The association of follicle stimulating hormone receptor (*FSHR*) gene polymorphism of on egg productivity in hybrid chicken (*Gallus gallus gallus*, Linnaeus 1758)

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Abstract. *Kurnia RR, Lesmana I, Ernanto AR, Perdamaian ABI, Trijoko, Daryono BS. 2021. The association of follicle stimulating hormone receptor (FSHR) gene polymorphism of on egg productivity in hybrid chicken (Gallus gallus gallus, Linnaeus 1758). Biodiversitas 22: 1221-1226.* Pelung chicken genetics Improvement by selective breeding to Layer Lohmann Brown was successfully developed F_1 which yielded 140 eggs for 300 days of production. BC_1 chicken derived from ♀ Layer and ♂ F_1 (♀ Layer vs ♂ *Pelung* chicken). Polymorphism on c*FSHR* gene promoter was potential genetic marker candidate to assist the selection. This research aims to study the BC_1 chicken egg-related traits performance for 16 weeks and study the correlation of c*FSHR* polymorphism to egg productivity. *FSHR* gene promoter was showed using sanger sequencing. Chicken were grouped based on their haplotype. Chicken were maintained in battery cage for observation of egg production. The results show that there is a difference of BC_1 chicken DOC weight from different egg weight. The six SNP polymorphisms exist on c*FSHR* gene promoter fragments on 10, 51, 59, 121, 233, 331 nucleotides and conducted 7 haplotype group. The highest egg production in BC_1 chicken on TTGCYA and lowest egg production on TTGYYG haplotype. Based on the correlation test there was a positive correlation at p> 0.05 between BC_1 chicken TTGCYA haplotype with egg production and positive correlation at p <0.05 between BC_1 TAGTTA haplotype with egg length.

Keywords: Backcross, c*FSHR* gene promoter, egg productivity, hybrid chicken

INTRODUCTION

Chicken eggs are one of the economically important products in Indonesia. Egg price is relatively affordable compared with other sources of animal protein. The consumption of chicken eggs increased in 2015 by 3.57% per year while the consumption of local chicken eggs during 1996-2015 on average decreased by 2.62% per year. Layer chicken egg consumption in Indonesia is higher than the local chicken eggs, this is because the price is relatively more expensive and limited in number.

Indonesia is known as the largest chicken germplasm center after China and India. This is evidenced by the presence of various types of local chickens spread in Indonesia with different morphological characters, such as *Pelung* chicken in Cianjur, West Java (Daryono et al. 2020), *Kokok-Balenggek* chicken in Solok District, West Sumatra, and *Kedu* chicken in Temanggung, Central Java (Ulfah et al. 2016). Local chicken in Indonesia has low productivity, so it has not been able to serve as a national food source. Therefore, it is necessary to perform genetic improvement to support the independence of the provision of food sources of animal protein.

Marker Assisted Selection (MAS) approach can accelerate the selection process by analyzing the interrelationship between quantitative traits and genetic variations. Chicken egg productivity was one of the interesting quantitative traits. Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada (UGM) since 2006 was perform breeding program to produce new chicken lines with local chicken qualitative character but have broiler fast growth rate (Utama et al. 2018; Tanjung et al. 2019; Perdamaian and Daryono 2020). Hybrid chicken (F_1) derived from female broiler vs male *Pelung,* has a faster growth rate than *Pelung*, but its plumage color varies and still resembles a broiler. Another breeding scheme was brown layer chicken to produce F_1 hybrid chicken, which possesses an appropriate growth rate (Perdamaian et al. 2017). It is also necessary to use brown layer chicken as female parent to produce hybrid chicken with high egg productivity.

In our previous study using High-Resolution Melt (HRM) approach, hybrid chicken derived from crossing Layer Lohmann Brown to male *Pelung* has improved follicular development than *Pelung* chicken (data not shown). Those follicular development related to the different frequency of alleles in *FSHR* gene promoter polymorphism. That hybrid chicken has high egg productivity which produces approximately 140 eggs during 300 days of production (unpublished data). Back Crossing hybrid chicken to Layer Lohmann Brown was necessary to produce First Back Cross (BC_1) chicken which

hopely possesses higher accumulated of egg quantitative traits.

One of importance genes related to eggs productivity, follicle-stimulating hormone receptor (*FSHR*) gene express protein which serve as receptor of follicle-stimulating hormone (FSH). The FSH role was to regulate follicle development and recruitment in the ovary (Lee et al. 2019; Guo et al. 2020). Polymorphism in the follicle-stimulating hormone receptor (*FSHR*) gene was widely reported to affect egg productivity-related traits in chicken (Lee et al. 2011; Lee et al. 2019). Polymorphism on the 181 A > T of *FSHR* positively correlates to the Beijing You chicken breed egg productivity (Lee et al. 2011).

In this research, *FSHR* gene polymorphism was investigated for egg quality and productivity in the BC_1 lines of Indonesian hybrid chicken.

MATERIALS AND METHODS

This research was conducted in April 2017-June 2019, Lohman Brown and F_1 hybrid chicken (Figure 1) mating were done in breeding facility located at Innovation Center of Agro Technology, Universitas Gadjah Mada (PIAT UGM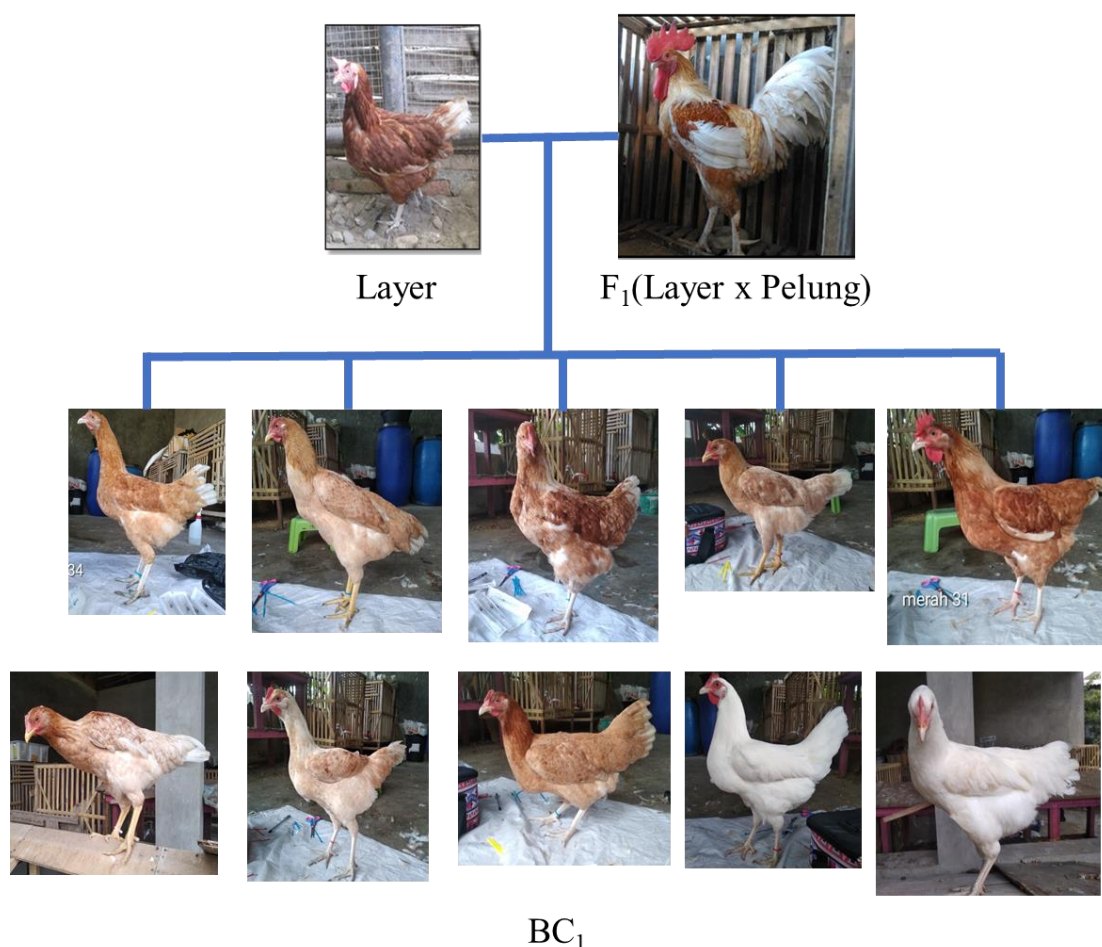), Yogyakarta, Indonesia. Eggs were collected and artificially incubated. Molecular analysis was done at Laboratory of Genetics and Breeding, and Central Laboratory (FALITMA) of Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Hens maintenance

Standard broiler chicken feed (AD II, Japfa, Indonesia) was used for raising Day Old Chicken (DOC) of BC_1 chicken. Ten chicken was given free access of food and water. Ten female chickens of adult BC_1 line were kept in semi-intensive 4x4 meters pens and adequate ventilation at PIAT UGM. Natural lighting with no special photoperiodization treatment was chosen. Chickens were maintained in battery cage for 4 months from the start laying period.

Egg weight

In this research, the weight of female Lohmann Brown chicken eggs was discriminated against for early selection of BC_1 chicken line. Eggs with more than or equal to 60 grams in insert in group A, while eggs less than 60 grams in insert in group B. This grouping aims to determine whether there is any influence of egg weight on the weight of DOC. Independent T-test was used to differentiate the two groups.

Figure 1. The picture of BC_1 chicken, female Layer and F_1 (Layer x Pelung)

Egg width and length

Egg produced from BC$_1$ chicken was collected and its weight and length recorded each haplotype. Chicken egg length and width were measured using vernier caliper.

Genomic DNA isolation

Genomic DNA was extracted using Chelex method followed our previous report (Ernanto et al. 2018). with the addition of TE Buffer in the sample, centrifuge was done at 13.000 rpm for 13 minutes. Supernatant was discarded, and 5% Chelex solution, 18 µL 0.05 M DTT, 2 µL proteinase K (10 mg /mL) was added to the remaining pellet. The solution was then vortexed prior to incubated at 56 ℃ for 2 hours with vortexed every 15 minutes. Incubation was prolonged into 100 ℃ boiling water for 8 minutes before homogenized for 30 seconds. Samples were centrifuged at 13,000 rpm for 3 minutes and its supernatant was transferred into new microtubes and stored at -20 ℃ before use.

Genomic DNA amplification

Gene of interest was amplified using thermocycler (BioRad, US) with specific primer (IDT, Malaysia). 25 µL cocktail consisted of 12.5 µL Master mix PCR kit (KAPATaqTM; US), 2.5 µL forward (5'-TCA-GCT-GAG-GCC-TGT-GAT-TTC-3) and reverse (5'-GGA-GAG-AAG-CGA-GGC-TGA-TTT-3') primer, 10 µL DNA template (±50 ng/µL), and 10 µL ddH2O.

The PCR reaction was performed on a thermal cycler with a pre-denaturation condition at 95 °C for 3 minutes. Then 30 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 20 seconds, and extension at 72 ° C for 25 seconds. Post extension for 5 minutes at 72 °C. Horizontal DNA electrophoresis was done at 2% agarose gel.

Sanger sequencing of *FSHR* gene

The obtained PCR product was quoted to Sanger sequencing (1st BASE, Malaysia). *FSHR* gene promoter (348 bp) were sequenced with forward (5'TGCCTGGGTGTAGGACATA-3') and reverse (5-'CACCCTTCCTTGAAGGTC-3') primer. DNA Baser

software was used to observe the presence of single nucleotide polymorphism (SNP).

Statistical analysis

The correlation test was done using SPSS 16.0 software to determine the relationship between the polymorphism of the c*FSHR* gene promoter and the productivity of the hybrid chicken egg backcross1 at a 95% confidence level.

RESULTS AND DISCUSSION

In this study, Backcross or BC$_1$ chicken line was derived from crossing female Lohmann Brown chicken with F$_1$ (female Lohmann Brown x male *Pelung*) chicken. Lohmann Brown chicken eggs weight were discriminated for early selection of BC$_1$ chicken. The average weight of group A and group B DOC were significantly different at 99% confidence level (p <0.01). The result of this selection is chicken with higher body weight and good egg productivity than Pelung chicken which had 500g body weight and around 30.17 eggs/23 weeks (Kilatsih et al. 2020).

Ten females of BC$_1$ chicken has six different variations of the feather which was white (9%), white and brown (27%), brown (37%), light brown (9%), dark brown (9%), and brown-gray color (9%). Genomic DNA was extracted using the phenol-chloroform technique and amplified by using a conventional thermal cycler (Figure 2).
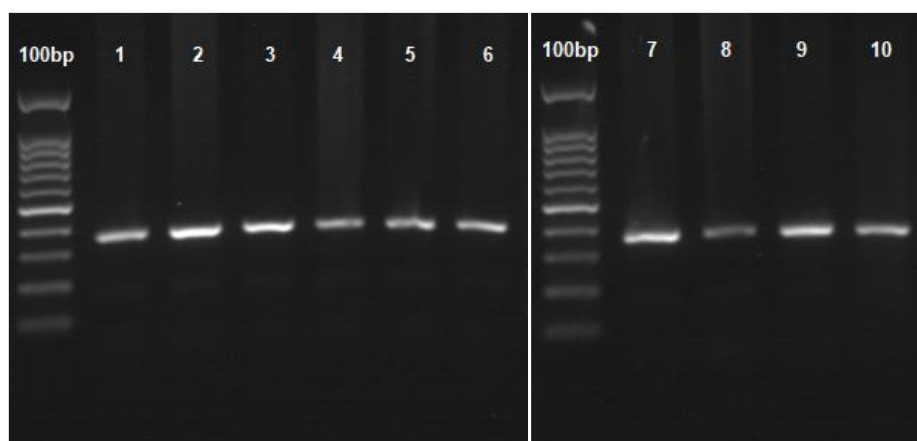
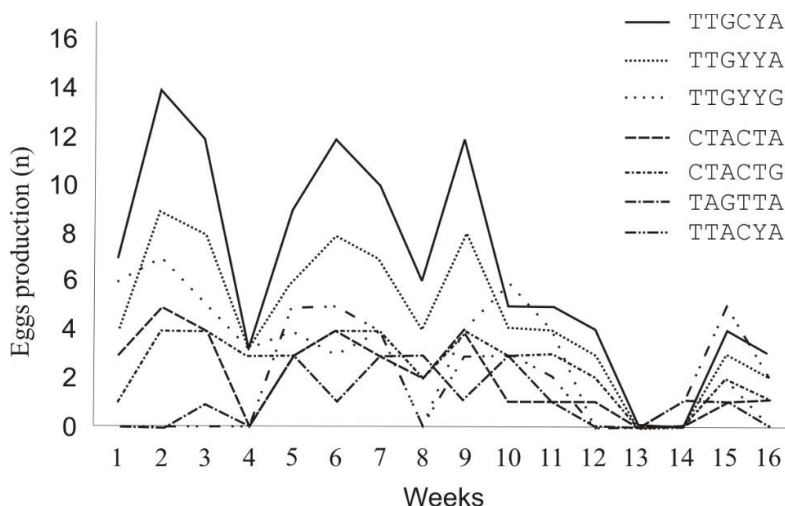After amplified using conventional thermal cycler, PCR products were Sanger sequenced (Figure 3).

**TTCAGCTTGCTCAGGATTCAAGTGAGTCAACATCAGCAG
AGTTACCCATGAAAATCCAGCTAGCGAGCTCACTTACCA
TGCAGATGACTTACAAGCTGTGCATATCTTAAACTTATA
ATAAATAAGGACTCAAAGATGGGGTTCAGTGTCCAGGCC
TCATGATCATGATAATGTCCTTGAAGCTATTTCTTGAAC
CTGTACCTCTTGTTGTGTAATAAATATTCATTCTGTTTA
TTTTCCAATGATAAAATTAGCTCACTGGTCTCC**

Figure 3. The partial sequence of *FSHR* promoter gene of BC$_1$ chicken

Figure 2. The DNA band of chicken *FSHR* gene visualized by 2% agarose gel electrophoresis shift. Line 1 to 10 indicate the BC$_1$ chicken

Figure 4. The egg productivity of BC_1 chicken each haplotype group

Table 1. The genotype of BC_1 chicken according to c*FSHR* gene polymorphism

Chicken tag	SNP position					
	10	51	59	121	233	331
1	CC	TT	AA	CC	TT	AA
2	TT	TT	AA	CC	CT	AA
3	TT	TT	GG	CT	CT	GG
4	TT	TT	GG	CT	CT	AA
5	TT	TT	GG	CC	CT	AA
6	TT	TT	AA	CC	CT	AA
7	TT	AA	GG	TT	TT	AA
8	TT	TT	GG	CT	CT	AA
9	CC	TT	AA	CC	TT	GG
10	TT	TT	GG	CC	CT	AA

Table 2. The haplotype of BC_1 chicken based on c*FSHR* gene polymorphism

Haplotype	Population percentage (%)
TTGCYA	20%
TTGYYA	20%
TTACYA	20%
CTACTG	10%
CTACTA	10%
TAGTTA	10%
TTGYYG	10%

FSHR gene sequences of ten BC_1 chickens were analyzed using DNA Baser software. The genotype of BC_1 chicken based on *FSHR* gene polymorphism can be seen in Table 1.
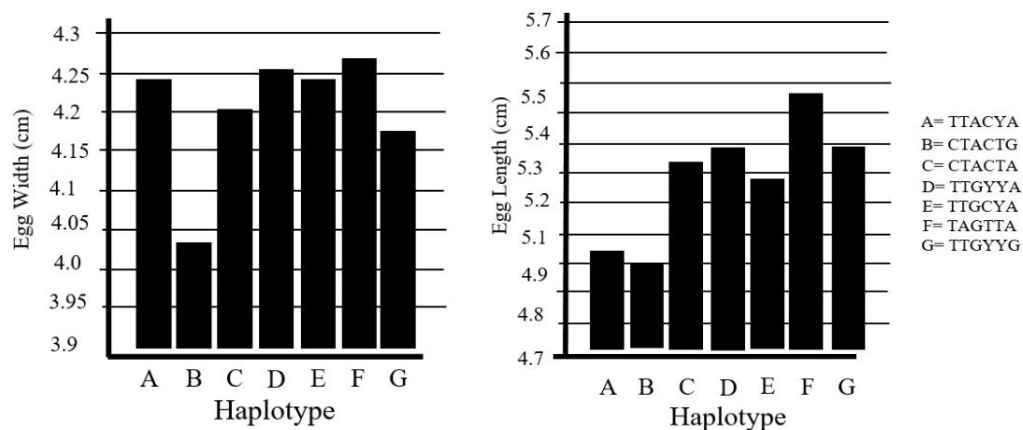
Based on Table 1. BC_1 chicken has six polymorphisms on fragment DNA of 10, 51, 59, 121, 233, and 331. The recapitulation of these SNP further grouped into seven haplotypes. The result of grouping of individuals based on haplotype can be seen in Table 2.

BC_1 chickens were comprised of seven haplotypes. Of which, three haplotypes consisted of two individuals, and four haplotypes consisted of one individual. The eggs productivity of BC_1 chickens of each haplotype was observed for 16 weeks. The eggs production BC_1 chickens each haplotype per week for 16 weeks is presented in Figure 4.
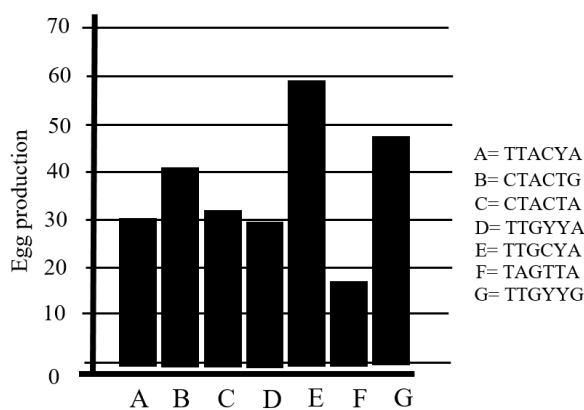
In this study, the egg production of BC_1 chickens began recorders after 11th week of egg production then observed for 16 weeks. There was a trend line showing the cumulative average number of eggs for 16 weeks. In general, BC_1 chickens with TTACYA haplotype has two production peaks at 6th and 15th week. In BC_1 chickens with CTACTG and CTACTA haplotypes have three peaks. First production peak of CTACTA occurring at the 2nd week while CTACTG at 3rd week of observation. The second and third peaks are the same at 9th and 15th week of observation.

BC_1 chickens with TTGYYA and TTGCYA haplotypes have the same 3 peaks at 2, 8, and 15 weeks. BC_1 chickens with haplotype TAGTTA have 2 peaks at 8 and 15 weeks. In haplotype TTGYYG of BC_1 chickens has 2 peaks that are on weeks 2 and 10. The average number of cumulative productions of BC_1 chicken in each haplotype group for 16 weeks presented in Figure 5.

The cumulative average production of BC_1 chicken egg for 16 weeks reached as many as 39.11. local chicken can produce as many as 25 eggs and the layer chicken can produce as many as 75 eggs at the same age which is maintained by the litter system. Figure 6 shows that the TTGCYA haplotype has an average high egg production compared to the other six haplotypes. The TTGYYG haplotype is the second-highest, followed by the CTACTG haplotype. There are 3 groups of haplotypes with the same amount of egg productivity: TTACYA, CTACTA, and TTGYYA. Then haplotype TAGTTA relatively low productivity eggs. Thus, the TTGCYA haplotype can be used as a marker or marker in egg productivity selection.

Figure 5. The average egg width (left) and length (right) of each haplotype group of BC_1 chicken

Figure 6. The average number of egg production each hybrid chicken BC_1 haplotype group

Based on Figure 3 it is known that the TAGTTA haplotype has a higher average egg length than the other six haplotypes. The correlation test using SPSS 16.0 shows a positive correlation (r = 0.725) and shows the level of significance at the level of trust $p < 0.05$. The correlation test between chicken haplotype and average egg width using SPSS showed that the positive correlation was very weak (r = 0.194) and did not show the significance level ($p > 0.05$).

Discussion

Chicken crossbreeding aims to produce hybrid which possesses heterosis effect (Isa et al. 2020). The breeding program for layer chicken was exhaustive job since heritability of egg-related traits is low to medium (Du et al. 2020). In this study, BC_1 chicken line was derived from crossing female Lohmann Brown chicken with F_1 (female Lohmann Brown x male *Pelung*) chicken. Originally, this crossing aim to produce a chicken with more homozygous plumage and eggs productivity related traits but BC_1 remains had plumage color variation.

There was a positive correlation between the weight of eggs against the resulting hatching weight. The weightier the egg will produce weightier DOC. This is because the eggs with a high weight have a high egg yolk and egg white's composition, while the eggs with a small weight have a yellow and egg whites are slightly composition. Egg yolk is a source of energy used for the growth of day-old chicken during the hatching process (Ayeni et al. 2020; Duman and Şekeroğlu 2017).

Haplotype is an interaction between alleles in DNA fragments. Haplotype analysis that known demonstrates the identification of more efficient genetic variations compared with one single nucleotide polymorphism (SNP) analysis. Grouping of haplotypes BC_1 chickens was performed on a battery cage for egg production observation for 16 weeks.

The productivity of BC_1 chicken was still relatively low around 39.11 egg/chicken, this is because not able to produce the same amount of egg production as its parent F_1, but its egg productivity has been above the productivity of Pelung chicken. Low egg productivity levels BC_1 chickens can be caused by different maintenance systems using a cage system or battery enclosure.

The promoter of *FSHR* gene was widely studied in poultry (Liu et al. 2015; Liu et al. 2018; Li et al. 2019; Sun et al. 2020; Du et al. 2020). Several studies found causative DNA mutations at promoter region of *FSHR* gene associated with egg-related traits. These mutations affect gene expression (Xu et al. 2017; Brady et al. 2020). The miRNA expression pattern in ovarian tissue was also reported to be linked to egg production (Wu et al. 2016). Study in Khorasan native fowl, mutation in exon 1 of IL-2 compromises the protein structure (Tohidi and Javanmard 2020). Expression level of *FSHR* is marker for human ovarian cancer (Wei et al. 2018).

In this study, correlation test result shows a weak positive correlation (r = 0.250) but does not show the level of significance ($p > 0.05$). This suggests that the presence of polymorphisms in c*FSHR* gene promoters although single nucleotide polymorphism will affect allele interactions in egg productivity.

The results showed a positive trend between polymorphism of c*FSHR* gene promoter and egg production although it did not show the level of significance at $p < 0.05$. This research needs to be further developed to see the promoter activity at mRNA level. It is known for its polymorphism impact on gene expression with a large chicken population. Further research must evaluate the Food conversion ratio (FCR) of BC_1 chicken since feed contributes up to 80% of total cost.

The eggs productivity of BC_1 chicken was higher than *Pelung* chicken, but lower than its predecessor F_1 and Lohmann Brown. There was polymorphism within six SNPs in the c*FSHR* gene promoter in the 10, 51, 59, 121, 233, and 159 nucleotides, and there was a positive correlation at $p > 0.05$ between polymorphism of c*FSHR* gene promoter fragment to egg number, egg weight, egg width and positive correlation at $p < 0.05$ between polymorphism of c*FSHR* gene promoter to egg length.

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