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Original Article

Genetic polymorphisms of *LOC101800257* gene and its relationship with egg characteristics in Kerinci ducks (*Anas Platyrhynchos*)

Polimorfismos genéticos do gene *LOC101800257* e sua relação com as características do ovo em patos Kerinci (*Anas platyrhynchos*)

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Abstract

The Kerinci duck is a breed native of Jambi Province in Indonesia, is noteworthy for its various eggshell color. This study's primary objective was to explore the connection between genetic variations in the *LOC101800257* gene and the eggshell color and egg weight of Kerinci ducks. For this investigation, a total of 46 Kerinci ducks were utilized for identification of the *LOC101800257* gene polymorphisms using Sequencing analysis. The association between the *LOC101800257* gene polymorphisms with the eggshell color and egg weight were analyzed using *General Linear Model* analysis. The research findings revealed the presence of two mutation sites, namely g.13993R (intron 11) and c.15504R / p.I435V (exon 9), within the *LOC101800257* gene of Kerinci ducks. These mutations exhibited high genetic diversity, characterized by high values of polymorphic informative content (PIC) and nucleotide diversity (Nd), both exceeding 0.30 and 2.00, respectively. Furthermore, both mutation sites displayed a significant association (P<0.05) with the GG genotype which had a higher effect on egg weight. However, it is essential to note that these genetic variations in the *LOC101800257* gene were not found to serve as genetic markers for eggshell color in Kerinci ducks. The *LOC101800257* gene can be employed as a genetic marker in molecular selection processes to enhance the egg weight of Kerinci ducks.

Keywords: eggshell color, haplotype, Kerinci ducks, *LOC101800257* gene, sequencing.

Resumo

O pato Kerinci é uma raça originária da província de Jambi, na Indonésia, que se destaca pelas diversas cores da casca do ovo. O principal objetivo deste estudo foi explorar a ligação entre as variações genéticas no gene *LOC101800257* e a cor da casca e o peso dos ovos dos patos Kerinci. Para essa investigação foi utilizado um total de 46 patos Kerinci para a identificação dos polimorfismos do gene *LOC101800257* através de análise de sequenciação. A associação entre os polimorfismos do gene *LOC101800257* com a cor da casca e o peso do ovo foi analisada através da análise do modelo linear geral. Os resultados da pesquisa revelaram a presença de dois locais de mutação, nomeadamente g.13993R (íntron 11) e c.15504R/p.I435V (éxon 9), dentro do gene *LOC101800257* dos patos Kerinci. Essas mutações exibiram uma elevada diversidade genética, caracterizada por elevados valores de conteúdo informativo polimórfico (PIC) e diversidade de nucleótidos (Nd), ambos superiores a 0,30 e 2,00, respetivamente. Além disso, ambos os locais de mutação apresentaram uma associação significativa (P < 0,05) com o genótipo GG, que teve maior efeito no peso do ovo. No entanto, é essencial referir que essas variações genéticas no gene *LOC101800257* não serviram como marcadores genéticos para a cor da casca do ovo nos patos Kerinci. O gene *LOC101800257* pode ser empregado como marcador genético em processos de seleção molecular para aumentar o peso dos ovos dos patos Kerinci.

Palavras-chave: cor da casca do ovo, haplótipo, patos Kerinci, gene *LOC101800257*, sequenciação.

1. Introduction

Kerinci ducks, classified as Anas platyrhynchos, represent one of Indonesia's indigenous duck varieties, originating specifically from the Jambi Province. Designated as Indonesia's native duck breed, this decision was formalized by the Indonesian Ministry of Agriculture (Supriawan et al., 2023). According to Khanza et al. (2021),

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Kerinci ducks, both male and female, exhibit various egg characteristics, including egg weight (71.80±1.27/67.20±1.92 g), egg circumference (158.95±8.92/146.36±2.66 mm), egg length (64.38±2.05/59.32±2.28 mm), egg width (50.59±2.86/46.59±0.80 mm), and egg volume (27.06±3.64/21.84±1.31 mL).

Of note is that Kerinci ducks are distinctive for producing eggs with two different shell colors, blue and white. However, several studies have indicated that eggshell color can impact factors such as the Haugh unit and egg weight (Lestari et al., 2015; Sastrawan and Erita, 2022). Huang (2016) found that Leizhou black duck eggs with blue shells tend to have an average shell thickness, higher yolk weight, increased protein content, and higher Haugh unit values than white-shell duck eggs. Kocetkovs et al. (2022) and Nurdin and Prayogi (2018) demonstrated a correlation between eggshell color and the strength and thickness of the shell, thereby affecting egg shelf life, where blue duck eggs are considered better than white. Therefore, some farmers in Indonesia, particularly in Java, consider their livestock's physiological status when making business decisions (Wibowo and Juarini, 2008).

The blue color of duck eggshells is typically determined by the presence of protoporphyrins and biliverdin, as evidenced by studies conducted by Badas et al. (2017) and Bai et al. (2019). Further investigation into the genetic factors influencing eggshell color through miRNA analysis revealed that genes such as ABCG2, FABP7, CD36, and SLC12A8 play a significant role, as Xu et al. (2018) documented. Additionally, the *LOC101800257* gene has been identified as a crucial factor in determining eggshell color in ducks, as indicated by research conducted by Zou et al. (2019). Interestingly, in chickens, the same *LOC101800257* gene is responsible for regulating the transport of biliverdin from the liver, which in turn controls the formation of blue eggshells, as elucidated by Wang et al. (2010).

The LOC101800257 gene in ducks is 29,363 base pairs long and is located on chromosome 1, consisting of 19 exons (NC_051772.1). Zou et al. (2019) noted the presence of two mutation sites in the intron 11 (g.13993R) and exon 9 (c.15504R) regions of the LOC101800257 gene in the Leizhou black duck. This study aims to identify the diversity of the LOC101800257 gene through two mutation locations and its association with the characteristics of Indonesian Kerinci duck eggs using sequencing analysis. It is hoped that the results of this research can be a reference in designing a molecular selection strategy for Kerinci ducks which aims to improve the quality and productivity of Kerinci duck eggs, which can be determined from the color of the eggshell.

2. Materials and Methods

2.1. Birds and experimental site

A total of 70 heads of Kerinci ducks were used in this study and collected from the farmers at Kerinci Regency, Jambi Province of Indonesia (Figure 1). After sexing, it was found that 46 female ducks were then reared to produce eggs and were observed further. The farm is located above 1000 m above sea level which at 01° 41' - 02° 26' South latitude and 101° 08' - 101° 40' East latitude. The climate conditions of the farm were recorded an average air temperature of 22.5 C, relative humidity of 83%, and rainfall of 112.6 mm³ per month.

Figure 1. The breeding tract of Kerinci ducks (*Anas platyrhynchos*) at Kerinci Regency, Jambi Province of Indonesia.

2.2. Management of birds and egg characteristics

Kerinci ducks were reared from the age of DOD to five months intensively in a cage size of 4 × 8 m^2. A commercial feed containing 4,100 kcal/kg with 21% of crude protein; 3-7% of fat; 0.9-1.1% of calcium; 0.6-0.9% of phosphorus. The ducks are given ND vaccine and vitamin C routinely at DOD age. Water consumption is provided ad libitum. Egg characteristics were measured refers to Putra et al. (2021) consist of egg weight (EWt), egg circumference (EC), egg length (EL), egg width (EW), egg index (EI), and eggshell color (blue/white). Measurement of egg characteristics was carried out immediately after the eggs were collected every day.

2.3. DNA analysis

At least 2 mL of blood samples were collected from each bird through the wing axillary vein using a venoject and vacutainer tube containing EDTA. The DNA extraction was performed using a Genomic DNA extraction kit (Geneaid, Taiwan) following the manufacturer's protocols. The primer pairs used in this study were specifically designed to target SNPs from the findings of Zou et al. (2019) which is located in intron 11 and exon 9. The primers used are presented in Table 1. The amplification of the *LOC101800257* gene was performed in a total volume of 30 µL consisting of 9 µL of DNA template, 0.6 µL of each primer, 15 µL of PCR master (Bioline, USA) and 4.8 µL of free-nuclease water. Therefore, the PCR reaction was performed in a mastercycler gradient (Eppendorf, Germany) with pre-denaturation at 95 °C for 5 min and followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 60.4 °C for 15 s, extension at 72 °C for 30 s and final extension at 72 °C for 3 min. The DNA electrophoresis with 1% agarose gel was performed in 100 V selama 30 min. Therefore, the forward sequencing analysis was performed on each PCR product by First Base Laboratory (Malaysia).

2.4. Data analysis

The Bioedit package (Hall, 1999) was used for sequence alignment of the *LOC101800257* gene, enabling the genotyping of a mutation site based on the chromatogram graphics profile. Subsequently, we calculated genetic diversity parameters such as genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He), polymorphic informative content (PIC), number of effective allele (ne), and Chi-square ($\chi 2$) values. PIC measures the ability of a marker to detect polymorphisms and is therefore of great importance in the selection of markers for genetic studies. Chi-square test is used to examine the role of chance in producing deviations between observed and expected values. These parameters are crucial in understanding the genetic diversity within our sample and were calculated concerning (Nei and Kumar, 2000). The General Linear Model was used to analyze the association between the *LOC101800257* gene polymorphism and the egg characteristic in Kerinci Duks. This model is often used to analyze the relationship between genetic diversity with a trait, as used by Harahap et al. (2024) (Equation 1):

$$Yij = \mu + Gi + Eij \tag{1}$$

where: Yij is the observed traits; µ is the common mean; Gi is the effect of the genotype; Eij is the experimental error.

3. Results

3.1. The LOC101800257 gene polymorphism in Kerinci ducks

The amplification of the *LOC101800257* gene was successfully achieved, resulting in two distinct amplicons of 502 bp and 457 bp, as depicted in Figure 2, on a 1% agarose gel. Consequently, this procedure revealed the presence of two transitional mutations, specifically g.13993R (intron 11) and c.15504R/p.I435V (exon 9), within the *LOC101800257* gene of Kerinci ducks, as illustrated in Figure 3.

The genetic diversity observed in the *LOC101800257* gene of Kerinci ducks is classified as high, with a polymorphic informative content (PIC) value surpassing 0.30, as documented in Table 2. Notably, the A allele predominated over the G allele at both mutation sites. The number of effective alleles (ne) in both mutation sites was approximately 2.00, indicating that the A and G alleles were the two prevalent alleles for the observed mutation sites.

Furthermore, it's noteworthy that both mutation sites within the *LOC101800257* gene were found to be in genetic equilibrium, with a Chi-square (χ^2) value falling below 5.99. This finding is a testament to the thoroughness of our study.

3.2. The association between the LOC101800257 gene polymorphism with egg characteristics in Kerinci ducks.

The analysis of the association between the *LOC101800257* gene polymorphism with egg characteristics revealed two mutation sites displayed a significant association with the egg characteristic, namely egg weight (EWt) of Kerinci ducks ($P<0.05$), as indicated in Table 3.

Table 1. Primer pairs to amplify *LOC101800257* gene of *Anas platyrhynchos*.

Region	Primer*	Amplicon (bp)	Gene Bank
Intron 11	F: 5'- ACA CAA CCC AAG GAT TAG GCT-3'	502	NC_051772.1
	R: 5'- GGC TGA CAC TCT GAT GAC CA-3'		
Exon 9	F: 5'- GGC TTG TGA GAT GGA TTT CGT AA-3'	457	
	R: R: 5'- AAT ACT TTA CTG ACT GTT CCC CA -3'		

*The primer designed using Primer 3 with the reference SNP accordance to Zou et al. (2019).

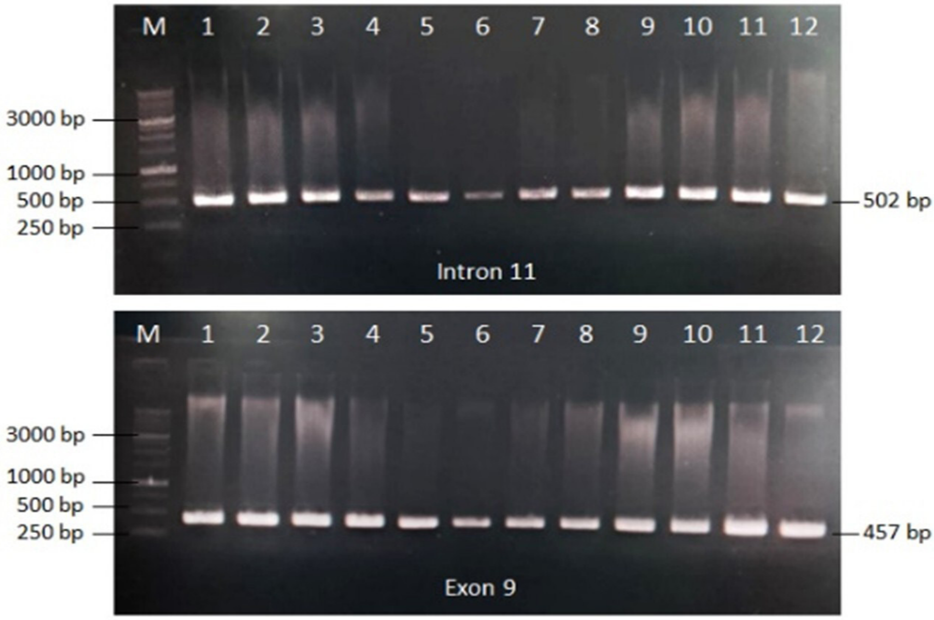

Figure 2. Two amplicons of *LOC101800257* gene in the intron 11 (502 bp) and exon 9 (457 bp) regions on 1% agarose gel. M: DNA ladder 1 kb; Line 1-12: DNA sample of Kerinci duck (*Anas platyrhynchos*).

Table 2. Genetic diversity in the *LOC101800257* gene of Kerinci duck (*Anas platyrhynchos*).

SNP	N	Genotype frequency			Allele frequency		H_o	H_e	PIC	n_e	χ^2
		AA	AG or GA	GG	A	G					
g.13993R	46	0.05	0.52	0.43	0.30	0.70	0.52	0.42	0.33	1.73	2.48*
c.15504R	43	0.21	0.56	0.23	0.49	0.51	0.56	0.50	0.37	2.00	0.59*

N: number of observation; AG genotype for g.13993R and GA genotype for c.15504R; H_o: observed heterozygosity; H_e: expected heterozygosity; PIC: polymorphic informative content; n_e: number of effective allele; χ^2: Chi-square value. *under in genetic equilibrium.

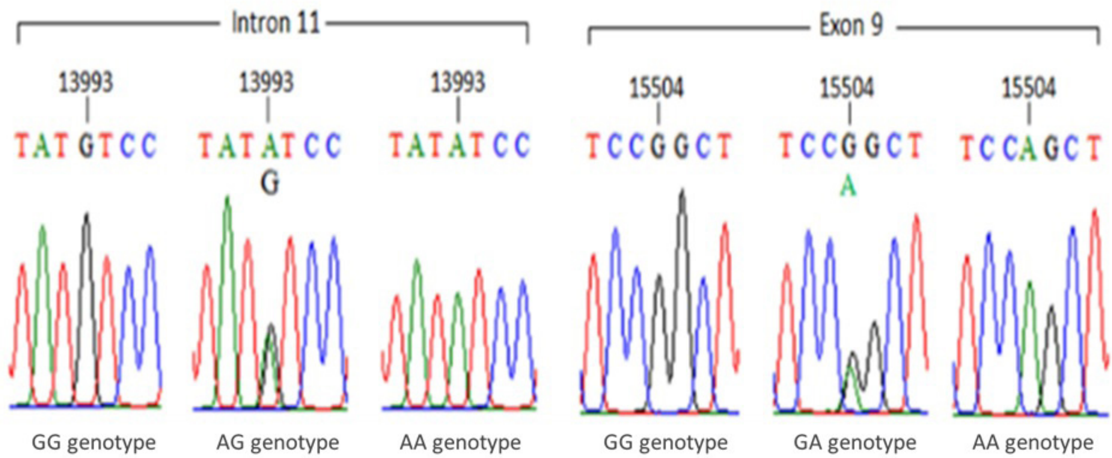

Figure 3. Detection of two mutation sites of g.13993R (intron 11) and c.15504R (exon 9) in the *LOC101800257* gene of Kerinci ducks (*Anas platyrhynchos*). R=A/G.

Examining Table 3, it becomes evident that the homozygous AA birds exhibited the lowest egg weight (EWt) compared to other genotypes, suggesting that the A allele may have a detrimental effect on the EWt trait in Kerinci ducks. However, it's worth noting that all genotypes for mutation g.13993R and c.15504R were observed in ducks with blue and white eggshells (Table 4). Nonetheless, the AA genotype for mutation g.13993R was conspicuously absent in white eggshell ducks. In the course of this study, a total of eight haplotypes were identified within the *LOC101800257* gene, and they were found to be significantly associated with the EWt trait in Kerinci ducks.

4. Discussion

In this study, two mutation sites of g.13993R and c.15504R were polymorphic and similar to the Leizhou black duck (Zou et al., 2019). Polymorphic Information Content (PIC) measures the ability of a marker to detect polymorphisms and is therefore of great importance in the selection of markers for genetic studies. The PIC value can be categorized into low (<0.10), moderate (0.10 - 0.30), and high (>0.30). Hence, two mutation sites in the *LOC101800257* gene can be used for the molecular selection of ducks. In addition, the genetic diversity in *LOC101800257* gene

Table 3. Effect of *LOC101800257* polymorphisms for egg quality traits of Kerinci ducks (*Anas platyrhynchos*).

Mutation	Genotype	Egg quality				
		Ewt	EC	EL	EW	EI
g.13993R	GG (N=20)	66.75±4.77[a]	146.64±4.67	58.82±3.17	46.70±1.48	77.26±3.79
	AG (N=24)	63.19±6.02[b]	147.00±3.13	59.74±2.20	46.81±1.00	78.29±2.06
	AA (N=2)	58.25±1.20[c]	147.00±0.00	60.45±0.64	46.80±0.00	78.70±0.14
c.15504R	GG (N=10)	65.72±4.16[a]	147.20±3.58	59.75±2.76	46.88±1.14	78.22±3.10
	GA (N=24)	65.22±5.65[b]	147.00±4.15	59.36±2.65	46.81±1.31	77.81±3.11
	AA (N=9)	60.92±6.06[c]	145.33±2.12	58.49±2.28	46.28±0.68	77.09±2.45
Haplotype	GG/AA (N=2)	58.75±12.51[c]	144.00±2.83	57.00±2.83	45.85±0.92	75.45±3.32
	GG/GG (N=7)	68.00±1.05[a]	147.29±4.39	59.48±3.32	46.91±1.40	77.97±3.77
	AG/GA (N=14)	64.43±6.47[b]	147.36±3.73	59.81±2.31	46.92±1.19	78.35±2.22
	GG/AG (N=8)	67.30±3.80[ab]	146.11±5.37	58.30±3.30	46.52±1.68	76.55±4.44
	AG/AA (N=6)	61.95±4.85[bc]	145.50±2.07	58.73±2.30	46.33±0.66	77.38±2.06
	GA/GG (N=1)	64.60	147.00	60.00	46.80	78.80
	AA/AA (N=1)	59.10	147.00	60.00	46.80	78.60
	AA/GG (N=1)	57.40	147.00	60.90	46.80	78.80

N: number of observations; EWt: egg weight (g); EC: egg circumference (mm); EL: egg length (mm); EW: egg width (mm); EI: egg index. Superscripts in the different columns differ significantly (P<0.05).

Table 4. Frequency of eggshell color in Kerinci ducks (*Anas platyrhynchos*) based on *LOC101800257* gene polymorphisms.

SNP	Genotype	Eggshell color	
		Blue	White
g.13993R	GG	15	5
	AG	18	6
	AA	2	0
c.15504R	GG	9	1
	GA	17	7
	AA	8	1
Haplotype	GG/AA	2	0
	GG/GG	6	1
	AG/GA	9	5
	GG/AG	6	2
	AG/AA	5	1
	GA/GG	1	0
	AA/AA	1	0
	AA/GG	1	0

was in genetic equilibrium. The genetic equilibrium in the population can be caused by random mating, selection, and migration (Lasley, 1978). Under some circumstances, a population can sustain Hardy-Weinberg equilibrium if its genotypes and allele frequencies hold steady over generations (Abramovs et al., 2020). The difference between actual and expected values is represented by the chi-square value, which is non-significant at the 5% significance level (0.05) (Akramullah et al., 2020). In this study, eight haplotypes of the *LOC101800257* gene were obtained in the present study. Zou et al. (2019) obtained nine haplotypes of the *LOC101800257* gene in Leizhou black ducks. Compared to the Leizhou black ducks, the AA/AG haplotype was absent in Kerinci ducks. In the birds under study, the AG/AG haplotype was frequently observed in blue and white eggshell ducks. In contrast to the Leizhou black duck, the AG/GA haplotype was often observed in the duck's blue eggshell. In comparison, the AA/AA haplotype was frequently observed in the white eggshells of Leizhou black ducks, its absence in the white eggshells of Kerinci ducks. This may be due to the *LOC101800257* gene mechanism which actually plays a role in determining eggshell, but in our study the limited number of samples meant that some individuals with the AA haplotype were not found.

Unfortunately, two mutation sites in the LOC101800253 gene can not be used as the genetic marker for the eggshell color of ducks. However, the conventional selection method by considering the eggshell of parents' duck in the crossbreeding stage may be applied to improve egg shell characteristics in ducks. Guo et al. (2020) reported a high heritability (h2) value for the eggshell color in laying hens, such as 0.65 for shell lightness, 0.42 for redness, and 0.60 for yellowness.

Despite of LOC101800253 gene, SLCO1C1, SLCO1B, ABCG2, and HMOX1 genes may be used as the genetic marker for eggshell color in ducks since these genes are expressed in chicken's shell glands (Wang et al., 2013; Wang et al., 2023). In the Jinding duck, CA2, ATP1B1, ATP2B2, and SLC14A1 genes were detected as the functional gene for eggshell color based on transcriptome analysis (Wang et al., 2017). Otherwise, *the ABCG2* gene has been reported as one of the functional genes influencing eggshell color in ducks (Chen et al., 2020).

The polymorphisms of the LOC101800253 gene were significantly associated with the EWt trait of the Kerinci duck. Unfortunately, there is no previous study informing the effect of genes under study on the egg characteristics in ducks. However, Kumalawati et al. (2023) reported no association between COLX and EWt traits in Indonesia's Alabio and Mojosari duck breeds. In White Leghorn laying chicken, haplotype of GnRHI and GnRHII genes are significantly associated with EWt trait (Bhattacharya et al., 2019). Li et al. (2009) reported that polymorphism in the intron 1 of the PRL gene was significantly associated with EWt trait in Chinese ducks. Growth family genes such as GH, GHR, and IGF1 were also significantly associated with the EWt trait in Ukrainian Poltava Clay and Rhode Island Red chicken breeds (Kulibaba et al., 2020).

The A allele in each mutation site of the *LOC101800257* gene may have harmed the EWt traits of the Kerinci duck when in homozygote genotype (AA) because it has the lowest EWt trait compared to another genotype. Fortunately, this undesirable allele was found with minor frequency in ducks under study. Surprisingly, the A allele was the major allele in Leizhou black ducks, as Zou et al. (2019) reported. Hence, Kerinci ducks have specific genetic characteristics that differ from Leizhou black ducks.

5. Conclusion

Two mutation sites in the *LOC101800257* gene in Kerinci were polymorphic. The AG/GA haplotype were frequently observed in blue and white eggshell colors. The *LOC101800257* gene significantly associated with egg weight (EWt). The ducks with the GG/GG haplotype in the gene have the highest EWt trait.

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