Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.17.3.170-174
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 17 Issue 3 July-September 2022

Polymorphism of Prolactin Gene (PRL/PstI) In Sikumbang Jonti Duck Using PCR-RFLP Methods

T. Rafian[1], Y. Yurnalis[2], Y. Fenita[3], and R. Iskandarsyah[2]

[1]Departement of Animals Husbandry, Faculty of Agriculture, Universitas Lampung. Indonesia
[2]Departement of Animals Production, Faculty of Animals Sciences, Universitas Andalas. Indonesia
[3]Departement of Animals Sciences, Faculty of Agriculture, Universitas Bengkulu. Indonesia
Corresponding author: teguh.rafian@fp.unila.ac.id

ABSTRACT

This study is aimed to determine polymorphism of the Prolactin gene (PRL|*Pst*I) in Sikumbang Jonti ducks using PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) method. This study used 56 Sikumbang Jonti ducks whose blood samples were taken. Gene amplification used a pair of primers forward 5' TGC AAA CCA TAA AAG AAA AGA 3' and reverse 5' CAA TGA AAA GTG GCA AAG CAA 3', which resulted in a 400 bp fragment in exon 5 of the Prolactin (PRL) gene. The amplification product was restricted using the *Pst*I enzyme, which recognizes the truncation site (5' G↓ACGTC 3'). From 56 samples of Sikumbang Jonti ducks identified, just one genotype was found, homozygous (-/-) with only one allele (-). Analysis of the restriction product in Sikumbang Jonti ducks obtained a uniform genetic variation of PRL|*Pst*I (monomorphic) with an allele frequency (-) of 100%.

Keywords: Local duck, Payakumbuh city, Sumatera Barat, genetic resources

INTRODUCTION

Indonesia has a wealth of livestock genetic resources that have the potential for livestock development. Various local breeds of livestock, well-known and unknown, can be found in each province with unknown numbers and potentials. These breeds have comparative advantages compared to imported livestock, such as good adaptability to tropical environments and good production characteristics due to of natural selection (Nova et al., 2020; Subekti et al., 2019).

One of the potential germplasms in the livestock sub-sector is ducks (Setyo Budi et al., 2015). Duck is one of the livestock that contributes animal protein to the community, such as producing eggs and meat. Ducks have several advantages when compared to other livestock ducks are more resistant to disease and have good feed efficiency (Nova et al., 2019; Saputro et al., 2016).

West Sumatra has four types of local ducks, one of which is the Sikumbang Jonti duck. Sikumbang Jonti duck is a germplasm native of West Sumatra, from Payakumbuh City, especially in Kenagarian Koto Baru Payobasuang. The Sikumbang Jonti duck has a white-black (male) or white (female) head feather color, a white-black (male) or white (female) neck feather color, a white chest coat color, and a white-black (male) or white (female) back coat color, green primary wing feathers color, white-black tail feathers color, and white thigh feathers color (Arlina et al., 2022).

However, the existence of Sikumbang Jonti ducks is starting to be replaced by ducks with better performance. Due to economic factors, many Sikumbang Jonti duck breeders must sell their ducks even though they are still in production. Therefore, it is necessary to increase the productivity of Sikumbang Jonti ducks through selection, especially genetically (Masti et al., 2021; Yurnalis et al., 2017). One method to identify gene polymorphism is using the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) method. The gene related to economic characteristics is the prolactin gene (PRL).

The PRL gene is a candidate gene that specifically controls variations in of egg production (Susanti, 2015; Susanti et al., 2012). The prolactin gene in ducks has been identified by Kansaku et al. (2005). The gene consists of five protein coding regions separated by four introns and codes for 229 amino acids. Many studies have examined the association between prolactin gene polymorphisms with egg production characteristics in Chinese ducks (Wang et al., 2011). Wang et al. (2011) found polymorphism in the exon five prolactin gene that was associated with egg production characteristics. This makes the basis for identifying the polymorphism of the exon five

prolactin gene in Sikumbang Jonti ducks to select duck egg production traits.

This study aimed to determine polymorphism of PRL genes in Sikumbang Jonti ducks using the PCR-RFLP method.

MATERIALS AND METHODS

This study used 56 samples of Sikumbang Jonti duck blood which were extracted using the genomic DNA purification kit procedure from Promega. PRL gene amplification was carried out by PCR (Polymerase Chain Reaction) method using a pair of primers forward 5' – TGC AAA CCA TAA AAG AAA AGA – 3' and reverse 5' – CAA TGA AAA GTG GCA AAG CAA – 3', which resulted in a 400 bp - long fragment (Wang et al., 2011). PCR amplification reagent using a master mix (ThermoSCIENTIFIC®) and PCR machine (Eppendorf® Mastercycler gradient) that was programmed with predenaturation of 95°C for 5 minutes, denaturation of 95°C for 45 seconds, annealing of 56°C for 45 seconds, extension 72°C for 1 minute, repeated 35 cycles, and final extension 72°C for 5 minutes. The results of the amplified PRL gene were electrophoresed using 1% agarose (ThermoSCIENTIFIC® TopVision Agarose#R0491) with ethidium bromide solution (MP Biomedicals®) staining and observed using a UV trans illuminator (SynGENE®G:BOX).

Determination of the genotype of the exon 5 prolactin gene using the enzyme *Pst*I buffer B (ThermoSCIENTIFIC®) and a water bath incubator at 37˚C for 6 hours. Visualization results of *Pst*I restriction products can be seen by electrophoresis of the product on 1% agarose gel (ThermoSCIENTIFIC® TopVision Agarose#R0491) and ethidium bromide solution (MP Biomedicals ®), which were then observed using a UV trans illuminator (SynGENE® G:BOX). The results will obtain an image of the cutting band with three possible genotypes:

1. Homozygous is not truncated (-/-), if there is only one measuring band along the amplification fragment.
2. Homozygous truncated (+/+), if there are two or more bands out of position/below the size of the amplification fragment.
3. Homozygous truncated (+/-), if there are two or more bands with one band at the position/size of the amplified fragment and another band under the position of the amplified fragment.

The variables observed in this study were the genotype and allele of the resulting genotype. The genotype and allele data obtained were analyzed in the form of:

1. The genotype frequency is calculated using the formula (Noor, 2010)

$$x_{ii} = \frac{n_{ii}}{N}$$

xii = frequencies of genotype to-i
nii = number of samples in genotype ii
N = number of samples

2. The allele frequency is calculated using the formula (Noor, 2010):

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

xi = frequencies of allele to-i
nii = number of samples in genotype ii (homozygous)
nij = number of samples in genotype ij (heterozygous)
n = number of samples

RESULTS AND DISCUSSION

Gene Amplification and Restriction (PRL|*Pst*I)

The results of the exon 5 PRL gene amplification (56 samples of Sikumbang Jonti ducks) along 400 bp are presented in Figure 1.

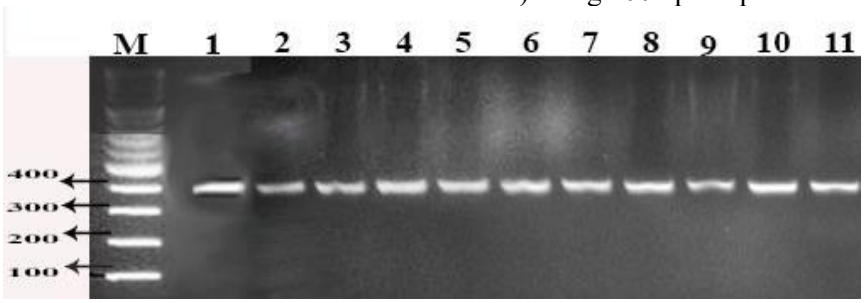

Figure 1. Results of PRL/*Pst*I Gene Amplification in Sikumbang Jonti ducks (M=Marker; No. 1-11=Individual Sample)

The length of the amplified fragment can be determined by matching the primer pair attachment site on the duck PRL gene sequence from Genbank with the access code NW_004676690 (Figure 2).

5707	**tgcaaaccat**	**aaaagaaaag**	**a**ctttatgagc	tgtacactac	tatctagcat	tcctcaagg
5767	ccagtatttc	ttagttctct	gtcctacata	cagtcagatt	cattattatc	cactacggta
5827	tcattttgtg	cctttaggtt	cattctggcg	acattggaaa	tgaaatttat	tctcagtggg
5887	aaggccttcc	atccttgcaa	cttgccgatg	aggactccag	actctttgcc	ttttacaacc
5947	tgctgcattg	cctccgcaga	gattcccaca	aaattgacaa	ctatctcaag	gttttgaagt
6007	gccgcctaat	acatgatagc	aattgctaag	tactcctggg	cttcatcgct	tactaaaatc
6067	attcatcatg	gtgttcttg**t**	**tgctttgcca**	**cttttcattg**		

Figure 2. The primers (forward and reverse) anneal to the bolded nucleotides

The polymorphism of the PRL|*Pst*I gene in the Sikumbang Jonti duck using the *Pst*I restriction enzyme which recognizes the cutting site on bases CTGCA↓G or G↑ACGTC is presented in Figure 3.

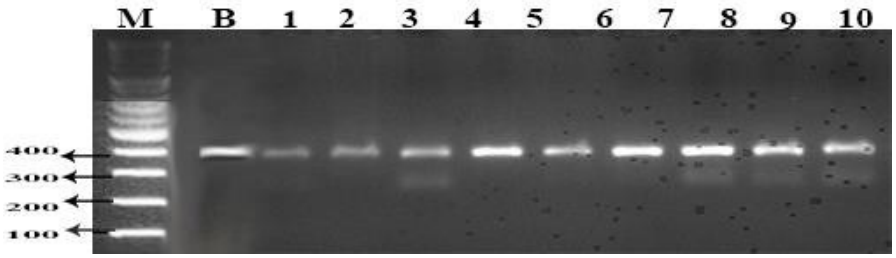

Figure 3. Results of PRL/*Pst*I gene Restriction in Sikumbang Jonti ducks (M=Marker; B=Blank; No. 1-11=Individual Sample)

Based on Figure 3, the restriction results of the PRL|*Pst*I gene exon 5 in Sikumbang Jonti ducks using the *Pst*I enzyme only obtained one band pattern along 400 bp. This shows that Sikumbang Jonti duck population has one genotype, which is homozygous (-/-). The results of this study are different from the research conducted by Wang et al. (2011) and Ghanem et al. (2017) which obtained three kinds of genotypes +/+, +/-, and -/- in Chinese local ducks and Peking ducks with same method. This is due to several factors, such as different breeds of livestock, selection of certain traits, gene mutations, and/or high levels of deep crossover (Noor, 2010).

Genotype and Allele Frequency of PRL|*Pst*I Gene

The results of the genotype and allele frequencies of the Sikumbang Jonti duck population are presented in Table 1.

Table 1. Genotype and allele frequencies of the PRL|*Pst*I gene in Sikumbang Jonti ducks

	N	Genotype Frequency			Allele Frequency	
		(-/-)	(+/-)	(+/+)	(-)	(+)
Total	56	56	0	0	56	0
Frekuensi (%)	100	100	0	0	100	0

N=number of populations

Based on Table 1, population of Sikumbang Jonti duck has one type of genotype, which is homozygous (-/-) with allele frequency (-) 1.00 (100%) or monomorphic (uniform). According to Noor (2010), a population is said to be monomorphic if it has one allele frequency equal to or less than 0.01 (1%). On the other hand, the results of this study are different from the results of research by Wang et al. (2011), which stated that the Jinjiang local Chinese duck has three genotypes, those are homozygous (+/+), heterozygous (-/+), and homozygous (-/-)

with genotype frequencies of (1.20), (32.60), and (66.30) respectively. The results of Ghanem et al. (2017) also showed that Peking ducks obtained three genotypes, those are homozygous (+/+), heterozygous (-/+), and homozygous (-/-) with a genotype frequency of (16.82), (48.38), and (34.80) respectively. This difference is due to the differences in the livestock breeds that was studied.

However, the research results of Purwantini et al. (2020), show that there is polymorphism in the PRL gene of Tegal and Magelang ducks. In addition, Indriati et al. (2015) also found a polymorphism of PRL genes in Mojosari Putih ducks. This shows that Indonesian local ducks still have the potential to be genetically selected through the PRL gene.

CONCLUSION

Based on the results of the study, it can be concluded that the PRL|*Pst*I gene in Sikumbang Jonti ducks is monomorphic and there is only a homozygous genotype (-/-).

REFERENCES

Arlina, F., Husmaini, S., Rhoudha, R., Sardi, W.R., Rafian, T., 2022. Keragaman Fenotipe Kualitatif dan Kuantitatif Itik Sikumbang Jonti sebagai Plasma Nutfah di Sumatera Barat. J. Ilmu Peternak. dan Vet. Trop. (Journal Trop. Anim. Vet. Sci.) 11, 291–299. https://doi.org/10.46549/jipvet.v11i3.173

Ghanem, H.M., Ateya, A.I., Saleh, R.M., Saleh, M., 2017. Artificial Insemination vs Natural Mating and Genetic PRL/*Pst*I Locus Polymorphism and Their Effect on Different Productive and Reproductive Aspects in Duck. Advances in Animal and Veterinary Sciences 5, 179–184. https://doi.org/10.17582/journal.aavs/2017/5.4.179.184

Indriati, M., Sumantri, C., Susanti, T., 2015. Analysis of Prolactin Gene Exon 4 Diversity in Peking, White Mojosari, and Peking White Mojosari Crossbreed. Med Pet 39, 14–19. https://doi.org/10.5398/medpet.2016.39.1.14

Kansaku, N., Ohkubo, T., Okabayashi, H., Guémené, D., Kuhnlein, U., Zadworny, D., Shimada, K., 2005. Cloning of duck PRL cDNA and genomic DNA. General and Comparative Endocrinology 141, 39–47. https://doi.org/10.1016/j.ygcen.2004.11.017

Masti, H., Yurnalis, Y., Nova, T.D., Kamsa, Z., Rafian, T., 2021. Keragaman Gen Growth Hormone Receptor (GHR) Ekson 10 pada itik Sikumbang Janti. JIPTHP 9, 15–19. https://doi.org/10.29244/jipthp.9.1.15-19

Noor, R.R., 2010. Genetika Ternak, 6th ed. Penebar Swadaya, Jakarta.

Nova, T.D., Anggraeni, S.D., Wardiansyah, M., Ramadhani, E.P., 2019. Frekuensi Pemberian Ransum Secara Periodik dan Level Protein Terhadap Karkas dan Gambaran Darah Itik Lokal Sikumbang Janti. JPI 21, 64. https://doi.org/10.25077/jpi.21.2.64-75.2019

Nova, T.D., Syahruddin, E., Zein, R., 2020. The Productivity of Duck in Different Temperature Cage Management. J.Nat.Indonesia 18, 43. https://doi.org/10.31258/jnat.18.1.43-61

Purwantini, D., Santosa, R.S.S., Santosa, S.A., Susanto, A., Candrasari, D.P., Ismoyowati, I., 2020. Prolactin gene polymorphisms and associations with reproductive traits in Indonesian local ducks. Vet World 13, 2301–2311. https://doi.org/10.14202/vetworld.2020.2301-2311

Saputro, B.E., Sutrisna, R., Santosa, P.E., Fathul, F., 2016. Pengaruh Ransum yang Berbeda pada Itik Jantan Terhadap Jumlah Leukosit dan Diferensial Leukosit. Jurnal Ilmiah Peternakan Terpadu 4, 176–181.

Setyo Budi, E., Yektiningsih, E., Priyanto, E., 2015. Profitabilitas Usaha Ternak Itik Petelur di Desa Kebonsari Kecamatan Candi, Sidoarjo. AGR 1, 32–37. https://doi.org/10.18196/agr.115

Subekti, K., Solihin, D.D., Afnan, R., Gunawan, A., Sumantri, C., 2019. Polymorphism of Duck HSP70 Gene and mRNA Expression under Heat Stress Conditions. International J. of Poultry Science 18, 591–597. https://doi.org/10.3923/ijps.2019.591.597

Susanti, T., 2015. Prolactin as a Candidate Gene Controlling Molting and Egg Production of Duck. Indonesian Bulletin of Animal and Veterinary Sciences 25, 23–28.

https://doi.org/10.14334/wartazoa.v25i1.1125

Susanti, T., Noor, R.R., Hardjosworo, P.S., Prasetyo, L.H., 2012. Relationship Between Prolactin Hormone Level, Molting and Duck Egg Production. J. Indonesian Trop. Anim. Agric. 37, 161–167. https://doi.org/10.14710/jitaa.37.3.161-167

Wang, C., Liang, Z., Liang, Z., Yu, W., Yu, W., Feng, Y., Feng, Y., Peng, X., Peng, X., Gong, Y., Gong, Y., Li, S., Li, S., 2011. Polymorphism of the prolactin gene and its association with egg production traits in native Chinese ducks. SA J. An. Sci. 41, 63–69. https://doi.org/10.4314/sajas.v41i1.66044

Yurnalis, Y., Husmaini, H., Sabrina, S., 2017. Polymorphisms of Growth Hormone Gene Exon 1 and their Associations with Body Weight in Pitalah and Kumbang Janti Ducks. International J. of Poultry Science 16, 203–208. https://doi.org/10.3923/ijps.2017.203.208