

The association of prolactin gene polymorphism with egg production traits in Alabio and Mojosari ducks

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Received April 4, 2022 ; Accepted July 5, 2022

ABSTRACT

Prolactin (PRL) affects egg production in duck as it induces broodiness and promotes follicles development. The objective of this study was to investigate the association between polymorphism of PRL gene and egg production traits in Alabio and Mojosari Ducks. Genomic DNA were isolated from 111 blood samples (51 Alabio and 60 Mojosari). PCR and sequencing were performed to identify polymorphisms and genotype of the animals. Data recording of the ducks including body weight at 16 weeks of age and at first egg, average weight of three first eggs laid and egg number laid up to 3, 6 and 12 months production were collected. Data were analyzed using independent sample t-Test. As a result, two single nucleotide polymorphisms (SNP) were detected in intron 4. Both SNP C-5796A and SNP T-5817C were found to have association with egg number laid up to three months ($P < 0,005$). The CA/TC genotype had higher egg number than CC/TT. These suggested that SNP C-5796A and SNP T-5817C could be potential markers for marker assisted selection to increase egg number in duck.

Keywords: Alabio, Mojosari, Prolactin, SNP, Egg production

INTRODUCTION

Prolactin (PRL) is a single chain polypeptide hormone that belongs to a group of growth hormones, mainly produced in the anterior pituitary gland and synthesized through gene transcription and mRNA translation in lactotrophs or somatotrophic cells (Kansaku *et al.*, 2008). PRL is involved in many biological functions in all vertebrates (Wang *et al.* 2011) and there are reported to be 300 functions of this hormone (Bole

-Feysot *et al.*, 1998). Studies in chickens indicate that the PRL gene is expressed in the hypothalamus, pituitary, oviduct and ovary (Wilkanowska *et al.*, 2014). The highest expression is observed in the pituitary gland (Li *et al.*, 2009). PRL gene transcription has been reported to be activated by the Pit-1 factor, but the mechanism is not yet clear (Ohkubo *et al.*, 2000). Kaggya *et al.*, (2012) reported that PRL secretion in poultry is mostly regulated by releasing factors consisting of vasoactive intestinal peptide (VIP),

dopamine (DA) and serotonin (5-HT). Vasoactive intestinal peptide (VIP) is secreted by the hypothalamus, VIP stimulates the release and expression of prolactin genes both in vitro and in vivo in poultry.

A high correlation between egg production and prolactin hormone level on duck was found (Susanti *et al.*, 2012). The level of PRL has a negative correlation with egg production, estradiol and progesterone (Reddy *et al.*, 2002). This hormone known to induce broody behavior in aves (Li *et al.* 2011). The plasma concentration of PRL in chicken increases during laying period and reaches its peak during brooding and then decreases after the hatch of the offsprings. The level of PRL mRNA in the anterior pituitary correlates with plasma PRL in the anterior pituitary (Kansaku *et al.*, 2005; Alipanah *et al.*, 2011). High concentration of PRL hormone will inhibit the function of pituitary gland, decreasing production of gonadotrophin hormone (follicle stimulating hormone and luteinizing hormone) then ovulation ceased. However, PRL is also reported to be an important hormone that regulates the follicular formation. It is therefore highly probable that PRL stimulates egg laying through enhancing LHR expression in follicular cells during ovarian follicular development (Li *et al.*, 2011).

PRL gene in ducks was cloned and sequenced by Kansaku *et al.* (2005). This gene consists of 5 exons and 4 introns, encoding 229 amino acids. Several studies have been focused on the association of PRL gene polymorphisms and phenotypes. Li *et al.* (2009) reported mutation T1326C in intron 1 region had positive association with egg weight in Gayou duck. Wang *et al.* (2011) 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.* (2012) found six SNPs in Brown Tsaiya ducks (T233C, T295CG, G309T, C381A, G3941T, A 3957C) located in non-coding region and all those SNPs were associated with egg weight at 40 weeks of age and fertility rate except for SNP T295C. SNP C2402T and C-2161G were significantly associated with egg production (number) and laying rate in native chicken in Iran (Bagheri *et al.*, 2013).

Ducks contribute 13.4% to the total poultry egg production in Indonesia (Directorate General of Animal Husbandry and Health, 2018). It shows ducks play important role in egg production in Indonesia. However it is important to increase duck contribution to egg production. Local ducks are important egg producers, thus improvement of egg production is still a need. Alabio and Mojosari ducks are Indonesian local ducks, which have been included as national germplasm by the Indonesian Ministry of Agriculture, with reference number of 2921/Kpts/OT.140/6/2011 (Alabio duck) and 2837/Kpts/LB.430/8/2012 (Mojosari duck) (Ministry of Agriculture, 2009; Ministry of Agriculture, 2011). Both of the ducks have been widely raised by smallholder in some regions for egg production. Recently, the use of molecular genetic technology is an important parts of the genetic quality improvement program for animals. A popular molecular genetic technology is marker assisted selection (MAS), which is a selection method based on phenotypic data information combined with genetic markers.

This study aimed to observe the association between polymorphism of PRL gene and egg production trait Alabio and Mojosari. The results then can be used as potential marker for selection to increase egg production in Indonesia local ducks.

MATERIALS AND METHODS

Ethics Statement

The methods for animals experiment were reviewed and approved by Ethics Committee of Faculty of Veterinary Universitas Gadjah Mada Yogyakarta (No. 0133/EC-FKH/Eks./2019).

Animals and DNA Isolation

Blood samples were collected from 60 Mojosari and 51 Alabio Duck in BPTU-HPT Pelaihari South Kalimantan. Genomic DNA was isolated from blood sample using SYNCTM DNA Extraction Kit (Geneaid, Taiwan). All the duck were 6th generation of a breeding program selected for egg production at BPTU-HPT Pelaiha-

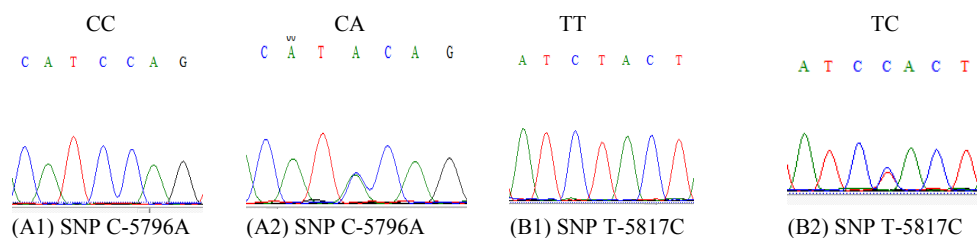


Figure 1. SNP identification of PRL gene in Mojosari and Alabio duck based on electrophoregram

ri South Kalimantan since 2009. The animals were individually identified. Rearing was carried out in groups until 16 weeks old, then transferred to individual cages to prepare for laying. Data records including body weight at 16 weeks of age (BW16) and at first egg (BW1stEGG), age of first egg (AGE1stEGG), average weight of three first eggs laid and egg number were collected. Total number of eggs produced during laying phase was classified into three categories including total number of eggs produced from months 1 to 3 (EN3), 1 to 6 (EN6), and 1 to 12 (EN12). In this study, the total number of eggs produced in a week, a month, and a year were the total number of eggs produced for seven days, four weeks (28 days), and twelve months (48 weeks or 336 days), respectively.

PCR and SNP Identification

PCR was performed to amplify the PRL Gen using primer forward : TGCAAACCA-TAAAAGAAAAGA and reverse: CAATGAAAAGTGGCAAAGCAA (Wang et al. 2011) located partially in between intron 4 and exon 5, based on Genbank no AB158611. The 20 µl PCR mixture included 2 µl 10x buffer, 1,6 µl dNTP, 0,2 µl primer taq, primer forward and reverse 0,8 µl of each , 12 µl DDW and 2 µl DNA genome. PCR protocol was as follows 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at annealing temperature 52 °C, 30 s at 72 °C, and a 10 s final extension at 72 °C Wang et al. 2011). The 400 bp PCR-products were electrophoresed on 1,5% agarose for visualisation and stained using Ethidium bromide. The result then documented using digital camera.

Sequencing of the 111 individual PCR products was then performed in LPPT Universitas

Gadjah Mada Yogyakarta to identify the SNP and genotype of the animals. Alignment DNA sequences of Alabio and Mojosari ducks were carried out using BioEdit to detect the polymorphism of PRL gene. Statistical analysis.

Hardy-Weinberg equilibrium was analyzed using Chi-Square's Person with this following model

$$X^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$$

Where

X^2 : Chi-Square value,

O_i = observed genotype frequency,

E_i = expected genotype frequency and

n = number of data.

The association between genotypes and egg production traits were analyzed by independent sample t-Test using IBM SPSS 8 (IBM).

RESULTS AND DISCUSSION

SNP Identification

In this study, polymorphism of PRL gene was detected using DNA sequencing methods from 111 individual PCR products. Two new SNPs were detected in Alabio and Mojosari ducks. The SNP C-5796A and T-5817C were located in intron 4. Each SNP produced two genotypes. SNP C-5796A has CC and CA genotypes, while SNP T-5817C has TT and TC genotypes (Figure 1.) in both Alabio and Mojosari ducks. Both mutations did not result in amino acid change due to both SNPs were located at intron 4.

In the previously study, Wang et al. (2011) found SNP in exon 5 (C-5961T) which resulted two genotypes CC and CT in six breeds of China

Table 1. The types and number of animals based on SNP C-5796A dan T-5817C

Breed	Number	SNP C-5796A	SNP T-5817C
Alabio	50	CC	TT
	1	CA	TC
Mojosari	54	CC	TT
	6	CA	TC

Table 2. Allelic and genotypic distribution in Alabio and Mojosari for SNP C-5796A

Breed	Total	Number of Genotypes			Allelic Frequency		X ²
		CC	CA	AA	C	A	
Alabio	Observed	50.00	1.00	0.00	0.99	0.01	0.025
	Expected	50.00	0.99	0.00			
Mojosari	Observed	54.00	6.00	0.00	0.95	0.05	0.166
	Expected	54.16	5.70	0.15			

Table 3. Allelic and genotypic distribution in Alabio and Mojosari for SNP T-5817C

Breed	Total	Number of Genotypes			Allelic Frequency		X ²
		TT	TC	CC	T	C	
Alabio	Observed	50.00	1.00	0.00	0.99	0.01	0.025
	Expected	50.00	0.99	0.00			
Mojosari	Observed	54.00	6.00	0.00	0.95	0.05	0.166
	Expected	54.16	5.70	0.15			

native ducks. Wang *et al.* (2011) also found SNP T-3988G and T-4009C in intron 4. Mutation of adenin insertion was found at 2001 bp location in intron two in Pekin, Mojosari and Pekin Mojosari Crossbred (Irma *et al.* 2014). Indriati *et al.* (2016) reported two SNPs INDEL 4031A and G-4110TC in the region of intron 4 in the White Mojosari dan Peking-White Mojosari crossbred duck. Bai *et al.* (2019) detected SNP A-412G in intron 1 of the PRL gene, with three genotypes: AA, AG, and GG in domestic China ducks (Jinding and Youxian). Chuekwon and Boonlum (2016) found SNP -359A in intron 1 and results in 3 genotypes GG, GT and TT in Khaki Campbell duck. However the polymorphisms of PRL gene in Alabio and Mojosari ducks was still unknown previously, thus further studies are still needed to explore the polymorphisms of this gene.

Based on two SNPs found in this study, there were two types of animals with different genotypes combination. The genotype combination were CC/TT and CA/TC for SNP C-5796A dan T-5817C. The types and number of animals

based on SNP C-5796A dan T-5817C were shown in Table 1. This study revealed that frequency of CC / CA genotype frequency was the same as TT / TC genotype frequency.

Allelic and Genotypic Distribution

Alleles and genotypes frequency of the PRL gene were calculated for each SNP in Alabio and Mojosari (Table 2 and 3). C allele was dominant in Alabio and Mojosari with allele frequencies 0.99 in Alabio and 0.95 in Mojosari for SNP C-5796A. The frequency of CC genotype in Alabio and Mojosari was 98.07% and 90.00%, respectively. The frequency of CA genotype was 1.92% in Alabio and 10% in Mojosari. T allele was in dominance in both ducks for SNP T-5817C. The frequency of TT genotype was 98.07% in Alabio and 90.00% in Mojosari. The frequency of TC genotype was 1.92% in Alabio and 10% in Mojosari. C and T alleles were in dominant for both duck. This was in line with the result of Wang *et al.* (2011) that found C and T alleles were dominant in F1 stock of China native ducks. Meanwhile Yurnalis (2019) reported that C and G al-

leles were dominant in Bayang duck in west Sumatera.

In this study indicated that the population of Alabio was monomorphic for SNP C-5796A and T-5817C due to C allele frequency in SNP C-5796A and T on SNP T-5817C are both 0.99. Moreover, the heterozygosity of both Alabio and Mojosari ducks are in low category: 2.06% and 9.50% (SNP 1 & SNP 2), respectively. This may due to continuous selection that have been done for years in the stock of Alabio used in this study. The duck have been selected for egg production in 6 generations. Chi square test results showed that the population of Alabio and Mojosari ducks were in Hardy-Weinberg equilibrium for both SNPs (Table 2 and 3). Association between PRL gene and egg production traits

The result of association analysis between PRL gene and egg production traits shows that SNP C-5796A dan T-5817C were associated with number of egg laid up to 3 months production ($P < 0,05$) (Table 4). Meanwhile there were no association between both SNP with other traits. In this study the frequency of CC/CA genotypes is the same as TT/TC genotypes.

PRL has wide biological functions in vertebrata, in Avian it is an important hormone that induces broodiness. This hormone is also known to promote ovarian follicle growth and egg-laying performance by increasing the expression of LH receptors (LHR) in gonad cells (Li *et al.* 2011). Based on its function PRL is a candidate gene that affects egg production traits. Some studies have shown that SNPs in coding and non-

coding region of the PRL gene have effects on egg production and egg quality. Similary, in this study, two new SNPs (The SNP C-5796A and T-5817C) which located in intron 4, were detected in Alabio and Mojosari ducks. This results is supported by Li *et al.* (2009) that reported the SNP T-1326C in intron 1 region had positive association with egg weight in Gayou duck. Another study also indicated SNP (A-412G) in intron 1 was associated with egg production and egg weight in which GG genotype had significantly greater egg production and egg weight than those with AG and AA genotype (Bai *et al.* 2019). However, Wang *et al.* (2011) indicated that SNP C-5961T located in exon 5 of PRL gene were also associated with annual egg production and egg weight in native China ducks. This mutation causes an AA conversion (Cys to Arg). Jiang *et al.* (2009) also reported a positive association of PRL gene with egg production in SNP A-401G, G-268A and T-266A located at 5'-proximal. The AA genotype had higher egg production than other genotypes. SNP T-884C and T-335C were positively associated with age at first egg, egg number at 59 weeks and egg number at 300 days. T allele of T-335C generates a putative binding site for GATA-2 in silico (Zhang *et al.*, 2015).

The current study showed that SNP C-5796A dan T-5817C in intron 4 region of PRL gene had effect on number of egg laid from first egg up to 3 month production in Alabio and Mojosari duck. Intron is a part of gene which does not code for amino acid, but intron is involved in

Table 4. Association analysis between SNP C-5796A dan T-5817C), and egg production traits in Alabio and Mojosari

Traits	Unit	CC/TT (104)	CA/TC(7)	P
BW16	Gram	1300.88±156.73	1325.71±83.03	0.680
AGE1 st EGG	Weeks	23.81±2.14	24.00±2.82	0.831
BW1 st EGG	Gram	1532.01±242.46	1520.00±79.16	0.897
BW1 st	Gram	59.09±4.53	56.57±4.85	0.159
EN3	Egg	70.63±7.71	76.4±4.09	0.048*
EN6	Egg	138.35±15.81	149.18±8.78	0.083
EN12	Egg	237.97±32.22	259.42±18.08	0.085

BW16 : body weight at 16 weeks, AGE1stEGG : age at first egg, BW1stEGG : body weight at first egg, EW1st : average weight of three first eggs laid, EN3 : number of egg laid from first egg up to 3 month production, EN6 : number of egg laid from first egg up to 6 month production, EN12 : number of egg laid from first egg up to 12 months production

*shows significant association at $P < 0.05$

every mRNA process. It has wide functions in transcription initiation, transcription termination, genome organization, time delays in transcribed intron, transcription regulation and alternative splicing (Chorev and Carmel, 2012). SNPs in non-coding regions affects gene expression by affecting regulatory elements and some intronic SNPs activate cryptic splice sites, leading to alternative splicing (Goto *et al.*, 2001). However, intron can increases gene expression at many different levels, including transcription, polyadenylation, mRNA export, translational efficiency, and the rate of mRNA decay by the act of their removal by the spliceosome (Nott, 2003). Shaul (2017) also reported that in many eukaryotes, introns can increase gene expression without functioning as a binding site for transcription factors. No single mechanisms were reported on by which introns affect gene expression. Different introns have different impacts on expression in different gene. Thus, further study is needed to explore the functions of these SNPs.

The result of association analysis showed that CA/TC genotypes possessed higher egg number laid up to 3 months production (EN 3) than CC/TT genotypes ($P = 0.048$). However estimated genetic correlation between egg number for 3 months (EN 3) and egg number for 12 months (EN 12) was positively high ($rg = 0.80 \pm 0.05$ and 0.95 ± 0.03 for Alabio and Mojo-sari, respectively) (Damayanti *et al.*, 2019). Positive genetic correlation shows linear relationship between EN 3 and EN12. This indicates that increasing EN 3 will increase EN 12 (Warwick *et al.*, 1990). The result observed the egg number laid up to 12-month egg production (EN 12) of CA genotype was higher than CC / TT genotype, although not significantly different.

CONCLUSION

In conclusion, two new SNPs of PRL gene were detected in Alabio and Mojosari duck. The SNP C-5796A and T-5817C were located in intron 4 region and found to have association with egg number laid up to three months. The CA/TC genotype had higher egg number than CC/TT.

This suggested that SNP C-5796A and SNP T-5817C could be potential markers for marker assisted selection for increasing egg number in duck. Conclusion should be written a maximum of 100 words.

ACKNOWLEDGMENTS

This research was supported by LPDP Ministry of Finance (No. FR19122018164960) and RTA (Rekognisi Tugas Akhir) fellowship funded by Universitas Gadjah Mada (No. 3294/UN/DITLIT/DIT-LIT/LT/2019). We would like to appreciate special thanks to BPTU-HPT Pelaihari South Kalimantan.

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