



■ Author(s)

Pratiwi N ⁱ	 https://orcid.org/0000-0001-5334-5772
Sartika T ⁱ	 https://orcid.org/0000-0002-1354-3229
Komarudin ⁱⁱ	 https://orcid.org/0009-0006-7560-0520
Haryati T ⁱ	 https://orcid.org/0000-0002-0839-3051
Kostaman T ⁱ	 https://orcid.org/0000-0003-1248-0614
Azizah N ⁱ	 https://orcid.org/0000-0003-0076-3596
Sukmara A ⁱ	 https://orcid.org/0009-0000-8406-3874
Brahmantiyo B ⁱ	 https://orcid.org/0000-0001-5095-3852
Isbandi ⁱ	 https://orcid.org/0009-0006-2179-6652
Zaenal H ⁱ	 https://orcid.org/0009-0006-9887-4516
Nuraini H ⁱⁱⁱ	 https://orcid.org/0000-0002-6834-987X
Khasanah H ^{iv}	 https://orcid.org/0000-0002-4475-343X
Ridhillah MF ⁱ	 https://orcid.org/0009-0003-9940-3706
Saputra F ⁱ	 https://orcid.org/0000-0002-7949-9777

ⁱ National Research Innovation Agency of the Republic of Indonesia (BRIN) Cibinong Sciences Center, Research Organization for Agriculture and Food, Research Center for Animal Husbandry, Cibinong, Bogor, 16915, Indonesia.

ⁱⁱ Indonesia Center for Livestock and Animal Health Instrument Standardization (ICLAHIS).

ⁱⁱⁱ IPB University Jl. Agatis, Darmaga Campus, Faculty of Animal Science, Bogor, 16680, Indonesia.

^{iv} University of Jember Jl. Kalimantan, Faculty of Agriculture, Department of Animal Science, No 37, Jember, 68121, Indonesia.

■ Mail Address

Corresponding author e-mail address

Tike Sartika

National Research Innovation Agency of the Republic of Indonesia (BRIN) Cibinong Sciences Center, Research Organization for Agriculture and Food, Research Center for Animal Husbandry, Cibinong, Bogor, 16915, Indonesia.

Phone: +62 821 1460 8880

Email: tikesartika@hotmail.com

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Characteristics of Egg Production and Discovery of the Prolactin Gene in Indigenous Tropical White Leghorn Layer Chickens of Indonesia

ABSTRACT

White Leghorn/WL chicken was imported to Indonesia in 2007. However, suboptimal production ensued due to inadequate maintenance management. This research aims to evaluate the egg production traits of WL chickens at the IRIAP (Indonesian Research Institute for Animal Production). A total of 185 WL chickens (5 batches) were utilized, with 47 DNA samples sequenced using specific primers for the PRL gene's exons 2 and 5. Various parameters were observed, including age at first laying (AFL), body weight at first laying (BWFL), egg weight at first laying (EWFL), total egg numbers (TEN), hen-day egg production (HEP), average egg weight (AEW), feed consumption (FC), feed conversion ratio (FCR), and mortality. The findings indicated that AFL, BWFL, and EWFL did not display significant differences across all batch groups ($p>0.05$). TEN differed significantly between WL chickens at 24 weeks in B2 and B3 compared to B4 and B5 ($p<0.05$). The HEP of WL chickens exhibited a steady increase weekly, reaching a production peak of 96.2% at 28 weeks. The highest AEW observed between 41-44 weeks was 54.71 g, with FC and FCR recorded at 102 g and 2.11 respectively. Mortality throughout the 24 weeks was 5.41%. A single nucleotide polymorphism (SNP) was identified in intron 4 at g.7883 C>T (AF288765), with AH013783.3 (WL) showing the allele C, albeit in a monomorphic state. In conclusion, the productivity of WL chickens demonstrates favorable performance. Further investigation with a larger population is necessary to explore SNPs in the PRL gene thoroughly.

INTRODUCTION

White Leghorn (WL) chickens were discovered by British scientists in the 19th century in Tuscany, Central Italy. WL chickens have the potential to increase the economic income of farmers due to their good production as laying hens (Balcha *et al.*, 2021). Furthermore, compared to indigenous chicken breeds in Indonesia, WL chickens exhibit an earlier onset of egg-laying (Sartika & Iskandar, 2019). Notably, research conducted by Kostaman *et al.* (2020) recorded an annual total egg production of 280 eggs, reaching an approximate range of 300-320 eggs, with the age at first laying recorded at 18 weeks.

Chickens commonly face stress from changes in climate, feed, and their surroundings. Nevertheless, White Leghorn (WL) chickens are renowned for their exceptional capability to adapt to environmental changes. When provided with ample feed, WL chickens display an egg production rate of approximately 56.21% and exhibit resilience to diverse environmental conditions, as evidenced by studies conducted by Garip *et al.* (2011) and Sebho (2016). Furthermore, Bhadauria *et al.* (2019) noted that WL chickens achieve a hen-day egg production rate



of 71.3% during summer, particularly between 32-36 weeks of age.

In Indonesia, the introduction of White Leghorn (WL) chickens began in 2007 under the auspices of the Indonesian Research Institute for Animal Production (IRIAP). However, suboptimal production ensued due to inadequate maintenance management. Consequently, in 2020, there was a concerted effort to intensively rear and study WL chickens. One strategy to enhance the productivity of WL laying hens involves genetic selection, particularly focusing on the prolactin gene (PRL), which is associated with egg production (Mohamed *et al.*, 2017; Sartika *et al.*, 2021). Numerous studies have demonstrated that prolactin plays a crucial role in various physiological processes in poultry and birds, including stimulating the reproductive system, regulating egg production, and maintaining homeostasis (Nagarajan *et al.*, 2023). Previous research has shown that variations in the PRL gene can lead to decreased egg production due to brooding behavior (Mohamed *et al.*, 2017). Sartika *et al.* (2021) discovered correlations between specific single nucleotide polymorphisms (SNPs), such as g.3838C>T and g.8052T>C of the prolactin gene in KUB chickens with egg production, threshold laying age, and body weight. Associations between the prolactin gene and age at sexual maturity and body weight at hatch have also been investigated, with exon 2 being implicated in these processes (Rashidi *et al.*, 2012). Furthermore, research conducted by Lumatauw and Mu'in (2016) and Rohmah *et al.* (2022) suggests that exon 5 influences egg production in local chickens, while exon 2 has been implicated in studies by Li *et al.* (2014).

The objective of this research is to assess the egg production characteristics of White Leghorn (WL) chickens under typical breeding practices at IRIAP. To establish a connection between phenotype data and genetic factors associated with egg production, it is imperative to identify and scrutinize the polymorphisms of the PRL gene in exons 2 and 5 of WL chickens.

MATERIALS AND METHODS

Ethical statement

Ethical clearance for this research has been issued by the Animal Welfare Committee of the National Research Innovation Agency of the Republic of Indonesia through Decree Number: 079/KE.02/SK/10/2022. WL chickens were reared at the Indonesia Research Institute for Animal Production, Ciawi, Bogor.

Animals care and experimental design

A total of 185 WL laying hens from 5 batches were used in this study to observe the egg production traits in the laying period. The laying hens were housed in individual cages measuring 35 cm x 40 cm x 40 cm, with unrestricted access to drinking water. They were provided with commercial layer feed containing approximately 16.5-18% protein, offered *ad libitum*. To enhance the productivity of WL chickens, 50 g per 100 liters of water of egg stimulant was added and administered continuously for five consecutive days. This stimulant was intensively administered during extreme weather conditions (e.g. excessively dry or rainy seasons and humid environments), and vaccination procedures were followed. The research location had an average temperature of 28.07 °C, humidity of 80.67 %, and a temperature humidity index of 27.19 (Margatama *et al.*, 2023).

The parameters observed in this study include the age at first laying (AFL), body weight at first egg laying (BWFL), egg weight at first laying (EWFL), total egg numbers (TEN), hen-day egg production (HEP), average egg weight (AEW), feed consumption (FC), feed conversion ratio (FCR), and mortality. All parameters were recorded daily, and the average was measured every four weeks during the laying period (24 weeks). The daily average HEP was measured every four weeks following the formula by Basant *et al.* (2013). HEP is the proportion of daily egg production divided by the number of chickens available at the time. FCR was calculated based on total feed consumption divided by the total number of eggs (kg).

DNA Extraction

A total of 47 chicken blood samples were utilized as DNA samples to identify the PRL gene. Blood was drawn from the axillary vein of White Leghorn (WL) chickens employing a 1 mL disposable syringe, and approximately 0.5-1 mL were collected. The blood was then transferred into a 1.5 mL microcentrifuge tube pre-filled with EDTA. Subsequently, these blood samples were preserved at approximately 4 degrees Celsius for subsequent use. DNA extraction was carried out using the Quick-DNA Miniprep kit (Zymo Research, USA), following the protocol provided by the kit.

DNA Amplification and Sequencing

The amplification of the Prolactin (PRL) gene fragment was conducted using a thermal cycler (Veriti Thermofisher, USA) with the primers specified in Table 1. Prior to the amplification process, a 2 µL



DNA sample was transferred to a 0.2 mL microtube. The PCR reaction was performed in a 40 µL volume, consisting of 20 µL of PCR reaction mix (MyTaq™ Red Mix Bioline, USA), 2 µL of primers, 2 µL of DNA template (50 ng/µL), and 16 µL of Nuclease-Free Water. The PCR program consisted of three steps: the first step involved a predenaturation cycle at 94°C for 5 minutes. The second step encompassed denaturation at 94°C for 30 seconds, annealing at 56°C for exon 2 of the PRL gene, and 60°C for exons 2 or 5 of the PRL gene for 30 seconds, and extension at 72°C for 30 seconds, with these processes being repeated for 30 cycles. The third step involved a final extension at 72°C for 5 minutes, followed by holding at 4°C. A 2% Agarose gel was prepared in 0.5x TBE buffer stained with FloroSafe (1st BASE, Singapore), and subjected to electrophoresis at 100V for 40 minutes. Subsequently, the agarose gel was visualized under UV light using a gel documentation system. Sequencing was carried out at 1st BASE.

Table 1 – Primer of the chicken prolactin gene.

Region	Primer	Sequence	Length of amplicon (bp)
Intron 1 & Exon 2	Forward	5'-CTG CCT CTG ACA GCT ATT TCC A-3'	294
	Reverse	5'-CAT GTT CTC ACT CCC AGG AAA A-3'	
Intron 4 & Exon 5	Forward	5'-CTG TTC TAC ACC CAG ACA GAT TGA-3'	609
	Reverse	5'-AAG GTA TAA GCC ATC CCA GCT ATT-3'	

Data Analysis

The phenotype data were analyzed by ANOVA using the software Minitab version 14 with the following model:

$$Y_{ij} = \mu + B_i + e_{ij}$$

Where: Y_{ij} = the Observed Variable, μ = the population mean, B_i = the fixed effect of i -th batch, e_{ij} = the residual error. Sequencing data were processed using MEGA6 (Tamura *et al.*, 2013) with GenBank reference (AH013783.3).

RESULTS

Early maturity performance of WL Chickens

The performance of adapted WL chickens at early maturity is shown in Table 2. These adapted WL chickens achieved an average age at initial maturity

of 156 days (AFL), exhibiting a body weight at first laying (BWFL) of 1247.91 g, and an egg weight at first laying (EWFL) of 39.94 g. In a previous study conducted by Shi *et al* (2023), the AFL of WL chickens was 144.26 days, Rhode Island Red chickens was 149.29 days, Columbian Plymouth Rock chickens was 147.5 days, Barred Plymouth Rock was 150.32 days, Synthetic Dwarf Line was 154.61 days and Beijing-You chickens was 170.98 days. This study observed the performance of adapted WL chickens for 5 batches, with an average number of rearing in each batch of 30-48 chickens. Parameters such as AFL, BWFL, and EWFL were not significantly different among batches ($p>0.05$). Notably, the B2 group demonstrated an earlier age at initial maturity (152 days) with lighter BWFL (1237.67 g) and EWFL (38.62 g) compared to the B5 group, which exhibited a slightly delayed initial maturity at 159 days, but with heavier BWFL (1276.48 g) and EWFL (40.56 g). These findings align somewhat with those of Indonesian local chickens, specifically the KUB-2 chicken reared at IRIAP under the same management system. The KUB-2 chicken line is an enhanced version of the local chicken derived from the KUB-1 line. KUB-2 was chosen for its increased egg production and distinctive yellow shanks. The KUB-2 chicken displayed AFL, BWFL, and EWFL of 156.2 days, 1788 g, and 31.32 g, respectively (Sartika & Iskandar, 2019).

Table 2 Age at first laying (AFL), body weight at first egg laying (BWFL), and egg weight at first laying (EWFL) of adapted WL Chicken.

Table 2 – Age at first laying (AFL), body weight at first egg laying (BWFL), and egg weight at first laying (EWFL) of adapted WL Chicken.

Batch	Number of hens (n)	AFL (days)	BWFL (g)	EWFL (g)
B ₁	34	154.91±16.34	1219.88±94.80	40.44±4.45
B ₂	30	152.53±11.15	1237.67±98.35	38.62±3.21
B ₃	36	154.28±9.05	1232.11±97.09	38.42±4.79
B ₄	37	159.08±10.59	1260.30±97.19	41.08±4.37
B ₅	48	159.67±10.71	1276.48±111.77	40.56±3.22
Average		156.47±11.93 ^{ns}	1247.91±102.09 ^{ns}	39.94±4.15 ^{ns}

^{ns}: no significance ($p>0.05$).

B₁: Batch 1; B₂: Batch 2; B₃: Batch 3; B₄: Batch 4; B₅: Batch 5.

Productivity Performance of WL Chickens

The production performance of White Leghorn (WL) chickens exhibited a substantial total egg number (TEN) percentage of 80.72%, accompanied by a 5.41% mortality rate during the 24-week observation



period (Table 3). Significant differences in TEN were noted between batches B2 and B3 compared to B4 and B5 ($p < 0.05$). The overall TEN in this study did not significantly differ from a previous investigation, which reported 136.32 eggs for laying chickens raised in cages (Liaqat *et al.*, 2023). The B2 group demonstrated the highest TEN and the lowest mortality among the batches. This group achieved the highest TEN due to the early onset of laying at 152 days of age. In contrast, although B5 had the highest initial and final number of laying hens at 48 and 46 chickens, respectively, it produced fewer eggs (TEN) due to the delayed Age at First Laying (AFL). The correlation between TEN in WL chickens and the age at first laying was evident, with earlier laying resulting in higher egg production and vice versa. The study indicated that batch B2, characterized by lighter body weight at first egg laying (BWFL), yielded a higher TEN of 146.64 eggs compared to B5, which produced 130.61 eggs with a heavier BWFL.

Table 3 – Observed Total Egg Number (TEN) for Adapted WL Chicken over a 24-weeks period.

Batch	Initial Number of hens (n)	Final Number of hens (n)	TEN (eggs)	TEN (%)
B ₁	34	32	136.84±20.54 ^{ab}	81.45±12.23 ^{ab}
B ₂	30	29	146.62±8.97 ^a	87.27±5.34 ^a
B ₃	36	34	140.68±12.71 ^a	83.74±7.57 ^a
B ₄	37	34	126.79±16.18 ^b	75.47±9.63 ^b
B ₅	48	46	130.61±14.92 ^b	77.74±8.88 ^b
Average			135.62±16.54	80.72±9.85

^{a,b}: Different superscripts in the same column indicate significant ($p < 0.05$).

B₁: Batch 1; B₂: Batch 2; B₃: Batch 3; B₄: Batch 4; B₅: Batch 5.

The age of laying hens had an impact on the HEP of WL chickens. Table 4 provides information on the HEP and EAW of WL chickens categorized by age groups. Egg production recording commenced at 21 weeks of age and extended for 24 weeks. The HEP at 21-24 weeks reached 54.30%, accompanied by an average AEW of 45.67 g. HEP exhibited a consistent weekly increase, reaching its peak at 29-32 weeks of age. This elevated level of production persisted up to 44 weeks, with results consistently exceeding 80%. Specifically, the zenith of WL chicken production transpired at 28 weeks of age, achieving a remarkable 96.2% egg production in B2 (see Figure 1). This ascending trend in HEP aligns closely with prior studies, where peak production occurred within the age range of 28-32 weeks (Bhadauria *et al.*, 2019).

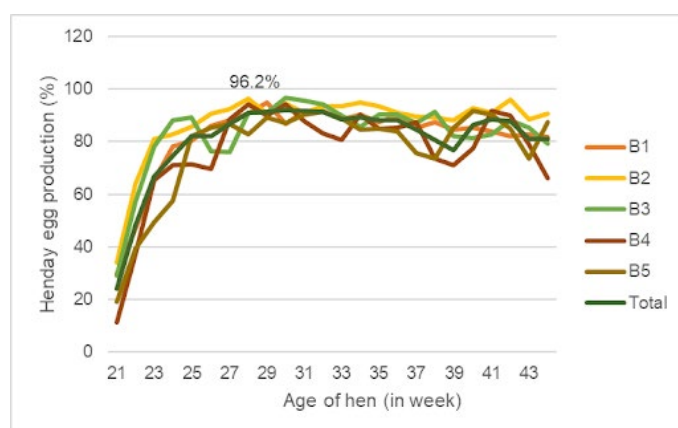


Figure 1 – Hen-day Egg Production over 24 weeks for adapted WL Chicken

Table 4 – Hen-day egg production (HEP/%) based on batch and age (weeks) of WL Chicken.

Batch	Age (weeks)						Total
	21-24	25-28	29-32	33-36	37-40	41-44	
B ₁	55.15±21.16	86.34±4.48	91.18±3.46	89.08±1.33	85.61±1.25	82.67±0.99	81.67±14.75 ^{ab}
B ₂	65.48±22.49	91.19±4.36	92.45±1.96	93.35±1.63	89.90±1.91	91.63±3.14	87.33±13.11 ^b
B ₃	63.09±26.16	83.23±7.93	94.37±2.55	89.01±2.21	85.40±4.58	83.93±3.75	83.17±14.22 ^{ab}
B ₄	46.33±27.51	81.02±12.26	88.76±4.60	85.29±3.96	77.33±7.17	81.62±11.84	76.73±18.82 ^a
B ₅	41.45±16.68	84.27±2.11	89.51±1.93	85.49±2.21	81.60±8.42	84.01±7.30	77.72±18.29 ^a

^{a,b}: Different superscripts in the same column indicate significant ($p < 0.05$).

B₁: Batch 1; B₂: Batch 2; B₃: Batch 3; B₄: Batch 4; B₅: Batch 5.

In terms of the batches, the average of HEP in B2 exhibited significant differences from the other groups ($p < 0.05$) (Table 5). WL chickens of B2 were known to have early AFL and showed positive results, with the highest HEP of 87.33%. In contrast, B5 showed late AFL, resulting in low HEP for the WL chickens. At 21-24 weeks of age, the chickens were still in the process of learning to produce eggs, with all batches of WL chickens recording a mean hen-day egg production (HEP) rate of 54.30%. Subsequently, post-25 weeks of age, HEP showed a notable increase, exceeding 80%. The HEP indicator of WL chickens in this study attested a robust egg production until the 44th week of age. Hen-day production of WL chickens was 180,81 eggs, Rhode Island Red chickens was 175,52 eggs, Columbian Plymouth Rock chickens was 169,53 eggs, Barred Plymouth Rock was 165,43 eggs, Synthetic Dwarf Line was 137,28 eggs and Beijing-You chickens was 116,48 eggs (Shi *et al.*, 2023). The daily egg production in WL chickens is influenced by multiple factors. In our investigation, we explored the Prolactin (PRL) gene's exons 2 and 5 in WL chickens, resulting in a monomorphic outcome. Previous reports have suggested the PRL gene's association with HEP and its utility for selecting laying hens (Rohmah *et al.*, 2022).



Table 5 – Hen-day egg production (HEP) and average egg weight (AEW) of adapted WL Chicken.

Age (weeks)	HEP (%)	AEW (g)
21-24	54.30±10.39	45.67±1.35
25-28	85.21±3.85	50.04±1.31
29-32	91.25±2.26	51.88±1.50
33-36	88.44±3.29	52.95±1.13
37-40	83.97±4.73	53.78±1.47
41-44	84.77±3.96	54.71±1.35

Food consumption (FC) and Feed conversion ratio (FCR) in WL chicken

The quantity of feed consumption (FC) exhibited a continuous increase, with the peak feed conversion ratio (FCR) being recorded during the 21-24 weeks age range, and the lowest observed in the period between weeks 41-44 (Table 6). In the initial weeks of hen age, White Leghorn (WL) chickens consumed a smaller amount of feed, specifically 92.05 g/d/h, leading to a high FCR of 3.48 g feed per gram of egg. As the chickens matured, their feed intake rose, resulting in a decrease in the FCR value. In this study, FCR denotes the ratio between FC and the eggs produced by WL chickens within a specified observation period. Previous studies on growth rates have explored the inverse relationship between heavier body weight in chickens and smaller FCR values (Muir *et al.*, 2023). However, in the context of this study that focuses on egg production, the diminishing FCR value (gram feed per gram of egg) could be attributed to an increase in weekly egg production. The higher FCR at 21-24 weeks might be explained by the relatively lower egg production during this early age compared to WL chickens at 25 weeks and older.

Table 6 – Feed consumption (FC) and feed conversion ratio (FCR) in adapted WL Chicken.

Age (weeks)	FC (g/d/h)	FCR (g feed/g egg)
21-24	92.05±7.24	3.48±0.78
25-28	98.91±3.80	2.25±0.21
29-32	102.79±5.48	2.16±0.16
33-36	100.43±6.93	2.14±0.19
37-40	101.25±5.54	2.23±0.27
41-44	102.28±6.38	2.11±0.14

Diversity in the PRL Gene across Exons 2 and 5

The White Leghorn (WL) chickens underwent amplification in exons 2 and 5 of the Prolactin (PRL) gene. The PCR product's length for PRL gene exon 2

was determined to be 294 bp (Figure 2), while for exon 5, it measured 609 bp (Figure 3). The examination of diversity within the exons 2 and 5 of the PRL genes was conducted through the direct-sequencing method. Specifically, no Single Nucleotide Polymorphism (SNP) was identified within the exon 2 PRL gene of the studied WL chickens. This investigation revealed the monomorphic nature of the PRL gene exon 2, characterized by a product length of 294 bp in White Leghorn chickens.

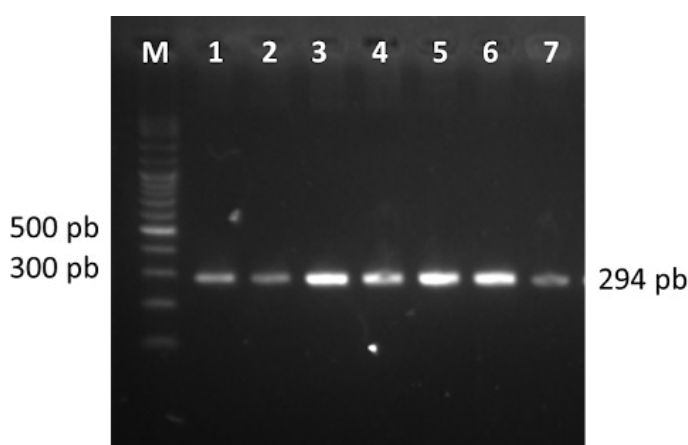


Figure 2 – Amplification PRL Gene Exon 2 in WL Chicken (line 1-7; 294 bp); M: 100bp ladder

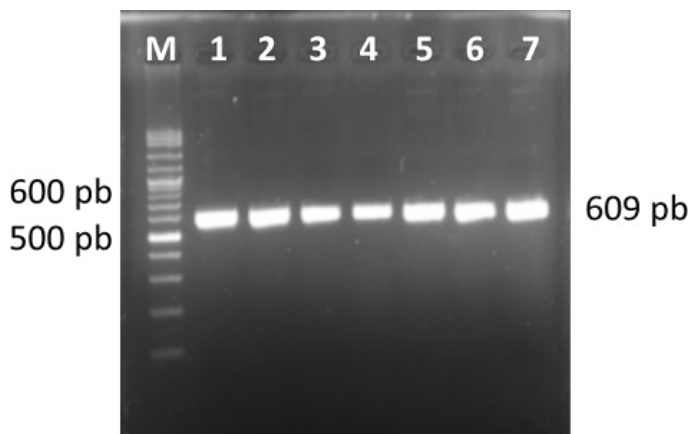
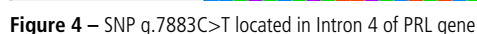


Figure 3 – Amplification PRL Gene Exon 5 in WL Chicken (line 1-7; 609 bp); M: 100bp ladder

This study demonstrated that within the PRL gene, Intron 4 in WL chickens based on GenBank with access number AH013783.3 (WL) exhibited the allele C, specifically at the position g.7883 C>T (aligned with GenBank AF288765). In contrast, the WL chicken population at the Indonesian Research Institute for Animal Production (IRIAP) presented the T allele in all 47 samples (Figure 4).





(HEP) and FCR. The prolactin gene in Magelang ducks is associated with egg production traits at SNP c.164G>A (Purwantini *et al.*, 2020). The process of laying eggs in WL chickens necessitates a sufficient supply of energy, high-quality protein, essential minerals, and vitamins from their diet to bolster egg production. It is noteworthy that the heritability value of FCR for laying hens ranges from 0.13 to 0.19 (Yuan *et al.*, 2015), while the heritability value for egg production at 45 weeks was reported to be 0.24 (Bogdanski *et al.*, 2023). Consequently, breeding programs tailored for laying hens should prioritize total egg numbers among its considerations.

In this investigation, exon 5 displayed monomorphism, indicating a lack of genetic variability. Notably, the observed variation in egg production among the 47 analyzed samples was minimal, amounting to 0.05%. Findings to the contrary were reported by Rohmah *et al.* (2022), who suggested that the presence of allele C in SNP g.8052T>C was associated with superior egg production, while Sartika *et al.* (2021) reported that allele T correlated with the highest egg production. The existence of the g.8052T>C mutation signifies a genetic alteration without changing amino acid composition. In summary, the interplay between alleles C and T influences chicken egg production under their linkage to specific genes and genetic variations that impact egg production and quality.

Moreover, Rohmah *et al.* (2020) identified four novel mutations—g.7823A>G, g.7835A>G, g.7886T>A, and g.8069T>C—with the first three located in Intron 4. Comparing this study with Rohmah *et al.*'s report, it becomes evident that Intron 4 harbors five SNPs. Introns play a pivotal role in diversifying protein structures through alternative splicing (Jo & Choi, 2015). Consequently, further exploration of the SNPs within Intron 4 and their influence on RNA splicing mechanisms is warranted. Ali *et al.*, 2024 study concluded that the GG genotype was significant with both eggshell thickness and weight indices throughout.

CONCLUSION

In conclusion, white Leghorn (WL) laying hens continue to exhibit commendable productivity in tropical conditions. The genetic analysis of WL chickens did not reveal any single nucleotide polymorphism (SNP) in exons 2 and 5 of the Prolactin (PRL) gene. These findings indicate that the PRL gene in exons 2 and 5 maintains a monomorphic nature. However, a

more extensive population of WL chickens is deemed necessary for a thorough investigation into the potential existence of SNPs in the PRL gene.

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AUTHOR CONTRIBUTIONS

NP; writing—original draft preparation and funding acquisition, TS; methodology, validation, writing—review and editing and supervision, K; formal analysis, TH; visualization and writing—review and editing, TK; writing—review and editing, NA; writing—original draft preparation and formal analysis, AS; laboratory analysis, BB; visualization and writing—review and editing, I; visualization and writing—review and editing, HZ; visualization and writing—review and editing, HN; visualization and writing—review and editing, HK; visualization and writing—review and editing, MFR; visualization and writing—review and editing and FS; software, formal analysis and data curation. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Derived data supporting the findings of this study are available from the corresponding author upon request.



CONFLICT OF INTEREST

The authors declare no conflict of interest.

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