Polymorphism of prolactine genes and its association with body weight in Bayang ducks, local duck from West Sumatera, Indonesia

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Abstract. This study aims to determine the polymorphism of Prolactin (PRL | XbaI and PRL | DraI) genes in Bayang ducks using the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) technique and its association with weight of ducks aged 1-10 weeks. This study used 200 Bayang duck blood samples consisting of 102 male ducks (♂) and 98 female ducks (♀). DNA from blood samples was isolated using the Genomic DNA Purification Kit (Promega) using protocol from the manufacture. The DNA was then amplified using two primers with F: 5'-AAA TTC CCT CTC ACA GTT ACA-3' and R: 5'-GAT GCA GAG ACA AGT TTC ACC-3' and F: 5'-GAATAGAACACTTGACCCTG -3' and R: 5'-TAGAGGGGCAAGCATAAG -3' which produces fragments with a length of 416 bp and 566 bp. Restriction with XbaI enzymes that recognized the binding site (5'-TT CTAGA-3') resulted 3 genotypes: homozygote (+/+), heterozygote (+/-) and homozygote (-/-) with frequencies 0.455, 0.495 and 0.050 respectively and with frequency allel (+) 0.702 and allele frequencies (-) 0.297. While the results of the restriction with enzyme DraI found three types of genotypes, namely (+/+), (+/-) and (-/-) with frequencies of 0.64, 0.35 and 0.01 respectively with frequency allele (+) 0.82 and allele frequencies (-) 0.18. From the results of the analysis, it was found that there was no relationship between these two diversity and weight ducks of duck.

Keywords – Bayang duck, PRL-XbaI, PRL-DraI, body weight, PCR-RFLP.

1. Introduction

West Sumatera, Indonesia has many species of local duck such as Pitalah, Bayang, Kumbang Jonti, and Kamang. All of this duck are layer with small body size and lower meat production. Bayang duck is the local duck found in Pesisir Selatan Distric, West Sumatra Province, Indonesia. Bayang duck is dual-purpose duck type with color of the feathers is predominantly white and brown, the body looks large and erect, short neck, black beak and Legs. Adult weight about 1.4 - 2.0 Kg. The eggs are light blue with a production of about 180 - 200 per year. Recently, the demand of duck meat in Indonesia are increasing together with the increasing of product diversification. To supply requirement and demand of duck meat, meat performance of native duck should be improved by selection.

The application of molecular genetics in identification of polymorphism in growth candidate genes that show association with specific economically relevant traits provide useful information to enhance genetic improvement programme in livestock and validation of genetic markers of growth traits is the initial and crucial step to establish a Marker Assisted Selection system (MAS).
**PRL** gene is one of the growth hormone gene family and is synthesised mainly in the anterior pituitary of all vertebrates [1]. The prolactin is a candidate gene that specifically controls the variation in the amount of egg production through reducing egg biosynthesis during the brood period [2]. The prolactin gene in ducks consists of five exon regions separated by four introns and encoding 229 amino acids [3]. Research on the prolactin gene been widely carried out by [1] [4] [5]. [1] found variations in exon two, four and five exons in local Chinese ducks. Other mutation points were identified in the non-coding part of the prolactin gene in Tsaiya and Gaoyou ducks [4] [5]. Research conducted by [4] in Chinese local ducks found T/C mutations at position 1326 bp in intron 1 region than can be identified using *Dra*I enzyme.

Prolactin is a single-chain polypeptide hormone synthesised in the anterior pituitary gland. The prolactin hormone in ducks is composed of 229 amino acids encoded by the prolactin gene [3]. Research on intron 1 duck prolactin gene polymorphism is associated with egg weight [4] while exon 5 shows the relationship with annual egg production [1]. Research on the polymorphism of intron one prolactin gene was associated with egg weight [4] while exon five showed a relationship with annual egg production [1]. Haplotype analysis shows that each mutation is related to egg production and reproductive traits [5]. The mutation of duck prolactin gene occurs in introns. Research conducted by [6] in Gaoyou ducks (Chinese local ducks) found prolactin gene SNP at intron 1, ie T/C mutation at position 1326 bp after sequencing was carried out three genotypes AA, AB and BB. And research conducted by [1] on several types of Chinese local ducks found SNP of prolactin gene in intron 1. They found C→A mutation at position 386 at introns which can be detected by *Xba*I enzyme. In Mulard ducks, **PRL**/**Xba**I polymorphism had an effect (*p < 0.05) on body weight aged 10 and 12 weeks [7].

The present study was carried out to study the polymorphism in Prolactine gene and its association with weight in Bayang duck and to analyse the potential of this gene to act as markers for selection tools for duck breeding.

### 2. Materials and method

#### 2.1. Research material

The material used in this study were 200 Bayang duck blood samples consisting of 102 male ducks and 98 female ducks. The fertile duck egg were collect from famer and hatched. Then the Bayang duck were kept intensively for 10 weeks and blood samples were taken through a brachial vein ± 1 ml. DNA isolation is carried out from a blood sample using the Genomic DNA Purification Kit from Promega based on the procedure from the manufacturer. PRL gene amplification was performed using primer 5'-GAATAGAACACTTGACCCTG F -3 ' and 5'-TAGAGGAGGCAAGCATAG R -3' and primers F 5 'AAATTCCCT CTCACAGTTACA'3 and R 5' GATGCAGAGACAAGTTTCACC '3 which produce fragment length 566 bp and 416 bp. PCR amplification using Master Mix (Thermo SCIENTIFIC®) with the following composition: DNA sample 2 ul, Master Mix 12.5 ul, each primer 2 ul and, Nuclease-Free Water (REF P1193) 7.5 ul done using PCR machine (ependorf® Mastercycler gradient) with pradenaturasi at a temperature of 95°C for 5 minutes, denaturation at a temperature of 95°C for 45 seconds, annealing at temperatures of 55°C for 45 seconds and extension 72°C for 1 minute, and a final extension of 72°C for 5 minutes with 35 cycles. Results of PRL gene amplification, visualisation on 1% agarose (Agarose TOPVISION ThermoSCIENTIFIC®) and stained by Ethidium Bromide Solution (MP Biomedicals®) and the results observed by using a UV trans-illuminator (SynGENE® G: BOX). The results of the subsequent amplification restricted to use 5 units of restriction enzyme *Dra*I (5 'TTTAAA↓ 3') and the enzyme *Xba*I (5- T↓CTAGA-3').

#### 2.2. Data analisis

Statistical analysis: The genotype and allele frequencies were calculated in each group of ducks. The genetic effects of the **PRL**/**Dra**I and **PRL**/**Xba**I polymorphisms on body weight were analyzed using a General Linear Model (GLM) procedure. The model used the Eq. 1:
$Y_{ijkl} = \mu + G_i + S_k + g_{ijkl}$ (1)

where, $Y_{ijkl}$ is the observed value of the dependent variable, $\mu$ is the overall mean, $G_i$ is the fixed effect due to genotype ($i = 1,2,3$), $S_k$ is the fixed effect due to sex ($k = \text{males or females}$) and $g_{ijkl}$ is the random residual error. The Hardy-Weinberg equilibrium was assessed with the Chi-square test. The statistical significance of differences among the means was calculated in accordance with the SAS/STAT Software, Release 6.12 (SAS Institute Inc., Cary, NC, USA).

3. Result and discussion

The amplification of the PRL gene fragments in Bayang ducks was carried out using the Polymerase Chain Reaction (PCR) method with two pairs of primers 5’- GAA TAG AAC ACT TGA CCC TG-3’ (forward) and 5’-TAG AGG AGG CAA GCA TAG-3’ (reverse) [4] and forward: 5’-AAA TTC CCT CTC ACA GTT ACA-3’ and reverse: 5’-GAT GCA GAG ACA AGT TTC ACC-3’ which results in fragments of length 566 bp and 416 bp. The results of gene amplification are presented in Figure 1.

4. Genotyping of PRL|DraI and PRL|XbaI

The PRL|DraI gene genotype can be detected by the PCR-RFLP technique using the DraI restriction enzyme. DraI restriction enzymes cut DNA by recognizing TTT↓AAA located in the intron 1. The following DNA restriction fragments were obtained for PRL-DraI polymorphism: 519bp/47bp for the TT genotype, 519/310bp/209bp/47bp for TC genotype, and 310bp/209bp/47bp for the CC genotype (Figure 2).

Frequencies genotypic and allelic frequencies of of PRL|DraI and PRL|XbaI were presented in Table 1
Table 1. Frequencies genotypic and allelic frequencies of of PRL|DraI and PRL|XbaI.

|       | Frequencies Genotype | Frekuensi Allel | n    |
|-------|----------------------|-----------------|------|
|       | CC                   | CT              | TT   | C   | T   |      |
| Male  | 0.684 (63)           | 0.293 (27)      | 0.021 (2) | 0.831 | 0.168 | 92   |
| Female| 0.602 (53)           | 0.397 (35)      | 0     | 0.801 | 0.198 | 88   |
| Sum   | 0.64 (116)           | 0.34 (62)       | 0.01 (2) | 0.816 | 0.183 | 180  |
|       |                      |                 |       |      |      |      |
|       | GG                   | GT              | TT   | G   | T   |      |
| Male  | 0.48 (49)            | 0.48 (49)       | 0.02 (4) | 0.728 | 0.272 | 102  |
| Female| 0.42 (42)            | 0.50 (50)       | 0.06 (6) | 0.673 | 0.327 | 98   |
| Sum   | 0.455 (91)           | 0.495 (99)      | 0.05 (10) | 0.702 | 0.297 | 200  |

The results of the analysis in Table 1 show that PRL|DraI in male Bayang ducks obtained three genotypes consisting of homozygotes (CC), heterozygotes (CT) and homozygotes (TT) with frequencies of 0.684, 0.293 and 0.21, respectively. While in female Bayang ducks, only obtained two genotypes consisting of homozygotes (CC) and heterozygotes (CT) with frequencies of 0.602 and 0.397. Male Bayang ducks have the most homozygous (CC) genotypes, 63 samples with a frequency of 0.684 compared to Bayang ducks, females have genotypes (CC) only 53 samples with a frequency of 0.602, while for the most heterozygous (CT) genotypes obtained by Bayang ducks females, which were 35 samples with a frequency of 0.397 compared to male Bayang ducks, only got 27 samples with a frequency of 0.293 and for homozygotes (TT) it was only owned by male Bayang ducks which were 2 samples 0.021. so that the allele frequency (C) is 0.801 higher than the frequency frequency (T) which is 0.183.

The results of RFLP characterization analysis using the XbaI restriction enzyme on the exson 1 intron 1 PRL gene fragment obtained three kinds of fragments, namely non-cut fragments (416 bp) known as homozygous genotypes (TT), fragments that can be cut (353 bp and 63 bp) which is known as genotype (GG), but the band with a base length of 63 pb is not visible because it is too short, and the combined fragments (416 bp, 353 bp, and 63 bp) or heterozygotes are known as genotypes (GT).

The proportion of the Prolactin (PRL|xbaI) gene fragment alleles in the Bayang duck studied showed that the frequency of the heterozygote (GT) genotype was slightly greater than the homozygous (GG) genotype frequency, ie the heterozygous (GT) genotype frequency of 0.495 and frequency genotype (GG) of 0.455. However, the frequency of the homozygous (TT) genotype is quite low, there is only a genotype frequency of 0.050. This causes the allele (G) frequency of 0.702 to be more dominant than the allele (T) frequency of 0.297. The proportion of alleles obtained in the Prolactin (PRL|xbaI) gene fragments in the Bayang duck studied has a frequency of genotype and allele not much different from the genotype and allele frequency values in the study of [1] in Shaoxing local Chinese ducks obtained frequency values of heterozygous (GT) genotypes of 0.500, homozygous (GG) genotypes of 0.416 and homozygotes (TT) of 0.024 with allele (G) frequency of 0.738 more dominant from the allele frequency (T) of 0.262. From the analysis no relation between this polymorphism with body weight Bayang duck.

5. References
[1] Wang C, Liang Z, Yu W, Feng Y, Peng X, Gong Y, Li S. 2011. Polymorphism of the prolactin gene and its association with egg production traits in native Chinese ducks. S Afr J Anim Sci. 41:64–69.
[2] Chen, C. F., Y. L. Shiue, C. J. Yen, P. C. Tang, H. C. Chang and Y. P. Lee. 2007. Laying traits and underlying transcripts, expressed in the hypothalamus and pituitary gland, that were associated with egg production variability in chickens. Theriogenol 68: 1305-1315.

[3] Kansaku, N., Ohkubo, T., Okabayashi, H., Guémené, D., Kuhnlein, U., Zadworny, and D., K. Shimada. 2005. Cloning of duck PRL cDNA and genomic DNA. Gener Compar Endocrinol: 39-47.

[4] Li, H., F. Zhu, W., Q. Chen, K., W. Zhang, and T., J., W. Song. 2009. Association of polymorphisms in the intron 1 of duck prolactin with egg performance. Turk J Vet Animals Science. 33, 193-197.

[5] Chang, M. T., Y. S. Cheng and M. C. Huang. 2012. Association of prolactin haplotypes with reproductive traits in Tsaiya ducks. Anim Rep Sci. 135: 91-96.

[6] Hui, F. L., Q. Z. We, W. C. Kuan, J. Z. Tang, and T. S. Wei. 2009. Association of polymorphisms in intron 1 of duck prolactin with egg performance, Turk J Vet Anim Sci. 33 (3): 193-197.

[7] Mazurowski, A, A. Frieske, A. Wilkanowska, D. Kokoszyński, S. Mroczkowski, Z. Bernacki and G. Maiorano. 2016. Polymorphism of prolactin gene and its association with growth and some biometrical traits in ducks. Italian Journal of Animal Science, 15, P 200-206.