Genetic evaluation of Central Javanese local duck based on the ovalbumin gene

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Abstract. Duck farm industry has a big contribution to the supply of animal protein sources in Indonesia. Reproductive efficiency in poultry is determined by the factor of the high ratio of hatching and fertilization, fertile duration, egg weight, and the number of eggs. Reproductive efficiency control genes play a major role in parent selection and provision of superior duck seeds. This study aims to evaluate the genetic quality of local ducks in Central Java based on the ovalbumin gene using PCR-RFLP technique. A total of 35 central Javanese local ducks were feathered on the inside of the right and left wings. Duck DNA is isolated from a feather sample. The ovalbumin gene was amplified with PCR techniques using specific primers TovaF1 and TovaR1. The product of the ovalbumin gene amplification (350 bp) is subsequently cut with restriction enzyme SspI (5' - AATATT - 3'). DNA bands from restriction enzyme cutting then analyzed its genotype. The results showed that 6 (17.14%) ducks had CC genotype, 16 (45.71%) with TT genotype, and 13 (37.14%) of CT genotype. Ducks with CC and TT genotypes showed higher hatchability compared to CT genotype. It was concluded that 22 (62.86%) central Javanese local ducks were genetically superior in terms of hatchability (genotype CC and TT).

1. Introduction

Duck farm industry has a big contribution to animal protein supply in Indonesia. Duck meat and egg have a unique and delicious taste that makes consumers love it. Along with the increasing demand for it, the need for superior quality duck type also increases. However, information about the quality of superior duck type is not followed by its availability. Although the duck species are relatively large and spread in almost all region of Indonesia, their productivity is still low, especially ducks that are traditionally farmed.

Local duck is one of the high genetic quality germplasm. Its diversity can be utilized optimally through the breeding program. It is necessary to conduct an inventory (collection), data collection (documentation), and conservation of existing germplasm, especially local varieties, in order to reduce or even prevent genetic erosion of the germplasm. Local livestock in an area or country needs to be maintained because these animals have adapted well in their natural environment. The preservation of local livestock genetic resources is necessary. However, until now, there has not been identified as the type of genetic diversity and genes that need to be maintained in the population. Huang et al. [1] state that
the genes needed for the genetic quality selection are genes controlling a trait, especially important traits that have economic uses. These genes are then referred to as marker assisted selection (MAS) [1].

The quality and quantity of superior duck types, among others, is determined by the nature of its reproductive efficiency. In the reproduction process, parental conditions significantly affect reproduction success and also the offspring’s health [2,3]. In poultry, reptiles, and fish, maternal factors are transmitted to the embryo through eggs [4]. Consequently, the absorption of maternal factors is limited only before and subsequent a few hatches. Reproductive efficiency in poultry is determined by factors (1) high ratio of hatching and fertilization, (2) period of fertile (fertility), (3) egg weight, and (4) total eggs produced. In poultry, several genes involved in reproductive efficiency are ovalbumin genes [1], type X collagen (X collagen; COLX) [5], ovomucoid [6], and prolactin [7].

Reproductive efficiency control genes play a major role in parent selection and provision of superior duck types. The latest science and technology development advance genetic selection efficiency, faster and more accurately than using the conventional method with morphological characters. Genes are the blueprint of living organisms. The results of genomic research are assured accuracy and registered in gene databases with an accession number. Utilization of molecular selection to get superior ducks based on genetic quality will achieve, easily.

This study aims to evaluate the quality of the Central Javanese local ducks based on ovalbumin gene using the PCR-RFLP method. Ovalbumin is the main protein (54%) compiler of albumin, other than ovotransferrin (12%), ovomucoid (1%) ovomucin (3.5%) and lysozyme (3.5%) [8]. Ovalbumin gene is related to the hatchability of Tsaiya duck (Anas platyrhynchos) [6]. The ovalbumin gene is a significant prospected gene as a marker-assisted selection to increase hatchability in Tsaiya ducks [1]. Identification of central Javanese duck’s hatchability genotype is the documentation process of potential genetic resources of livestock. The data can be used to determine strategic policies about direction and livestock management program, utilization, and conservation of duck genetic resources.

2. Methods
The sample of this research was 35 domestic ducks in Central Java. The feather sample used for DNA isolation is the rachis and calamus part about 4-5 cm. Because the calamus and rachis are filled with marrow and have many tissues. DNA isolation employed gSYNC™ DNA Extraction Kit.

PCR with the target of the ovalbumin gene was carried out using Thermocycler GeneAmpR 2400 (Perkin Elmer). Ovalbumin gene is amplified using forward primer TovaF1 (5’-ACA GCA AGA CCC AAA GCA CT-3’) and reverse primer TovaR1 (5’-GCC TGG TCA TGC TTA ATT GG-3’) [1]. Ovalbumin gene amplification was conducted using Dream Taq polymerase master mix (Thermo Scientific) with the composition of the 12.5 ul PCR master mix; 1.2 ul of forwarding primer; 1.2 ul of reverse primer, 8.1 ul of ddH2O, and 2.0 ul of DNA samples (50 ng). The primer sequences that amplified approximately 350 bp fragments were done by following program (1) initiatory denaturation at 95°C for 2 min, (2) 35 cycles at 94°C for 45 sec, annealing temperature at 57°C for 50 sec, and 72°C for 60 sec (3) and the final extension at 72°C for 7 min. The expected 350 bp PCR product was visualized by 1.2 % agarose gel.

The genotype of ovalbumin gene identification was conducted by restriction fragment length polymorphism (RFLP), as many as 10 μl amplicons were mixed with 1 U/μg DNA of SspI restriction enzyme at 37 °C for overnight, followed manufacturer instruction. Then, 2 % agarose in TBE buffer was used to visualize the digested fragments. DNA bands from PCR-RFLP were analyzed for size based on DNA markers. The band characters are analyzed for their genotype, first is a CC-genotype with 350 bp fragment length. Second is two bands with 236 bp, and 114 bp fragment length in one column showed TT-genotype, and three bands with 350 bp, 236 bp, and 114 bp showed a CT genotype [1]. Genotype data from each duck DNA sample then analyzed descriptively based on duck types.
3. Result and Discussion

Ovalbumin is the main protein of egg white. In eggs, protein is found in egg whites and yolks, while lipids are mainly concentrated in yolks. Proteins in yolks consist of phosvitin (8%), livetins (38%), lipovitellins (36%), and low-density lipoproteins (17%). The yolks also contain 1% carotenoids, which form yellow color [9]. White eggs composition consist of water (88%) and protein (11%), with the remainder consisting of ash, carbohydrates, and a few amounts of lipids (1%). The prime proteins in white eggs were ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3%), and ovomucin (3%). Minor proteins found in egg whites were ovomacroglobulin (0.5%), avidin (0.05%), cystatin (0.05%), ovoflavoprotein (0.8%), ovoglycoprotein (1.0%), and ovoinhibitor (1.5%) [10].

The ovalbumin has a molecular weight of 45 kDa with 386 amino acids. Ovalbumin has no classic N-terminal ladder sequence [11], but there are 3 post-synthetic modification sites in addition to the N-terminal acetyl group. Ovalbumin has a unique amino acid composition, where N-terminal amino acids are glycine acetylation, and C-terminal is proline. The composition is also known as glycoprotein and contains the carbohydrate group attached to N-terminals. Ovalbumin consists of 3 components, A1, A2, and A3 contains 2, 1, and no phosphate groups, respectively. The relative proportion of subcomponents is 85: 12: 3 [9].

All Central Javanese local duck DNA samples were generated 350 bp PCR products, as depicted in Figure 1. PCR-RFLP analysis of the ovalbumin gene revealed two alleles (C and T) with three genotypes (CC, TT, and CT). Analysis of RFLP with SspI digestion showed that the Central Java local duck genotype consists of three variations, namely CC, TT, and CT. A fragment with 385T was sliced by restriction endonuclease SspI (5’-AATATT-3’), whereas a fragment with the cytosine polymorphism (5’-AACATT-3’) was not cut by SspI. The CC genotype only shows 350-bp fragments that are not cut by SspI, so it only has a band as long as 350-bp that visualized using electrophoresis. The genotype TT and CT were contained 350-bp, which was cut by SspI. After cutting process, it was generated 236-bp and 114-bp band fragment for TT genotype, and 350-bp, 236-bp, 114-bp for CT genotype (Figure 2).

![Figure 1. Results of ovalbumin gene amplification in duck DNA samples showing 350 bp PCR product](image-url)
Figure 2. The visualization of PCR-RFLP products of duck ovalbumin genes cut by SspI in 2% agarose

In this research, a variety of genotypes on different duck types is showed by PCR-RFLP results (Table 1). The PCR-RFLP technique is effective as a breed selection method. The PCR-RFLP method was successfully used to determine the genotype of various chicken breeds and Javanese backyard waterfowl based on the Mx gene [12-13]. The PCR-RFLP was also successfully used to analyze the polymorphisms in duck OIH (Ovoinhibitor) [14].

The results of this research showed that of the 35 ducks analyzed, 6 (17.14%) ducks with CC genotypes, 16 (45.71%) TT genotypes and 13 (37.14%) CT genotype (Table 1). Huang et al. reported that ducks with CC and TT genotype had significantly higher hatchability than CT ducks. However, ducks with CC, TT, and CT genotypes did not show significant differences in other reproductive traits [1].

Most types of ducks in this study have varied genotypes. However, all Peking and Blorong duck have homozygous genotypes (CC and TT), with high hatchability. All Pengging duck have heterozygous (CT) genotypes, tend to low hatchability. From the perspective of hatchability, ducks with CC and TT genotypes (17.14% and 45.71%, respectively) ought to retained, whereas the CT genotype ducks (37.14%) needs to be excluded. However, in addition to hatchability, many other factors also determine good reproductive performance, such as fertility and its period, egg mass, and the number of eggs.

In the current condition, it is too hard to determine and eliminate heterozygous individuals using traditional selection based on phenotypes or crossover methods. It requires many efforts in time and accuracy. It is caused by heterozygous alleles that difficult to distinguish based on their phenotype [1]. However, with assisted selection markers, as in this study, heterozygous CT genotyped individuals can be easily identified and eliminated from the population.

| Ducks Types            | Size sample | Genotype |
|------------------------|-------------|----------|
|                        |             | CC       | TT       | CT       |
| Tegal Branjangan duck  | 5           | 1        | 2        | 2        |
| Peking duck            | 5           | 5        | 0        | 0        |
| Pengging duck          | 5           | 0        | 0        | 5        |
| Tegal Jarakan duck     | 5           | 0        | 2        | 3        |
| Tegal Blorong duck     | 5           | 0        | 5        | 0        |
| Tegal Lemahan duck     | 5           | 0        | 4        | 1        |
| Magelang duck          | 5           | 0        | 3        | 2        |
| Total                  | 35          | 6 (17.14%) | 16 (45.71%) | 13 (37.14%) |
Huang et al. [1] reported that a high expression level of ovalbumin could be reduced hatchability. Ovalbumin is a reserve protein that acts as one of the determinants of embryonic poultry development. Ovalbumin determines embryo development through the mechanism of the interferon 6 (IRF6) regulating factor [15], and its mutation results in embryonic death [16]. Ovalbumin is one of the main determinant factors of success embryonic development. Overexpression of it, increase exceed egg white’s protein that, decreasing egg hatchability. Identical cases have also been illustrated for other proteins that are excessive in number, will affect the function of vital organs [17].

A C385T nucleotide substitution in the ovalbumin gene can cause missense substitution of histidine amino acids to tyrosine [1]. Amino acid mutations are part of the protein evolution process. Amino acid mutation can modify functional sites and protein interactions more dramatically [18]. The study of the effects of amino acid substitution on the structure and function of proteins continues to be developed. Several methods were developed to predict changes in protein structure due to non-synonym single nucleotide polymorphism (nsSNP) [19]. Protein-protein interactions are important in most cellular processes and functions [20-21]. Understanding interaction interfaces and their evolution is an important basis for pharmaceutical applications in drug discovery [22]. Because nsSNPs can occur in drug binding proteins, and they can affect treatment response.

Of the diseases caused by missense mutations, the highest is associated with changes in protein structure and functions. Analysis of all nsSNP in about 2000 proteins in more than 8,000 cancer samples show an increase in cysteine, histidine, and tryptophan at the expense of arginine. Arginine has a prominent function on tumor suppressor proteins such as p53 [23]. The research result of Milenkovic et al. shows that the A147T mutation significantly alters the flexibility and stability of the 18 kDa translocator protein (TSPO). Expression of TSPO is upregulated in activated microglia in various neuroinflammatory, neurodegenerative, and neoplastic disorders [24]. Another research result reported that most human HSD3B2 (3β-hydroxysteroid dehydrogenase type 2) related diseases are caused by nsSNP in functional residues that are highly conserved in this protein [25].

The role of histidine in ovalbumin needs to be studied further. The study of the role histidine in essential proteins has many benefits, including the development of live vaccines. Histidine 264 as a key metal binding ligand in metalloenzyme mannose-6P isomerase (ManA) *Escherichia coli* can be replaced with MeH (3-methyl-l-histidine) [26]. Zhang et al. research revealed that replacement of HxD-histidine with phenylalanine or arginine in the catalytic core of protein kinase eliminates catalytic activity and autophosphorylation. However, replacement of histidine-to-tyrosine results in loss of catalytic activity without interfering auto-phosphorylation. HxD-histidine mutations can also be concerned in the pathogenesis of several diseases, including cancer [27].

4. Conclusion
The genetic quality of local ducks in Central Java can be determined by the PCR-RFLP method on the ovalbumin gene. The genotypes of ovalbumin genes (CC, TT, and CT) in ducks indicates hatchability levels. The analysis of PCR-RFLP in this study revealed that 22 (62.86%) central Javanese local ducks were genetically superior in terms of hatchability (genotype CC and TT).

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