Exploring the Relationship Between Growth Hormone Secretagogue Receptor (GHSR) Gene and Body Proportions in Bangkok Chickens: Insights from DNA Sequencing and Hin6I Enzyme-Restricted PCR-RFLP Analysis

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Abstract

The purpose of this study was to determine the diversity of the growth hormone secretagogue receptor (GHSR) gene and also to analyze the association of the GHSR gene with the body proportions of Bangkok chickens. A total of 125 Bangkok chickens were reared, and blood samples were taken. Bangkok chickens were kept in colony cages with ad libitum feeding and drinking. The GHSR gene polymorphism was determined using the PCR-RFLP method. The restriction enzyme used in this study was Hin6I. The data analyzed were indicators of body proportions, which included data on body weight, carcass weight, and commercial cut weight. Single nucleotide polymorphism (SNP) identification using the Molecular Evolutionary Genetics Analysis 7 program with reference number AB095994.1. Allele frequency values, genotype frequencies, and Hardy-Weinberg balance values were also analyzed. The association between the GHSR gene and the traits observed in Bangkok chickens was analyzed using the T-test. The results of the study showed that the GHSR gene in Bangkok chickens had two genotypes, namely TT and CT. The values for Ho and He were 0.224 and 0.198, respectively. The genotype frequencies of TT and CT were 0.776 and 0.224, respectively. The two genotypes were associated with body weight, carcass weight, and commercial weight ($p < 0.05$). For all measured criteria, the TT genotype showed a higher weight value than the CT genotype. The GHSR gene has the potential to be used as a genetic marker for the selection process on body weight, carcass weight, and commercial weight traits.

Keywords: GHSR gene, Bangkok chicken, PCR, RFLP

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INTRODUCTION

Indonesia has a large number of local chicken livestock with significant development potential. At least 34 local chicken species with various morphological traits can be found in Indonesia (Ningsih *et al*., 2022). Indonesia boasts a diverse array of local chicken breeds, encompassing both original varieties and those modified over decades or even centuries. Among them is the Bangkok Chicken, a descendant of Thailand's regional chicken, historically known as the ancestor of the King's chicken. Having been cultivated for an extensive period and widely adopted by Indonesians since 1960, the Bangkok Chicken is officially recognized as a local Indonesian breed. Its popularity stems from numerous advantages, such as a robust body structure and exceptional endurance, making it a preferred choice for various applications. (Badaruddin *et al*., 2017). In addition to that, Bangkok chickens possess high economic value. It is not surprising that breeders maintain them for business purposes, rather than merely as a hobby and source of pride. Another notable attribute of Bangkok chickens is the exceptionally good quality of their sperm (Hijriyanto, 2017). This quality promote Bangkok chickens suitable as male candidates in the process of establishing new superior local chicken strains.

Despite these advantages, Bangkok chickens also have disadvantages, such as a relatively slow growth rate. Almost all types of local Indonesian chickens tend to share this drawback, exhibiting

low productivity that falls below their genetic potential (Nataamijaya, 2010). Muryanto *et al*. (2002) stated that the development of local chickens has constraints on the slow rate of growth. Therefore, it is necessary to improve the genetic quality of Bangkok chickens to increase the efficiency of this type of chicken cultivation. One method that can be used is selection based on specific genes that affect growth traits.

The growth hormone secretagogue receptor (GHSR), or growth hormone secretagogue receptor, is involved in many physiological functions, including pituitary growth hormone secretion, food intake, and energy expenditure (Fang *et al*., 2010). The GHSR gene was identified as being associated with growth performance in kampung chickens (Syaikhullah *et al*., 2017). The GHSR gene is a gene that plays an important role as the main receptor in the process of GH secretion, or what is often called growth hormone (Kojima and Kangawa, 2005). The GHSR gene consists of 2 exons and 1 intron (Niarami *et al*., 2014). In another study, the GHSR gene was shown to affect the fatty properties of meat in Xinghua chickens. GHSR is also related to the level of obesity in humans and mice. GHSR interacts with GHRL in the process of bioactivation through the neuroendocrine pathway (Lei *et al*., 2007). According to Nie *et al*. (2009), the GHSR gene is also involved in GH secretion, food intake, and energy homeostasis. Fang *et al*. (2010) found a diversity of GHSR gene loci in intron 1 in White Recessive Rock and Xinghua chickens, which was associated with weights at 28 and 90 days of age, chest and thigh muscle weights. This diversity is caused by a mutation of the base T to C.

This study was conducted to investigate the diversity of GHSR gene genotypes in Bangkok chickens and the association of these genes with body weight, carcass weight, and commercial weight in Bangkok chickens.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Research Ethics Commission of Experimental Animals and Health, Politeknik Negeri Jember, Number: 298/PL17.4/PG/2024.

Study Period and Location

This study was conducted in July–December 2022. The locations of this study were the Teaching Laboratory, Department of Animal Science, Politeknik Negeri Jember, as a place of object cultivation, and the Genetic Molecular Laboratory, Faculty of Animal Science, IPB University, for the genetic observations.

Livestock Samples

The sample used in this study was DOC Bangkok chickens, totaling 125 roosters, kept in colony cages. From the chicken population, 125 blood samples were collected from Bangkok chickens raised from DOC until 12 weeks. Food and drink were given *adlibitum*.

Genotype Analysis

The total genome was obtained by the phenol-chloroform method (Sambrook and Russell, 2001). The use of primers to amplify the GHSR gene is referred to (Fang *et al*., 2010) as modified. Forward primer (5'-CCC ACA AAG TTA GCT GCA GAC-3') and reverse primer (5'-CAC CTC TCC ATC TGG CTC ATT-3'). The primer was able to amplify the 470 bp GHSR gene in intron 1 (Figure 1).

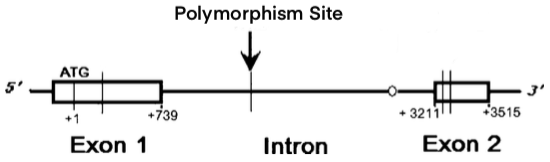

Figure 1. Genomic organization and polymorphism sites of the chicken GHSR gene.

DNA amplification was carried out in a total volume of 15 µL consisting of 1 µL DNA, 0.3 µL primer, 7.5 µL master mix, and 6.2 µL distilled water. PCR was carried out at the initial denaturation at 94ºC for 4 minutes, which was carried out for 1 cycle. denaturation at 94ºC for 10 seconds, annealing at 60ºC for 20 seconds, extension at 7ºC for 30 seconds (35 cycles), and final extension at 72ºC for 7 minutes.

Determination of the genotype on the GHSR gene using the RFLP method. 5 µL of the product from the GHSR gene PCR process was cut using a 2 µL restriction endonuclease mix consisting of 1 µL dH2O, 0.7 µL buffer, and 0.3 µL cutting enzyme, then incubated for 16 hours at 37ºC. The cutting enzyme or restriction enzyme used for the GHSR gene is Hin6I, which can recognize the G|CGC cut site. Electrophoresis was carried out on a 2% agarose gel with 0.5 TBE buffer (Tris Borat EDTA), which was supplied with an electric current of 100 volts for 40 minutes. The visualization of electrophoresis results was carried out using a UV transilluminator.

Data Analysis

The data analyzed was in the form of body proportion indicators, which included data on body weight, carcass weight, and commercial cuts (breast, thigh, drumstick, wings, and lumbar). Sequencing data from the GHSR gene were analyzed using the Bioedit program (Hall, 1999). Single Nucleotide Polymorphism (SNP) identification was carried out using the Molecular Evolutionary Genetics Analysis 7 (MEGA 7) program with reference number AB095994.1 (Tamura *et al*., 2011). Allele frequency values, genotype frequencies, Hardy-Weinberg equilibrium values, observational heterozygosity, and expected heterozygosity were calculated based on Allendorf *et al*. (2012) method. The association between the GHSR gene and the observed traits in Bangkok chickens was analyzed using the T-test.

RESULTS AND DISCUSSION

GHSR Gene Amplification

The amplification of the GHSR gene in Bangkok chicken was successfully carried out with an initial denaturation process at 94ºC for 4 minutes for 1 cycle. denaturation at 94ºC for 10 seconds, annealing at 60ºC for 20 seconds, extension at 72ºC for 30 seconds (35 cycles), and final extension at 72ºC for 7 minutes. The GHSR gene amplification process produces a product of 470 bp (Figure 2). The results of this amplification were similar to the amplification process of the GHSR gene in Kampung chickens in the study of Syaikhullah *et al*. (2017). The results of the amplification were then cut using the restriction enzyme Hin6I and produced 2 types of genotypes, namely CT and TT. RFLP results for the GHSR gene showed that the CT genotype had 3 bands of 470 bp, 354 bp, and 116 bp, while the TT genotype had 1 band of 470 bp (Figure 3). These results are quite different when compared to the results of Fang *et al*. (2010) where the GHSR gene was found in Chinese local chickens (Xinghua) with as many as 3 genotype variants, namely CT, TT, and CC.

The sequencing results of the GHSR gene in Bangkok chickens found a deletion at the primary position of 134 bp, which caused the loss of base C (Figure 4). The alignment of the GHSR gene in Bangkok chickens indicates a mutation, where the T base changes to C. This can be referred to as a transversion mutation, specifically, a change from a purine base to a pyrimidine. SNP transversion has a high chance of causing amino acid changes resulting in non-synonymous mutations (Allendorf *et al*., 2012).

According to Fang *et al*. (2010), mutations in the GHSR gene were observed to occur in intron 1. The intron also contains DNA sequences that bind enhancers that might affect the transcription process. Introns also contain regulatory RNA sequences that can affect the translation process and mRNA stability (Syaikhullah *et al*., 2017). When a mutation occurs, RNA can change the product of a gene. Mutations in this area can affect the function of gene activity. In the process of translation, introns are also involved in the process of regulating protein production activity. Mutations in this region have the potential to affect the amount of protein production, function, or gene expression (Perdew *et al*., 2006).

Diversity, Heterozygosity, and HW Equilibrium of the GHSR Gene

Genotype frequency indicates the ratio of the number of a genotype to a population by calculating the ratio between the number of certain genotypes in each population, while allele frequency is the ratio of an allele to the total

number of alleles in an SNP in a population (Noor, 2010).

Table 1 showed that the frequency of the TT genotype in the GHSR gene is greater than that of the CT genotype, which was 0.776, while the frequency of the CT genotype was 0.224. In the GHSR gene, the frequency of the T allele was 0.888, and the frequency of the C allele was 0.112. The single nucleotide polymorphism (SNP) of the GHSR gene was indicated to be polymorphic. If an SNP has an allele frequency value of ≤ 0.99 in a large population and ≤ 0.95 in a smaller population, it can be stated that the SNP is polymorphic (Allendorf *et al*., 2012).

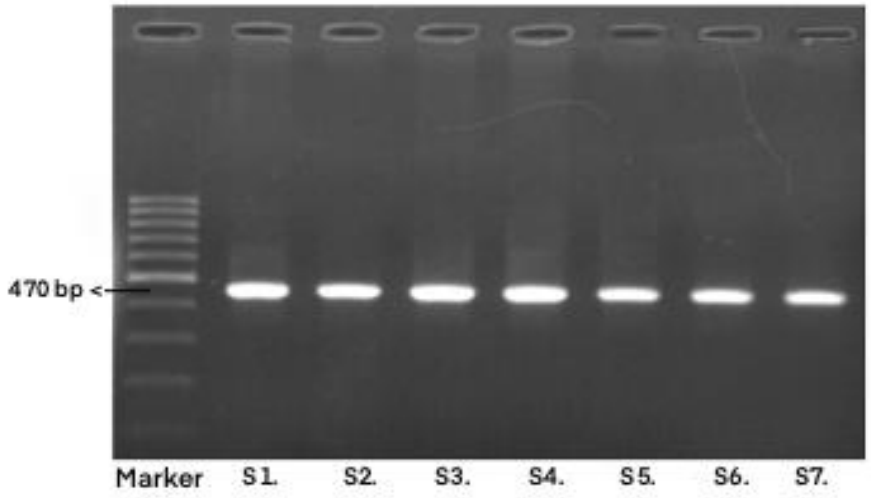

Figure 2. Results of GHSR gene amplification in Intron 1 (S1–7 = Bangkok chicken blood sample).

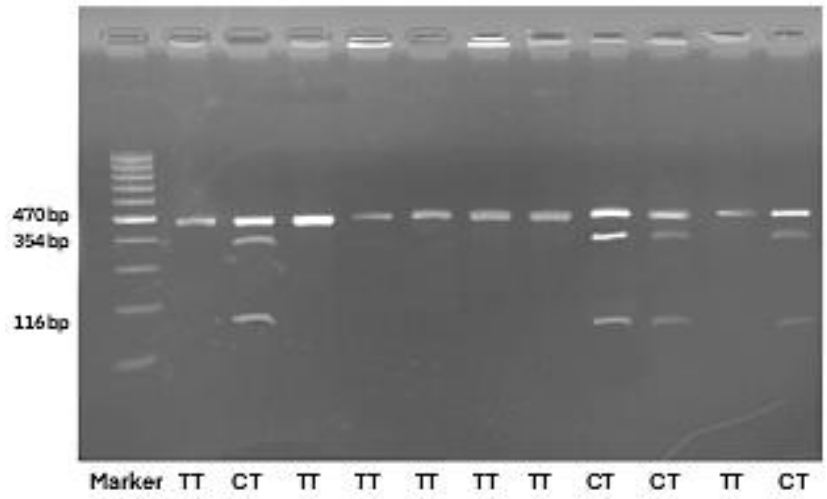

Figure 3. RFLP results of the GHSR gene restricted by Hin6I enzyme; M: 100 bp; CT: Genotype CT (470 bp, 354 bp, 116 bp); TT: Genotype TT (470 bp).

Figure 4. Spot C base deletion at position 134 bp in Bangkok chicken based on reference with GenBank access number AB095994.1.

Table 1. Genotype frequency, allele frequency, heterozygosity, and Hardy-Weinberg balance at the GHSR locus

Gen	Genotype Frequency		Allele Frequency		Ho	He	$\chi 2$
	TT (n = 97)	CT (n = 28)	T	C			
GHSR	0.776	0.224	0.888	0.112	0.224	0.198	1.99*

* not significant ($p > 0.05$, $\chi 2$ (0.05;1) < 3.84.

Table 2. Association of GHSR Intron 1 gene with body weight and carcass weight

Parameters	Genotype	
	TT	CT
Body weight (g)	774.61 ± 86.15[a]	613.36 ± 32.39[b]
Carcass weight (g)	463.25 ± 78.79[a]	353.94 ± 31.09[b]
Carcass percentage (%)	59.81 ± 2.8	57.71 ± 3.2

[a,b] different superscripts in the same row indicate significant difference ($p < 0.05$).

Table 3. Association of GHSR Intron 1 gene with commercial weight

Parameters commercial weight	Genotype	
	TT	CT
breast (g)	117.92 ± 21.81[a]	84.53 ± 7.73[b]
thigh (g)	84.47 ± 13.52[a]	67.15 ± 4.94[b]
drumstick (g)	82.17 ± 12.32[a]	55.73 ± 4.51[b]
wing (g)	77.15 ± 11.14[a]	55.64 ± 5.96[b]
lumbar (g)	119.46 ± 23.93[a]	86.15 ± 16.84[b]

[a,b] different superscripts in the same row indicate significant difference ($p < 0.05$).

Allendorf *et al*. (2012) explained that the diversity of a gene can be used as an indicator in determining a breeding program. The gene diversity number can be used as a tool to determine selection measures if the population is heterogeneous and attempts at crossing if the population is homogeneous (Fikri and Purnama, 2020). The value of heterozygosity is an indicator of the genetic diversity of a population that can be used for selection programs. An SNP is indicated to have high diversity if the heterozygosity value is > 0.50 (Allendorf *et al*., 2012; Wardhana *et al*., 2019). The Ho value from Table 1 showed less than 0.50, which indicates that the genotypic diversity of the Bangkok chicken population in this study was considered low. Based on Table 1, the observed heterozygosity value (Ho) was 0.224, while the expected heterozygosity value (He) was 0.198. This study indicates that in the Bangkok chicken population under examination, there were no inbreeding cases. A lower Ho value than He indicates inbreeding (Nassiry *et al*., 2009).

Association of the GHSR Gene with Body Proportion in Bangkok Chickens

The association of the GHSR gene with body weight and carcass weight was presented in Table 2. Bangkok chickens showed that the TT genotype had higher body weights and carcass weights compared to the CT genotype Bangkok chickens. However, the carcass percentage of the two genotypes did not show a significant difference at the $p < 0.05$ level. This shows that the GHSR gene is associated with body weight and carcass weight in Bangkok chickens.

These results follow the study of Syaikhullah *et al*. (2017), who found that the TT genotype was more dominant than the CT genotype in the body weight and carcass weight parameters of Kampung chickens. The GHSR gene at location rs16675844 had a significant effect on the characteristics of feed consumption and feed conversion, while the GHSR gene at location rs14678932 showed a significant association with the characteristics of breast weight and feed consumption (Jin *et al*., 2014; Kartikasari *et al*., 2019). The GHSR gene in chickens is the ghrelin

receptor (GHRL), while the GHSR gene is a peptide hormone produced by the chicken proventriculus, which stimulates the release of growth hormone (GH) and food intake (El-Magd *et al*., 2016; Wardhana *et al*., 2021). The GHSR gene can affect the growth and weight of chicken carcasses because these genes have an important role in biological response processes such as cell development and proliferation (Tschöp *et al*., 2000).

Commercial weight is also one of the indicators used to observe the body proportions of chickens. The association of the GHSR gene with commercial cut weight can be seen in Table 3.

The results of statistical analysis showed that there were significant differences between the two genotypes ($p < 0.05$) in breast weight, upper thigh weight, lower thigh weight, wing weight, and lumbar weight in Bangkok chickens. The TT genotype has a greater weight than the CT genotype in all commercial weight parameters. This shows that the GHSR gene is associated with commercial weight in Bangkok chickens. GHSR has also been extensively studied in several domestic animals, and SNPs are related to productivity performance. GHSR gene mutations were significantly ($p < 0.05$) associated with several fatty properties such as crude protein content, thigh muscle weight, and thickness of subcutaneous fat in chickens and ducks (Nie *et al*., 2009) Diversity in the GHSR gene was also found in a study conducted by Kaczor *et al*. (2016). The GHSR gene affects weight and daily weight gain in broiler chickens. A study conducted by El-Magd *et al*. (2016) found another mutation in the GHSR gene in exon 2 (G244A), which was a non-synonymous mutation, where there was a change in the amino acid product from lysine to arginine.

CONCLUSION

The results of this study suggest that the Growth Hormone Secretagogue Receptor (GHSR) gene restricted by the Hin6I enzyme in Bangkok chickens has 2 genotypes, namely TT and CT. The two genotypes were associated with body weight, carcass weight, and commercial weight ($p < 0.05$). The TT genotype had a higher weight value than the CT genotype for all observed parameters. The GHSR gene has the potential to be used as a genetic marker for the selection process based on body weight, carcass weight, and commercial weight traits.

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AUTHORS' CONTRIBUTIONS

GS: Conceptualization and drafted the manuscript. GS, RTH, and MA: Performed sample evaluation. GS and MA: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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