ISSN 0126-0472 EISSN 2087-4634
Accredited by Directorate General of Research and Development
Strengthening No: 36b/E/KPT/2016

Media Peternakan, August 2017, 40(2):78-87
DOI: https://doi.org/10.5398/medpet.2017.40.2.78
Available online at http://medpet.journal.ipb.ac.id/

The Associations of GH and GHR Genes with Carcass Components in Indonesian *Kampung* and Broiler Chicken Cross

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(Received 16-05-2017; Reviewed 19-07-2017; Accepted 31-07-2017)

ABSTRACT

The chicken growth hormone (GH) and its receptor (growth hormone receptor, GHR) play important roles in chicken performances due to their crucial functions in growth. The variations of GH and GHR genes were then thought to be associated with the variations of the performances. This experiment was designed to identify the g.2248G>A GH and the g.565G>A GHR loci polymorphisms and to evaluate their associations with carcass components in *Kampung* and broiler chicken cross. A total of 215 chickens including 4 chicken populations (*Kampung*, Cobb broiler, F1, and F2 *Kampung* x broiler chicken cross) were screened to identify polymorphism using PCR-RFLP technique with *Eco*RV and *Eco*72I restriction enzyme for GH and GHR loci, respectively. The carcass components were recorded at 26 weeks of age on F2 *Kampung* and broiler chicken cross (42 chickens) for association study. Both the g.2248G>A GH and the g.565G>A GHR loci were polymorphic with two alleles (G and A) and three genotypes (GG, AG, and AA). The GG genotype and the G allele of GH locus were predominant in all chicken populations. While in GHR locus, the AA genotype and the A allele were found to be higher in all chicken populations. The association study showed that the g.565G>A GHR locus polymorphism had significant effect on carcass components, including live weight, carcass weight, breast weight, thighs weight, breast muscle weight, and thighs muscle weight. There was no significant association was found between the g.2248G>A GH genotype and carcass components. It could be concluded that the g.2248G>A GH and the g.565G>A GHR loci were polymorphic in *Kampung* and broiler chicken cross and the g.565G>A GHR locus was accosiated with carcass components. This g.565G>A GHR SNP might be an important candidate marker for chicken growth and muscle mass improvement.

Keywords: Kampung chicken cross, growth hormone, growth hormone receptor, gene polymorphisms, carcass

ABSTRAK

Hormon pertumbuhan dan reseptor hormon pertumbuhan memiliki peran penting dalam performa pertumbuhan ayam. Tujuan penelitian ini adalah mengidentifikasi keragaman gen hormon pertumbuhan (lokus g.2248G>A GH) dan gen reseptor hormon pertumbuhan (lokus g.565G>A GHR) dan mengevaluasi pengaruhnya pada komponen karkas pada ayam silangan *Kampung* dan broiler. Penentuan genotipe dilakukan pada 215 ekor ayam, yang terdiri atas 4 populasi (*Kampung*, ras pedaging *strain Cobb*, silangan F1 dan F2 Kampung x ras pedaging *strain Cobb*) menggunakan enzim pemotong *Eco*RV dan *Eco*72I masing-masing untuk fragmen lokus g.2248G>A GH dan lokus g.565G>A GHR. Analisis asosiasi dengan komponen karkas dilakukan pada populasi ayam silangan F2 *Kampung* x ras pedaging strain *Cobb* umur 26 minggu. Kedua lokus tersebut bersifat polimorfik dengan dua alel yang ditemukan (alel A dan G) dan tiga genotipe (genotipe GG, AG, dan AA). Genotipe GG dan alel G pada lokus g.2248G>A GH ditemukan dalam frekuensi tertinggi di semua populasi yang diuji. Sementara pada lokus g.565G>A GHR, genotipe AA dan alel A ditemukan paling dominan. Analisis asosiasi menunjukkan adanya pengaruh signifikan pada lokus g.565G>A GHR dengan komponen karkas (bobot hidup, bobot karkas, bobot potongan karkas bagian

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dada, bobot potongan karkas bagian paha atas, bobot otot dada, dan bobot otot paha atas). Tidak ditemukan pengaruh signifikan pada lokus g.2248G>A GH dengan komponen karkas. Simpulan dari studi ini adalah lokus g.2248G>A GH dan g.565G>A GHR bersifat polimorfik pada ayam *Kampung* **dan silangannya, serta terdapat asosiasi signifikan antara lokus g.565G>A GHR dengan komponen karkas ayam silangan F2** *Kampung* **x ras pedaging strain** *Cobb.* **Hal ini menunjukkan bahwa lokus g.565G>A merupakan kandidat penciri komponen karkas ayam untuk tujuan peningkatan pertumbuhan dan massa otot.**

Kata kunci: ayam silangan Kampung, growth hormone, growth hormone receptor, keragaman genetik, karkas

INTRODUCTION

Native chickens do not only contribute to the conservation of poultry genetic resources, but also play an important role in rural economies in most of the developing and undeveloped countries (Moharrery & Mirzaei, 2014; Padhi, 2016). *Kampung* chicken is Indonesian native chicken, which can be found easily in rural area with strong economic and social relations to community (Nataamijaya, 2010). Zein & Sulandari (2012) reported that *Kampung* chicken's population is getting expanded with high genetic diversity. However, growth is the main challenge for Indonesian native chicken production. By intensive rearing system, Indonesian *Kampung* chicken reaches slaughter weight in 4.5 months or more (FAO, 2008).

Improvement of the performance of *Kampung* chicken could be done by selection and crossbreeding or by utilization of both selection and crossbreeding (Sheng *et al*., 2013; Padhi, 2016). In order to improve the growth and carcass performance of *Kampung* chicken, an F2 intercross between *Kampung* and commercial broiler chicken was designed and then followed by selection based on genes controlling growth. Abdurrahman *et al*. (2016) reported that the local chicken crossbreds had lower fat and cholesterol contents due to a higher muscular contraction compared to modern breeds. Meanwhile, at the gene level, molecular genetics provides rapid and accurate identification and selection tools for individuals to improve their performances permanently (Fulton, 2008; Padhi, 2016). Furthermore, in poultry breeding program, selection in molecular approach could be done before the traits were expressed or shortly after hatching (Fulton, 2008).

Chicken growth is mainly regulated by the growth hormone (GH). The chicken growth hormone is widely known as a major hormone that acts directly on chicken development and metabolism (Kim, 2010) and relates to egg production (Su *et al*., 2014; Vu & Ngu, 2016). GH regulates a variety of physiological functions such as growth, body composition, egg production, aging, reproduction, sexual maturation, and functionality of the central nervous system (Gosney *et al*., 2012; Hrabia *et al*., 2008; Hull & Harvey, 2014; Stephen *et al*., 2001). The GH/IGF-1 signaling pathway is also believed to be capable of promoting growth of selected types of cancer (Kopchick *et al*., 2014). Activity of GH is greatly influenced by its receptors by forming the GH-GHR complex (Kuhn *et al*., 2002). Dimerization of GHR, which is caused by GH binding, activates a variety of signaling molecules that contributes to the GH-induced changes in enzymatic activity, transport function, and gene expression (Ciftci *et al*., 2013). Study of Xu *et al.* (2013) proposed that lacking of the GHR gene might be involved in dwarfism formation in chicken.

Studies in broiler chicken shows that the GH gene is located in chromosome 27, has 4.1 kb overall length and consists of five exons and four introns (Stephen *et al*., 2001; Nie *et al*., 2005). The GHR gene, which is located in chromosom Z, has 4.0 kb length and consists of ten exons and nine introns (Nie *et al*., 2005). Nie *et al.* (2005) reported that 46 SNPs in GH gene and the g.2248G>A GH (G+1705A) polymorphism in intron 3 had strong associations with growth traits in F2 White Recessive Rock and Xinghua chicken. A number of 33 SNPs in GHR gene was reported by Nie *et al*. (2005), and the g.565G>A GHR polymorphism was reported had significant associations with fatness and muscle fiber traits in Chinese local chickens (Lei *et al*., 2007). Studies in other poultries reported the associations of the GH gene with growth and carcass traits in Pitalah and Kumbang Janti Indonesian ducks (Yurnalis *et al*., 2017), Huoyan Chinese native goose (Zhang *et al*., 2014), and Japanese quail (Johari *et al*., 2013). In other farm animals, the GH gene was reported in cattle breeds (Dolmatova & Ilyasov, 2011), pig (Ashok *et al*., 2014), salmonids fish (Kamenskaya *et al*., 2015), sheep (Jia *et al*., 2014), goat (An *et al*., 2011), camel (Shawki *et al*., 2015), and passerine bird (Arai & Iigo, 2010). The effect of GH and GHR gene polymorphisms on carcass component in *Kampung* and broiler chicken cross has not been examined yet. The objectives of this study were to identify the g.2248G>A GH and the g.565G>A GHR loci polymorphisms using PCR-RFLP and to evaluate their effect on carcass components in *Kampung* and broiler chicken cross. This polymorphism information might serve as a platform for development of molecular marker in carcass and meat selections in chicken.

MATERIALS AND METHODS

Animal and Phenotypic Data Collection

Animal care procedures were approved by the Animal Care and Use Committee of Bogor Agricultural University (No. 22-2016 IPB). A number of 215 individuals consisting of 4 chicken populations: *Kampung* (49), commercial *Cobb* broiler (30), F1 *Kampung* x *Cobb*

broiler cross (43), and F2 *Kampung* x *Cobb* broiler cross (93) were obtained from Animal Breeding and Genetics Division, Faculty of Animal Science, Bogor Agricultural University (IPB), Indonesia, were used in this experiment. The F2 cross was set by crossing individuals from the *Kampung* and commercial *Cobb* broiler lines as parental (P). *Kampung* chicken was selected as roosters, collected from traditional farmers in Ciawi, District of Bogor, West Java. Commercial *Cobb* broiler parent stock was used as hen. The F1 was generated by crossing *Kampung* and broiler lines. The F1s were then bred to generate F2. Blood sample from each individual (P, F1, and F2) was obtained from the wing vein and collected in tube containing EDTA. The experimental chickens were raised in the same environmental condition and had free access to feed and water.

F2 *Kampung* x *Cobb* broiler cross (42 chickens: 20 males and 22 females) were slaughtered at 26 weeks of age to collect carcass component data. Carcass component measurement included live weight, carcass weight, commercial cuts (breast, thighs, drum sticks, and wings) weights, muscle (breast muscle, thigh muscle, and drum sticks muscle) weights, and percentage of carcass, commercial cuts and muscles. The phenotypic of carcass measurement is provided in the Table 1.

Genomic DNA Extraction

Genomic DNA was isolated from blood. The DNA extraction protocol was performed according to Sambrook & Russel (2001) with minor modification. Briefly, each 20 µL of blood sample was added with 800 µL RBC lysis buffer, homogenized, and centrifuged (800 rpm) for 5 min. Then, the supernatant part was removed. The precipitation part was added with 40 µL 10% SDS, 10 µL Proteinase K 5 mg/mL, and 300 µL 1 x STE, and slowly shaken at 55°C for 2 h. Then, each sample was added with 400 µL phenol solution, 400 µL CIAA, and 40 µL NaCl 5M, and slowly shaken at room temperature for an hour, and centrifuged (12000 rpm) for 5 min. About 400 µL liquid from top layer was removed into a new tube and added with 800 µL 96% EtOH and 40 µL NaCl 5M. Then, the sample was frozen overnight. DNA molecule was centrifuged (12000 rpm) for 5 min, and the supernatant part was discarded. For the DNA precipitation, the pellet was air dried and 100 µL 80% TE was added. DNA sample was frozen stored for long term usage.

Table 1. The phenotype of carcass measurement

Trait	Mean (g)	Standard deviation (g)	Min (g)	Max (g)
LW	2,195.811	488.007	1124	2987
CW	1,457.976	358.350	715	2253
BW	379.381	109.381	180	640
TW	267.929	71.956	132	393
DW	256.143	66.520	133	370
WW	203.381	46.740	117	325
BMW	271.000	88.645	120	511
TMW	192.690	54.634	96	331
DMW	165.024	46.206	90	273

Note: LW= Live Weight; CW= Carcass Weight; BW= Breast Weight; TW= Thighs Weight; DW= Drum Sticks Weight; WW= Wings Weight; BMW= Breast Muscle Weight; TMW= Thighs Muscle Weight; DMW= Drum Sticks Muscle Weight.

Amplification and Genotyping

Specific fragments of GH and GHR genes were produced with the polymerase chain reaction (PCR) method using thermocycler machine (GeneAmp® PCR System 9700, Applied Bio SystemsTM, Foster City, USA). Primers (Table 2) were designed using Primer Designing Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The PCR process was run with 35 cycles consisting of denaturation at 95°C for 10 s, annealing at 60-61°C for 20 s, and extension at 72°C for 30 s. Amplification was performed with a total volume of 25 µL containing 50 ng/µL DNA sample, 0.5 pmol primer, 0.5 unit GoTaq Green Master Mix (Promega, Madison, USA), and water.

Genotyping was done using a restriction fragment length polymorphism (RFLP). PCR product and restriction enzyme (Thermo Fisher Scientific, EU, Lithuania) were incubated at 37°C for 12 h. Genotype was visualized through 2% agarose gel electrophoresis (v/w), which was stained with FluoroSafe DNA Staining (1st Base, Singapore) above UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA). Primers annealing position and restriction site of GH and GHR were shown in Figures 1 and 2, respectively. To confirm the mutations occurred, direct sequencing was performed on an ABI-PRISM3730 sequencer (1st Base, Singapore) in three samples per genotype per locus.

Table 2. The primer sequences

Gene	SNP target position	Primer sequence (5′–3′)*	PCR product	Annealing	Restriction enzyme	Product pattern (Genotype)	Reference
GH	Intron 3 (g.2248G>A GH)	F: ATGTCTCCACAGGAACGCAC R: GCTCTGTAAGCTGAGCACCAC	339 bp	60 °C	*Eco*RV (GAT|ATC)	GG: 339 bp AA: 148 and 191 bp AG: 148, 191, and 339 bp	Nie *et al.* (2005)
GHR	Intron 5 (g.565G>A GHR)	F: TCTGCAGAGTCGGGATATTTAGCA R: ACTCTCCATCAGAATTTATCCCG	326 bp	61 °C	*Eco*72I (CAC|GTG)	AA: 326 bp GG: 114 and 212 bp AG: 114, 212, and 326 bp	Lei *et al.* (2007)

Note: *F= Forward, R= reverse

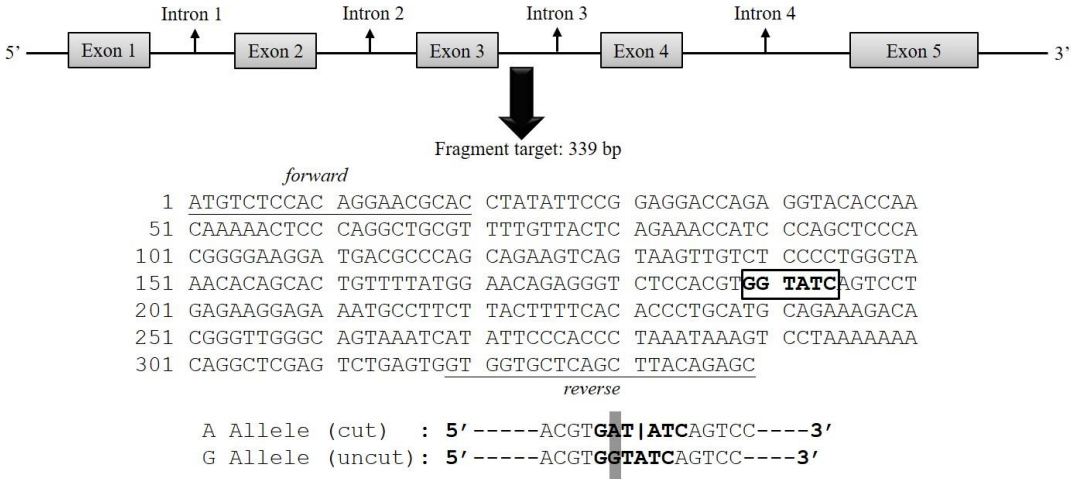

Figure 1. Fragment target of the g.2248G>A GH locus located in intron 3, and resulted the A and G allele. Underline shows forward and reverse primer annealing position; bold shows *Eco*RV restriction site (GenBank accession number: AY461843.1).

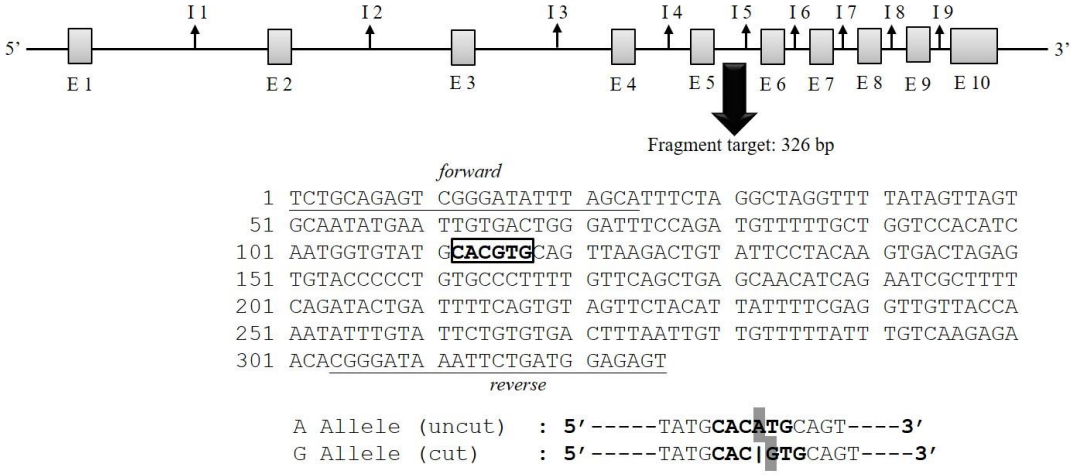

Figure 2. Fragment target of the g.565G>A GHR locus located in intron 5, and resulted the A and G allele. Underline shows forward and reverse primer annealing position; bold shows *Eco*72I restriction site; E= exon; I= intron (GenBank accession number: AJ506750.1).

Data Analysis

Polymorphism information and sequencing. Polymorphism parameters (genotype frequency, allele frequency, and the Hardy-Weinberg Equilibrium) were analyzed according to Nei & Kumar (2000). All sequence results (ABI trace files) were analyzed in Molecular Evolutionary Genetic Analysis (MEGA) 6.0 according to Tamura *et al*. (2013) and BioEdit (Hall, 2011). The Basic Local Alignment Search Tool (BLAST) was used to identify similarity (homology) with genes data in GenBank (www.ncbi.nhl.nih.gov./BLAST).

Statistical analysis. The association of genotype with carcass components was analyzed using SAS GLM procedure (SAS Institute, 2008) and Duncan multiple range test. The genetic effects were analyzed using this model:

$$y_{ijk} = \mu + S_i + G_j + \varepsilon_{ijk}$$

where y_{ijk} was the observed phenotypic trait (carcass components) for k^{th} individual with i^{th} sex (i= male and female) and j^{th} genotype (j= AA, AG, and GG), μ was the overall mean, S_i was the genetic effect of i^{th} sex, G_j was the genetic effect of j^{th} genotype, and ε_{ijk} was the normally distributed residual error.

RESULTS

Polymorphisms of GH|*Eco*RV and GHR|*Eco*72I Loci

The partial fragment of GH and GHR genes in all individuals were successfully amplified and showed a 339 and 326 bp bands, respectively (Figure 3). The lengths of PCR products were in good agreements with the reference sequences (GenBank accession number: AY461843.1 and AJ506750.1 for GH and GHR, respectively). Genotyping analysis of GH locus was performed

using *Eco*RV restriction enzyme. This genotyping identified two alleles (A and G) and three genotypes (AA, AG, and GG). The A allele was indicated by 191 and 148 bp bands (restricted), while the G allele was indicated by 339 bp (unrestricted, Figure 4). Mutation was found in 190 bp from G to A nucleotide, (Figure 5). According to GenBank (accession number: AY461843.1), this SNP is located in g.2248G>A GH. Moreover, *Eco*72I enzyme was used for genotyping of GHR locus. The genotyping of GHR|*Eco*72I locus generated the same allele and genotype with GH|*Eco*RV locus. The A allele was indicated by a 326 bp (unrestricted), while the G allele was indicated by 212 and 114 bp (restricted, Figure 4). Mutation from G to A nucleotide was occurred in the position of 115 bp (Figure 6). According to GenBank (accession number: AJ506750.1), this SNP is located in g.565G>A GHR.

All chicken populations were polymorphic for both GH and GHR loci (Table 3). The GG genotype and the G allele of GH locus were predominant in all chicken populations. The AA genotype was barely found (0.005) in overall population. The AA genotype was only found in *Cobb* population (0.033), not in *Kampung*, F1, and F2 chicken populations. While in GHR locus, the AA genotype and the A allele were found to be higher in all chicken populations. The x^2 analysis of GH locus showed that all chicken populations were in the Hardy-Weinberg equilibrium. In the GHR locus, the *Cobb* broiler and F2 cross chicken populations were in the Hardy-Weinberg disequilibrium. This result indicated that the allele and genotype frequencies were not constant from generation to generation in these two populations (Allendorf *et al*., 2013).

Effect of Gene Polymorphism on Carcass Components

Association analyses showed that the g.565G>A GHR locus polymorphism had significant effect on chicken carcass components, but no significant associations between g.2248G>A GH genotype and carcass components were observed (Table 4). The GG genotype of g.565G>A GHR locus had higher live weight, carcass weight, breast weight, thigh weight, breast muscle weight, and thigh muscle weight than the AA genotype ($P<0.05$) in F2 *Kampung* x *Cobb* broiler chicken cross. No significant differences were found in carcass percentage, commercial cuts percentages, and muscle weight percentages.

DISCUSSION

The essential roles of GH and GHR genes in chicken have been explored since decades for improving meat productivity of chicken. The GH molecule has two binding sites which each interacts with the extracellular region of the performed GHR dimer. This binding leads a functionally dimerized complex that induces intracellular signaling (Kopchick, 2016; List *et al*., 2013). The GH-GHR complexes are then resulted in the endoplasmic reticulum (van den Eijnden & Strous, 2007). This complexes travel to the cell surface and activate the JAK2 via the STAT5/MAPK pathway (Sedek *et al*., 2014).

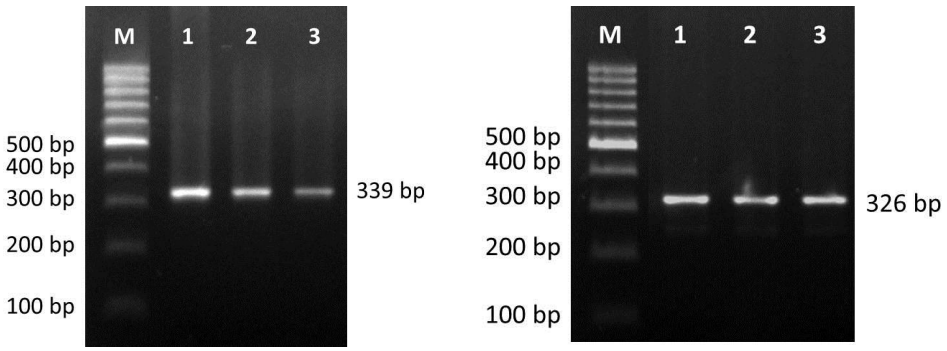

Figure 3. Visualization of GH (left) and GHR (right) fragment genes amplification in 1.5% agarose gel. (M= marker; 1-3= sample).

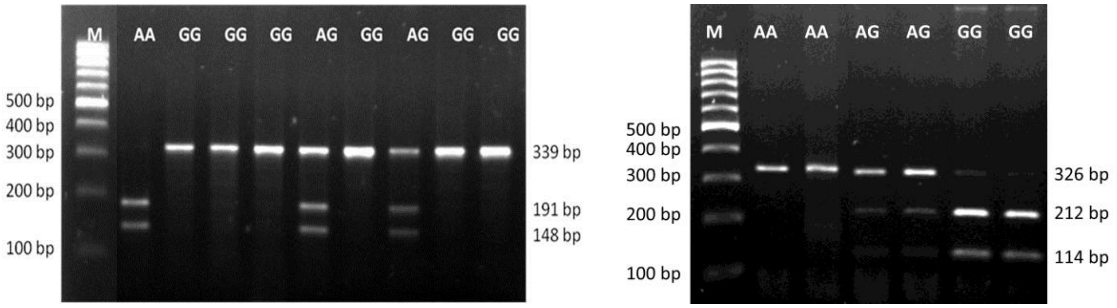

Figure 4. Visualization of GH|*Eco*RV (left) and GHR|*Eco*72I (right) genotyping in 2% agarose gel (M=marker; GG, AG, AA = genotype)

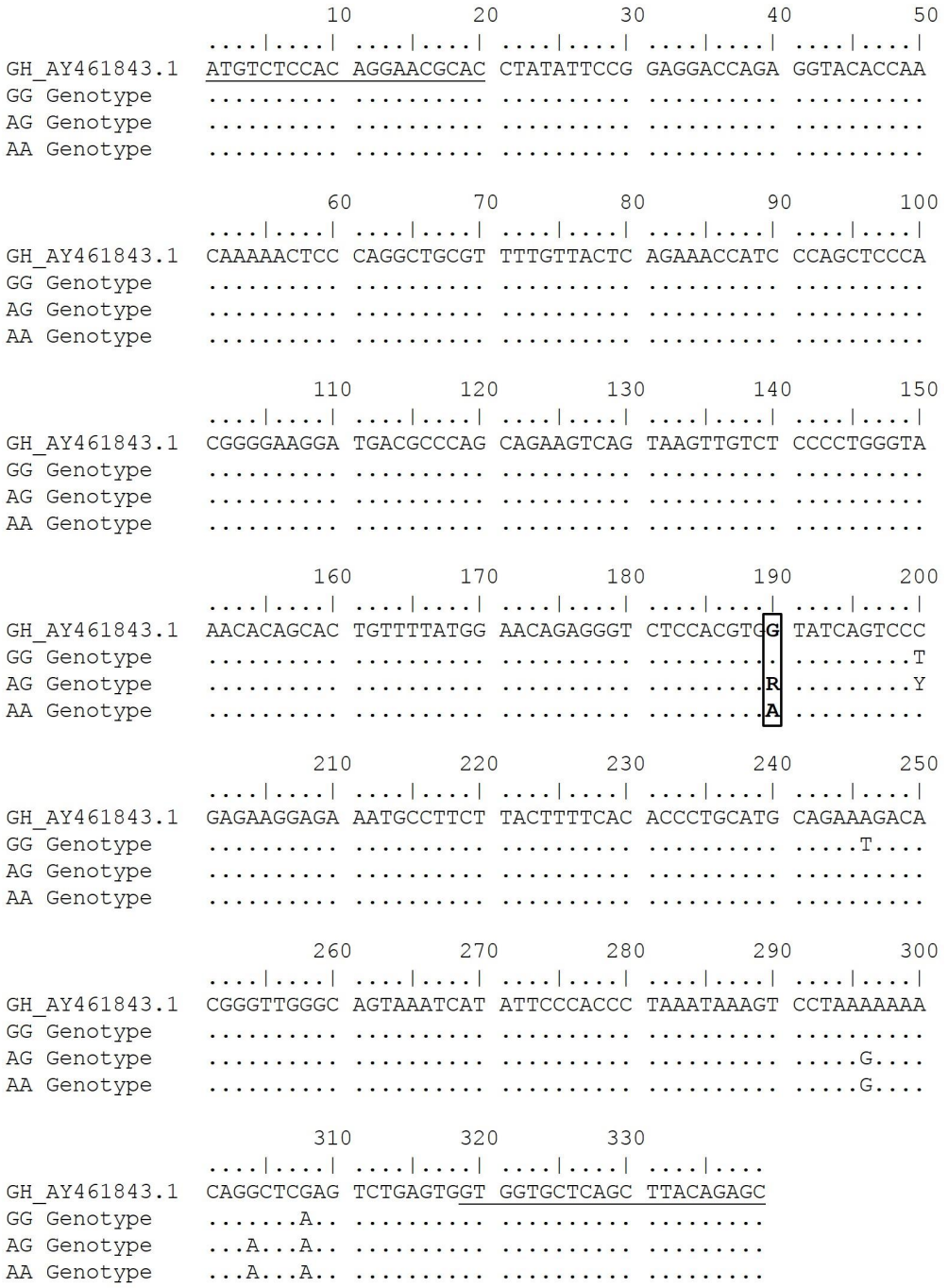

Figure 5. Partial intron 3 nucleotide sequence of chicken GH gene. Underline shows forward and reverse primer annealing positions; bold shows *Eco*RV restriction sites; box shows g.2248G>A GH SNP target (GenBank accession number: AY461843.1) .

Kim *et al*. (2010) explain that the GH-GHR complexes activate hepatic IGF-I secretion, which stimulates the differentiation and proliferation of bone and muscle cell. Our study in the GH|*Eco*RV genotyping generated the GG genotype predominantly (0.749), the AG genotype as a second majority (0.247), and almost no AA genotype was found (0.005) in overall population. The AA genotype was detected only in *Cobb* broiler population in a very low frequency. Similarly, in the F2 population generated from White Recessive Rock and Xinghua chicken, 18 chickens with the AA genotype were found from total 451 chickens (AA genotype freq= 0.04; Nie *et al*., 2005). Higher GG genotype frequency was also reported by Lei *et al*. (2007) in Xinghua (0.67) and White Plymouth Rock (1.00) chickens, Zhang *et al*. (2007) in Mountainous Black-Bone (0.70), Caoke (0.70), Sanhuang (0.73), and a commercial crossbred (0.83) chickens, Al-Khatib & Al-Hassani (2016) in Cobb (0.47) and Hubbard (0.61) chickens, and Anh *et al.* (2015) in broiler x Khai Mook Esarn chicken (0.65).

Association study was performed at 26 weeks of age, when *Kampung* chicken reached the sexual

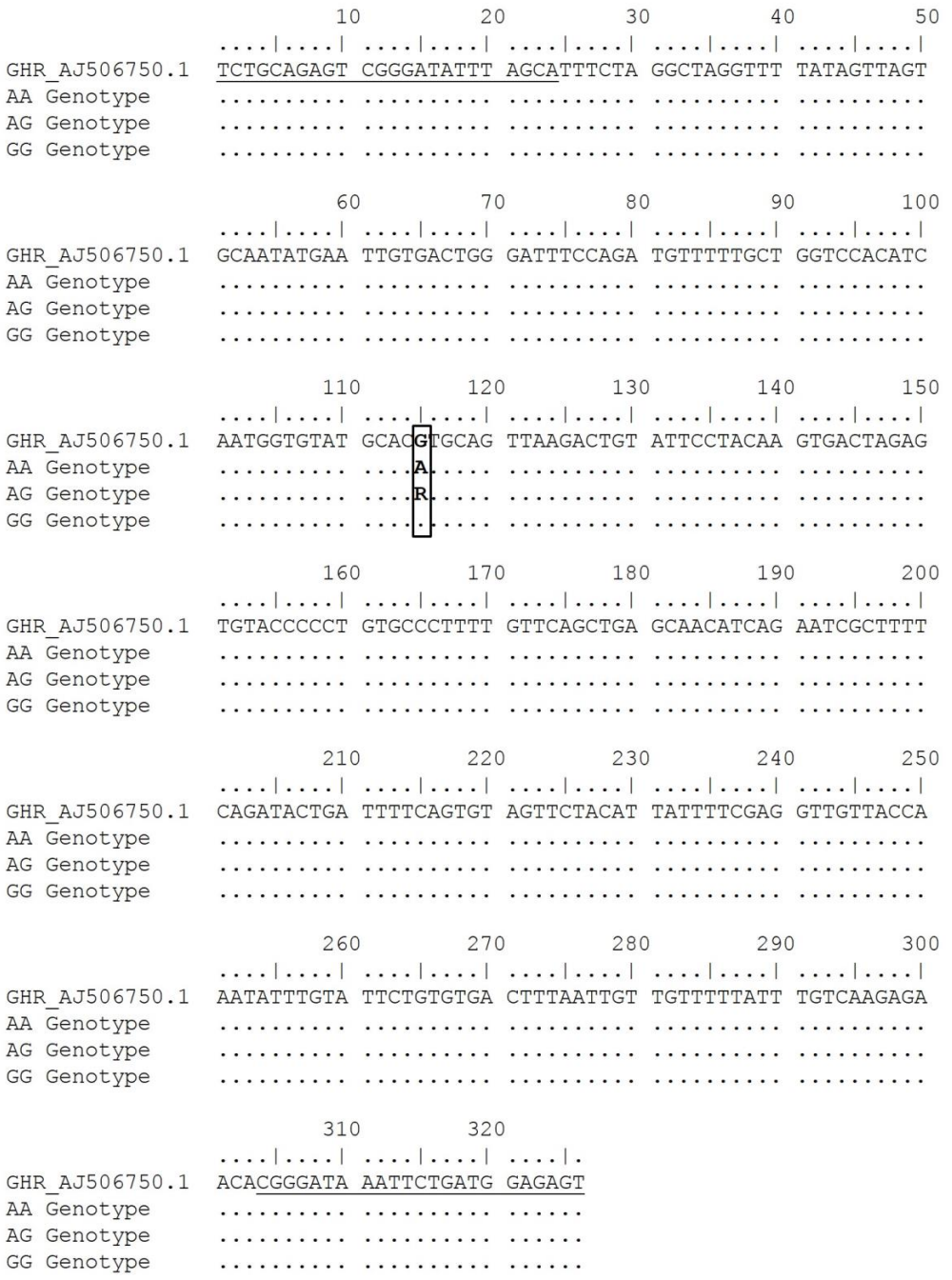

Figure 6. Partial intron 5 nucleotide sequence of chicken GHR gene. Underline shows forward and reverse primer annealing positions; bold shows *Eco*72I restriction sites; box shows g.565G>A GHR SNP target (GenBank accession number: AJ506750.1).

maturity in semi-intensive rearing system (Yuwanta & Fujihara, 2000). At the age of 26 weeks, chicken growth is in the stationary phase. Thus, in this phase, chicken has reached the maximum growth (Knízetová *et al*., 1995). Results of our study showed no significant effect between the g.2248G>A GH polymorphism and chicken carcass components. This result was in line with Zhang *et al*. (2007) in the Chinese indigenous chicken populations. Different to our findings, Nei *et al*. (2005) and Anh *et al*. (2015) found any associations of this SNP with chicken growth and carcass traits. Nie *et al*. (2005) reported that the AA genotype had positive effects (higher body weight, shank length, and ADG) in a F2 population derived from a cross of a fast-growing line, White Recessive Rock, and a slow-growing line, Xinghua. However, Anh *et al*. (2015) found that the AA genotype negatively affected carcass traits (lower body weight and ADG) in Thai broiler chicken. From those various studies, effects of this g.2248G>A GH polymorphism in different chicken breeds generated varied results. Kansaku *et al*. (2008) presumed that this indicated the various involvements of GH in production parameters. Moreover, Harvey (2013) concludes from large literature that pituitary GH has no effect on avian growth.

Table 3. The polymorphism information of the g.2248G>A GH and the g.565G>A GHR loci in *Kampung* chicken and cross

Locus/ Population	n	Genotype frequency			Allele frequency		$\chi 2$
		GG	AG	AA	G	A	
GH|*Eco*RV							
1. *Kampung*	49	0.939 (43)	0.061 (3)	0.000 (0)	0.969	0.031	0.049
2. *Cobb* Broiler	30	0.600 (18)	0.367 (11)	0.033 (1)	0.783	0.217	0.193
3. F1 *Kampung* x *Cobb* Broiler Cross	43	0.558 (24)	0.442 (19)	0.000 (0)	0.779	0.221	3.458
4. F2 *Kampung* x *Cobb* Broiler Cross	93	0.785 (73)	0.215 (20)	0.000 (0)	0.892	0.108	1.350
Overall population	215	0.749 (161)	0.247 (53)	0.005 (1)	0.872	0.128	2,369
GHR|*Eco*72I							
1. *Kampung*	49	0.000 (0)	0.020 (1)	0.980 (48)	0.010	0.990	0.005
2. *Cobb* Broiler	30	0.300 (9)	0.067 (2)	0.633 (19)	0.333	0.667	21.675*
3. F1 *Kampung* x *Cobb* Broiler Cross	43	0.000 (0)	0.372 (16)	0.628 (27)	0.186	0.814	2.247
4. F2 *Kampung* x *Cobb* Broiler Cross	93	0.140 (13)	0.194 (18)	0.667 (62)	0.237	0.763	20.035*
Overall population	215	0.102 (22)	0.172 (37)	0.726 (156)	0.188	0.812	41.095*

Note: n= number of sample, * = significantly different ($\chi^{20.05} = 3.84$)

Table 4. The association of the g.2248G>A GH and the g.565G>A GHR loci polymorphisms and carcass components in F2 *Kampung* x broiler chicken cross

Trait	g.2248G>A GH		g.565G>A GHR		
	GG (n=31)	AG (n=11)	GG (n=4)	AG (n=10)	AA (n=28)
LW (g)	2211.53 ± 505.44	2151.52 ± 455.08	2598.50 ± 51.08^{a}	2286.05 ± 431.80^{ab}	2106.06 ± 513.62^{b}
CW (g)	1471.35 ± 376.01	1420.27 ± 316.61	1746.25 ± 75.08^{a}	1570.30 ± 342.30^{ab}	1376.68 ± 362.60^{b}
BW (g)	389.94 ± 117.60	349.64 ± 79.03	491.00 ± 81.05^{a}	433.50 ± 103.51^{ab}	344.11 ± 98.21^{b}
TW (g)	269.13 ± 73.87	264.55 ± 69.56	328.25 ± 37.12^{a}	283.10 ± 58.56^{ab}	253.89 ± 75.75^{b}
DW (g)	259.26 ± 66.80	247.36 ± 68.11	285.75 ± 44.62	277.90 ± 56.28	244.14 ± 70.62
WW (g)	204.29 ± 47.67	200.82 ± 46.15	231.25 ± 32.44	218.50 ± 48.46	194.00 ± 46.11
BMW (g)	279.87 ± 95.42	246.00 ± 63.00	341.00 ± 48.96^{a}	305.10 ± 97.98^{ab}	248.82 ± 82.46^{b}
TMW (g)	196.68 ± 58.67	181.45 ± 41.53	241.50 ± 48.59^{a}	198.70 ± 44.41^{ab}	183.57 ± 56.22^{b}
DMW (g)	167.19 ± 46.20	158.91 ± 47.90	191.50 ± 31.37	172.40 ± 37.59	158.61 ± 49.95
CP (%)	66.27 ± 5.35	66.03 ± 3.60	67.24 ± 3.79	68.44 ± 3.60	65.26 ± 5.27
BP (%)	26.56 ± 4.21	24.60 ± 0.82	28.20 ± 5.29	27.59 ± 2.35	25.19 ± 3.72
TP (%)	18.31 + 1.89	18.49 ± 1.23	18.85 ± 2.54	18.09 ± 1.16	18.38 ± 1.82
DP (%)	17.74 ± 2.16	17.27 ± 1.60	16.37 ± 2.53	17.80 ± 1.66	17.73 ± 2.07
WP (%)	14.08 ± 1.65	14.16 ± 1.01	13.20 ± 1.35	13.94 ± 0.74	14.29 ± 1.69
BMP (%)	18.97 ± 3.61	17.27 ± 1.36	19.60 ± 3.41	19.24 ± 3.10	18.12 ± 3.32
TMP (%)	13.33 ± 1.68	12.99 ± 2.33	13.85 ± 2.88	12.68 ± 1.33	13.36 ± 1.87
DMP (%)	11.39 ± 1.31	11.08 ± 1.35	10.95 ± 1.67	11.02 ± 0.99	11.46 ± 1.38

Note: LW=Live Weight; CW=Carcass Weight; BW=Breast Weight; TW=Thighs Weight; DW=Drum Sticks Weight; WW=Wings Weight; BMW=Breast Muscle Weight; TMW=Thighs Muscle Weight; DMW=Drum Sticks Muscle Weight; CP=Carcass Percentage; BP=Breast Percentage; TP=Thighs Percentage; DP=Drum Sticks Percentage; WP=Wings Percentage; BMP=Breast Muscle Percentage; TMP=Thighs Muscle Percentage; DMP=Drum Sticks Muscle Percentage; n=number of sample; different superscript indicates significantly difference at the P<0.05 levels for chickens with different genotypes of a given locus.

Polymorphism study in the g.565G>A GHR locus found that the AA genotype had the highest frequency (0.726) than the AG (0.172) and GG (0.102) in overall population. Similar result was reported in Vietnamese (0.86, Khoa *et al*., 2013), Noi (1.00, Khoa *et al*., 2013), Xinghua (1.00, Lei *et al*., 2007), and White Plymouth Rock (1.00, Lei *et al*., 2007) chickens. Ouyang *et al*. (2008) could not detect the g.565G>A GHR polymorphism using DHPLC detection in Leghorn layer, White Recessive Rock broiler, Taihe Silkies, and Xinghua chickens. This study evidenced the association of the g.565G>A GHR genotype with live weight, carcass weight, breast weight, thigh weight, breast muscle weight, and thigh muscle weight, for the first time. Our finding proposed that the GG genotype of the g.565G>A GHR had positive effect on chicken carcass and yielded proportional body composition. The inhibition of normal human and animal skeletal muscle growths and fat depositions was reported to be caused by mutation in GHR gene by causing the inhibition of GH signal transduction (Lin *et*

al., 2012). However, the molecular mechanism of GHR introns and its effect on muscle growth in chicken is unclear. It is believed that the genetic potential of *Kampung* chicken can be improved through crossbreeding and selection. In this study, the G allele of the g.565G>A GHR locus tends to have a positive effect on body and carcass weight in F2 *Kampung* and broiler chicken cross. This locus can be recommended as a good candidate to select chickens with better growth and heavier carcass weights. However, further analysis in larger populations and study on the gene expression and protein level is necessary to be proven to reinforce this hypothesis.

CONCLUSION

This study investigated the polymorphisms of GH and GHR genes and emphasized that the g.565G>A GHR locus polymorphisms had significant association with carcass components in F2 *Kampung* and broiler chicken cross. Here, GHR is a potential marker for carcass traits in chicken.

ACKNOWLEDGEMENT

Authors thank to PMDSU Research Grant 2016 (Batch I) from The Ministry of Research, Technology, and Higher Education, Republic of Indonesia, which financially supported this work.

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