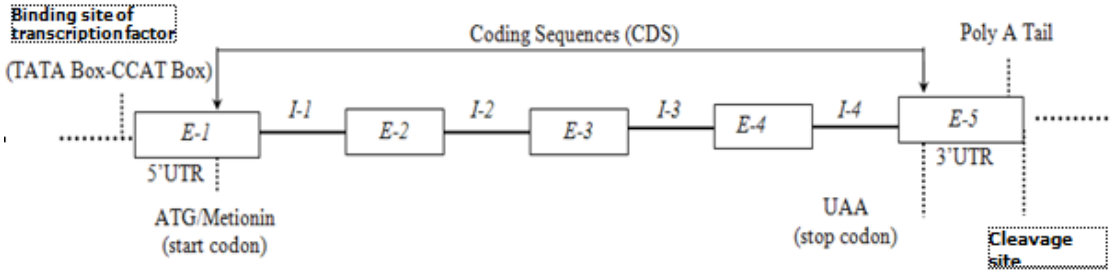

Figure 1. Illustrations portions of prolactin gene in ducks (modified from Kansaku *et al.*, 2005)

The duck prolactin gene had length 6.33 kb and were composed of five exons and four introns, encoding 229 amino acids; and the duck prolactin cDNA shares 92.0, 91.7 and 91.4% sequence homology to chicken, turkey and quail prolactin respectively (Kansaku *et al*., 2005). This prolactin gene sequences could provide the basis for investigating the effect on gene traits based on polymorphisms - trait association. Wang *et al*. (2011) also had conducted an analysis of prolactin gene in 6 types of ducks that come from China and discovered the existence of 12 single nucleotide polymorphism (SNP) in the 5 'flanking region, intron 1, exon 2, intron 2, exon 4, intron 4, exon 5, and 3 'flanking region. These studies mostly using peking duck as material research that could be categorized as a stable strain as selection results. While in Indonesia the ducks with phenotypic diversity was still very high. It was necessary to be done own research on the diversity of prolactin gene and its association with the properties of production and reproduction. In this paper would discuss the association of exon 5 of prolactin gene with egg production on PMp ducks.

MATERIALS DAN METHODS

The materials in this study were 3 breed ducks that were kept in the Indonesian Research Institute for Animal Production (IRIAP). They consisted of 25 Peking duck, 22 white Mojosari and 47 the crossbred PMp. Peking duck and white Mojosari were kept in a litter cage, so that the records of egg production individually were not available. While the PMp ducks were kept in a cage, so that every individual can be observed their egg production. Feed given equal between Peking duck, white Mojosari and PMp namely the feed for laying ducks with a protein content of 19% and EM 3200 kcal/kg. The drinking water was provided ad libitum. Phenotypic observations consisted of egg production was carried out for 73 weeks and it was be associated with the diversity of exon 5 of the prolactin gene.

The observation of prolactin gene diversity, genomic DNA was extracted from whole blood of the Pekin, Mojosari putih and PMp ducks using Genomic DNA Mini Kit (GeneAidTM DNA Isolation Kit) according to the manufacturer's protocol. Kit contained RBC lysis buffer, cell lysis buffer, protein removal buffer and DNA hydration buffer. DNA qualities were evaluated by

spectrophotometer (purity and concentration).

Amplification of prolactin gene was carried out using Polymerase Chain Reaction technique. Amplification of exon 5 was done by KAPA®PCR Kit. Composition of the PCR reaction were 25 ml of PCR kit master mix, 23 ml of distilled water, 1 ml of sample DNA and primers 1 ml, so that the final volume were 50 ml. The conditions of PCR was 2 min pre-denaturation at 94°C, 30 s denaturation at 94°C, 20 s annealing at 60°C, elongation of 40 s at 72°C and a final extension stage 10 min at 72°C.

The design of primer was necessary stage prior to the amplification of exon 5 of prolactin gene on ducks. In this study, the primary design was done with the help of a web-based program namely the Integrated DNA Technology (IDT DNA), Primer 3, and the National Center for Biology Information (NCBI). One pair of gene specific primer was designed based upon the published prolactin sequence gene of duck (Genbank accession no: AB158611). The primer information was listed in the Table 1.

Table 1. The primer sequences and product size of exon 5 of duck prolactin gene

Code	Primer sequences (5' – 3')	Product size (bp)	Amplification region	Annealing Temperature (°C)
PRL - F5	GCATTCCTCAAGGCCAGTAT	343	5844 - 6035	60
PRL - R5	TGGCAAAGCAACAAGAACAC			

Visualization of PCR product was analyzed by gel electrophoresis. Gel electrophoresis contained agarose gel 1.5% in TBE 0.5X with 2µl fluorosave. Universal ladder (KAPPATM) was used as a DNA marker. Gels were run out at 100V for 30-45 min. Individual banding patterns were determined under visible light by using UV transiluminator. A total of 30 µl of PCR product from each PCR samples were sequenced for forward sequence in Macrogene, Korea.

Single nucleotide polymorphisms were identified by comparing individual alignment to current prolactin published sequence for *Anas plathyrinchos* (Code Access GenBank: AB158611.1) using *Moleculer Evolutionary Genetics Analysis* (MEGA 5) and chromatograms were individually examined via *BioEdit Sequence Alignment Editor*. Analysis of nucleotide composition was used with BioEdit Sequence Alignment Editor and Moleculer Evolutionary Genetics Analysis (Tamura et al., 2007). Polymorphism – trait association analyses was performed with PROC GLM procedur (SAS Institute, 2002). Association between the SNP and egg traits were analyzed according to the following model: $Yij = \mu + Gi + eij$, where Y is the observed values of egg number traits; µ is the population mean, Gi is genotype and e is random error.

RESULT AND DISCUSSION

DNA purity and concentration from blood method were high, there were 1.81 and 169 µg/ml respectively. DNA extracts from blood showed in light bands (Figure 2). The results of analysis based program MEGA5 have been revealed two SNPs in the 3' flanking region of prolactin gene of duck PMp namely T6049- and T6052A (Figure 3).

The point mutation of 6049 and 6052 were in the 3' flanking region and they were also called non-coding regions. SNPs located in non-coding regions have effects on gene expression

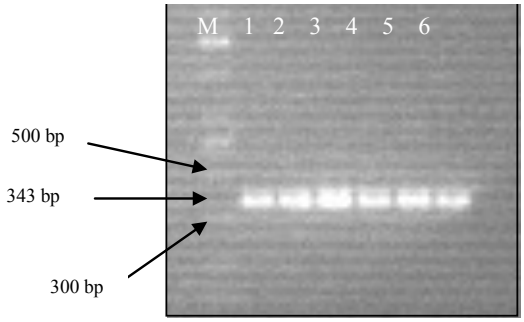

Figure 2. The PCR visualization on the part of samples of exon 5 of prolactin gene

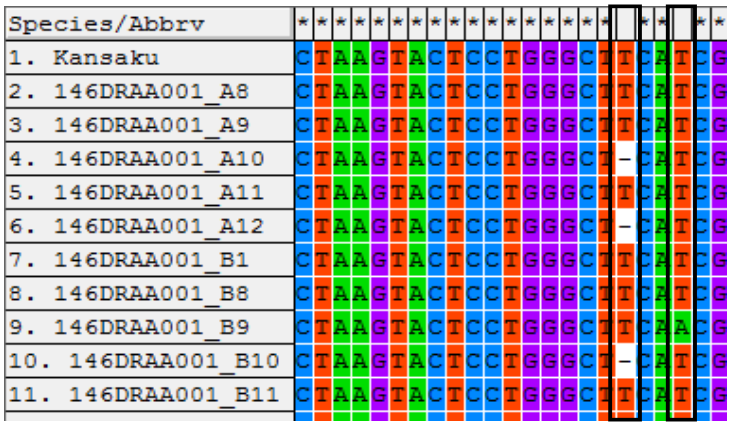

Figure 3. The part of samples that show the mutation point at T6049- and T6052A

By affecting regulatory elements and some intronic SNPs actives cryptic splice sites, leading to alternative splicing (Alberobello *et al*., 2011). The results of the study were virtually identical as that found on local ducks China. Wang *et al*. (2011) found a mutation point in the 3 'flanking region namely T6052A on local ducks in China. The mutation point was exactly the same as those found in ducks PMp as the local ducks in Indonesia. Wang *et al*. (2011) also found other mutation point in exon 5 namely C5961T and shown to have an influence on the number and weight of eggs produced by the Chinese local ducks. However, the mutation point was not found in ducks PMp.

Based on the current results, all SNPs in non-coding regions were found and the associations of exon 5 of prolactin genotype combinations with egg number were identified. The results of association analysis between the single SNPs and the phenotypic traits in the PMp ducks were presented in Table 2.

Table 2. The average egg production of PMp duck for 73 weeks based on genotype of mutation point T6049- and T6052A

The point mutation	Genotype	Egg production (egg)
T6049-	Delesi T (n = 9)	138.12a
	TT (n = 33)	179.56a
T6052A	TT (n = 4)	143.9a
	TA (n = 2)	252.0a
	AA (n = 36)	149.8a

Based on Table 2, there is no real relationship between the mutations that occur with egg production in PMp ducks. The absence of relationship is likely due to the number of samples used little or as a result of inbreeding. As it is known that the PMp ducks as material in this study is the fourth generation from crosses inter-se, so that inbreeding was a very high possibility. Genesis inbreeding can result in reduced genetic diversity in a population (Muir and Aggrey, 2003).

CONCLUSION

In the present study has been found two SNPs of exon 5 of prolactin gene namely T6049- and T6052A. The results of analysis, there was no association between the SNPs found with PMp duck egg production. That can be concluded that there were SNPs in 3' flanking region of prolactin gene on PMp duck, but they cannot be used as marker genes for egg production, because all genotypes showed the same level of production. Further investigations on more duck populations with large sample sizes are needed to confirm this finding.

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