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Diversity of PRL Gene as a Candidate Genetic Marker for Egg Production Performance of Sikumbang Jonti Ducks

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ABSTRACT

This study was conducted to obtain genetic markers in the PRL gene as one of the basic foundations of selection methods for the egg production performance of Sikumbang Jonti ducks. DNA extraction was performed using Promega's Genomic DNA Purification Kit procedure. Amplification of the PRL gene was performed using the Polymerase Chain Reaction (PCR) method using a pair of forward and reverse primers for each fragment. Furthermore, the results of PRL gene amplification were electrophoresed using agarose media, which was as much as 1.5% with Ethidium Bromide staining. Finally, sequencing of PCR products of PRL gene fragments of Sikumbang Jonti ducks was carried out using the services of First BASE Laboratory in Singapore to be analyzed by direct sequencing using Dideoxy Sequencing ABI 3730 XL Automated DNA Sequencer. The results showed that there were six (6) mutations in the PRL gene at the position of intron 2 (g.1997 G>A, g.2090 C>A, g.2300 G>C), exon 4 (g.3778 T>A), and exon 5 (g.5929 C>A, g.5963 G>A) which were in Hardy-Weinberg equilibrium and associated with total egg production and length of egg stopping time. So, it can be concluded that the diversity of the PRL gene is used as a candidate genetic marker for egg production of Sikumbang Jonti ducks.

Key words: Local Duck, Indonesian, Performance Reproduction, Sequencing

INTRODUCTION

One factor that can affect egg production in ducks is the prolactin (PRL) hormone. PRL hormone has an ovarian regression effect on ducks, a condition of lysing the ovary or follicle so that ovulation does not occur. This is because PRL hormone levels that exceed the needs can inhibit the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. GnRH is a hormone that stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) needed for follicular development and oviposition. Low levels of LH and FSH in the body can cause follicles not to develop and be reabsorbed by the body. However, the PRL hormone is also needed to form the egggland shell during production (Susanti et al. 2012). In addition, the PRL hormone also affects the length of the process of stopping eggs and the speed of re-starting laying eggs. Based on this dual function of PRL hormone, it can be concluded that PRL hormone plays a vital role in egg production in ducks. Based on this, PRL hormone levels in the body can affect the egg production of Sikumbang Jonti ducks. PRL hormone is a peptide hormone influenced by the PRL gene as a trait carrier. Thus, the diversity in the PRL gene is thought to affect the diversity in egg production of Sikumbang Jonti ducks. Therefore, selection that can increase the egg production of Sikumbang Jonti ducks is selection using genetic markers, especially in the PRL gene. Arlina et al. (2024) and Suardana dan Suyasa (2024) stated molecular markers can identify genetic differences directly at the DNA level as a genetic component because all characteristics displayed by individuals reflect the gene characteristics possessed by these individuals.

Genetic markers mark genes or DNA sequences linked to a trait usually described as an observed variation. Selection using genetic markers is an efficient method because the selection process can be done early without waiting for phenotype results. Moreover, the selection method using genetic markers can be passed on to offspring so that the next population generation does not need to be selected again. Suhartati et al. (2020), stated advances in molecular genetic biotechnology through genetic markers based on functional genes can be an effective, accurate, and efficient alternative for selection. Yurnalis et al. (2024)

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stated molecular livestock selection is done by evaluating the nucleotide sequence profiles of genes in the DNA that affect livestock productivity. In addition, the results of Li et al. (2020) of real-time PCR of the PRL gene were expressed in the hypophyseal glands, ovaries, and uterus during ducks' egg-laying and brooding periods. This supports that the PRL gene does work during these periods. Thus, the PRL gene can be used as a candidate gene for selection in duck egg production.

Yurnalis et al. (2019) stated that there was diversity in the PRL gene in local ducks in West Sumatra. In addition, Mazurowski et al. (2016) stated that the PRL gene can be used as a candidate for duck genetic markers. This is also supported by some previous research results, which show that the PRL gene in intron 1 (Chuekwon and Boonlum 2017) and exon 5 (Wang et al. 2011; Ghanem et al. 2017) is associated with duck egg production traits. However, research on the PRL gene as a genetic marker in local ducks in Indonesia is still very minimal, so further research needs to be done to find genetic markers in the PRL gene for local duck egg production in Indonesia, especially in Sikumbang Jonti ducks as a way to fulfill the availability of broiler duck breeds in West Sumatra.

On the other hand, research related to the PRL gene as a selection method in local Indonesian ducks, especially in Sikumbang Jonti ducks, has not been studied. So it is necessary to conduct research to obtain genetic markers in the PRL gene as one of the basic selection methods for the egg production performance of Sikumbang Jonti ducks.

MATERIALS AND METHODS

Ethical approval

Animal experiments were conducted following the Republic of Indonesia Law No. 18 of 2009 (section 66), which addressed animal keeping, raising, killing, and proper treatment and care.

Materials

The tools used were a 2mL Eppendorf tube, micropipette, centrifuge (ThermoSCIENTIFIC® HERAUEUS Pico17centrifuge), vortex (TRADERaypa® MIXTUB), 0.2mL PCR tube, micropipette, PCR machine (Eppendorf® Mastercycler gradient), electrophoresis machine (ThermoSCIENTIFIC® OWL A5), and UV Trans Illuminator machine (SynGENE® G: BOX).

The materials used were blood samples of female Sikumbang Jonti ducks, cell lysis solution (Promega® REF A793A), nucleus lysis solution (Promega® REF A7941), protein precipitation solution (Promega® REF A795A), isopropanol (MERCK®), 70% ethanol (MERCK®), DNA rehydration solution (Promega® REF A796A), sterile wipes, master mix (ThermoSCIENTIFIC®), nuclease-free water (Promega® REF P1193) and PRL gene forward and reverse primers (Table 1; Fig. 1), TBE Buffer (ThermoSCIENTIFIC®), Agarose (ThermoSCIENTIFIC® TopVision Agarose # R0491) and Ethidium Bromide solution (MP Blomedicals®).

Methods

DNA extraction was performed using Promega's Genomic DNA Purification Kit procedure. Next, the PRL gene was amplified using the PCR (Polymerase Chain Reaction) method using a pair of forward and reverse primers for each fragment. PRL gene amplification reagent using a master mix. In-vitro amplification was performed using a PCR machine programmed with pre-denaturation at 95°C for 5min, denaturation at 95°C for 45s, annealing at the temperature according to the primer for 45s each, extension at 72°C for 1min, and final extension at 72°C for 5min. The denaturation to extension cycle was repeated for 35 cycles. Furthermore, the results of PRL gene amplification were electrophoresed using 1.5% agarose media with Ethidium Bromide staining for evaluation. Finally, the sequencing of PCR products of PRL gene fragments of Sikumbang Jonti ducks was carried out using the services of First BASE Laboratory in Singapore for direct sequencing analysis using the Dideoxy Sequencing ABI 3730 XL Automated DNA Sequencer.

Genotyping results of PRL gene fragment sequencing were aligned using the MUSCLE technique in the MEGA X program (Kumar et al. 2018) with reference sequences according to access codes JQ677091.2. The mutated nucleotide sequencing results were viewed using the FinchTV1.4.0 program (Geospiza, Inc.).

Data analysis

The results of allele data were analyzed in the form of allele frequency using the formula. (Noor 2008):

$$x_i = \left(2n_{ii} + \sum_{j \neq i} n_{ij} \right) / 2n$$

Description:
xi = frequency of allele i
nii = number of individuals with genotype ii (homozygous)
nij = number of individuals with genotype ij (heterozygous)
n = number of samples

Deviations in genotype frequency from Hardy-Weinberg equilibrium were analyzed using the chi-square ($\chi 2$) test based on the formula (Noor 2008):

$$\chi^2 = \sum_{i=1}^{k} \frac{(o_i - e_i)^2}{e_i}$$

Description:
$\chi 2$ = chi square distribution
o_i = observed frequency i
e_i = expected frequency i

Association analysis of PRL gene diversity on total egg production and time to stop laying using the General Linear Model method processed manually using Ms. Excel (Microsoft) with the following equation:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij}$$

Description:
Y_{ij} = egg production performance
μ = general mean
G_i = effect of allele i
ε_{ij} = effect of treatment error

RESULTS AND DISCUSSION

Diversity of PRL gene

The results of the PRL gene diversity study in Sikumbang Jonti ducks found six (6) mutations, namely at the position of intron 2 (g.1997 G>A, g.2090 C>A, g.2300 G>C), exon 4 (g.3778 T>A), and exon 5 (g.5929 C>A, g.5963 G>A). The PRL gene mutations of Sikumbang Jonti ducks are presented in Fig. 2 to 7.

Table 1: Prolactin gene primers in the study

Fragment	Primer Name	Primer Sequence (5'-3')	Length Product (bp)	Tm (ºC)
Exon 1	PRLE1DL	AGCTGCCGTTATCCTTCTCT	694	61
	PRLE1DR	ACTCGAAAACTGGATGTCTTGG		
Exon 2	PRLE23DL	TCAAGAGTCAGCACAGGAGA	939	61
Exon 3	PRLE23DR	TGTCAGCAAATGTCTTTTCAGTG		
Exon 4	PRLE4BDL	TGCTCTAAATGCCTCCTAACAC	385	59
	PRLE4BDR	TGAGAACTTTGCAGCTATCTTGT		
Exon 5	PRLE5ADL	CCTCAAGGCCAGTATTTCTTAGT	591	61
	PRLE5ADR	TGGTGTTATGTGGTTTTGGATTT		

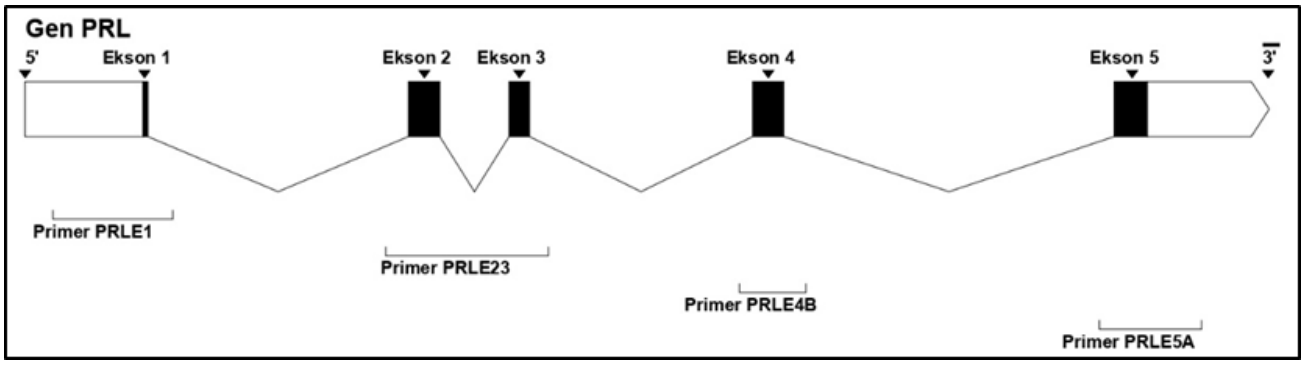

Fig. 1: PRL gene structure reconstruction in ducks (access code: NC_040047.1).

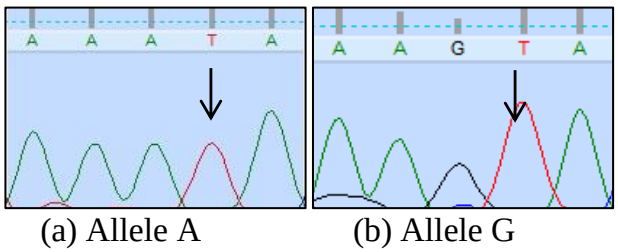

Fig. 2: PRL gene cut chromatogram g.1997 G>A.

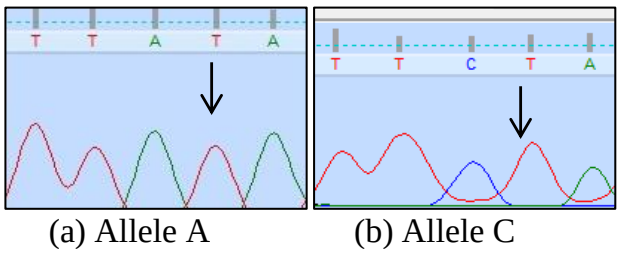

Fig. 3: PRL gene cut chromatogram g.2090 C>A.

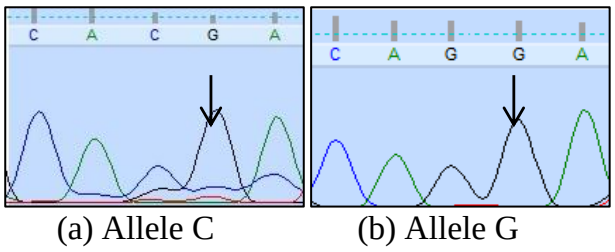

Fig. 4: PRL gene cut chromatogram g.2300 G>C.

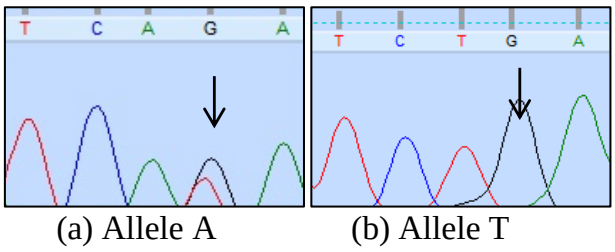

Fig. 5: PRL gene cut chromatogram g.3778 T>A.

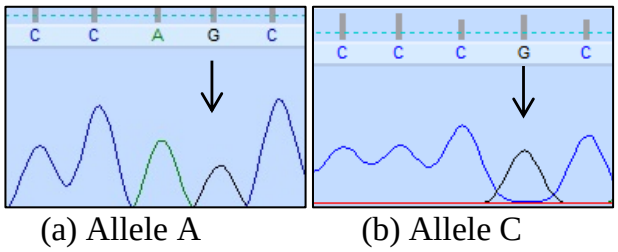

Fig. 6: PRL gene cut chromatogram g.5929 C>A.

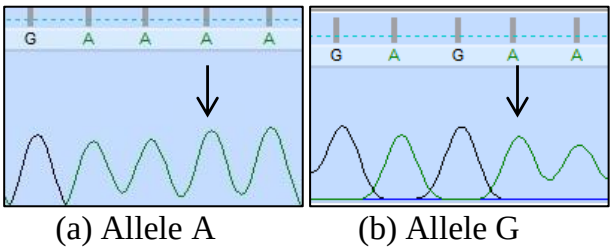

Fig. 7: PRL gene cut chromatogram g.5963 G>A.

The results of this study differ from the results of Rafian et al. (2022), which stated that they did not find mutations in exon 5 of the RPL gene of Sikumbang Jonti ducks. However, the results of this study are the same as the research of Irma et al. (2014) which stated that there was a mutation in intron 2 of the duck PRL gene, Indriati et al. (2016), which stated a mutation in exon 4 of the duck PRL gene and Ghanem et al. (2017), Astuti (2019) and Nguyen et al. (2023), who stated a mutation in exon 5 of the duck PRL gene. Moreover, Wang et al. (2011) also stated the presence of PRL gene mutations in local Chinese ducks at intron 2, exon 4, and exon 5.

On the other side, this study did not find mutations in intron 1 of the PRL gene like the results of previous studies, such as the research of Bai et al. (2019) on local Chinese ducks, the research of Chuekwon and Boonlum (2017) on local British ducks (Khaki Chambell ducks), the research of Susanti and Yuniastuti (2020) on ducks in Central Java, and the research of Yurnalis et al. (2019) on local West Sumatra ducks (Bayang ducks).

The results of allele frequency and Hardy-Weinberg equilibrium value of the PRL gene in the Sikumbang Jonti duck population in this study are presented in Table 2. Based on Table 2, mutations in the PRL gene are diverse. According to Noor (2008) an allele is said to be diverse if it has an allele frequency value below or equal to 0.99. Allele frequency is the ratio of the number of alleles to the total population (Novel et al. 2010). The calculation of the chi-square test shows the diversity in the PRL gene is in Hardy-Weinberg equilibrium. Noor (2008) states that the calculation of chi-square value is used to determine the value of Hardy-Weinberg equilibrium. This indicates that the study population is in a constant state, mating occurs randomly, no mutation occurs, the population size is unlimited, and no genetic drift occurs. Hardy-Weinberg law states that the frequency of alleles and genotypes in the gene pool of a population remains constant over several generations unless certain influences disturb the balance (Novel et al. 2010). This is also supported by the opinion of Wang et al. (2022) and Rehman et al. (2020), who stated that the Hardy-Weinberg equilibrium test can be used to study genetic variation in natural populations. Gene and genotype frequencies will remain constant after random mating episodes if no evolutionary forces are acting on the population. Saputra et al. (2020) stated selection becomes a factor that quickly changes the balance in the population.

Table 2: Allele frequency dan chi^2 test PRL gene of Sikumbang Jonti ducks

SNPs	Allele	Amino Acid	Population	Allele Frequency	Chi^2 test
1997	G	-	32	0.73	ns
	A	-	12	0.27	
2090	C	-	24	0.55	ns
	A	-	20	0.45	
2300	G	-	28	0.64	ns
	C	-	16	0.36	
3778	T	Leucine	33	0.75	ns
	A	Glutamine	11	0.25	
5929	C	Proline	22	0.50	ns
	A	Glutamine	22	0.50	
5963	G	Glutamate Acid	30	0.68	ns
	A	Lysine	14	0.32	

Note: ns = not significant

Noor (2008) stated that the influences that disturb the Hardy-Weinberg equilibrium are non-random mating, mutation, selection, migration, limited population size, and genetic drift. Selection is the process of determining which livestock can be bred in the next generation to increase the desired frequency and decrease the frequency of unwanted genes. Mutation is a change in gene chemistry that results in changes in gene function, and genetic drift is a process that causes gene frequencies to change suddenly (Noor 2008).

Association of PRL gene to total egg production

Based on Fig. 8, the diversity of PRL genes (g.1997 G>A, g.2090 C>A, g.2300 G>C, g.3778 T>A, g.5929 C>A, g.5963 G>A) showed significantly different results ($P<0.01$). The results of this study are the same as those of Purwantini et al. (2020), Bai et al. (2019), Ghanem et al. (2017), Chuekwon and Boonlum (2017) and Wang et al. (2011), which stated that PRL gene diversity was associated with total egg production. The results of research by Purwantini et al. (2020), showed that the PRL gene is associated with the total egg production of several local Indonesian ducks. Likewise, the results of Ghanem et al. (2017) and Wang et al. (2011), which stated that the PRL exon 5 gene was also associated with the total egg production of Entok-Peking cross ducks and five local Chinese ducks. In addition, the results of research by Bai et al. (2019) and Chuekwon and Boonlum (2017) also, the intron 1 PRL gene was associated with total egg production, although in this study, there was no mutation in the intron 1 position.

Prolactin (PRL) hormone is synthesized by the anterior hypophysis gland, which has a role in protecting several physiological functions of poultry (Kansaku et al. 2005; Hiyama et al. 2015), which can cause ovarian regression or indirectly by competing with the hormone progesterone produced by the ovaries (Anwar and Safitri 2005). Ovarian regression is a condition in the ovary in which follicular growth is lysed and absorbed by the body, so there is no follicular growth or ovulation does not occur (Anwar and Safitri 2005; Chuekwon and Boonlum 2017). This is because very high levels of PRL hormone will inhibit the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, resulting in decreased luteinizing hormone (LH) and FSH production from the hypophysis (Susanti 2015). Gonadotropin hormones such as FSH and LH are required for follicular development and oviposition because low levels of FSH and LH can cause follicular growth not to be formed and birds do not lay eggs (Anwar and Safitri 2005). According to Anwar and Safitri (2005), the success of egg production from a bird is determined by the number of follicles formed and ovulated. So, when PRL hormone levels are very high in the blood, follicles will not develop. This shows that PRL levels in the blood affect egg production in poultry. Susanti et al. (2012) also added that high concentrations of PRL hormone in the egg production phase affect duck egg production, the higher the PRL hormone in the blood, the higher the duck egg production, this is because the PRL hormone in the blood in the production phase is needed for the process of eggshell formation.

Association of the PRL gene with the length of time to stop laying eggs

The results of the analysis of variance of the length of time to stop laying eggs of Sikumbang Jonti ducks on the diversity of PRL genes are presented in Fig. 9. Based on Fig. 9, the diversity of PRL (g.1997 G>A, g.2090 C>A, g.2300 G>C, g.3778 T>A, g.5929 C>A, g.5963 G>A) showed significantly different results ($P<0.01$). According to Suhendro et al. (2022) genes that have significant associations can be considered as candidates for selection markers.

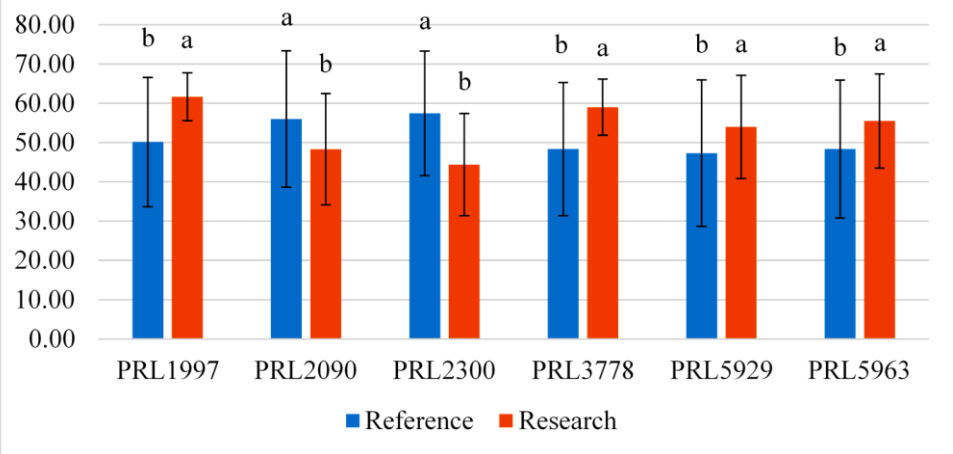

Fig. 8: Association of total egg production with the PRL gene of Sikumbang Jonti ducks during the study. Different alphabets indicate significantly different results (P<0.01)

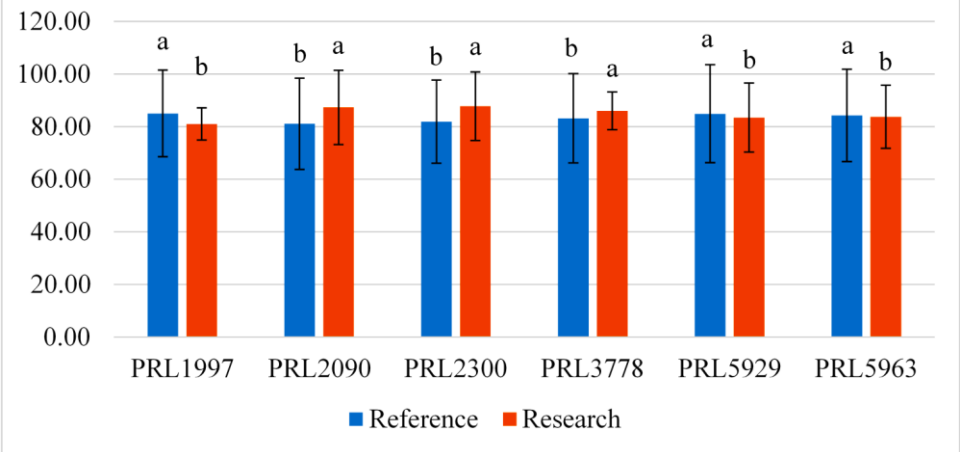

Fig. 9: Association of time length to stop laying eggs (days) with the PRL gene of Sikumbang Jonti ducks during the study. Different alphabets indicate significantly different results (P<0.01)

Chuekwon and Boonlum (2017) stated that PRL hormones affect the molting process and the speed of starting to lay eggs. Molting is the feather loss phase of a bird followed by a phase of stopping laying eggs for 3-4 months (Anwar and Safitri 2005). During the molting process, the ovaries of poultry shrink and cause egg production to stop (Susanti 2015). However, Susanti et al. (2012) stated that total egg production is not associated with the duration of molting. According to Susanti et al. (2012), the speed of starting to lay eggs again in ducks that have high PRL levels is faster than those with low PRL levels at the first time of production. This is because the PRL hormone in the production phase which is needed for the formation of egg shells switches functions for the process of forming new feathers in the molting phase Susanti et al. (2012). However, the levels of PRL hormone needed during the molting phase are not as much as during the egg production phase (Susanti 2015). The results of research Susanti et al. (2012), showed that the concentration of PRL hormone in AP and PA ducks during the egg production period was 196.50 ± 17.56 and 166.50 ± 8.85 ng/mL, while during the molting period was 75.38 ± 11.84 and 79.18 ± 4.98 ng/mL. This is thought to be a factor in the length of time to stop laying eggs both before and after molting.

On the other side, Susanti (2015) also stated that PRL hormone levels that exceed the levels required for the process of making eggs in the reproductive tract can inhibit the secretion of other reproductive hormones such as LH and FSH, and can cause birds to stop laying eggs. This is following the research of Anwar and Safitri (2005), which showed that the administration of anti-prolactin can inhibit the molting process and affect the speed of egg laying. Based on the dual function of the PRL hormone, it can be concluded that the PRL hormone is instrumental in duck egg production.

Conclusion

From the results of this study, it can be concluded that six (6) mutations in the PRL gene at the position of intron 2 (g.1997 G>A, g.2090 C>A, g.2300 G>C), exon 4 (g.3778 T>A), and exon 5 (g.5929 C>A, g.5963 G>A) are in Hardy-Weinberg equilibrium and are associated with total egg production and length of egg stopping time, so they can be used as candidates for genetic markers of egg production of Sikumbang Jonti ducks.

Conflict of interest

There is no conflict of interest with any organization about the discussed in this manuscript.

Acknowledgments

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Author's contribution

Teguh Rafian, Yurnalis Yurnalis, Husmaini Husmaini, and Firda Arlina designed the concept of this study and

searched for funding. All authors have read and approved the final manuscript.

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