JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(2): 107-116, June 25, 2022

Research Paper, PII: S2322455X2200012-12
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DOI: https://dx.doi.org/10.36380/jwpr.2022.12

Phenotypic Characters and *TYRP1* Polymorphism of F_4 Golden Kamper Hybrid Chickens (*Gallus gallus domesticus* Linnaeus, 1758)

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Received: 07 April 2022
Accepted: 28 May 2022

ABSTRACT

Golden Kamper is a local meat-typed chicken with four generations of Pelung male and Layer female selective breeding. This chicken has various plumage colors and patterns. Therefore, the desired plumage color is red barred plumage (*B1*). In chickens, the missense mutation in the Tyrosinase-related-proteins 1 (*TYRP1*) causes a chocolate color plumage (*choc*) with an epistatic effect on barred plumage. The current study aimed to observe the growth of 16 chickens from hatching until 49 days of age to investigate the phenotypic characteristics, especially plumage color at 49-day-old chickens, then to determine the effect of the *TYRP1* polymorphism on F_4 Golden Kamper phenotypes. The methods used in this study included selective breeding among F_3 Golden Kamper, collection of F_3 Golden Kamper's eggs, then rearing the day-old chickens of F_4 Golden Kamper. Phenotypic data were collected and blood collection was performed for DNA isolation, DNA amplification, and sequencing. Of 16 F_4 Golden Kamper, all chickens had a uniform comb type of single (rprp, 100%). The produced shank colors were white (31.25%), yellow (62.5%), and blackish gray (6.25%). The plumage colors were red barred (12.5%), white barred (12.5%), brown (68.75%), and chocolate (6.25%). The bodyweight of F_4 Golden Kamper at the age of 7 weeks reached 597.3 g. The morphometric results indicated that F_4 Golden Kamper had the same posture and body proportions as Pelung chickens, however, with a higher weight. Fourteen substitutions were found in the *TYRP1* fragment of F_4 Golden Kamper. The single nucleotide polymorphisms (SNP) had no correlation with the chocolate plumage phenotype in F_4 Golden Kamper. The evaluated SNPs in *TYRP1* were not associated with the brown plumage color phenotype.

Keywords: Chicken, Golden Kamper, Phenotype, Polymorphism, *TYRP1*

INTRODUCTION

Pelung is one of the Indonesian local meat-type chickens that originated from Cianjur, West Java province, Indonesia. Pelung chicken has a remarkable superiority in terms of body weight, compared to other local breeds (Henuk and Bakti, 2018). Local chickens have drawbacks in terms of low productivity. Therefore, the farmers prefer to generate commercial broiler and layer chickens for their profits (Ahn et al., 2015; Nurfadillah et al., 2018).

Crossbreeding and selective breeding are genetic approaches that can be used to improve the quality of local chickens. Two individuals who each have superior traits are mated, then the offspring (filial; F_1) are selected based on the desired phenotypic character (Damayanti et al., 2019). Gama Ayam Research Team from the Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University, has conducted selective breeding since 2013 to improve the egg productivity of the local Pelung chickens but retain its the phenotypic characters (Kilatsih et al., 2020; Kurnia et al., 2021).

The hybrid is called the F_1 Kamper chicken which has various phenotypic characteristics. Therefore, selective breeding has been carried out on F_1 Kamper chickens to produce a more uniform F_2 population and continued to derive a uniform F_4 population. The prospective F_1 Kamper and its progenies for full-sib mating are selected based on the character of the red barring trait, brown combined sex-linked barring gene plumage color, and

heavyweight to produce a chicken called F_2 and other types. Crosses between relatives or commonly referred to as inbreeding can lead to a decrease in genetic variation, resulting in uniformity of homozygosity in a population (Antos et al., 2013).

The brown plumage colors in Golden Kamper breeds are common unwanted expressed traits. Brown plumage is derived from genetics and environmental factors interplay. Many scientists had investigated several genes associated with brown plumage (Yu et al., 2017; Makarova et al., 2019; Olori, 2019; Zheng et al., 2020). The Golden Kamper plumage color resembles the chocolate plumage trait in the Orpington breed, a brown layer chicken (Li et al., 2019), and a Rhode Island Red breed. However, the reports of causative mutation of brown plumage (which is also similar to Golden Kamper) at Rhode Island Red are not yet available. Regarding the red barring traits, *TYRP1* is a more precise target, compared to other major brown color genes. Dark brown plumage in Golden Kamper is visually more similar to Chocolate plumage trait Tyrosinase-related-proteins 1 (*TYRP1*) than dark brown (SOX10, Gunnarsson et al., 2011), yellow (SOX10, Zhu et al., 2022), and buttercup (MC1R, Kerje et al., 2003).

Red barred plumage is a black-brown strip caused by dilution of sex-linked barring with brown genes. The barring plumage traits (B0, B1, B2) are caused by a mutation in Cyclin-dependent kinase inhibitor 2A (CDKN2A). The CDKN2A and *TYRP1* are located in Z chromosome (Hellström et al., 2010; Schwochow et al., 2017; Li et al., 2017). Furthermore, Tyrosinase (TYR) which is involved in the same melanin pathway as *TYRP1* has an epistatic effect on barred plumage (Hua et al., 2021).

In chickens, c.640C > A polymorphisms in the exon 3 of *TYRP1* are associated with the appearance of dark brown plumages (chocolate color trait). The current research aimed to investigate the association of *TYRP1* gene polymorphism on F_4 Golden Kamper dark brown plumage color and assess body weight inheritance.

MATERIALS AND METHODS

Ethical consideration

All procedures in this research (rearing, and blood collection) were conducted in accordance with standard chicken care guidelines. No experimental action was conducted in this research.

Chicken breeding and day-old chicken maintenance

The present research was conducted in Center for agrotechnology Innovation (Pusat Inovasi Agroteknologi; PIAT), Kali Tirto, Berbah, Sleman Regency, Yogyakarta, Indonesia. The parental mating was conducted in a cage (8 m^2) and fed with a commercially available pellet as a standard adult feeder (AD-II; Japfa Comfeed) and water *ad libitum*. A total of 16 chickens used in this study were days old chickens (DOCs) of F_4 Golden Kamper produced from female F_3 and male F_3 Golden Kamper mating. All eggs were artificially incubated and the hatched DOCs were transferred into a rearing cage. Adaptation was done for one day and the rearing cages were warmed before DOCs were deployed. Adaptation of DOCs was performed by adding 5mg anti-stress (Vita stress, Medion Farma) and 5 mg multivitamin supplement (vitamins A, B1, B2, B6, B12, C, D3, E, K3 as well as calcium-D-pantothenate, nicotinic acid, natrium butirat) of Vitachick (Medion Farma) in every 7 liters drinking water for a day. The DOCs were reared with lighting and heater using 10 watts light bulb for 24 hours and fed with a crumble standard broiler grower (BR-I; Japfa Comfeed) and water *ad libitum*. As can be seen in Table 1, the quantitative characteristics observed in the current study were body weight which was measured once every week with a digital scale KrisChef EK9350H for 7 weeks and the qualitative characters were measured using a tape measure (Metline) based on Damayanti et al. (2019).

Blood collection and DNA isolation

In the current study, a whole blood sample (1 ml) was drawn from a wing vein using a 3 ml syringe with a 23G needle from all chickens. The collected blood samples were stored in a vacutainer and preserved at -20°C. The DNA was extracted using the Chelex method according to the previous study by Ernanto et al. (2018). Blood was absorbed into Whatman filter paper and then incubated in lysis buffer (200 µL 5% chelex; 18 µL 0.05 M Dithiothreitol (DTT), 2 µL proteinase K [10 mg/mL]) at 100ºC for 8 minutes. The tube was vortexed and incubation was prolonged at 56°C for 2 hours with vortexed and spun down every 15 minutes. Incubation continued at 100°C for 8 minutes then vortexed. Tubes were centrifuged (Gyrozen Mini Centrifuge GZ-1312, South Korea) at 13000 × g for 3 minutes and its supernatant was transferred into clean microtubes. Tris-EDTA (TE) Buffer (1:1) was added to the tube and stored at -20°C.

Table 1. Morphological characters of chickens

Characteristic	Detailed procedure
Chicken height	Measured from the digit/hallux to the tip of the comb
Body height	Measured from the digit/hallux to the end of the distal vertebrae
Beak width	Measured from articular to dexter
Beak length	Measured from the base of the angular process to the end of the mandibular symphysis
Head length	Measured from the supraorbital bone to premaxilla
Head width	Measured from quadratojugal sinister to dexter
Comb height	Measured from the highest tip of the comb to the base of the comb
Comb length	Measured from the back to the front of the comb
Body length	Measured from the tip of the first thoracic vertebra to the base of the pygostyle
Body width	Measured from the base of the femoral bone to dexter
Chest circumference	Measured from the sternal of the keel in a circle
Dorsal length	Measured from the thoracic vertebrae to the caudal vertebrae end
Wingspan	Measured from the base of the humerus to the end of the carpus
Neck length	Measured from the base of the atlas to the tip of the thoracic vertebrae
Tibia length	Measured from the tip of the femur to the base of the tibiotarsus
Femur length	Measured from the end of the patella to the base of the femur
Shank	Measured from the tarsus to the base of the patella

Modified from Damayanti et al. (2019).

Fragment gene of interest amplification and sequencing

The fragment gene of interest was amplified using a gradient thermocycler (BioRad, US) with a specific primer (IDT, Malaysia). A 25 µL cocktail consisted of a 12.5 µL Master mix PCR kit (KAPATaqTM; US), 2.5 µL forward (5'-TCTCATTATTATTCCGTCAGG-3), and reverse (5'-GCAAAGTTCCAGTAGGGTAG-3') primer (Zheng et al., 2020), 2 µL DNA template (± 50 ng/µL), and 8 µL ddH_2O. The amplification protocol was performed as one cycle of pre-denaturation condition at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 30 seconds. Post extension for 5 minutes at 72°C. The PCR product quality was verified by electrophoresis using 2% gel agarose. This step was mandatory to verify the PCR product purity and size before undergoing Sanger sequencing.

The PCR product was sequenced using sanger sequencing (1st BASE, Malaysia) to visualize the single nucleotide polymorphism (SNP). The *TYRP1* (1500 bp) was sequenced with the same primers for PCR. Gene Studio (GeneStudio ver. 2.2.0) and Clustal Omega (2022) were used to observe the presence of SNP.

Data analysis

All data from F_1 to F_4 generation were tabulated and compared with ANOVA and followed by post hoc Tukey HSD using IBM SPSS (version 25) software to assess the significance between generations from hatching to 49 days of age. The observation of plumage color, shank color, and comb shape character were performed at 7 weeks of age. Data were presented in tables and figures. The correlation between SNP and brown plumage color was analyzed using Fisher's Exact Test. P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Pedigree and quantitative phenotype of F_4 Golden Kamper

A cross between F_3 Golden Kamper produced 16 F_4 Golden Kamper DOCs consisting of 7 males and 9 females (Figure 1). The weights of F_4 Golden Kampers were compared with F_1 Kampers (Lesmana, 2016; unpublished data), F_2 Golden Kamper (unpublished data), Pelungs, and layer chickens (Figure 2). As can be seen, F_4 Golden Kampers at the age of 7 weeks had a higher weight (597.3 gr), compared to F_2 Golden Kampers (435.7 gr), Pelungs (472.6 gr), and layers (424.9 gr). These data could indicate that hybridization and selective breeding methods led to positive results in body weight. However, the average weight of F_4 Golden Kampers was still lower than F_1 Kampers (771.3 gr) which was the first filial of a cross between a Pelung rooster and a Layer female. The body weight of F_4 Golden Kamper was lower than F_1 Kamper, which could be influenced by intrinsic and extrinsic factors. Intrinsic factors are factors that influence from within the body, such as genetic factors. However, extrinsic factors are factors that influence from outside the body, such as environmental conditions, exposure to stress, and the amount of consumed nutrients.

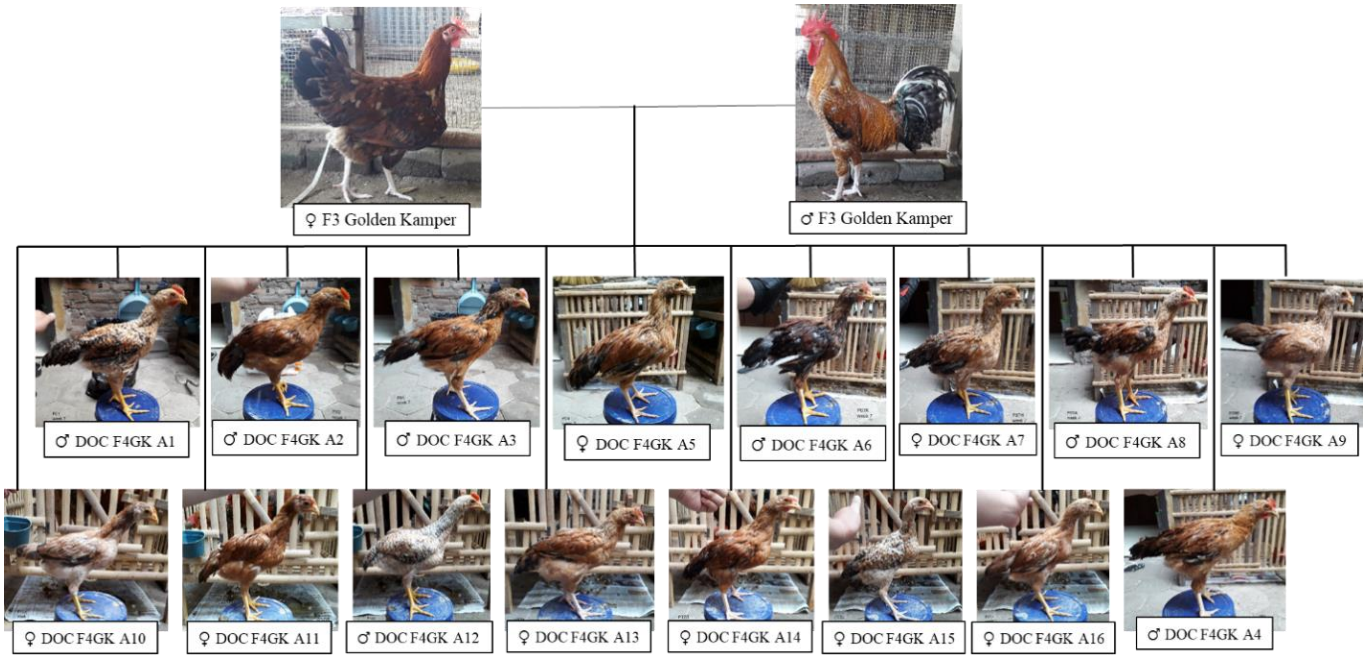

Figure 1. Pedigree and plumage phenotype of 16 evaluated F_4 Golden Kampers

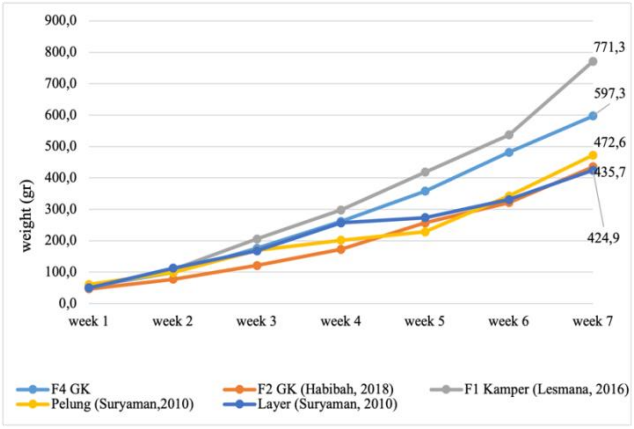

Figure 2. The body weight of chickens from hatching to 49 days of age

The average weight of the five groups of chickens (Pelung, Layer, F_1 Kamper, F_2 Golden Kamper, and F_4 Golden Kamper) was normally distributed. Regarding ANOVA analysis, the average weight of the five groups of chickens was not significantly different ($p > 0.05$). Therefore, although the average body weight of F_4 Golden Kamper chickens was lower than their grandparents (F_1 Kamper), F_4 Golden Kamper chickens remained a prospective local meat-type chicken breed candidate due to the higher body weight, compared to pure Pelung chicken. Body size is a factor that can affect the selling value of local chickens in Indonesia. The proportion of chicken body size can be observed by taking morphometric or zoometric quantitative data of chickens into account (Alsudany et al., 2017). The results of the morphometric measurements of F_4 Golden Kamper can be seen in Table 2.

Table 2. The morphometric characters of F_4 Golden Kamper chicken at 49 days of age

Number	Parameters	Value (cm)
1	Chicken height	32.50
2	Body height	21.94
3	Beak width	1.06
4	Beak length	2.22
5	Head length	1.61
6	Head width	2.03
7	Comb height	2.08
8	Comb length	0.62
9	Body length	8.07
10	Body width	14.31
11	Chest circumference	8.41
12	Dorsal length	5.26
13	Wingspan	20.49
14	Neck length	8.86
15	Tibia length	6.19
16	Femur length	8.97
17	Shank	6.30

F_4 Golden Kamper had similar total height, body height, femur length, and tibia length with Pelung chickens (Mahardhika and Daryono, 2019). Considering total height, F_4 Golden Kamper reached 32.50 cm while

Pelung only 32.13 cm. Regarding body height, F_4 Golden Kamper was higher than Pelung (21.94 versus 20.5 cm), however, F_4 Golden Kamper was shorter than Pelung (6.19 versus 6.79 cm) in terms of femur length. F_4 Golden Kamper had longer tibia than Pelung (8.97 versus 8.90 cm). In case of chest diameter, F_4 Golden Kamper has a larger chest circumference than Pelung (20.49 versus 18.59 cm, Mahardhika and Daryono, 2019). These morphometric data showed that the F_4 Golden Kamper chicken had posture and body proportions that resembled Pelung Chicken, but with a higher weight. This shows that the F_4 Golden Kamper chicken has high potential as an ideal local meat-type chicken because it has the posture and body proportions as Pelung chickens but has a higher body weight.

Qualitative phenotype of F_4 Golden Kamper

In addition to the quantitative characteristics, qualitative characteristics were also investigated in 16 F_4 Golden Kamper chickens. The qualitative characteristics included the shape of the comb, the color of the legs or shank, and the color of body hair (Serpico, 2020). The results obtained from the observation of qualitative characters can be seen in Table 3.

Table 3. Qualitative characters of F_4 Golden Kamper chickens at 49 days of age

Qualitative character	Phenotype (Genotype)	Number	Percentage
Comb Shape	Single (rprp)	16	100
Shank Color	Yellow (*wwZ^{Id}-)*	10	62.5
	White (*W-Z^{Id}-)*	5	31.25
	Blackish-grey (*wwZ^{id}*)	1	6.25
Plumage Color	red-barred *(B1-e^{b})*	2	12.5
	White-barred *(B1-)*	2	12.5
	Brown (*NNe^{b}*-)	11	68.75
	Chocolate- (*NNchoc*)	1	6.25

(A) (B) (C) (D)

Figure 3. Phenotype of F_4 Golden Kamper. A: Red barred, B: White barred, C: Brown, and D: Black-Brown (chocolate).

Based on observations, there are three groups of shank colors on F_4 Golden Kamper, namely white, yellow, and blackish-gray. The group of white shanks consisted of 5 individuals (31.25%), then the yellow shank consisted of 10 individuals (62.5%), and only 1 (6.25%) was categorized as blackish-grey shank chicken. Shank color in chickens is influenced by many different allele genes, including autosomal and sex-linked genes (Jin et al., 2016; Jiguo et al., 2017; Shen et al., 2019). The autosomal dominant *W* gene produces a white color because it inhibits lipochrome, while the recessive *w* allele for *W* produces lipochrome which causes a yellow color in the epidermal layer of the shank. The sex chromosome-linked gene, namely *Id*, acts as a melanin inhibitor, and the recessive allele, namely *id* highlights the black color in the dermis layer of the shank (Daryono and Perdamaian, 2019). Based on observations, it can be concluded that the genotype of a female F_4 Golden Kamper chicken with a blackish-grey shank is *wwZ^{id}*. Then, the genotype of F_4 Golden Kamper with white shank was *W-Z^{Id}Z^{Id}* or *W-Z^{Id}Z^{id}* in males and *W-Z^{Id}* in females. The genotypes of F_4 Golden Kamper with yellow shanks were *wwZ^{Id}Z^{Id}* or *wwZ^{Id}Z^{id}* in males and *wwZ^{Id}* in females. Both parents used in the current study had white shanks, so the genotype of

the female F_3 Golden Kamper shank was WwZ^{Id} and the F_3 Golden Kamper male was $WwZ^{Id}Z^{id}$.

The plumage color of F_4 Golden Kamper is red barring traits ($B1e^b$, 12.5%), White barring traits (B1, 12.5%), Brown (NNE^b, 68.75%), and Black-Brown (chocolate traits; NNchoc, 6.25%). The red barring motif is a plumage pattern of horizontal stripes of two different colors caused by complex temporal and special gene activity (Schwochow et al., 2017). The red barring motif on the F_4 Golden Kamper chicken consists of two or more colors, namely white, black, and brown. This barring motif is inherited from the Pelung *blirik* (white sex-linked barring) which was selected to be used as an ancestor in the first cross with female layer chickens. The *blirik* pattern can mainly be found on the tail, neck plumages, and wing plumages. The color of the golden-brown *blirik* plumage is the color that becomes the target character of the Golden Kamper. The emergence of F_4 Golden Kamper individuals with almost entirely brown body plumage color can be caused by the reappearance of Layer plumage phenotype characteristics. The Lohmann Brown layer chicken elder which was chosen as the ancestor in the previous cross had a brown body color with no *blirik* motif.

Polymorphisms of *TYRP1* in F_4 Golden Kamper

The PCR product of 16 F_4 Golden Kamper and both parental (F_3 Golden Kamper) is illustrated in Figure 4. In both parental and filial, the length of DNA fragment was 1500 bp. This is the length of the nucleotide where the specific primer for *TYRP1* is attached.

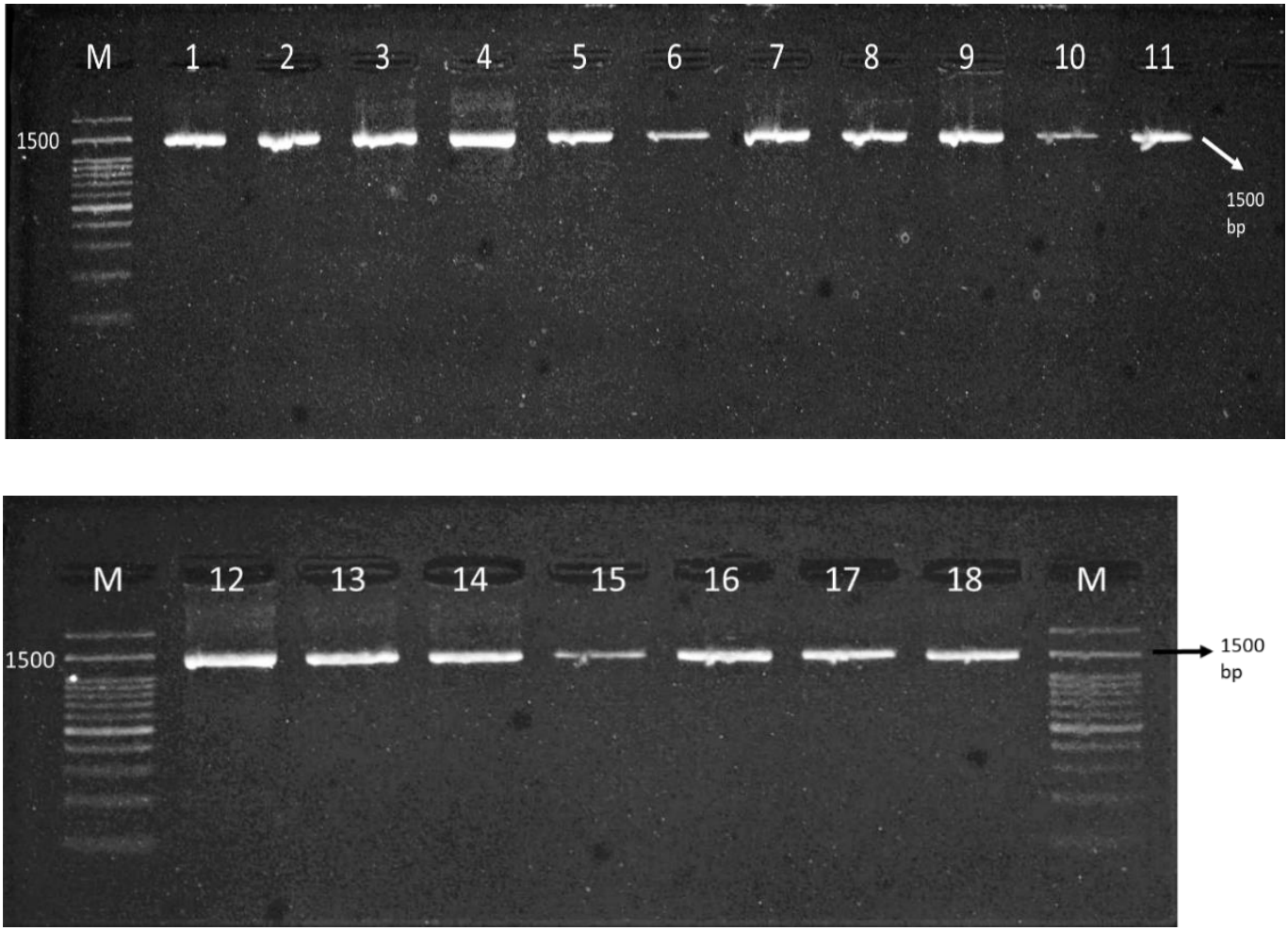

Figure 4. Visualization of 1500 bp PCR product *TYRP1*. M: DNA ladder, 1-16: F_4 Golden Kamper chicken, 17: Male F_3 Golden Kamper, 18: Female F_3 Golden Kamper.

Table 4. The 4-haplotype derived from 14 single nucleotide polymorphisms at *TYRP1*

	Polymorphism of *TYRP1*						
	Reference	**A1**	**A2**	**A6**	**A11**	**P Male**	**P Female**
Single nucleotide polymorphism							
G3536A	G	G	G	G	A	A	A
G3587A	G	G	G	G	A	G	A
C3695T	C	C	C	C	T	C	T
G3738A	G	G	G	G	A	A	A
C3775T	C	C	C	C	T	C	T
T4053G	T	T	T	T	G	G	G
G4186T	G	G	G	G	T	G	T
C4251G	C	C	C	C	G	C	G
A4273G	A	A	A	A	G	A	G
G4460A	G	G	G	G	A	G	A
A4486T	A	A	A	A	T	A	T
C4513T	C	C	C	C	T	T	T
G4773T	G	G	G	G	T	T	T
A4856C	A	A	A	A	C	C	C
Haplotipe	Reference	Reference	Reference	Reference	1	2	1
Plumage Color	-	Red barred	Brown	Chocolate	Brown	Red barred	Chocolate

A: Allele, P: Parental

Based on the results of sequencing and alignment of the *TYRP1* gene, it can be observed that there are 14 mutation points in F_4 Golden Kamper and its parental (F_3 Golden Kamper). The polymorphism points of the *TYRP1* gene are presented in detail and divided into haplotypes (Table 4). Based on Table 4, it can be observed that all polymorphisms that occur in F_4 Golden Kamper and F_3 Golden Kamper brooders are substitution polymorphisms. Substitutions that occur are transversion substitution and transition substitution. All the single nucleotide polymorphisms (SNPs) obtained formed two haplotypes in Golden Kamper and its parental. Chicken codes A1, A2, and A6 have the same DNA sequence as the GeneBank reference (Gene ID: 395913), so it can be assumed that there is no polymorphism in these individuals. The A11 has the same haplotype as the female parent (P Female), while the male parent (P Male) has a different haplotype from the A11 and P Female. Based on the haplotype analysis of the sequencing results of the *TYRP1* gene above, A11 inherits the *Z* chromosome from the female parent because *TYRP1* gene located in *Z* chromosomes (Table 4). The sex of chickens is determined by the *Z* and *W* chromosomes. In contrast to the human sex chromosomes, chickens that have heterogametic chromosomes are female (*ZW*) while male chickens have homogametic sex chromosomes (*ZZ*, Lawal et al., 2020).

The correlation between changes in the nucleotide arrangement due to the presence of polymorphisms with the appearance of the brown plumage color phenotype in F_4 Golden Kamper chickens can be analyzed by Fisher's Exact Test. Correlation test was carried out at each point of the polymorphism of the plumage color of F_4 Golden Kamper. The results of Fisher's exact test are shown in Table 5.

The obtained results indicated that all 14 SNPs in the *TYRP1* were not correlated with the appearance of the brown plumage color phenotype in F_4 Golden Kamper chickens. Therefore, the *TYRP1* gene cannot be used as a molecular marker of the brown plumage color phenotype, an unwanted color that appears in Golden Kamper chicken breeds.

The absence of a correlation between the *TYRP1* gene polymorphism and the brown plumage color phenotype in F_4 Golden Kamper chickens can be caused by multiple factors. In this report, no mutation in the previously reported site (c.640C > A) of *TYRP1* was responsible for the chocolate plumage trait. In chickens, c.640C > A polymorphisms in the exon 3 of *TYRP1* substitute histidine for asparagine amino acid. This mutation occurs in the ZnA region which interacts with zinc metal ions as a cofactor (Solano, 2018) and has a negative effect on the function of the *TYRP1* protein (Li et al., 2019). This mutation is associated with the appearance of dark brown plumages (chocolate color trait) in Orpington chickens.

Chicken plumage color is influenced by a complex variety of genes (Makarova et al., 2019). In addition to the *TYRP1* gene, there are also mutations in other genes that can cause the brown plumage color phenotype in chickens. Schwochow et al. (2021) reported that a 15-bp deletion in the *PMEL17* gene causes a grayish-brown color (dun) in chickens crossed between Red Junglefowl males and White Leghorn females. Meanwhile, based on research from Gunnarsson et al. (2011), an 8.3-kb deletion in the *SOX10* gene causes a dark brown phenotype in hybrid red

jungle fowl chickens. According to a study by Zhang et al. (2015), several genes that control plumage color and skin color in chickens can be specific in certain populations.

Table 5. Correlation test of *TYRP1* polymorphism to brown plumage color phenotype

Polymorphisms	Genotype	Genotype frequency	Brown plumage frequency
G3536A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
G3587A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
C3695T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
G3738A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
C3775T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
T4053G	TT	0.75	0.05
	TG	0	0
	GG	0.25	0.05
G4186T	GG	0.75	0.05
	GT	0	0
	TT	0.25	0.05
C4251G	CC	0.75	0.05
	CG	0	0
	GG	0.25	0.05
A4273G	AA	0.75	0.05
	AG	0	0
	GG	0.25	0.05
G4460A	GG	0.75	0.05
	GA	0	0
	AA	0.25	0.05
A4486T	AA	0.75	0.05
	AT	0	0
	TT	0.25	0.05
C4513T	CC	0.75	0.05
	CT	0	0
	TT	0.25	0.05
G4773T	GG	0.75	0.05
	GT	0	0
	TT	0.25	0.05
A4856C	AA	0.75	0.05
	AC	0	0
	CC	0.25	0.05

CONCLUSION

In conclusion, the obtained results of the current research indicated the benefits of cross breeding and genetics selection for improving the body weight of the local chickens. However, the results revealed that the inheritance fashion of plumage color was complex. It is, therefore, important to conduct further experiments using more target genes associated with eumelanin synthesis .

DECLARATIONS

Acknowledgments

This research was funded by the Ministry of Higher Education Republic of Indonesia (Kemenristekdikti) through the Excellent Applied Research for Higher Education (Penelitian Terapan Unggulan Perguruan Tinggi) Fund 2021 (No.: 7260/UN1. DITLIT/DIT-LIT/PT/2021). Authors also would like to express their gratitude to Gama Ayam Research Team, Mr. Suryadi, and Pusat Inovasi Agroteknologi (PIAT) UGM for their kind assistance during this research work.

Authors' contribution

Gilang Ilham Firmansyah conducted the experiment, writing the original article. Ayudha Bahana Ilham Perdamaian wrote and revised the manuscript. Budi Setiadi Daryono designed the experiment, supervised the study, and revised the manuscript. All authors checked the data and the final draft of the manuscript.

Competing interests

The authors have no competing interests.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical consideration

The authors checked for ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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