Potency of papua local chickens as egg producers: a molecular review

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Potency of papua local chickens as egg producers: a molecular review

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Abstract. This research aims to evaluate potency of Papua Local Chickens as egg producers through allelic frequency estimation of 24-bp indel (insertion-deletion) on the prolactin gene promoter (cPRL) owned by the population. A total of 68 Papua Local Chickens (ALP) are used in this research, and blood samples are taken individually for DNA isolation. DNA fragment target size 130 or 154 bp contain the 24-bp indel locus is successfully amplified using Polymerase Chain Reaction (PCR) method with a pair of specific primer. Electrophoresis analysis on 1.5 % agarose gel indicates three genotypes: II, ID and DD with the frequency of 8.82 %, 41.18 % and 50.00 %, respectively. The I and D allele frequencies are 0.29 and 0.71. This result estimate shows that the I allele frequency is categorized medium in Papua Local Chickens population. Therefore, the Papua Local Chickens is potential in producing eggs. Their egg producer potency can be increased by eliminating the broodiness behaviour of the hens in the population or by increasing the I allele frequency through a controlled mating.

1. Introduction

Papua local chickens, known as native chickens or non-commercial breed, are found hanging around in the area of Manokwari Regency, West Papua Province of Indonesia. This kind of local chickens is strongly suspected carrying genes that come from layer commercial breeds (the male hybrids) rose by an agricultural research institute during the era of Dutch colonialism. The institute was intentionally spread out the male layer hybrids (the Australorp and the Barred Plymouth Rock) to farmers in the region, let them randomly mated with the female local chickens and at the end formed today 'Papua Local Chickens' called 'Ayam Lokal Papua' (ALP). Based on this history, it is assumed that the ALP chickens therefore are potential as egg producers [1].

Laying activity in birds cannot be separated from prolactin hormone function. This hormone (chicken prolactin hormone, cPRL) plays an important role in bird brooding behavior. Increase of the cPRL in the blood plasma will induce the hen chicken to broodiness and stop the laying activity [2], consequently decrease the egg production [3]. The increase of the cPRL in the blood plasma will cause regression in chicken ovary [4] and it affects the production of eggs [5].

At molecular aspect, the cPRL is coded by a gene that is located at the chromosome number 2. It has the size of 9,536 bp. This cPRL is divided into a promoter part with 5 exons, 4 introns and a flanking region [6, 7]. The promoter consists of -1 (330 bp) promoter, -2 (287 bp) promoter and -3 (314 bp) promoter [8]. The exon part consists of -1 (81 bp) exon, -2 (182 bp) exon, -4 (180 bp) exon [9], -3 (59 bp) exon [6], and -5 (418 bp) exon [10]. The intron part consists of -1 (714 bp) intron, -2 (406 bp) intron, -4 (744 bp) intron [11], and -3 intron with no size information yet. The cPRL gene, specifically the promoter part, constitutes as a candidate gene for chicken broodiness [5,12], and egg

production [13]. The cPRL promoter becomes an important part for the cPRL gen to be expressed. This is the part that functioning in early transcription activation of the cPRL gen expression [14]. Therefore, any mutation happens to this promoter area will cause the cPRL gen to be unable to express its product. This condition affects the female bird to loss her broodiness but gives an impact on the increase of egg production.

In chickens, a mutation in the form of insertion (I)-deletion (D) or Indel has been found. It contains 24-bp on the promoter region at site -358. Mutation at this locus produces two alleles, I and D. The I allelic frequency in non-commercial breed is vary from low to moderate [13, 15]. On the other hand, the I allele is found to be a common allele in layer breed of chickens. There was even only the I allele in White Leghorn [13]. Further investigations find out that the presence of the I allele in a population contributes a positive impact to the egg production [13, 15, 16]. The I and D allele frequencies in Iran native chickens are as much as 0.59 % and 0.41 %, respectively [15]. In quails, the I and D allelic frequencies were almost equal, 0.52 % and 0.48 %, respectively [17]. It was reported that when native chickens had selected based on prolactin genotype promoter as a genetic marker (MAS) to serve as a basic population, selection response of the egg production would be faster and accurate [18]. Chickens with homozygote Insertion genotype (II) would decrease the cPRL expression, therefore no brooding behavior found [19]. They concluded that the cPRL promoter could be used as a genetic marker for brooding behavior in chickens. This research aims to investigate the potency of Papua local chickens as egg producers through the estimation of the I and D allelic frequencies at 24-bp indel locus in prolactin gen promoter.

2. Materials and method

A number of 68 unsexed ALP chickens obtained from farmers of Manokwari Regency, West Papua Province, Indonesia, were used in this research. About 1 ml blood samples were taken individually from the chicken experiment thru brachial vein by using disposal gauge and placed in a 3 ml tube contained K3EDTA. The blood samples were brought to Biotechnology Research Study, The Gadjah Mada University for DNA isolation and analysis. The DNA isolation of the blood samples was carried out using Dneasy Blood & Tissue Kit. The DNA isolation process was done following the available procedure of the selected kit. The obtained DNA samples were quantified on a spectrophotometry to evaluate their concentrations and purity. In a 1.5 µl micro tube, as much as 2 µl of DNA solution were mixed with 98 µl aqua bides. The solution was read on spectrophotometer at OD_{260} and OD_{280}. The DNA concentration was the OD_{260} x 50 x 50 µg and the purity was the value of OD_{280} divided by the value of the DNA sample with the concentration $\geq$ 100 µg. The purity of 1.7-2.2 was used in this research.

DNA fragment contained 24-bp Indel loci with a pair of restricted forward primer 5'-TTT-AAT-ATT-GGT-GGG-TGA-AGA-GAC-A-3', and reverse: 5'-ATG-CCA-CTG-ATC-CTC-GAA-AAC-TC-3'(4), was amplified using PCR. The amplification process was initiated by preparing a mixed solution in a 25 µl PCR micro tube with 2 µl DNA solution, 2 µl forward primers, 2 µl reverse primer, 12.5 µl Go Taq Green and 6.5 µl water free nuclease. The tube contained the mixed solution then was placed in a PCR machine, programmed at 94^{o}C for 5 minutes, and amplified for 35 cycles. Each cycle had a denaturation temperature of 94^{o}C for 30 seconds, annealing 54^{o}C for 30 seconds, extension of 72^{o}C for 30 seconds, and final extension at 72^{o}C for 5 minutes.

The obtained DNA fragment size 130 bp or 154 bp from the amplification process (amplicon) was further subjected to the next analysis stage, electrophoresed on 1.5 % agarose gel in TBE buffer. The electrophoresis process was initiated by mixing 5 µl amplicon with 2 µl loading buffer, and put in gel wells used a micro pipet. The gel was run under 100 voltage power pressures for 30 minutes together with the DNA marker size 100-300 bp. Genotype identification of the 24-bp Indel/cPRL loci was based on the banding patterns showed by the electrophoresis result of the amplicon. The II genotype (Insertion-Insertion) was characterized by a single DNA banding pattern size 154 bp, the ID genotype (Insertion-Deletion) characterized by two DNA banding patterns size 154 bp and 130 bp, and the DD genotype characterized a single DNA banding pattern size 130 bp. Allele and genotype frequencies of

the experimental chickens were estimated by using a formula [20] as follow: Xi = (2nii + Σnij) /(2n) to estimate the allele frequency and Xii = nii/n to estimate the genotype frequency. Xi= the i^{th} allele frequency Xii=the ii^{th} genotype frequency, nii=total of samples with ii genotype, nij=total of samples with ij genotype, and n= the total of all samples.

3. Results and discussion

In this research, a DNA fragment target size 130 bp and/or 154 bp of 68 DNA samples of the ALP chicken experiment contained 24-bp Indel locus on site -358 that located on the promoter region, and restricted by a pair of specific primer [13] was successfully amplified. The results of the amplifications (amplicons) were electrophoresed and determined three kinds of genotypes: II, ID and DD (figure 1). The total of chickens with the II, ID and DD genotype were 6, 28 and 34 heads, respectfully. The three kinds of these genotypes were the result of the presence of mutation on the promoter area that formed 2 alleles: the I allele (Insertion) size 154 bp and the D allele (Deletion) size 130 bp as much as 24 bp [13, 15].

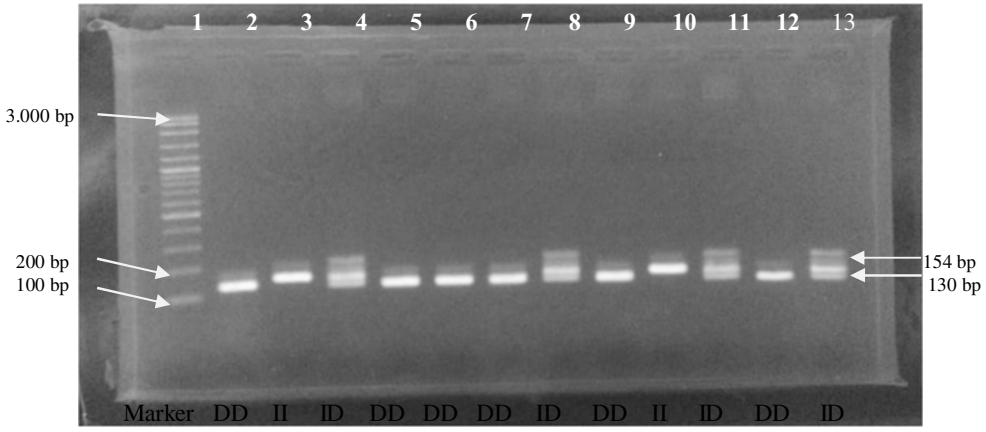

Figure 1. Amplicons contain 24 bp Indel on site -358 promoter region of the prolactin gen of the experimental chickens.

The sequence of DNA fragment size 154 bp (the I allele) and size 130 bp (the D allele) in *gallus gallus* were presented in figure 2 and 3, respectively.

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                              forward primer              insertion (24-bp)
                                    ↓                          ↓
2221 tttctagaaa tagctagaat tttaatattg gtgggtgaag agacaaggaa gacaagaaga
2281 gacaagacaa ggaaggaaga gaagacacct gcaggcaggg agaataacat tttacaaaca
2341 tagaggataa cagtctcaga attgacaact ggagttttcg aggatcagtg gcatttgcaa
                                          ↑
                                    reverse primer
```

Figure 2. The DNA sequence of the I allele size 154 bp in prolactin gen of gallus gallus (GenBank: AB011438.2).

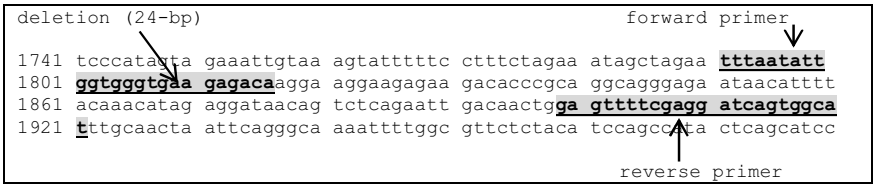

Figure 3. The DNA sequence of the D allele size 130 bp in prolactin gen of *gallus gallus* (GenBank: AB011438.2).

By using a formula [20], it was computed that the allele and genotype frequencies estimation of 24-bp Indel loci of APLP cPRL gen for II, ID and DD were 8.82 %, 41.18 % and 50.00 %, respectively. The I and D allele frequencies were 0.29 and 0.71. The I allele frequency value of 0.29 found in ALP chicken population was classified as moderate. This was in accordance with previous study [13, 15, 16] that the I frequency in non-commercial chickens varied from low to moderate. On the other hand, the I frequency in commercial laying hens was classified high. The I allele in White Leghorns, Yangshan, Taihe Silkies-1, White Rock, Nongdahe, and Taihe Silkies-2 were 1.00, 0.05, 0.20, 0.22, 0.17, and 0.02, respectfully [13]. This experimental result showed that the White Leghorn, a type of commercial laying hen, had no other allele except the I allele. The frequency of the I allele (0.29) and the II genotype (8.82) identified in ALP chickens were still much lower than that of the one found in the native chickens of Iran. The Iran's native chickens possessed the frequencies of the I allele of 0.59 and the II genotype of 39% [15].

The above research results explained that the I allele (Insertion) in chickens caused a positive effect on the eggs production. On the other hand, the D allele (deletion) caused a negative effect on the production of eggs. It had been proved by several researchers that the genotype of 24-bp Indel on the cPRL gen promoter region had a significant relationship with the production of eggs [13, 15, 16]. Chickens with the II homozygote of 24-bp loci on the cPRL promoter region decreased the expression of CPRL [17] and that kind of chickens showed no brooding characters. Based on that information, it could be concluded that the Iran's native chickens are more potential in producing eggs as compared to the ALP chickens. With the finding of the moderate I allele frequency in ALP chickens, this could be mentioned that the ALP chickens potency as egg producers is easy to increase by applying a controlled mating system in their population. The formation and reproduction of ALP chickens with the II genotype and a wide distribution to the farmers are needed to be performed in order to accelerate the increase of the potency of ALP chickens as egg producers. Spreading the ALP chickens with the II genotype to the farmers community will result in a gradual increase of the I allele frequency of the chicken population. Therefore, the brooding behavior of the ALP chickens would be minimized but the ability of hens in producing egg would be increased. In turn, the high egg production of ALP chicken population will be formed. The roles and concerns of the government and other stake holders to realize the above idea are highly expected.

4. Conclusion

The potency of the ALP chickens as egg producers is still need to be improved because of the presence of the I allele of 24-bp Indel (Insertion-Deletion) of the prolactin promoter gen in the population categorize moderate (the I allele frequency was 0.29). The efforts to increase this potency can be done through the formation, reproduction and distribution of the ALP chickens with the II genotype to the farmer communities.

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