Jurnal Biologi Tropis

Original Research Paper

Identification of Single Nucleotide Polymorphism (SNP) in Exon 5 of the Prolactin Gene in Native Chickens

Tapaul Rozi[1], Maskur[1*], Rahma Jan[1], Lalu Muhammad Kasip[1], Muhammad Muhsinin[1]

[1]Department of Animal Breeding and Genetics, Faculty of Animal Science, University of Mataram, Indonesia;

Article History
Received : November 28th, 2024
Revised : December 10th, 2024
Accepted : December 18th, 2024

*Corresponding Author:
Maskur,
Department of Animal Breeding and Genetics, Faculty of Animal Science, University of Mataram, Indonesia
Email: maskur@unram.ac.id

Abstract: The prolactin gene (PRL) in chickens is one of the genes that regulates broodiness and egg production in laying hens. Single Nucleotide Polymorphism (SNP) is a form of single nucleotide variation in the DNA base sequence at specific locations in the genome that can influence differences in quantitative traits in livestock. This study aimed to identify SNPs in the prolactin gene of native chickens. The research methods included DNA extraction, PCR amplification, and DNA sequence analysis for genotype determination of individuals. The results revealed four mutation points at positions g.8052 bp T>C, g.7886 T>A, g.7823 A>G, and g.8069 T>C. All mutation points in exon 5 were substitution mutations. Statistical analysis showed that all loci were polymorphic and the population was in Hardy–Weinberg equilibrium.

Keywords: PRL gene, egg production, native chickens, Marker-Assisted Selection

Introduction

The low egg production of native chickens in Indonesia is influenced by several factors, including the type of feed used, traditional management practices, poor genetic quality, and a high broodiness trait in native chickens (Fadillah, 2016). High egg-producing native broiler hens are essential to support poultry farming because the fertilized eggs they produce will be hatched to produce day-old chicks (DOC), which are the primary component of poultry farming.

To date, efforts to improve the genetic quality of native chicken stock have been carried out through conventional selection based on production and reproductive performance. Studies to identify genes related to egg production variation have been widely conducted, particularly in native chickens. However, the use of biomarkers for selection in native chickens with high production and reproduction performance is still limited.

One of the genes correlated with egg production in chickens is the prolactin gene (Cui et al., 2006). Prolactin is a candidate gene that specifically regulates variations in egg production through reduced egg biosynthesis during the brooding period (Chen et al., 2007). Supporting selection efforts for native Indonesian chickens to develop new strains as female lines can be achieved by utilizing the prolactin gene.

Prolactin in aves has been reported to play an important role in regulating physiological processes, including egg production, stimulating and maintaining broodiness, osmoregulation, and gonadal cell development (Sharp et al., 1979). Information on prolactin gene diversity in native poultry in Indonesia is still limited to native chicken species. The prolactin gene in native chickens is known to be polymorphic and associated with egg production traits through brooding mechanisms (Sartika, 2004).

The SNP at exon 5 of the prolactin gene has been previously studied, with the SNP at position g.8052T>C identified in native Chinese chickens such as Qingyuan partridge and recessive white chickens, which impacts the total egg production during the first 300 days of laying. Similar findings were observed in

commercial layer chickens such as White Leghorn and Hy-Line Brown, where this SNP significantly influenced daily egg production percentage (Erehehuara, 2003; Li et al., 2013).

The prolactin gene (PRL) at exon 5 has been shown to be associated with egg production traits in chickens, making it a potential genetic marker for selection programs in Indonesian native chickens. Analyzing prolactin gene diversity in Indonesian native chickens is essential to identify genetic markers as a foundation for selection programs. Furthermore, association analysis between genetic diversity and egg production traits will be conducted.

Material and Method

Experimental Animals and DNA Isolation and DNA Isolation

A total of 46 female chickens were used in this study to identify prolactin gene diversity. All samples were obtained from native farms in West Lombok, West Nusa Tenggara, Indonesia. Genomic DNA was extracted from blood samples using the Genomic DNA Mini Kit from Geneid, following the manufacturer's protocol (ISO 9001:2008 QMS).

Primer Design

Primers were independently designed based on DNA sequence data for avian species available at the National Center for Biotechnology Information (NCBI) using the Molecular Evolutionary Genetics Analysis (MEGA7) program. The primers were designed and validated using web-based tools, including Bioinformatic and Thermo Fisher Scientific Multiple Primers. For identifying single nucleotide polymorphisms (SNPs) in the prolactin gene, the primers used were: Forward (F): 5'-TGGAGGAGGCCAAAAAGAGATG-3' and Reverse (R): 3'-CAGCCCACAGGTACTTAGCAA-5'.

Polymerase Chain Reaction

DNA amplification was performed using PCR under the following conditions: an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C, annealing at 60°C, and extension at 94°C for 1 minute each. PCR products were electrophoresed on 2% agarose gels and visualized using a UV Transilluminator. The polymorphic sites of the prolactin gene fragments obtained from PCR were confirmed through sequencing using the Sanger method (Sanger et al., 1977). Chromatographs were analyzed using BLAST, FinchTV, and the MEGA X program (Kumar et al., 2018).

Data Analysis

Genotype data were analyzed using PopGene software version 1.31 to calculate genotype frequencies, allele frequencies, homozygosity, and heterozygosity. The chi-squared test (χ^2) was used to examine deviations in genotype frequency from Hardy–Weinberg equilibrium (Nei and Kumar, 2000). Observed heterozygosity and expected heterozygosity were calculated based on allele frequency using the Weir (1996) formula.

Results and Discussion

Prolactin Gene Amplification

The prolactin gene in native chickens was successfully amplified with a 100% success rate using a thermocycler (ESCO) under an annealing temperature of 57°C for 20 seconds. The success of amplification depends on the correct primer design and annealing temperature. Annealing temperatures can vary depending on the primer length and sequence. Gunawan et al. (2017) stated that annealing is one of the stages in the PCR process where the primer binds to the DNA template. Generally, annealing temperatures range from 37°C to 60°C (Handoyo and Rudiretna, 2001). The length of the PCR product from the amplification process was 557 bp, as visualized using agarose gel electrophoresis (1.5%). Visualization of the amplification results for the prolactin gene in the exon 2 region of native chickens is shown in Figure 1.

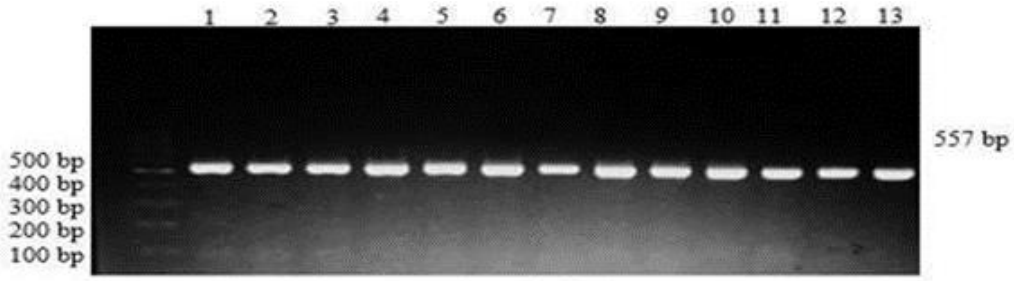

Figure 1. Prolactin Gene Amplification Results in Native Chickens; M = 100 bp DNA marker; lane 1–13 = samples

The success of DNA amplification is also influenced by the precise interaction of PCR components, including annealing temperature, primer concentration, amplification duration, and target DNA concentration (Viljoen et al., 2005). Visualization results indicate that the amplified product of the prolactin gene segment, covering exon 5 and part of intron 4, has a length of 557 bp. This matches the target region amplified by primers at positions 7565–8122 bp. In the chicken genome, the prolactin gene sequence spans 9,536 bp, consisting of 5 exons and 4 introns, and encodes 229 amino acids located on chromosome 2 (Alipanah et al., 2011).

Prolactin Gene Variation

The prolactin gene variation in native chickens was identified using sequencing. Sequencing results revealed mutation points at positions g.8052 bp T>C and g.7886 T>A, consistent with findings by Rohmah et al. (2022), Osman et al. (2017), Erehehuara (2003), and Li et al. (2013). Additionally, two novel mutation points were identified at base positions g.7823 A>G and g.8069 T>C. All mutation points identified in exon 5 in this study g.7823 A>G, g.7886 T>A, g.8052 T>C, and g.8069 T>C were substitution mutations. The positions of these mutations in exon 5 of the prolactin gene are shown in Figure 2.

Nucleotide base substitution mutations are categorized into transition mutations and transversion mutations. Mutations at positions g.7823A>G, g.8052T>C, and g.8069T>C represent transition mutations, while the mutation at g.7886 T>A represents a transversion mutation. Transition mutations involve the replacement of a purine base with another purine (A>G) or a pyrimidine base with another pyrimidine (C>T). In contrast, transversion mutations occur when a purine is replaced by a pyrimidine or vice versa (Luo et al., 2016).

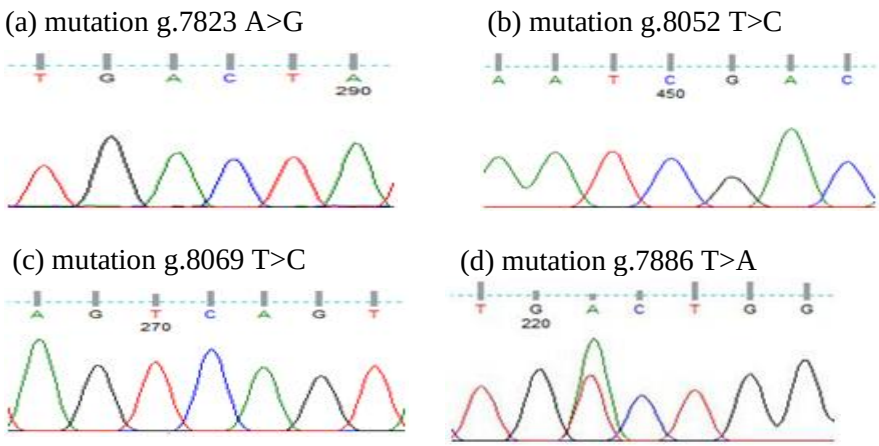

Figure 2. Partial sequence of the prolactin gene showing substitution mutations. (a) transition g.7823 A>G; (b)transition g.8052 T>C; (c)transition g.8069 T>C;(d) transversion g.7886 T>A

In terms of base bonding structure, transition mutations do not alter hydrogen

bonding, whereas transversion mutations change the hydrogen bond structure from a double to a triple bond or vice versa (Muladno, 2010). Hydrogen bonds are non-covalent interactions with low free energy (2-6 kJ mol^{-1} in water), making the weak bridges easily broken and re-formed (Petsko and Ringe, 2004). Changes in hydrogen bond structures can influence DNA transcription and alter the structure or conformation of the resulting protein molecule. According to Luo et al. (2017), transversion mutations have a greater impact on structural or regulatory functions of a gene compared to transition mutations.

Each mutation can affect one or more roles of the encoded protein. The possible roles influenced by mutations include protein stability or folding, ligand binding, catalysis, allosteric regulation and other mechanisms, as well as post-translational protein modifications (Wang and Moult, 2001;Teng et al., 2008).The complete amino acid changes derived from the translated prolactin gene mRNA sequence are presented in Table 1.

Table 1. Amino Acid Changes from Translated Prolactin Gene mRNA Sequence

Mutation Position	Original Codon	Mutated Codon	Original Amino Acid	Mutated Amino Acid	Mutation Type
g.7823 A>G	AAA	AGA	Lysine (Lys)	Arginine (Arg)	Non-synonymous
g.7886 T>A	ACA	ATA	Threonine (Thr)	Isoleucine (Ile)	Non-synonymous
g.8052 T>C	GGT	GGC	Glycine (Gly)	Glycine (Gly)	Synonymous
g.8069 T>C	TCA	TCC	Serine (Ser)	Serine (Ser)	Synonymous

Mutations in base g.7823 A>G (Lys>Lys) and g.8052 T>C (Ile>Ile) are synonymous mutations that do not alter the amino acid sequence. Synonymous mutations encode the same amino acid sequence but can still influence protein structure and function by affecting RNA transcription mechanisms, mRNA structure, and translation speed (Komar 2007; Supek et al. 2014; Zuben and Chava 2011). Mutations in base g.7886 T>A (Asp>Val) and g.8069 T>C (Leu>Ser), on the other hand, are non-synonymous mutations that change the amino acid sequence. Non-synonymous mutations alter DNA base sequences and can significantly impact protein structure and function (Castejon et al. 2011). Transition mutations are generally more frequent and tend to result in synonymous mutations, whereas transversion mutations, being non-synonymous, often lead to amino acid changes that affect the biochemical properties of the protein. This phenomenon is subjected to natural selection and DNA repair mechanisms (Denver et al. 2004; Pauly et al. 2017).

Based on the prolactin gene's mode of action, DNA sequence mutations may alter the resulting protein structure, potentially disrupting the prolactin signaling pathway, particularly the interaction between prolactin and its receptor. The prolactin signaling pathway is initiated when prolactin binds to its receptor (Binart et al. 2000). This interaction activates JAK (Janus kinase), with the prolactin receptor inducing signals through kinases interacting with its cytoplasmic tail. The JAK gene facilitates signal transduction and activates the STAT (signal transducer and activators of transcription) protein (Dasilva et al. 1996).

The STAT protein dissociates from the receptor and translocates to the nucleus, where it binds to the promoter region of target genes, including prolactin regulatory sites. This binding governs the activation and deactivation of the prolactin gene (Radhakrishnan et al. 2012). DNA sequence mutations altering protein structure could lead to prolactin gene inactivation, disrupting this crucial signaling pathway.

Allele and Genotype Frequencies of The Prolactin Gene in Native Chicken

The chi-square test (χ^2) was conducted to determine whether the population is in Hardy-Weinberg equilibrium. This test is essential because the offspring from experimental crosses often deviate from Mendelian inheritance patterns. A population is considered in equilibrium if the calculated χ^2 value is smaller than the table χ^2 value. A population in

equilibrium indicates it has not undergone mutation, migration, non-random mating, selection, or significant changes in sample size (Noor 2010).

Heterozygosity is a parameter used to measure the level of genetic diversity within a population based on allele frequencies at each locus (Anggraeni 2009). Observed heterozygosity (Ho) is the average proportion of individuals with polymorphic loci based on observations, whereas expected heterozygosity (He) is an estimate of genetic diversity calculated from allele frequencies. If the observed heterozygosity value is greater than the expected heterozygosity, it indicates a diverse population. A population is considered to have high genetic diversity if the heterozygosity value exceeds 0.50 (Allendorf and Luikart 2007). The results of genotype frequency, allele frequency, heterozygosity values, and chi-square test (χ^2) for the prolactin gene at various loci are presented in Table 2.

Table 2. Genotype Frequency, Allele Frequency, Heterozygosity, and Chi-Square Test (χ^2) for SNPs in the Prolactin Gene (PRL) of native Chickens

SNP Position	N	Genotype Frequency(n)	Allele Frequency	χ^2 Test	Ho	He
g.7823 A>G	46	AA = 0.50(23) AG = 0.37(17) GG = 0.13(6)	A = 0.68 G = 0.32	0.95tn	0.43	0.37
g.7886 T>A	46	TT = 0.91(42) TA = 0.09(4) AA = 0.00(0)	T = 0.96 A = 0.04	0.14tn	0.09	0.08
g.8052 T>C	46	TT = 0.69(32) TC = 0.22(10) CC = 0.09(4)	T = 0.80 C = 0.20	4.27*	0.22	0.31
g.8069 T>C	46	TT = 0.26(12) TC = 0.57(26) CC = 0.17(8)	T = 0.46 C = 0.54	0.89tn	0.57	0.50

N: Total number of samples; (n): Number of samples with the specific genotype; $\chi^2(0.05;1) = 3.84$; χ^2 Test=significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$); tn: indicates no significant deviation ($P > 0.05$); Ho: Observed heterozygosity; He: Expected heterozygosity.

In this study, four SNPs were identified in the prolactin gene of native chickens: g.7823 A>G, g.7886 T>A, g.8052 T>C, and g.8069 T>C. At the g.7823 A>G locus, we found three genotypes: AA, AG, and GG. The AA genotype had the highest frequency (50.00%), followed by AG (36.96%) and GG (13.04%)(Table 2). Genetic diversity in the population is depicted by three diversity indices: the proportion (percentage) of polymorphic loci in the population, the average heterozygosity at Hardy-Weinberg equilibrium, and the allele frequencies (Nei and Kumar, 2000). The high frequency of the AA genotype leads to a higher allele A frequency (0.68) compared to allele G frequency (0.32), indicating that this locus is polymorphic. Allele frequency is the relative frequency of an allele in a population, reflecting genetic diversity. An allele is considered polymorphic if its frequency is less than or equal to 0.99 (Hartl and Clark, 2000). The results of the Hardy-Weinberg equilibrium test showed no significant difference ($P > 0.05$), indicating that the population is in Hardy-Weinberg equilibrium. This finding aligns with the heterozygosity calculations for native chickens, which reveal that the observed heterozygosity (Ho) is higher than the expected heterozygosity (He). This suggests that the population at the g.7823 A>G locus is genetically diverse, potentially due to mutations at this locus.

The SNP at the g.7886 T>A locus was found to have only two genotypes: TT (91.30%) and TA (8.70%). The high frequency of the TT genotype results in a higher frequency of allele T (0.96) compared to allele A (0.04), indicating polymorphism at this locus. The genotype frequencies at the g.7886 T>A SNP locus, based on the chi-square test (χ^2), show that the native chicken population is in Hardy-Weinberg equilibrium ($P > 0.05$). This is further supported by the heterozygosity calculations, which

indicate that the observed heterozygosity (Ho) is greater than the expected heterozygosity (He), signifying that the population at this locus is genetically diverse.

The SNP at the g.8052 T>C locus was identified with three genotypes: TT (69.57%), TC (21.74%), and CC (8.70%) (Table 2). The high frequency of the TT genotype results in a higher frequency of allele T (0.80) compared to allele C (0.20), indicating that the g.8052 T>C locus is also polymorphic in the native chicken population. The Hardy-Weinberg equilibrium test using the chi-square test (χ^2) indicates that this population is in Hardy-Weinberg equilibrium ($P > 0.05$).

The g.8069 T>C locus was also identified with three genotypes: TT (26.09%), TC (56.52%), and CC (17.39%) (Table 2). The frequency of allele C (0.54) is higher than allele T (0.46), indicating polymorphism in the prolactin gene in native chickens. The allele frequencies of the four identified SNPs have values ≤ 0.99, confirming that the prolactin gene in native chickens is polymorphic. An allele is classified as polymorphic if its frequency is ≤ 0.99 (Hartl, 2000). The Hardy-Weinberg equilibrium test shows that the population is in equilibrium ($P > 0.05$), meaning that the observed heterozygosity (Ho) is higher than the expected heterozygosity (He). This indicates that the population with mutations at the g.7823 A>G locus exhibits diversity. This diversity may result from factors such as the suboptimal implementation of selection practices in native chickens. Noor (2010) stated that large populations do not change from one generation to the next unless affected by factors such as selection, migration, mutation, or genetic drift. The prolactin gene SNPs in exon 5 have been studied previously and were also identified.

The SNPs at positions 8052 T>C and 8113 G>C were also identified in native Chinese chickens, such as the Qinyuan Partridge and Recessive White breeds (Erehehuara, 2003; Li et al., 2013). Additionally, a non-synonymous SNP at position 7921 bp (C>T), which changes the amino acid from serine to proline, and a synonymous SNP at position 8187 bp (C>T) were reported in Hubbard F15 chickens (Osman et al., 2017). The identification of the 8052 T>C SNP in this study aligns with the findings of Li et al. (2013) and Erehehuara (2003). Li et al. (2013) found that the 8052 T>C and 8113 G>C SNPs were significantly associated with the age of first egg-laying and egg production within the first 300 days of laying. Similarly, Erehehuara (2003) reported that these SNPs greatly influence egg production in White Leghorn and Hy-Line Brown chickens. Additionally, the findings of Rashidi et al. (2012) showed a significant association between exon 5 SNPs and egg production in Mazandaran chickens.

Conclusion

A total of four SNPs were identified in the prolactin gene of native chickens: g.7823 A>G, g.7886 T>A, g.8052 T>C, and g.8069 T>C. The allele frequencies for all four SNPs indicate polymorphism (≤0.99). The higher observed heterozygosity values compared to expected heterozygosity values suggest that these populations are genetically diverse. Factors such as the lack of selection, migration, mutation, and genetic drift in large populations contribute to this equilibrium. The identified SNPs in the prolactin gene demonstrate genetic polymorphism in native chickens, with potential applications in improving production traits. Future selection programs could leverage these polymorphisms to optimize traits such as egg production and reproductive performance.

Acknowledgments

This research was funded by the University Research Grand with the contract number: 2258/UN18.L1/PP/2023. Thank you to the rector and everyone who has assisted in this research.

References

Alipanah M, Shojaian K, & Bandani HK. (2011). The polymorphism of prolactin gene in native chicken zabol region. J Anim Vet Adv. 10:619-621.

Bhattacharya TK, Chatterjee RN, Sharma RP, Niranjan M, Rajkumar U, & Reddy BLN. (2011). Polymorphism in the prolactin promoter and its association with growth traits in chickens. Biochem Genet. 49:385-94.

Chen CF, Shiue YL, Yen CJ, Tang PC, Chang HC, & Lee YP. (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.

Cui JX, Du HL, Liang Y, Deny XM, Li N, & Zhany XQ. (2006). Association of polymorphism in the promoter region of chicken prolactin with age production. Poult Sci. 85: 26–31.

Erehehuara (2003). Studies on the SNP of the chicken's prolactingene. Wuhan: Huazhong Agricultural University.

Fadillah A. (2016). Performa produktivitas induk hasil seleksi pada persilangan ayam pelung ras pedaging sentul kampung [skripsi]. Bogor: Institut Pertanian Bogor.

Food and Agriculture Organization of The United Nations (2008). Native chicken genetic resources and production systems in Indonesia. Prepared by Muladno. GCP/RAS/228/GER Working Paper No. 6. Rome (IT): FAO.

Halley C, & Visscher P. (2000). DNA marker and genetic testing in farm animal improvement: Current applications and future prospects. In: Depth Rev Read Inst cell, Anim Popul Biol Univ Edinburgh. Edinburgh (Scotland): University of Edinburgh.

Iskandar S, & Susanti T. (2007). Karakter dan Manfaat Ayam Pelung di Indonesia. Wartazoa. 17 (3): 128-136.

Jiang RS, Xu GY, Zhang XQ, & Yang N. (2005). Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. Poult Sci. 84:839-845.

Lewin B. (1997). Genes are mutable units. In: Genes VI. New York (US): Oxford University Press. p. 51-70.

Li HF, Shu JT, Du YF, Shan YJ, Chen KW, Zhang XY, Han W, & Xu WJ. (2013). Analysis of the genetic effects of prolactin gene polymorphisms on chicken egg production. Mol Biol Rep. 40:289–294.

Montaldo, H. H. & C. A. M. Herrera. (1998). Use of molecular markers and major genes in genetic improvement of livestock. J. Biotech. 1 : 2.

Muladno (2002). Seputar Teknologi Rekayasa Genetika. Pustaka Wirausaha Muda dan USESE Foundation, Bogor.

Nataamijaya AG. (2000). The native of chiken of Indonesia. Bull Plasma Nutfah. 6(1): 1-6.

Nataamijaya AG. (2010). Pengembangan potensi ayam lokal untuk menunjang peningkatan kesejahteraan petani. J Litbang Pertanian. 29(4): 131-138.

Sartika T, Duryadi D, Mansjoer SS, Saefuddin A, & Martojo H. (2004). Gen promotor prolaktin sebagai penanda pembantu seleksi untuk mengontrol sifat mengeram pada ayam kampung. JITV. 9:239-245.

Sartika T, & Iskandar S. (2007). Mengenal plasma nutfah ayam Indonesia dan pemanfaatannya. Sukabumi (ID): Kepraks.

Sharp PJ, Scanes CG, Williams JB, Harvey S, & Chadwick A. (1979). Variations in concentrations of prolactin, luteinizing hormone, growth hormone and progesterone in the plasma of broody bantams (Gallus domesticus). J. Endocrinol. 80: 51–57.

Sumantri, C., A. Anggraeni, A. Farajallah & D. Perwitasari (2007). Keragaman mikrosatelit DNA sapi perah FH di Balai Pembibitan Ternak Unggul; Baturaden. J. ITV 12: 124-133.

Susanti T, Noor RR, Hardjosworo PS, & Prasetyo LH. (2012). Relationship between prolactin hormone level, molting and duck egg production. J Indonesian Trop Anim Agric. 37:161-167.

Watahiki M, Tanaka M, Masuda N, Sugisaki K, Yamamoto M, Yamakawa M, Nagai J, & Nakashima K. (1989). Primary structure of chicken pituitary prolactin deduced from the cDNA sequence. Conserved and specific amino acid residues in the domains of the prolactins. J Biol Chem. 264:5535-5539.

Williams, J. L. (2005). The use of marker-assisted selection in animal breeding and biotechnology. Rev. Sci. Tech. of. Int. Epiz., 24:379-391.