

Egg Production Characteristic and the Study of Follicle-stimulating Hormone Receptor Gene on Various of Sentul Chicken

Ismoyowati Ismoyowati^{1*}, DM Saleh¹, and I Suswoyo¹

¹Faculty of Animal Science, Universitas Jenderal Soedirman, Purwokerto, Indonesia

*Corresponding author. Email: ismoyowati@unsoed.ac.id

ABSTRACT

The study was conducted to compare differences in the characteristics of egg production and quality, as well as the identification of FSHR gene polymorphisms in various sentul chickens. The research was conducted by experimental methods using a completely randomized design. The research material used 100 female Sentul chickens aged 16 weeks. The treatment tested was a fixed factor of various Sentul chickens consisting of: "Abu" Sentul, "Emas" Sentul, "Geni" Sentul, "Debu" Sentul, "Batu" Sentul. The variables measured include hen day production, egg weight, and egg quality. The data obtained were analyzed of variance. The primary design used Primer3 based on a database from GeneBank (NM_205079.1), *Gallus gallus* follicle stimulating hormone receptor (FSHR). The primary base sequence of the FSHR gene was forward/sequence: 5'-CGTGATTCACACACCCTGAC-3' and reverse/sequence: 5'-CCACCTGGACACTTTCCTGT-3'. The DNA sequences were aligned by using BioEdit version 7.7 for identification of the single nucleotide polymorphism (SNP). The analysis of variance showed that various Sentul chickens had insignificant different egg production and quality characteristics ($P>0.05$). The sequencing results at base length 64 bp showed a mutation from guanine to adenine, and at base 66 bp showed a mutation from cytosine to adenine, so that there were genotypes AA, AC, GA, and GC. The heterozygosity value based on the FSHR gene was 0.594. The results showed that the high heterozygosity of the FSHR *Gallus gallus* had no effect on the diversity of egg production and quality of various Sentul chickens.

Keywords: *Sentul Chicken, FSHR, egg production, egg quality, SNP*

1. INTRODUCTION

Indonesia has much local poultry that has a high potential for livestock development. The local chicken population is mostly in rural areas. Most of the chickens (70 percent) were raised traditionally (extensive) and only 30 percent were reared by following the domestic chicken intensification program (INTAB). In 2019 the temporary population will reach 311,912 million heads. The contribution of local chickens in donating eggs amounts to 4.03% of the total national egg production or 220.2 thousand tons [1]. This indicates that local chickens have a fairly large role in the development of livestock in Indonesia, as well as the economic base of rural farmers. Sentul chicken is a local chicken that grows in Ciamis Regency, is one of the original genetic resources and has been designated as a leading

commodity in West Java. The advantages of Sentul Chicken include relatively fast growth and high egg production compared to other local chickens. This advantage allows Sentul Chicken to be used as a community industrial commodity or to be further developed into superior local chickens. The development of Sentul Chicken is very important to explore its potential, given its dwindling population, and is local Indonesian chicken germplasm.

Therefore, it is necessary to explore the potential of Sentul Chickens, both that are raised by breeders semi-intensively or through experimental research carried out intensively in the laboratory. Efforts to identify and characterize Sentul chickens are still very much needed. Identification can be carried out primarily on phenotypic traits, both qualitatively, quantitatively and

biomolecularly to determine genetic diversity. Genetic diversity is one of the bases for determining the rate of change in the value of successful selection in a population and can also be used in determining the origin of livestock. Genetic diversity can be seen by using allele characters from a particular locus originating from body fluids or tissues such as blood, egg white, and egg yolk. The study of genetic diversity and genetic distance can be carried out by several methods. One of the methods is the analysis of molecular genetic diversity which is quite rapidly developing with DNA analysis techniques, one of which is the single nucleotide polymorphism (SNP). SNP or microsatellite markers are very limited in their target enzymes so that they are specific. Follicle-stimulating hormone (FSH) is a glycoprotein that is synthesized and secreted by gonadotropic cells from the anterior pituitary gland, plays an important role in the gonadal function and fertility [2]. FSH in the circulatory system plays a physiological role by binding to specific transmembrane receptors (FSHR) on target cells. FSHR cDNA sequences were first successfully cloned from chicken ovarian tissue [3]. Sequence analysis and integrated results of the chicken FSHR gene were demonstrated in 2005 [4]. Studies have shown that FSHR is selectively expressed in Sertoli cells and ovarian granulosa cells [5] and the level of its expression is closely related to germ cell differentiation and maturation [6]. Gene promoters play an important role in transcription regulation [7, 8]. This study aims to compare differences in egg production and quality characteristics, as well as identification of FSHR gene polymorphisms in various sentul chickens.

2. MATERIALS AND METHODS

The research material used 100 female Sentul chickens aged 16 weeks. The layer mash feed contains nutrients: 16% crude protein, 12% moisture content, 3% fat, 8% crude fiber, 3.5% Ca, 0.4% P and 2700 kcal ME/kg. The materials used to assess the polymorphism of the FSHR gene consisted of materials for DNA isolation and PCR. PCR uses a Primary base sequence of the FSHR gene was forward: 3'-CGTGATTCACACACCCTGAC -5 'and Reverse FSHR: 5'- CCACCTGGACACTTTCCTGT-3'.

The research was conducted by using experimental methods with a completely randomized design. The treatment tested was a fixed variance factor of Sentul chicken consisting of "Abu" Sentul, "Emas" Sentul, "Geni" Sentul, "Debu" Sentul, "Batu" Sentul. Each experimental unit was filled with 4 chickens and repeated 5 times so that it involved 100 chickens. The variables measured include Hen day production, egg weight, and egg quality. Observation of Sentul Chicken egg production begins when the hens start laying eggs up to 3 months of production. The data obtained were analyzed with analyzed variance.

DNA isolation was done using a DNA kit, then electrophoretic using 1% agarose gel. PCR application was carried out by targeting the FSHR gene in a total reaction volume of 25 µl consisting of 12.5 µl of My Taq HS Ready mix PCR kit, 9.5 µl of dH₂O / pure water, primary FSHR *Gallus gallus* Forward FSHR-F: 3' - CGTGATTCACACACCCTGAC -5 'and Reverse FSHR: 5'- CCACCTGGACACTTTCCTGT-3' ', 1 µl each, and finally 1 µl of genomic DNA. The amplification stages include: the pre-denaturation stage is carried out for 5 minutes at 94°C, then denaturation is carried out for 30 seconds at 94°C, and followed by annealing for 45 seconds at 64°C, elongation at 72°C for 1 minute and post-elongation for 5 minutes at 72°C. The PCR reaction was repeated 35 cycles to get maximum results, the results of the PCR reaction (PCR product) were then electrophoresed on 1% agarose gel and visualized using Ultra Violet light. Sequencing PCR products were aligned by using BioEdit version 7.7 for identification of the single nucleotide polymorphism (SNP). The results of genotype identification, then the gene frequency and genotype were calculated based on Pirschner [9].

$$FAn = \frac{\sum GH A gene}{\sum GH A gene + \sum GH n gene}$$

Note: FAn = frequency of gene A at the nth locus.

Genetic diversity was determined using the heterozygosity formula based on Nei [10]:

$$h = 1 - \sum_{i=1}^m x^2$$

Information:

m = number of alleles,

X_i = frequency of the ith gene

3. RESULTS AND DISCUSSION

3.1. Production and Quality of Eggs of various Sentul chickens

In chickens, phenotypic variation is seen not only in traits with Mendelian inheritance such as feather color, but also in quantitative characteristics such as egg production [11]. Egg production of various Sentul chickens showed a difference (P <0.05). Differences in hen day production are caused by genetic and environmental factors. Genetic factors are factors that determine production capability, while environmental factors are supporting factors, so that livestock can produce according to their abilities [12]. Sentul chicken is a local chicken that has a high genetic diversity, one of which can be seen from the color of its different feathers. "Abu" Sentul chickens have the highest egg production compared to other chickens.

Table 1. Egg production and quality of various sentul chickens

Sentul Chicken	Hen day production (%)	Egg weight (g)	Haugh Unit	Egg shell thickness (mm)
“Abu”	41.19±10.38 ^a	42.84 ±0.98	80.98±3.84	0.39±0.02
“Emas”	31.45 ±5.37 ^{ab}	44.04 ±1.52	83.87±4.03	0.39±0.02
“Geni”	38.99±12.11 ^{ab}	42.84 ±2.18	82.77±2.72	0.43±0.01
“Debu”	28.17±4.42 ^b	46.58 ±2.76	85.57±2.54	0.41±0.01
“Batu”	30.82±4.46 ^{ab}	45.2 ± 3.82	85.96±4.41	0.40±0.01

Note: Superscripts that are different in the same column indicate significantly different (P <0.05).

Egg production is strongly influenced by maternal genetic and environmental factors in which the hen is reared [13]. Estimates of the moderate heritability value of egg production traits determine the inheritance of these traits to the offspring, due to the influence of additive genes [14]. Therefore the selection to increase egg production can be applied to Sentul chickens. Hormonal factors also affect egg production. Follicle-stimulating hormone (FSH) plays a role in regulating follicle development and selection of dominant follicles to arrive at the ovulation stage. Follicle stimulating hormone has been reported to be important for steroidogenesis, follicular development and growth, as well as increasing the rate of granulosa cell division associated with follicular selection in chicken ovaries [15, 16]. FSH stimulates follicles by binding to their receptors (FSHR). The chicken FSHR gene was mapped to *Gallus gallus* autosome 3 and covered a 77.6 kb region consisting of 10 exons. FSHR can be selected as a physiological candidate gene for the performance of laying hens based on its role in the transmission of signals on the endocrine axis [17].

In this study, egg quality, which included egg weight, shell thickness and HU value in all Sentul chicken varieties, was relatively the same (P > 0.05). Egg quality is determined by genetic and environmental factors. Even though Sentul chickens consist of 5 varieties based on feather color, genetically they all include *Gallus gallus*. The environmental factor that most influences egg quality is feed. The same feed, both in the amount and nutrient content of the feed, resulted in relatively the same egg weight, specific gravity and HU value. Egg weight has an important role in hatching success and DOC weight. The greater the egg weight, the greater the DOC, so it will affect the weight of adult chickens. The thickness of the shells of this study was higher than the results of previous studies [18], the thickness of Sentul

chickens shells ranged from 0.33 to 0.34 mm. Eggs with thicker shells show better quality to protect egg contents both from microbial contamination and against impact in the distribution process. The value of Haugh obtained is of AA quality. Haugh unit is the quality or quality of egg whites measured based on egg white height and egg weight. The Haugh unit value has a positive correlation with egg quality, meaning that the higher the Haugh unit value indicates good egg quality. The Haugh unit value of an egg is influenced by the ovomucin content

3.2. FSHR Gene Polymorphisms in Sentul Chickens

The FSHR gene encodes the FSH receptor, which controls the development and recruitment of follicles in the ovaries. The FSHR gene is a gonadotropin glycoprotein that is produced in the anterior pituitary. This hormone binds to receptors on the ovaries to regulate gametogenesis and steroidogenesis processes in the gonads [17]. The results of the alignment process of the sequencing show that at base length 64 bp shows a mutation from guanine to adenine and at base length 66 bp shows a mutation from cytosine to adenine (Figures 1 and 2), this indicates a transition mutation. Transitional mutations occur due to the substitution of one purine base (adenine and guanine) with another purine base or between one pyrimidine base (thymine and cytosine) with another pyrimidine base [19].

The sequencing results showed that the FSHR gene of sentul chickens was polymorphic by obtaining SNPs which produced four types of genotypes, namely AA, AC, GA and GC (table 2). All types of Sentul chickens show polymorphic except for “Debu” Sentul chickens, which only have the AA genotype, and the most diverse is the “Geni” Sentul chicken.

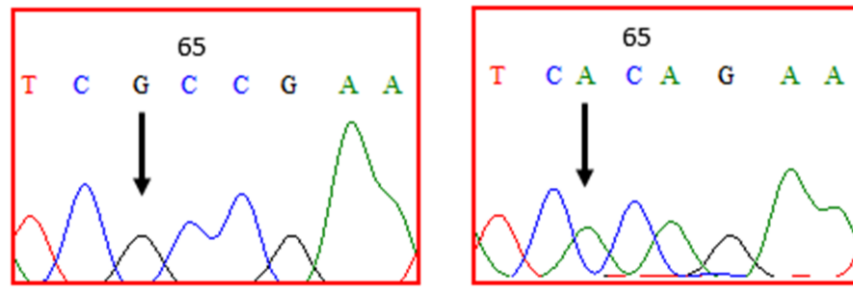


Figure 1. Change in SNP at 64 bp position, namely c.64G> A

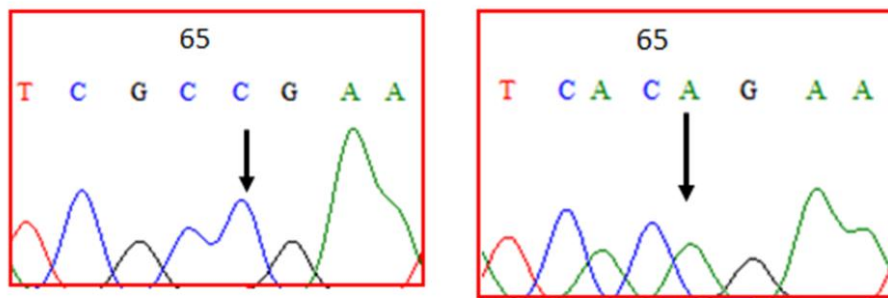


Figure 2. Change in SNP in the 66 bp position, namely c. 66C> A.

Table 2. . Genotypes of sentul chickens based on the results of DNA sequencing of the FSHR *Gallus gallus* gene

No. of chickens	Sentul Chickens				
	“Geni “	“Batu”	“Emas”	“Abu”	“Debu”
1	GC	GA	AA	AA	AA
2	AA	AC	GC	AC	AA
3	GC	AC	GC	GA	AA
4	AC	GA	GC	GA	AA
5	AA		AA	AA	
6	AA		GC	GA	
7	AC		GC	AC	
8	GC		GC	GA	
9	AA		AA		
10	GC		AA		

Table 3. Genotype and gene frequency and heterozygosity value of sentul chickens based on FSHR *Gallus gallus*

Genotype	Total	Genotype frequency	Gene frequency	Estimation heterozygosity
AA	14	0.388	A = 0.554 C = 0.223 G = 0.223	0.594
AC	6	0.167		
GA	6	0.167		
GC	10	0.278		

The heterozygosity value of various sentul chickens was 0.594. Heterozygosity (h) is the most accurate way to measure genetic variation; heterozygosity is also known as genetic diversity, while genetic diversity in a population is measured by means of heterozygosity (H) if more than one locus is observed [20]. This diversity is relatively high if seen from the PIC (Polymorphic Informative Content) value, namely the diversity is low if the value is less than 0.25, is moderate if the value ranges from 0.25 to 0.5, and is high if the value is higher from 0.5. Besides being able to be used as a basis for determining the level of genetic information, the PIC value can also be used for the purpose of determining the existence of polymorphic alleles, which is the same function as the heterozygosity value. The heterozygosity value is always higher than the PIC value, because the PIC value is the corrected heterozygosity value [21].

The relatively high genetic diversity in the various sentul chickens studied was probably due to the adaptability obtained from natural selection and the ability to survive in sufficient conditions, besides that in the population, there was the possibility of many random mating, which led to high heterozygosity values. Several factors that can cause the high value of heterozygosity include migration, mutations and crosses [22]. The high heterozygosity indicates a relatively high diversity in various types of Sentul chickens. Genotypic differences in Sentul chickens had a significant effect on hen day production, but had no significant effect on egg quality. These results suggest that genetic factors influence egg production more than environmental factors. On the characteristics of egg quality, environmental factors, feed have a higher effect.

4. CONCLUSIONS

“Debu” Sentul chickens have lower egg production characteristics than other Sentul chickens, while egg quality is relatively the same. The estimated heterozygosity of the FSHR gene in Sentul chickens was relatively high, but there was no association between FSHR gene genotype and egg production traits.

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