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Polymorphism of Insulin-Like Growth Factor-1 (IGF-1) Gene on Bayang Ducks Using PCR-RFLP Method

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ABSTRACT

This study aims to determine the polymorphism of the IGF-1 gene in Bayang ducks using the PCR-RFLP method. This study used 120 blood samples of Bayang ducks in Kampung Binuang Dalam, Padang City, West Sumatra Province. Bayang duck blood samples were taken through the brachial vein for ± 1 mL. DNA extraction was taken from the blood using the Genomic DNA Purification Kit (Promega) Protocol. DNA was amplified using a pair of primers F: 5'- CCA GGA ATA TCT TTG GAA GCT GT-3 and R: 5'- TGC TAC GTT ACC AGC CTT GA -3 'which produced a 433 bp fragment of exon 3 IGF-1 gene, and F: 5'- CTG GAG CAG GCA GGA AAA TT - 3 'and R: 5'- TCC AGG GAC AGT GAC TCA AC -3' which produced 801 bp fragments of exon 4 IGF-1 gene. The amplification product was restricted by the DdeI enzyme, which recognized the C↓TNAG slashing site (N = G, A, T, C) for the exon three and MnII regions, which recognized the CAC↓GTG cutting site for the exon four regions. The cutting of the IGF-1 exon three gene product in Bayang ducks using DdeI as a restriction enzyme only recognized the C↓TNAG cutting site. The cutting results visualized with 2% agarose gel showed two genotypes: homozygous (+ / +) of 41 samples and the heterozygote (+/-) genotype of 60 samples. The cutting of the IGF-1 exon four gene product in Bayang ducks using MnII as a restriction enzyme only recognized the CAC↓GTG cutting site. The cutting results visualized with 2% agarose gel showed three types of genotypes, namely four samples homozygous (+/+), 67 samples heterozygous (+/-), and 49 samples homozygous (-/-). Based on the results of the study, it can be concluded that the IGF-1 gene in Bayang ducks is polymorphic, with the frequency of the IGF-1|DdeI genotype being in a hardy-weinberg imbalance, and the IGF-1|MnII genotype in a hardy-weinberg equilibrium.

Keywords: Enzym Restriction, Indonesian, Local Duck, Sumatra Barat

INTRODUCTION

One of Indonesia's germplasms in the livestock sub-sector is ducks. Local ducks are scattered in several regions of Indonesia with different climatic conditions. According to (Maharani et al., 2019), local ducks in Indonesia are divided into three based on their natural habitat. Ducks are livestock with a high diversity level in terms of types and production potential (Rafian et al., 2023). Ducks also have the potential to be developed because they have reasonably good adaptability (Nova et al., 2020; Subekti et al., 2019) and have efficiency in changing the feed into good meat (Saputro et al., 2016). Moreover, ducks play an important role as producers of eggs (Rafian et al., 2022) and meat to support the availability of cheap and readily available animal protein (Nova et al., 2016).

Breeders dominate duck farming with a traditional maintenance system, where ducks are grazed in rice fields or places with lots of water (Apriyantono, 2011). The increasing demand for broiler ducks has caused many breeders to switch to raising male ducks for meat ducks (Matitaputty and Bansi, 2018). One of the local types of meat is the Bayang duck. Bayang duck is a local duck located in Bayang District, Pesisir Selatan Regency, West Sumatra. The Decree of the Minister of Agriculture in 2012 regarding the determination of livestock clumps reports that quantitatively, the Bayang duck has a relatively high body weight, namely the male of 1.8 ± 0.3 kg and the female of 1.6 ± 0.2 kg. Egg production is 184-215 eggs/year with an average egg weight of around 65 grams, and peak production reaches 85%. Bayang Duck has reproductive traits: age of sexual maturity around 5.5 ± 0.6 months and duration of egg production 2.5-3 years.

Bayang ducks have the potential to be developed because these ducks are adaptive to the environment and have high reproductive. However, the system of raising Bayang ducks in the community is still traditional, and there has been no treatment to improve the performance of the Bayang ducks in a better direction. Based on this, there is a need to improve the performance of the Bayang ducks. One of the ways to increase the performance of the Bayang duck is by selecting

the population of the Bayang duck to be developed. Selection can be done via genetic markers or genes (Yurnalis et al., 2017; Yurnalis et al., 2019).

Genes that affect livestock growth performance include IGF-I, GH, and GHR (Liu et al., 2020; Yurnalis et al., 2019; Masti et al., 2019). Insulin-like growth factors are transport proteins in the blood. Insulin-like growth factors are given to this molecule because of its structural similarity to the hormone insulin. Insulin-like growth factor-I (IGF-I) is a polypeptide that increases cell proliferation (Svoboda and Van Wyk, 1983) and sugar uptake by cells (Poggi et al., 1979). Genetic improvement in the quality of seedlings is determined by genetic variation and the structure of the parent population. Knowledge of genetic data is essential for breeding. The development of molecular techniques, such as the Polymerase Chain Reaction (PCR) technique, is an amplification technique to increase the concentration of DNA strands to be high enough to be analyzed. Identification of genes can be performed by the RFLP method (Restriction Fragment Length Polymorphism). According to (Suhartati et al., 2020) and (Suhendro et al., 2021), the analysis of the Restriction Fragment pattern resulted in DNA being digested by the polymerase enzyme.

Based on the description above, it is necessary to know the diversity of the IGF-1 gene using PCR-RFLP markers for basic information on the selection of Bayang ducks through Marker Assisted Selection (MAS).

MATERIALS AND METHODS

This study used 120 samples of Bayang duck blood from several duck breeders in Kampung Binuang Dalam, Padang City, West Sumatra. Bayang duck blood was taken through the brachial vein using a Disposable Syringe (± 1 mL), and then the blood sample was inserted into a vacutainer tube containing EDTA. Blood samples are stored at -20 ºC until they are used. DNA isolation was carried out from blood samples using a genomic DNA purification kit from Promega. Furthermore, the results of DNA extraction will be electrophoresed on 1% agarose gel to identify the success of the DNA extraction process. The isolated DNA was amplified using a pair of primers, as shown in Table 1.

The PCR amplification reagent used MasterMix (Thermo Scientific) with the following composition:

a. DNA sample as much as 2 µl,
b. the master mix of 12.5 µl,
c. a primary mixture of F and R as much as 3 µl, and
d. 7.5 µl of nuclease-free water.

Invitro amplification was performed using a PCR machine (Eppendorf Mastercycler gradient) with a pre-saturation program at 95 ºC for 45 seconds, annealing at each primer temperature for 45 seconds, extension 72 ºC for 1 minute, and final extension 72 ºC for 5 minutes, which was performed for 35 cycles.

The IGF-1 gene amplification electrophoresis results using agarose 1.5 % were stained by ethidium bromide, which results were observed using a UV transilluminator. IGF-1 gene amplification is successful if the agarose gel shows the bands at the position/size of the well-containing PCR product DNA samples according to the target length of each primer by comparing the positions of the DNA ladder bands.

Enzyme restriction was performed by taking 15 µl of PCR product, adding 10 µl of enzyme mixture, and incubating for 4 hours. DNA cutting products were visualized on a 2% agarose gel with 0.5 × TBE buffer (Tris Borate EDTA) stained with ethidium bromide and run using an electrophoresis power supply at 100 volts for 2 hours. The results of electrophoresis were observed with the help of UV transilluminator light. The DNA band that appeared was compared with the marker to determine the length of the fragment.

Table 1. Primer and enzyme restriction were used in this research

Fragments	Sequence Primer (5'-3')	Product	Tm (ºC)	Enzym Restriction
Exon 3 Regions	CCA GGA ATA TCT TTG GAA GCT GT TGC TAC GTT ACC AGC CTT GA	433 bp	55	DdeI
Exon 4 Regions	CTG GAG CAG GCA GGA AAA TT TCC AGG GAC AGT GAC TCA AC	801 bp	60	MnII

Based on the observation of the electrophoresis results with the UV Transilluminator, an image of the cut band with three possible genotypes will be obtained:

a. Homozygous not truncated (-/-) if only one band is the same size as the amplification fragment.
b. Homozygous truncated (+/+) if two or more bands are out of position/below the size of the amplification fragment.
c. Heterozygous (+/-), if two or more bands with one band are in the position/size of the amplified fragment and the other band is below the position of the amplified fragment.

The genotype diversity of everyone can be determined from the gene DNA strands found. Each sample is compared with the same size (marker), and the allele frequency is:

$$\chi_{i=}\frac{2nii+\sum_{j\neq 1} nij}{2N}$$

While the genotype frequency is calculated by the formula (Nei and Kumar, 2000):

$$\chi_{ii=}\frac{\sum_{i=1}^{n} ni}{2N}$$

Note:
χ i = Frequency of the i allele;
χ ii = Frequency of genotype;
nii = number of samples with genotype ii;
nij = number of samples with genotype ij;
N = Number of samples

The Hardy-Weinberg equilibrium test was conducted to determine whether the allele frequency and genotype frequency in the reared duck population were still in p2+2pq+q2 equilibrium tested by chi-square (χ2) according to (Nei and Kumar, 2000) with the following formula:

$$\chi^2 = \sum \frac{(0-E)^2}{E}$$

Note:
χ2 = chi-square
O = number of observations of the i genotype
E = expected number of the i genotype

RESULT AND DISCUSSION

Polymorphism of GH Gene

DNA product of Shadow duck blood samples amplified using primary pairs F: 5'-CCA GGA ATA TCT TTG GAA GCT GT-3 'and R: 5'-TGC TAC GTT ACC AGC CTT GA-3' produces 433 bp long fragment of IGF-1 gene in exon three regions, then visualized with 1.5 % agarose electrophoresis which is presented in Figure 1.

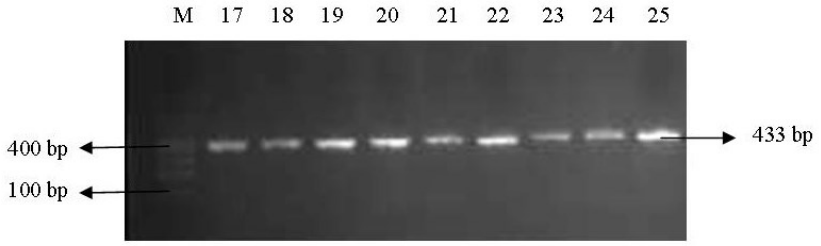

Figure 1. The results of PCR product amplification of the IGF-1 gene of Bayang ducks along 433 bp (M = marker Kapa Universal 100bp); No 17-25 = individual samples)

The amplification of the primary pair F: 5'-CTG GAG CAG GCA GGA AAA TT-3 'and R: 5'-TTC AGG GAC AGT GAC TCA AC-3' produces 801 bp long fragment of IGF-1 gene in exon four region, then visualized with 1.5% agarose electrophoresis which is presented in Figure 2.

Figure 1 and Figure 2 show the results of the amplification of the IGF-1 gene applied explicitly because there is one DNA band with a size that matches the primary design. Amplified fragment length results are known by matching primers annealing sites on IGF-1 gene sequences ducks. An exemplary amplification result is influenced by several factors, such as the purity of the DNA extraction results, the accuracy of selecting the primers used, and the accuracy of the PCR conditions. Primers are an important part of PCR because primers are the initiator of target DNA synthesis (Viljoen et al., 2005). To prepare a primer, 20 bases and 50% G / C content must be met. The accuracy of PCR conditions is determined by the accuracy of the reaction mixture and the accuracy of the temperature conditions in each cycle (Rahayu et al., 2006).

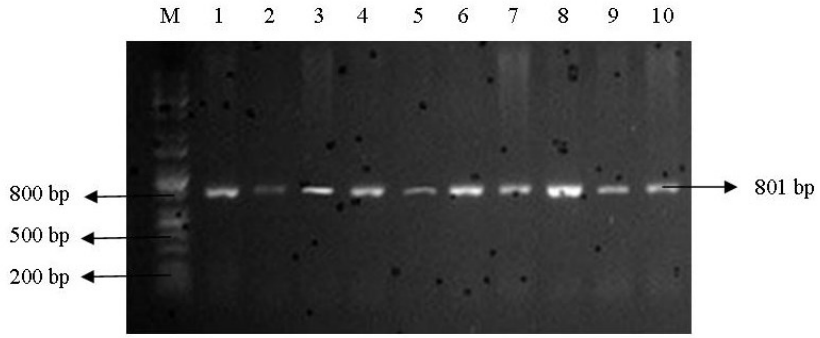

Figure 2. The results of PCR product amplification of the IGF-1 gene for Bayang Ducks along 801 bp (M = marker (Kapa Universal 100 bp); No. 1-10 = individual samples)

The cutting of the IGF-1 exon three gene product in Bayang ducks using DdeI as a restriction enzyme only recognized the C↓TNAG cutting site. The cutting results visualized with 2% agarose gel showed two genotypes: homozygous (+ / +) of 41 samples and the heterozygote (+/-) genotype of 60 samples.

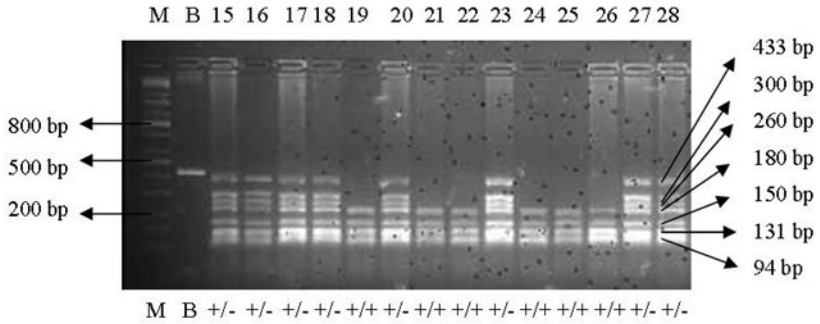

Figure 3. Results of the restriction of the IGF-1 | gene fragment DdeI (M = Marker (Kapa Universal 100 bp); B = Blank; No. 15-28 = individual samples)

The first pattern genotype has four (4) fragment pieces called homozygous (+/+) with fragment sizes of 180 bp, 150 bp, 131 bp, and 94 bp, and the second pattern has seven (7) fragment pieces called heterozygous (+/-) with fragment sizes of 433 bp, 300 bp, 260 bp, 180 bp, 150 bp, 131 bp, and 94 bp.

The cutting of the IGF-1 exon four gene product in Bayang ducks using MnII as a restriction enzyme only recognized the CAC ↓ GTG cutting site. The cutting results visualized with 2% agarose gel showed three types of genotypes, namely four samples homozygous (+/+), 67 samples heterozygous (+/-), and 49 samples homozygous (-/-).

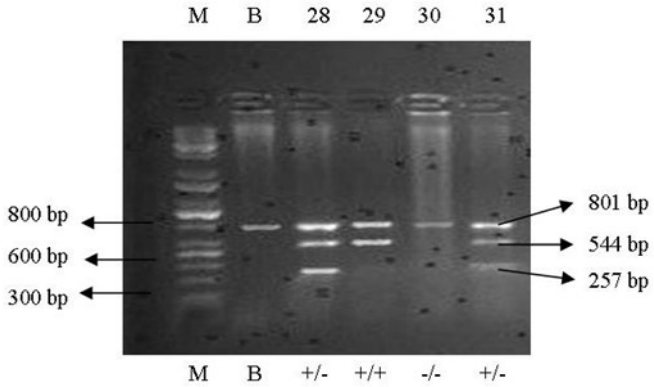

Figure 4. Results of the restriction of IGF-1 | gene fragments MnII. (M = Marker (Kapa Universal 100 bp); B = Blank; No. 28-31 = individual samples)

The first pattern genotype has three (3) fragments called heterozygous (+/-) with a band size of 801bp, 544 bp; 257 bp; the genotype of the second pattern was two fragments called homozygous (+/+) with a fragment size of 801 bp and 544 bp, the genotype of the third pattern did not contain pieces called (-/-).

Genotype frequency and allele frequency

The results of the genotype frequency in ducks the shadows of the research are presented in Table 2 and Table 3. In Table 2, the ducks with a homozygous genotype (+ / +) were 41 samples or 0.41%, and the heterozygous genotype (+/-) was 60 samples. or 0.59%, and homozygous (- / -) as much as 0 samples or 0.00%. In Table 3, the samples of ducks with homozygous genotypes (+ / +) were four samples or 0.03%, 67 samples or 0.56% heterozygous genotypes, and homozygous genotypes (- / -) 49 samples or 0.41%.

Table 2. Frequency of genotype and allele of IGF-1|DdeI Bayang duck

Population	N	Genotype (n)			Genotype Frequency (%)			Allele Frequency (%)	
		(+/+)	(+/-)	(-/-)	(+/+)	(+/-)	(-/-)	(+)	(-)
Bayang ducks	101	41	60	0	0,41	0,59	0,00	0,70	0,30

The allele frequency results showed a diversity (polymorphism) in the IGF-1 gene of Bayang ducks. It is because there are frequencies of two alleles, namely allele (+) and allele (-), which have a value of less than 99%—following the opinion (Nei and Kumar, 2000), which states that genetic variation occurs when two or more alleles are less than 99%.

Table 3. Frequency of genotypes and alleles of IGF-1|MnII Bayang duck

Population	N	Genotype (n)			Genotype Frequency (%)			Allele Frequency (%)	
		(+/+)	(+/-)	(-/-)	(+/+)	(+/-)	(-/-)	(+)	(-)
Bayang ducks	120	4	67	49	0,03	0,56	0,41	0,31	0,69

Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium is used to determine whether the observed observations are deviant. Hardy-Weinberg equilibrium was calculated using the chi-square test ($\chi 2$). The chi-square test ($\chi 2$) was performed on the condition that p2+2pq +q2=1. The results of testing the Hardy-Weinberg equilibrium law on the Bayang duck population are presented in Table 4.

Table 4. Chi-square test of HW Equilibrium IGF-1 in Bayang ducks

Population	N	Frequency Observation			Frequency Expectation			Chi-Square (χ^2)
		+/+	+/-	-/-	+/+	+/-	-/-	
IGF-1|*Dde*I	101	0,41	0,59	0,00	0,49	0,42	0,09	*
IGF-1|*Mn*II	120	0,03	0,56	0,41	0,09	0,43	0,48	ns

Based on the chi-square test results in Table 4, the genotype frequency of the results of this study, the genotype IGF-1|DdeI was significantly different. In contrast, the genotype IGF-1|MnII was not significantly different from the frequency of the Hardy-Weinberg genotype. It shows the genotype frequency of IGF-1|DdeI is in hardy-Weinberg equilibrium while genotype IGF-1|MnII is in hardy-Weinberg equilibrium. Hardy-Weinberg imbalance can be caused because the ducks used have been selected, the population is small, and the mating is not random. Following the opinion (Noor, 2008), the things that affect the imbalance of the Hardy-Weinberg law are mutations, gene flow, migration, selection, genetic drift, and mating occurs non-randomly. Vasconcellos et al. (2003) Added that several events, such as the accumulation of genotypes, mutations, selection, migration, and mating in the same group/population (endogamy), can cause population imbalance.

CONCLUSION

Based on the results of the study, it can be concluded that the IGF-1 gene in Bayang ducks is polymorphic, with the frequency of the IGF-1|DdeI genotype being in a hardy-weinberg imbalance, and the IGF-1|MnII genotype in a hardy-weinberg equilibrium.

REFERENCES

Apriyantono, A. 2011. Pedoman Budidaya Itik Pedaging yang Baik. Gramedia.

Harvey, S.C., G. Scanes, WH. Daughaday. 1995. Growth Hormone. Boca Raton: CRC Press.

Liu G., M. Zeng, X. Li, Y. Rong, Z. Hu, H. Zhang, X. Liu. 2020. Expression and analysis of ESR1, IGF-1, FSH, VLDLR, LRP, LH, PRLR genes in Pekin duck and Black Muscovy duck. Gene Vol 769: Article145183. Doi: https://doi.org/10.1016/j.gene.2020.145183 .

Maharani, D., D.N.H. Hariyono, D.D.I. Putra, J. Lee, J.H.P. Sidadolog. 2019. Phenotypic Characterization of Local Female Duck Populations in Indonesia. Journal of Asia-Pacific Biodiversity 12(4): 508–514.

Masti H, Y. Yurnalis, T.D. Nova, Z. Kamsa, T. Rafian. 2021. Keragaman Gen Growth Hormone Receptor (GHR) Ekson 10 pada itik Sikumbang Janti. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan. 9(1):15–19. Doi: https://doi.org/10.29244/jipthp.9.1.15-19

Matitaputty, P.R., Bansi H. 2018. Upaya Peningkatan Produktivitas Itik Petelur Secara Intensif dan Pemberian Pakan Berbahan Lokal di Maluku. Jurnal Peternakan Sriwijaya. 7(2):1-8.

Murray, R.K., D.K. Granner, and P.A. Mayer. 2013. Harper's biochemistry. Prentice-Hall Internasional Inc.

Nei, M., and S. Kumar. 2000. Molecular Evolution and Genetics. Oxford University Press.

Noor, RR. 2008. Genetika Ternak. Penebar Swadaya.

Nova, T.D., E. Syahruddin, R. Zein. 2020. The Productivity of Duck in Different Temperature Cage Management. J.Nat.Indonesia 18(1): 43-61.

Nova, T.D., Y. Yurnalis, A.K. Sari. 2016. Keragaman Genetik Gen Hormon Pertumbuhan (GH|MboII) pada Itik Sikumbang Janti Menggunakan Penciri PCR-RFLP. Jurnal Peternakan Indonesia 18(1): 44-52.

Poggi, C., B. Le-Marchand, J. Zapf. 1979. Effects of binding of insulin-like growth factor-I in the isolated soleus muscle of lean and obese mice: Comparison with insulin. Endocrinology 105:723.

Rafian, T., Y. Yurnalis, H. Husmaini, F. Arlina. 2023. Polymorphism of Sikumbang Jonti ducks Growth Hormone (GH) Gene using PCR-RFLP Methods. Agritropica: Journal of Agricultural Science. 6(1): 8-13.

Rafian, T., Y. Yurnalis, Y. Fenita, R. Iskandarsyah. 2022. Polymorphism of Prolactin Gene (PRL/PstI) In Sikumbang Jonti Duck Using PCR-RFLP Methods. Jurnal Sain Peternakan Indonesia 17(3): 170-174.

Rahayu S., S.B. Sumitro, T. Susilawati, S. Soemarno. 2006. Identifikasi Polimorfisme Gen GH (Growrth Hormone) Sapi Bali dengan Metode PCR-RFLP. Berk. Penel. Hayati: 12: 7-11.

Saputro, B.E., R. Sutrisna, P.E. Santosa, F. Fathul. 2016. Pengaruh Ransum yang Berbeda pada Itik Jantan Terhadap Jumlah Leukosit dan Diferensial Leukosit. Jurnal Ilmiah Peternakan Terpadu 4(3): 176–181.

Subekti, K., D.D. Solihin, R. Afnan, A. Gunawan, C. Sumantri. 2019. Polymorphism of Duck HSP70 Gene and mRNA Expression under Heat Stress Conditions. Int J Poult Sci. 18(12):591–597. https://doi.org/10.3923/ijps.2019.591.597

Suhartati L, I. Khaerunnisa, A. Gunawan, R. Rukmiasih, S. Darwati, C. Sumantri, R. Rizqan. 2020. Short communication: Identification of the exon 1 myostatin gene polymorphism and its association with slaughtered weight in Indonesian Kampung and broiler chicken. Biodiversitas. 21(8):3893–3897. https://doi.org/10.13057/biodiv/d210860

Suhendro, I., J. Jakaria, R.R. Noor. 2021. The genetic diversity of heat shock protein 70 gene at promoter and 5 untranslated regions in beef cattle. J Indones Trop Anim Agric.

46(2):136–144. https://doi.org/10.14710/jitaa.46.2.136-144

Svoboda, M.E., and J.J. Van Wyk. 1983. Purification of somatomedin-C/insulin-like growth factor I. Methods in Enzymology. 109:798.

Vasconcellos, L.P.M.K., D.T. Talhari, A.P. Pereira, L.L. Coutinho, and L.C.A. Regitano. 2003. Genetic characterization of Aberdeen Angus cattle using molecular markers. J. Genet. Mol. Biol. 26: 133-137.

Viljoen, G.J., L.H. Nel, and J.R. Crowther. 2005. Molecular Diagnostic PCR Handbook. Springer, Dordrecht, Netherland.

Yurnalis, Y., A. Arnim, D.E. Putra, Z. Kamsa, T. Afriani. 2019. Identification of GH Gene Polymorphisms and Their Association with Body Weight in Bayang Duck, Local Duck from West Sumatra, Indonesia. In: IOP Conf Ser Earth Environ Sci. Vol. 347. https://doi.org/10.1088/1755-1315/347/1/012032

Yurnalis, Y., H. Husmaini, S. Sabrina. 2017. Polymorphisms of growth hormone gene Exon 1 and their associations with body weight in pitalah and kumbang janti ducks. Int J Poult Sci. 16(5):203–208. https://doi.org/10.3923/ijps.2017.203.208