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Single Nucleotide Polymorphism (SNP) Exon 4 Proclatine Gene on Local Duck

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ABSTRACT

This study uses the sequencing method to determine the genetic diversity of the prolactin gene (PRL) in the exon four region in Bayang ducks. This study used 118 blood samples of Bayang ducks. Blood samples were isolated and then sequenced using a pair of primers L: 5'- GCA CAG TTG TTC TTA CTA GTT CG -3' and R: 5'- TCT GAG AAC TTT GCA GCT ATC T -3', which produced a 586 bp fragment in the Prolactin (PRL) gene exon 4. The amplification product was sequenced using First Base Singapore's services. The results showed that there were 16 polymorphisms in the exon four region and parts of introns 3 and 4 in the Bayang duck sample, namely two deletions at position -223C>del and -218A>del and 14 mutations at position -136G>A, 157G>A, +9T>G, +11G>A, +16T>C, +20A>C, +25T>C, +29A>C, +35T>A, +38A>G, +43C>A, +71G>A, +84G>A, +91T>A. Based on the study results, it can be concluded that the diversity of the prolactin gene (PRL) of Bayang ducks in the exon four region is polymorphic and can be used as a candidate for marker-assisted selection in Bayang ducks.

Keywords: Genetic diversity, Indonesian, local duck, sequencing, Sumatra Barat

INTRODUCTION

Ducks are one of the germplasms in the poultry sector that has considerable potential as egg and meat producers, as an alternative to the availability of animal protein in Indonesia. West Sumatra Province has several local ducks that have developed as genetic resources, such as Pitalah ducks (Yurnalis et al., 2017), Kamang ducks (Arlina et al., 2021; Rafian et al., 2023a), Sikumbang Jonti ducks (Rafian et al., 2023b) and Bayang ducks (Rafian and Yurnalis, 2023c). According to Rafian and Yurnalis (2023c), the Bayang duck is a local duck raised by farmers in the Pesisir Selatan Regency. She has the potential to be developed because it can adapt to environmental limitations well and produce good eggs.

Improvements in maintenance management, feed, and genetic improvement need to be made to increase livestock productivity. Genetic improvement can be done by selection and crossbreeding. Conventional selection has contributed significantly to discovering new breeds with superior traits that exist today. However, along with the development of technology, selection can be done based on DNA characterization of genes so that selection can be done accurately and faster (Bilyaro et al., 2023).

In chickens, efforts have been conducted to identify genes associated with variations in egg production. One gene that correlates with egg production in chickens is prolactin (Cui et al., 2006). The prolactin gene is a candidate gene that explicitly controls variations in the amount of egg production through the reduction of egg biosynthesis during the brooding period (Chen et al., 2011). Prolactin gene information in local chickens is polymorphic and associated with egg production traits through brooding (Sartika, 2004). Prolactin genes in birds have been reported to play an essential role in the regulation of physiological processes, including egg production, spurring and maintaining brooding traits, osmoregulation, immune modulation, function and development of gonadal cells (Sharp et al., 1979).

The prolactin gene in ducks was identified by Kansaku et al. (2005), which is 10 kb in size and consists of five (5) protein-coding regions (exons) separated by four introns and encodes 229 amino acids. Studies linking prolactin gene polymorphisms to egg production traits have been conducted in ducks in Taiwan and China, with point mutations identified in the protein-coding part of exon 2, exon 4 and exon 5 in local Chinese ducks (Wang et al., 2011).

Li et al. (2009) reported that duck prolactin gene polymorphism in intron one was associated with egg weight. Wang et al. (2011) found Chinese local duck Prolactin (PRL) gene polymorphism in intron 1 associated with eggshells. Also, they found Chinese local duck

Prolactin (PRL) gene polymorphism in exon 5 due to C5961T mutation associated with annual egg production and egg weight. Rashidih et al. (2012) found a polymorphism in the Prolactin (PRL) gene of chickens in exon 2 associated with first egg laying weight and age at sex maturity. Point mutations were identified in the non-coding part of the prolactin gene in Tsaiya and Gaoyou ducks (Li et al., 2009; Chang et al., 2012). Data on prolactin gene diversity in local poultry in Indonesia is still limited to native chicken species. Prolactin gene information in local chickens is polymorphic and related to egg production traits (Sartika, 2004). Meanwhile, prolactin (PRL) gene diversity data is still unavailable in local ducks.

In the duck prolactin gene polymorphism study, intron one was associated with egg weight (Li et al. 2009). Haplotype analysis showed that each mutation was associated with egg production and reproductive traits (Chang et al., 2012). The prolactin gene in chicken has been found at chromosome 2, while its location on the duck chromosome has not been identified. Research conducted by Hui et al. (2009) on Gaoyou ducks (local Chinese ducks) found prolactin gene SNP in intron 1. This study found T / C mutation at position 1326 pb after sequencing produced three genotypes: AA, AB and BB. The results of most minor square analyses showed that BB-genotyped ducks had higher egg weights than AB-genotyped ducks ($P<0.001$), and the percentage of double yolk incidence of AB ducks was higher than BB ducks ($P<0.05$). Still, there was no significant difference in the number of eggs and body weight parameters at first laying.

Based on the description above, research is needed to determine the genetic diversity of the prolactin gene (PRL) of Bayang ducks, especially in the exon four region, as a foundation in efforts to select superior Bayang ducks.

MATERIALS AND METHODS

Materials

The materials used in this study were 118 blood samples of 8-week-old female Bayang ducks. The blood samples were taken from Binuang Kampung Dalam Village, Pauh District, Padang City. The materials used were Bayang duck blood samples, Cell lysis, Nuclei lysis, Protein Precipitation solution, Isopropanol, 70% Ethanol, DNA rehydration, Master Mix (Dream taq Green), Nuclease-Free Water (REF P119), 2% agarose, 100 pb marker, TBE 1x (1 M Tris; 0.9 M Boric Acid; 0.01 M EDTA pH 8.0), and ethidium bromide. The primers used in this study were a pair of L primers: 5'- GCA CAG TTG TTC TTA CTA GTT CG -3' and R: 5'- TCT GAG AAC TTT GCA GCT ATC T -3' (Yurnalis et al., 2018).

The tools used were Eppendorf tubes, micropipette microtip, Eppendorf centrifuge 5417, vortex, sterile tissue, Eppendorf master cycle gradient machine, PCR tubes, glass bottles, measuring cups, stirrer, agar media mould, power supply, electrophoresis (Thermos Scientific), UV photo-transilluminator, UV glasses and gloves. PCR products of Prolactin (PRL) gene fragment exon 4 of Bayang duck samples were sequenced using the services of First Base in Singapore.

Methods

This research was conducted by genetic exploration method at the Livestock Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University and PCR product sequencing using the services of First Base in Singapore to observe the diversity of the Prolactin (PRL) gene from the Bayang duck population.

DNA isolation was performed from blood samples using a genomic DNA purification kit from Promega using the procedure suggested by the producer. Amplification of prolactin gene was performed using PCR (Polymerase Chain Reaction) method with a pair of primers L: 5'- GCA CAG TTG TTC TTA CTA GTT CG -3' and R: 5'- TCT GAG AAC TTT GCA GCT ATC T -3' which produced a fragment band of 586 bp in the exon 4 region. PCR amplification reagent of Prolactin (PRL) gene using Master Mix (ThermoSCIENTIFIC ®) with a composition of 2 µl DNA sample, 12.5 µl Master Mix, 3 µl L and R primer mix and 7.5 µl Nuclease-Free Water. In vitro, amplification was performed using a PCR machine (Eppendorf® Mastercycler gradient) programmed: presaturation at 95°C for 5 minutes, denaturation at 95°C for 45 seconds, annealing at 59°C for 45 seconds and extension 72°C for 1 minute, repeated 35 cycles and final extension 72°C for 5 minutes. To view the amplified fragments of the prolactin gene (PRL), PCR products were electrophoresed using 1% agarose (ThermoSCIENTIFIC ® TopVision Agarose #R0491) with Ethidium Bromide Solution staining (MP Blomedicals ®) and the results were observed using a UV transilluminator (SynGENE ® G: BOX).

Sequencing of PCR products was carried out using the services of a genetic company, 1st Base Singapore. Sequencing was carried out based on the principle of Sanger method sequencing using an Automatic DNA Sequencer with primers. DNA data from the Bayang duck Prolactin (PRL) gene sequencing results in chromatograms to determine the quality of sequences and analyzed using Geneious Basic 5.6.3 software. Genotyping of sequencing results for nucleotide base sequencing was analyzed using SeqmanTM version 4.00 DNASTAR by analyzing electroferogram data.

Data Analysis

The genotyping and allele data obtained were analyzed in the form:

Genotype frequencies were calculated using the formula (Nei and Kumar, 2000):

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

Description:
x_{ii} = frequency of genotype i
n_{ii} = number of individuals with genotype ii
N = total number of individual's sample

Allele frequency was calculated using the formula (Nei and Kumar, 2000):

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

Description:
x_i = frequency of allele i
n_{ii} = number of individuals with genotype ii (homozygote)
n_{ij} = number of individuals with genotype ii ij (heterozygote)
n = total number of individual samples

RESULT AND DISCUSSION

Amplification of Prolactin Gene (PRL)

Amplification of the Prolactin (PRL) exon four gene fragment was performed on 118 Bayang duckling samples using a PCR machine (Eppendorf Master cycler Gradient) at 59 °C annealing conditions with a pair of primers L: 5'-GCA CAG TTG TTC TTA CTA GTT CG -3' and R: 5'-TCT GAG AAC TTT GCA GCT ATC T -3' which resulted in a fragment band of 586 bp. This primer was designed using Primer3 with a target DNA length of 586 bp based on the DNA sequence information of the Prolactin gene (PRL) in GenBank (access number: NW_004676690). Amplification products were visualized through 1.0% agarose gel electrophoresis. Prolactin gene amplification results in Bayang ducks are presented in Figure 1.

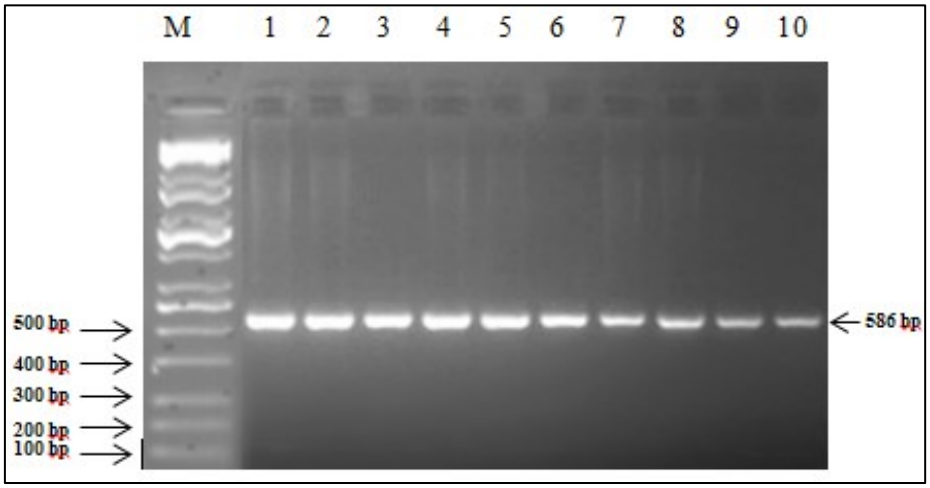

Figure 1. The results of PCR product amplification of Prolactin (PRL) gen exon four on Bayang duck (M = marker Kapa Universal 100bp); No 1-10 = individual samples)

PCR products of 118 samples were sequenced using the services of 1st Base in Singapore. All samples were successfully sequenced and analyzed using SeqManTM version 4.00 DNASTAR. The reference sequence was GenBank's Prolactin (PRL) gene sequence with access number NW_004676690. The sequencing results showed six variable sites in the exon four region.

Nucleotide diversity is an accurate parameter to describe genetic diversity. Positive elements using nucleotide diversity are independent of sample size and DNA length (Nei, 1987; Hartl and Clark, 1997). Small

samples can provide a broader picture of the actual population conditions. The obstacle that often occurs in the analysis is the number of samples because it is closely related to the available funds. In addition, the length of DNA fragments analyzed by choosing the suitable marker can provide good information.

Genetic diversity or genetic polymorphism is the presence of more than one form or type of genotype in the population. The source of genetic diversity is caused by sequence repeats, insertions, deletions and recombinations in DNA sequences between individuals, groups or a population (Nei and Kumar, 2000). Prolactin gene diversity and detection of mutation events are shown in Tables 1 and 2.

Based on the results of alignment analysis on prolactin sequences, 16 polymorphisms were found in the exon four region and parts of introns 3 and 4 in the Bayang duck sample. The analysis showed that almost all mutations in this study occurred in the partial region of intron three and intron 4. Based on the results of research related to the polymorphism of the Prolactin (PRL) gene in ducks, one of which is research by Indriati (2014) states the polymorphism of the Prolactin (PRL) gene exon 4 in Peking ducks, Mojosari White ducks and PMP ducks with the results found there are five mutation points namely INDEL 3724A, T3941G, C3975A, INDEL 4031A and T4110C are polymorphic and have a significant effect on egg production traits, percentage fertility and hatchability of duck eggs. The accumulation of base differences in 118 individuals gave six haplotypes (Table 1) that describe the differences among individual Bayang ducks and diversity within the population.

The results of this study based on the composition of base differences (haplotypes) also clearly show differences between individual livestock so that the Prolactin gene region in the study can be referred to as a molecular marker in ducks and support taxonomic research. Different haplotypes in each livestock can be used as a reference when identifying individual ducks. The more diverse the composite type of haplotypes in a population, the higher the level of genetic diversity and vice versa (Akbar et al., 2014).

Electroferograms of sequencing results showing the Prolactin (PRL) gene mutation in Bayang ducks are presented in Figures 2 to 7.

Table 1. PRL exon four gene polymorphisms aligned with Gene Bank reference (access code/number NW_004676690) in the Bayang duck population

H	Sequence number of the control region													
	-136	157	+9	+11	+16	+20	+25	+29	+35	+38	+43	+71	+84	+91
Rf	G	G	T	G	T	A	T	A	T	A	C	G	G	T
A	.	.	.	.	.	.	.	.	.	.	A	.	.	.
B	A	.	G	.	.	.	.	.	.	.	A	.	.	.
C	A	.	G	.	.	.	.	.	.	.	.	.	.	.
D	.	.	G	.	.	.	.	.	.	.	A	.	A	A
E	.	.	G	.	.	.	.	.	.	.	.	.	.	.
F	.	A	.	A	C	C	C	C	A	G	.	A	.	.

Description: H = Haplotype, Rf = Reference

Table 2. Mutation detection of Prolactin gene in Bayang ducks

No.	Mutation	Position	Mutation Variation	No.	Mutation	Position	Mutation Variation
1	Del C	-223	Deletion	9	T → C	+25	Transition
2	Del A	-218	Deletion	10	A → C	+29	Transversion
3	G → A	-136	Transition	11	T → A	+35	Transversion
4	G → A	157	Transition	12	A → G	+38	Transition
5	T → G	+9	Transversion	13	C → A	+43	Transversion
6	G → A	+11	Transition	14	G → A	+71	Transition
7	T → C	+16	Transition	15	G → A	+84	Transition
8	A → C	+20	Transversion	16	T → A	+91	Transversion

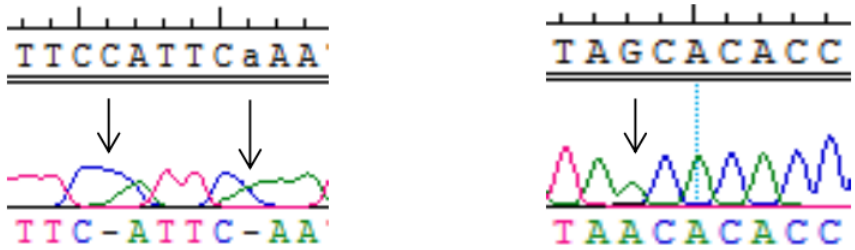

Figure 2. Deletion C on position -223, Deletion A on position -218, and Mutation G→A on position -136

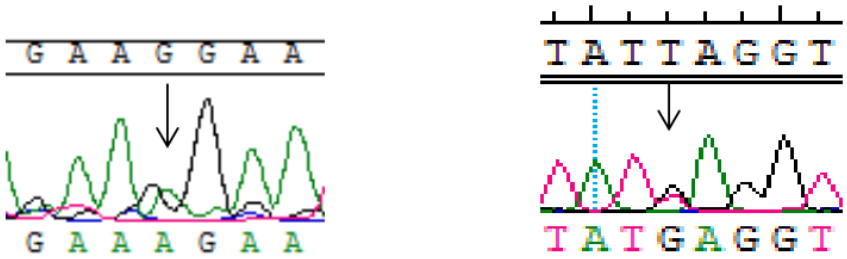

Figure 3. Mutation G→A on position 157 and Mutation T→G on position +9

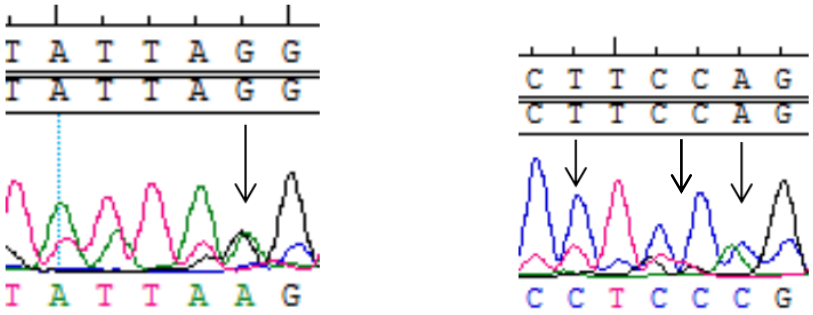

Figure 4. Mutation G→A on position +11, Mutation T→C on position +16, and Mutasi A→C on position +20

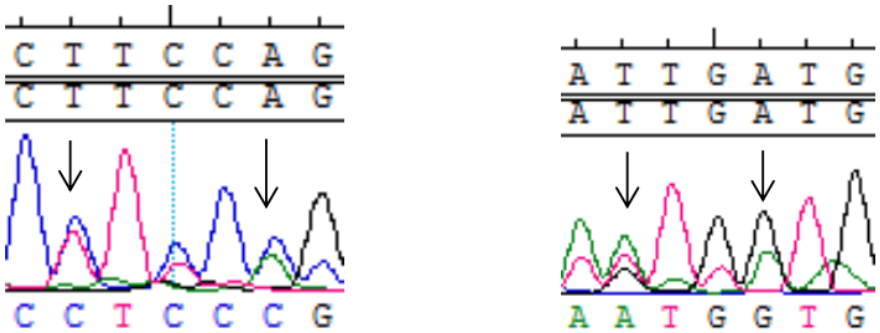

Figure 5. Mutation T→C on position +25, Mutation A→C on position +29, Mutation T→A on position +35, and Mutation A→G on position +38

Deletion-type mutations occur because specific base pairs disappear, causing a change in the arrangement of nucleotide bases (Yunita, 2009). The alignment analysis showed two deletion mutations, namely at position -223pb missing one cytosine base (deletion C) and at position -218pb missing one adenine base (deletion A). Transversion-type mutations occur due to a change between purine and pyrimidine bases. In this study, six transversion-type mutation points were found.

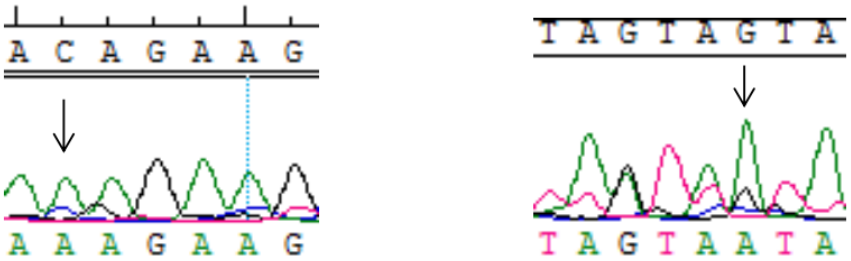

Figure 6. Mutation C→A on position +43 and Mutation G→A on position +71

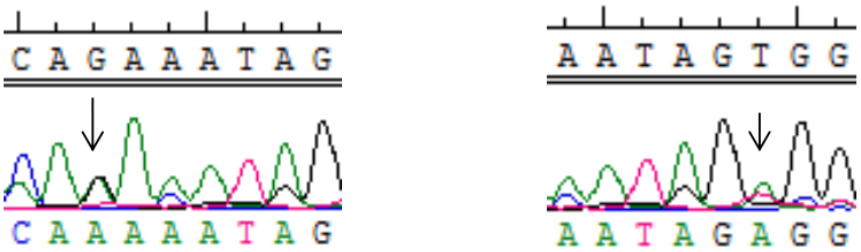

Figure 7. Mutation G→A on position +84 and Mutation T→A on position +91

Position +9pb occurred T→G mutation, namely the change of pyrimidine base (thymine) to purine (guanine); at position +20pb, occurred A→C mutation, namely the change of purine base (adenine) to pyrimidine (cytosine), at position +29pb occurred A→C mutation, namely the change of purine base (adenine) to pyrimidine (cytosine), at position +35pb there is a T→A mutation, which changes the pyrimidine base (thymine) to purine (adenine), at position +43pb there is a C→A mutation, which changes the pyrimidine base (cytosine) to purine (adenine), at position +91pb there is a T→A mutation, which changes the pyrimidine base (thymine) to purine (adenine).

In the transition type mutation, 8 mutation points were found, namely at position -136pb a G→A mutation occurred, namely a change in purine base (guanine) to purine (adenine), at position 157pb a G→A mutation occurred, namely a change in purine base (guanine) to purine (adenine), at position +11pb a G→A mutation occurs, which is a change in the purine base (guanine) to purine (adenine), at position +16pb a T→C mutation occurs, which is a change in the pyrimidine base (thymine) to pyrimidine (cytosine), at position +25pb a T→C mutation occurs, which is a change in pyrimidine base (thymine) to pyrimidine (cytosine), at position +38pb an A→G mutation occurs, which is a change in purine base (adenine) to purine (guanine), at position +71pb a G→A mutation occurs, which is a change in purine base (guanine) to purine (adenine), at position +84pb a G→A mutation occurs, which is a change in purine base (guanine) to purine (adenine). Transitional mutations occur due to changes between purine bases (adenine and guanine) with other purine bases or between pyrimidine bases (thymine and cytosine) with other pyrimidines (Nei, 1987).

Changes in the DNA base sequence will cause changes in the protein encoded by the gene. The sequence in the exon 4 region was transformed to amino acids using Geneious Basic 5.6.3 software, as presented in Figure 9.

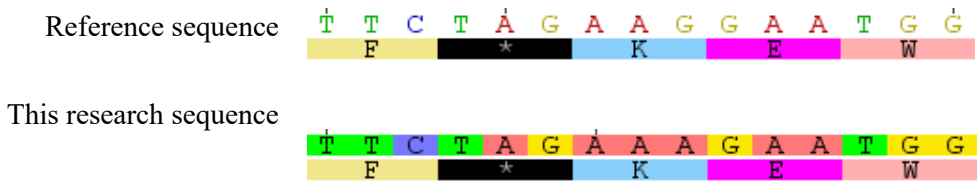

Figure 9. Amino acid sequence comparison of intron 4 region

Based on Figure 9, the mutation at position 157 will change the sequence from TTCTAGAAGGAATGG to TTCTAGAAAGAATGG, which only changes the AAG genetic code to the AAA genetic code, and there is no change in the amino acids Phenylalanine, stop, Lysine, Glutamate and Tryptophan so that mutations at this position are called silent mutations. Silent mutation, which is a change in a base pair in a gene (at position three codon) that causes a change in one genetic code but does not result in changes in the coded amino acids, is usually caused by transitional mutations and transversions (Stansfield et al., 2003).

Genotype and Allele Frequency

Genotyping frequency is the ratio of the number of genotypes to the total population. Genotypic diversity in individual livestock can be determined through the DNA waves found. Allele frequency is the relative ratio of an allele to all alleles at a locus in the population (Nei and Kumar, 2000).

Gene frequency in a population can change if there are evolutionary forces, which are factors that play a role in changing the frequency of alleles and genotypes, including mutation, migration (gene flow), non-random mating, genetic drift and natural selection (Arisuryanti & Daryono, 2007). Changes in allele frequencies and genotypes of a population indicate microevolution, an evolution that occurs at a small level (genes). Campbell and Mitchell (2003) state that if allele or genotype frequencies deviate from the expected value of the Hardy-Weinberg equilibrium, then the population is said to be evolving. Hardy-Weinberg law states that the genotype frequency of a large enough population will always be in a state of equilibrium if there is no selection, migration, mutation, or genetic drift.

The genotype frequency and allele frequency values that determine the diversity of the Prolactin (PRL) gene in Bayang ducks are presented in Table 3.

Table 3. Genotype and allele SNP on Prolactin (PRL) gene

No	Posisi SNP	N	Frekuensi Genotip			Frekuensi Alel	
1	-223 C→del	118	-	-	-	-	-
2	-218 A→del	118	-	-	-	-	-
3	-136 G→A	118	GG 0.90 (106)	GA 0.02 (3)	AA 0.08 (9)	G (0.91)	A (0.09)
4	157 G→A	118	GG 0.98 (115)	GA 0.00 (0)	AA 0.02 (3)	G (0.98)	A (0.02)
5	+9 T→G	118	TT 0.80 (94)	TG 0.02 (3)	GG 0.18 (21)	T (0.81)	G (0.19)
6	+11 G→A	118	GG 0.98 (115)	GA 0.00 (0)	AA 0.02 (3)	G (0.98)	A (0.02)
7	+16 T→C	118	TT 0.98 (115)	TC 0.00 (0)	CC 0.02 (3)	T (0.98)	C (0.02)
8	+20 A→C	118	AA 0.98 (115)	AC 0.00 (0)	CC 0.02 (3)	A (0.98)	C (0.02)
9	+25 T→C	118	TT 0.98 (115)	TC 0.00 (0)	CC 0.02 (3)	T (0.98)	C (0.02)
10	+29 A→C	118	AA 0.98 (115)	AC 0.00 (0)	CC 0.02 (3)	A (0.98)	C (0.02)
11	+35 T→A	118	TT 0.98 (115)	TA 0.00 (0)	AA 0.02 (3)	T (0.98)	A (0.02)
12	+38 A→G	118	AA 0.98 (115)	AG 0.00 (0)	GG 0.02 (3)	A (0.98)	G (0.02)
13	+43 C→A	118	CC 0.88 (103)	CA 0.02 (3)	AA 0.10 (12)	C (0.89)	A (0.11)
14	+71 G→A	118	GG 0.98 (115)	GA 0.00 (0)	AA 0.02 (3)	G (0.98)	A (0.02)
15	+84 G→A	118	GG 0.98 (115)	GA 0.00 (0)	AA 0.02 (3)	G (0.98)	A (0.02)
16	+91 T→A	118	TT 0.98 (115)	TA 0.00 (0)	AA 0.02 (3)	T (0.98)	A (0.02)

Descrption: SNP = Single Nucleotide Polymorphism; (N) = Total number of samples

The analysis results showed that the genotype frequency of the Prolactin gene sequence was found at the -136G>A locus, namely the genotype frequency of GG 0.90, GA 0.02 and AA 0.08. At the 157G>A, +11G>A, +71G>A, and +84G>A loci, the same genotype frequency was GG 0.98, GA 0.00, and AA 0.02. At the +9T>G locus, the genotype frequencies were TT 0.80, TG 0.02, and GG 0.18. The +16T>C and +25T>C loci had the same genotype frequencies of TT 0.98, TC 0.00, and CC 0.02. At loci +20A>C and +29A>C, the same genotype frequencies are AA 0.98, AC 0.00 and CC 0.02. At the +35T>A and +91T>A loci, the same genotype frequencies were TT 0.98, TA 0.00 and AA 0.02. The genotype frequencies at the +38A>G locus are AA 0.98, AG 0.00 and GG 0.02. The +43C>A locus has a genotype frequency of CC 0.88, CA 0.02 and AA 0.10.

Allele frequency analysis at the -136G>A locus showed that the G allele had a frequency value of 0.91, and the A allele had a frequency value of 0.09. At locus 157G>A, +11G>A, +16T>C, +20A>C, +25T>C, +29A>C, +35T>A,

+38A>G, +71G>A, +84G>A have the same allele frequency values of 0.98 and 0.02. The +9T>G locus has a T allele frequency value of 0.81 and a G allele frequency of 0.19. The +43C>A locus has a C allele frequency value of 0.89 and an A frequency of 0.11.

Based on the results of the analysis, it was found that there was a diversity of Prolactin genes in Bayang ducks in the target fragment area. The results of this analysis are generally polymorphic. According to Falconer and Mackay (1996), an allele is said to be polymorphic if it has an allele frequency equal to or less than 0.99. Genetic diversity occurs when two or more alleles exist in a population < 99% (Nei, 1987).

It is in line with the statement of Nei and Kumar (2000) that an allele is said to be polymorphic if the frequency of one of the genes below is equal to 0.95 or 0.99. Likewise, the statement of Allendorf and Luikart (2007) that an allele is said to be monomorphic if it has a frequency value of one of the alleles at the same mutation point reaches 1.00. According to Hartl and Clark (1997), it is essential to analyze polymorphic properties because a gene must be used as a genetic marker.

Potential of Restriction Enzyme

Information on SNP diversity can be seen with DNA sequence alignment, so the livestock population's genotype and allele frequency can be determined from this information. DNA sequence alignment is also used to determine the potential of restriction enzymes with the help of several bioinformatics software. Restriction enzymes are enzymes that work to cut DNA fragments at specific sites (Sunatmo, 2009).

Suharsono and Widyastuti (2006) stated that recombinant DNA molecules could not be made quickly without two types of enzymes, namely, restriction endonuclease enzymes that act as scissors to cut DNA at specific sites and DNA ligase, which acts as the glue that glues two DNA molecules in a test tube. The recognition sequence or recognition site is a DNA sequence to which the restriction enzyme attaches and cuts the sequence. One of the software used to see the potential of cutting enzymes on DNA sequences is CLC MainWorkbench 8. The potential cutting enzymes found from the use of CLC MainWorkbench software can be seen in Table 4.

Table 4. The potential of restriction enzyme

No	Position of SNP	The potential of restriction enzyme
1	-223 C>del	Not founding
2	-218 A>del	*TasI* (aatt↓), *TspEI* (aatt↓)
3	-136 G>A	*BmUI* (ac↓tggg), *BmrI* (ac↓tggg)
4	157 G>A	*XbaI* (tcta↓ga), *XspI* (ctag↓)
5	+9 T>G	*MnlI* (cc↓tc)
6	+11 G>A	*MseI* (ttaa↓), *TrugI* (ttaa↓)
7	+16 T>C	*MnlI* (cc↓tc), *BsrI* (act↓gg)
8	+20 A>C	*HpyAV* (cc↓ttc), *Hin4II* (cc↓ttc)
9	+25 T>C	*MnlI* (cc↓tc), *BstllI* (ac↓tgg)
10	+29 A>C	*SmUI* (cccg↓c), *BspACI* (ccgc↓)
11	+35 T>A	*BsmRI* (tgta↓ca), *PabI* (gt↓ac)
12	+38 A>G	*BstAuI* (tgta↓ca), *AauI* (tgta↓ca)
13	+43 C>A	Not founding
14	+71 G>A	Not founding
15	+84 G>A	Not founding
16	+91 T>A	*Mn1I* (cc↓tc)

Information on enzymes that have the potential to be used as restriction enzymes can be used in other studies that look at the genetic diversity of Bayang ducks using the PCR-RFLP method. According to Sidah (2019), genetic characteristics resulting from cutting with restriction enzymes are influenced by the type of restriction enzyme and the DNA sample being cut; the type of restriction enzyme used usually refers to the genomic sample to be analyzed. Restriction enzymes with specific cutting patterns will cut DNA in a sequence that matches its identifying sequence, affecting and producing DNA fragment cutting patterns with a specific size and limited length due to cutting DNA. The more cutting patterns of DNA fragments

produced with a specific size, the more site differences are obtained, thus indicating that the restriction enzyme used is suitable for the analysed DNA sample.

CONCLUSION

The results showed that 16 mutations were found in the exon four region of the Prolactin (PRL) gene of Bayang ducks and were polymorphic, so they have the potential to become genetic markers selection (MAS).

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