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Identification of mutation points in the prolactin (PRL) gene as genetic marker candidate in Kedu chicken

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ABSTRACT

This research aimed to identify point mutations in the PRL gene in Kedu chickens. The materials used were 15 female Kedu chickens aged 6-7 months, consisting of red comb black Kedu, black comb black Kedu, and white Kedu chickens. The research included blood sampling, DNA extraction, amplification of the PRL gene through Polymerase Chain Reaction (PCR), electrophoresis, sequencing, and data analysis. The parameters observed were the identification of point mutations, genotype frequency, allele frequency, and heterozygosity. Data analysis was performed using MEGA 12 and dnaSP.v6. The results showed a point mutation in the PRL gene in Kedu chickens located at site 454. The genotype frequency of CC was 0.067, while CT and TT were 0.666 and 0.267, respectively. The frequency of allele C was 0.4 and allele T was 0.6. The heterozygosity value was 0.48, which is considered moderate. Based on the results, it can be concluded that a mutation was found at point 454 C→T in the presence of alleles C and T, forming the genotypes CC, CT, and TT. The observed heterozygosity value was moderate, indicating that the studied population still shows relatively diverse genetic variation. Further research is needed to determine its association with the reproductive traits of Kedu chickens.

Introduction

Kedu chicken is a native local chicken breed from Kedu District, Temanggung Regency, Central Java, which can be utilized for its eggs and meat. Kedu chickens possess genetic advantages in growth and egg production, which are superior to those of other local chicken breeds (Arianto *et al*., 2019). According to Rukmana (2003), mature Kedu chickens can reach a weight of 2-2.5 kg and can produce up to 226 eggs per year. At the age of 180 days, Kedu chickens are capable of laying their first eggs. According to Habsari *et al*. (2019), the age at first laying in Kedu chickens is 6-7 months. This reproductive trait is important for the sustainability of egg-laying chicken farming and therefore needs to be maximized as it can influence egg production.

Reproductive traits can be optimized through genetic selection. Conventional genetic selection based on phenotypes takes a long time and is less efficient, therefore faster and more accurate molecular research using genetic markers is needed, one of which is the identification of point mutations in genes related to the reproductive traits of Kedu chickens. A mutation is a change in genetic sequence that can involve alterations, additions, or deletions of nucleotides, thus increasing the diversity of an organism (Ochtavia *et al*., 2024). A point mutation occurs when one or more nucleotide pairs in the genome sequence change, resulting in sequence variation in Deoxyribonucleic Acid (DNA). Mutations can potentially affect protein function and gene expression (Fauziah, 2025). Point mutations can act as genetic markers for specific traits, such as reproductive traits, because of their ability to trigger trait variation.

Prolactin (PRL) is a gene encoding prolactin hormone in the adenohypophysis gland, which plays a role in reproductive function. PRL influences the reproductive function of chickens by regulating their brooding behavior. An increase in prolactin levels that triggers brooding behavior can lead to ovarian regression and decreased egg production (Kilatsih *et al*., 2020). According to Rohmah *et al*. (2022), the exon 5 region of PRL gene can be used as a genetic marker in selection programs for Indonesian local chickens. Association studies of PRL gene with reproductive traits in chickens were previously conducted by Rosalinda *et al*. (2025) on crosses between Merawang chickens and KUB and between Murung Panggang chickens and KUB. Research on the association of the PRL gene with reproductive traits in Kedu chickens has never been reported, so identification of mutation points in the PRL gene needs to be carried out to determine its potential as a candidate genetic marker for reproductive traits in Kedu chickens. The aim of this research was to identify mutation points in the PRL gene of Kedu chickens that have the potential to become Single Nucleotide Polymorphisms (SNP), which can serve as genetic markers and the benefit gained is to provide information on SNP candidates as genetic markers for Kedu chickens that may contribute to selection and breeding programs related to reproductive traits, thereby increasing Kedu chicken egg production.

Materials and methods

Chicken groups

The study included 15 female Kedu chickens aged 6-7 months from different parental lines, consisting of red comb black Kedu chicken (n.=9), black comb black Kedu chicken (n.= 4), and white Kedu chickens (n.= 2).

Blood collection

A blood sample of 3 ml was collected from the Kedu chicken through the brachialis vein located under the chicken wing using a 3 ml syringe. The collected blood was placed in an EDTA vacum tube and immediately homogenized to prevent coagulation. The blood was then stored in a cooling box with ice gel and afterwards kept in a freezer until laboratory analysis was performed.

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DNA extraction

DNA extraction was performed using Geneaid-gSYNCTM DNA Extraction Kit according to the poultry protocol provided by the kit. Electrophoresis was performed using a 1% agarose gel to identify the DNA that had been successfully extracted by inserting 3 µl DNA genome isolate that had been homogenized with 1 µl loading dye into the gel well. Electrophoresis was performed at 100 volts for 20 minutes. The results were verified by inserting the gel into the gel documentation system.

Gene amplification

The PRL gene was amplified by Polymerase Chain Reaction (PCR), and the extracted DNA isolate was used as the template for PCR. The pair primer that used for PCR were primer forward 5′-CTGTTCTACACCCAGACAGATTGA-3′ and primer reverse 5′-AAGGTATAAGCCATCCCAGCTATT-3. The primer design was based on research by Rosalinda *et al.* (2025), the PCR mixture consisted of 5 µl DNA template, 25µl my taq polymerase HS red mix, 1 µl each of forward and reverse primers, and 18 µl nuclease-free water, all of which were placed in a 50-microliter PCR tube. The denaturation, annealing, and extension steps were repeated for 35 cycles followed by a final extension step for 5 minutes. The success of PRL gene amplification was evaluated by electrophoresis. Electrophoresis was performed using 1% agarose by inserting 3 µl of the PCR isolate into the well, and 3 µl DNA ladder was inserted at the end of the gel to evaluate the success of target gene amplification. Electrophoresis was performed at 100 volt for 30 minutes. The results were verified by inserting the gel into the gel documentation system. The PCR product was 553 bp and consisted of a small part of intron 4, exon 5, and terminator region.

Sequencing

Sanger Sequencing was performed to obtain the nucleotide sequence of the amplified PRL gene. DNA sequencing was conducted through the services of PT. Genetika Science Indonesia by sending the amplified samples along with forward and reverse PRL gene primers.

Data analysis

Sequencing data analysis was conducted using Molecular Evolutionary Genetics Analysis version 12 (MEGA 12) software to align and edit the sequences to identify the mutation points. Alignment was performed using the ClustalW option in the MEGA12 software. Analysis of point mutations and segregating sites was performed using DnaSP.6 software. Allele frequencies were calculated according to Prastowo *et al.* (2021), using the following formula:

$$X_i = ((2ni + \sum nij))/2N$$

The frequency of the i-th allele is expressed by xi, ni represents the number of individuals with genotype i, and nij represents the number of samples with genotype ij. N represents the total number of individuals in the sample. Genotype frequencies were calculated according to Nei and Kumar (2000) using the following formula:

$$X^i = ni/N$$

The frequency of the i-th genotype is expressed by Xi, ni represents the number of individuals with genotype i and N represents the total number of samples observed. Heterozygosity was calculated according to Prastowo *et al.* (2021) using the following formula:

$$H = 1 - \sum_{i=1}^{n} (Pi)^2$$

Heterozygosity is expressed by H, N represents the number of alleles, i represents the Allele, and Pi represents the Frequency of the i-th allele.

Results

Amplification of the PRL gene in Kedu chickens successfully produced a DNA fragment of approximately 600 bp (Fig. 1), consistent with primer analysis using Primer-BLAST, which showed a fragment length of 609 bp. The amplified fragment was located at positions g.7868–g.8420 of the complete PRL gene sequence (GenBank accession number AF288765.2). The sequencing results were trimmed and aligned for further analysis. Sequence analysis revealed a mutation at position 454 (C→T) or g.8321. Three genotypes were identified at this mutation site: CC, CT, and TT. The mutation site, genotypes, and chromatograms are presented in Fig. 2. Allele frequency analysis showed that the C allele had a frequency of 0.4, while the T allele had a frequency of 0.6. Genotype frequency calculations revealed CC= 0.067, CT= 0.666, and TT= 0.267. The heterozygosity value obtained was 0.48. Based on allele frequencies and heterozygosity, the Kedu chicken population analyzed in this study was classified as polymorphic.

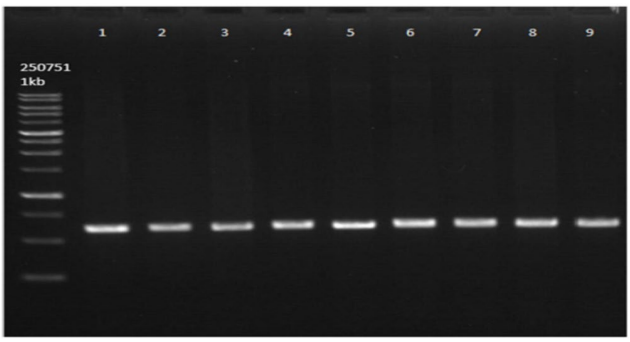

Fig. 1. Visualization of PRL gene amplification by agarose gel electrophoresis.

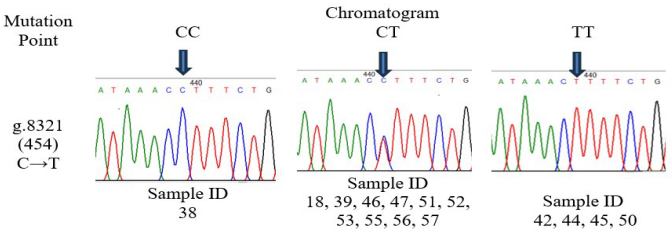

Fig. 2. Chromatogram and position of PRL gene mutation points.

Discussion

The C→T mutation detected at position g.8321 was located in the 3′ untranslated region (3′UTR) of the PRL gene, a critical hub for post-transcriptional regulation. Although the 3′UTR is not translated into amino acids, it plays an essential role in controlling gene expression by influencing mRNA stability, translation efficiency, localization, and decay. This region contains binding sites for microRNAs (miRNAs) and RNA-binding proteins (RBPs), which regulate mRNA fate through interactions with miRNA response elements (MREs). Mutations within the 3′UTR can disrupt these regulatory mechanisms, thereby altering protein production (Bug *et al.*, 2021; Mountford *et al.*, 2022; Fu *et al.*, 2024).

The C→T mutation identified in this study was also reported by Rosalinda *et al.* (2025) in crossbred Merawang × KUB (MXKUB) and Marung Panggang × KUB (MPXKUB) chickens, although additional mutation points were observed in that study. The difference in the number of mutations may reflect differences in population structure, genetic diversity, or selective pressures.

Interestingly, the allele frequencies observed in Kedu chickens differ from those reported by Rosalinda *et al.* (2025). In the present study, the mutant allele (T) was more frequent (0.6) than the wild-type allele (C),

whereas in MXKUB and MPXKUB chickens, the C allele was more dominant. According to Nei and Kumar (2000), a population is considered polymorphic when allele frequencies are less than 0.99, which was confirmed in this study. Such allele frequency patterns can be influenced by genetic mutations, outbreeding, or admixture of different populations (Afriani *et al*., 2022). The relatively high proportion of individuals carrying the mutant allele suggests that the T allele may confer beneficial effects on the population. This supports the potential use of the PRL gene as a genetic marker associated with reproductive traits. Derks and Steensma (2021) emphasized that molecular selection can increase the frequency of advantageous alleles while reducing deleterious ones.

Genotype distribution revealed that heterozygotes (CT) were predominant (66.6%), while homozygous mutants (TT, 26.7%) outnumbered homozygous wild types (CC, 6.7%). The dominance of heterozygotes may reflect a heterozygote advantage, provide superior traits such as greater viability or disease resistance (Bui *et al*., 2023), or indirectly being maintained by farmers due to the economic advantages associated with this genotype. The higher frequency of TT compared to CC may also indicate selective pressure against CC individuals, potentially due to reduced fitness or survival. The heterozygosity value (0.48) indicated moderate genetic diversity (Kolompoy *et al*., 2020). This value was higher than those reported by Rosalinda *et al*. (2025), who found heterozygosity values of 0.40 in MXKUB chickens and 0.30 in MPXKUB chickens. Such diversity is advantageous, as it increases population resilience and provides more variation for selection programs. Nabilla (2024) highlighted that genetic selection is more effective in populations with high genetic diversity, improving the likelihood of obtaining superior genotypes.

Conclusion

Based on the results of the research, it can be concluded that a mutation was found at position 454 C→T with alleles C and T, forming the genotypes CC, CT, and TT. The heterozygosity value obtained was moderate. The identified mutation site has the potential to serve as a candidate genetic marker in Kedu chickens.

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Conflict of interest

The authors have no conflict of interest to declare.

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