Association of GHR|*Alu*I gene polymorphism with body weight parameters in Grati-PO cattle

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ABSTRAK

Gen *growth hormone receptor* (GHR) merupakan anggota dari famili reseptor sitokin kelas I yang penting dalam regulasi pertumbuhan termasuk pada sapi. Penelitian ini bertujuan untuk menganalisa asosiasi antara gen GHR|*Alu*I dan parameter bobot badan pada populasi sapi PO Grati. Sebanyak 304 sampel darah sapi PO dikoleksi dari kandang percobaan Loka Penelitian Sapi Potong yang dilengkapi dengan 304 data fenotipiknya. Sampel darah diisolasi menggunakan zymo extraction kit. Deteksi keragaman gen GHR menggunakan metode polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) dengan enzim pemotong *Alu*I. Indikator polimorfisme meliputi frekuensi genotipe, frekuensi alel, Hardy-Weinberg Equilibrium, heterozigositas pengamatan (Ho), heterozigositas harapan (He), polymorphism information content (PIC) dan jumlah alel efektif (Ne). Analisis asosiasi dilakukan menggunakan linier mixed model. Performans bobot lahir, bobot sapih, bobot setahun dan bobot umur 18 bulan masing-masing adalah 25,4 ± 0,2kg; 113,8 ± 2,3 kg;159,9 ± 2,7 kg; dan 213,7 ± 3,6kg. Hasil analisis asosiasi signifikan (p<0,05) pada bobot sapih, bobot setahun dan bobot umur 18 bulan. Gen GHR berpotensi sebagai penanda genetik untuk sifat pertumbuhan dan dapat digunakan sebagai kandidat penanda genetik untuk seleksi sapi PO-Grati.

Kata kunci: Bobot badan, Growth hormone receptor, Polimorfisme, Sapi PO-Grati

ABSTRACT

The growth hormone receptor (GHR) gene is member of the class I cytokine receptor family that important in growth regulation including in cattle. This study aimed to analyze the association between GHR|*Alu*I gene with body weight, parameters in Grati-PO cattle population. A total of 304 blood samples were collected from the experimental station at the Beef Cattle Research Station, which

were also equipped with 304 phenotypic data. DNA was extracted using a zymo extraction kit. Detection of of the polymorphism of GHR gene using PCR-RFLP method with *Alu*I restriction enzymes. polymorphism indicators include genotype frequency, allele frequency, Hardy-Weinberg Equilibrium, observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC) and number of effective alleles (Ne). Association analysis was carried out using a linear mixed model. The performance of birth weight, weaning weight, yearling weight and 18 months weight were 25.4 ± 0.2 kg; 113.8 ± 2.3 kg; 159.9 ± 2.7 kg and 213.7 ± 3.6 kg, respectively. The result of association analysis was significant ($P<0.05$) on weaning weight, yearling weight and 18 months weight. It could be concluded that the GHR gene has potential to be a candidate genetic marker for growth traits in Grati-PO cattle.

Keywords: Body weight, Growth hormone receptor, Polymorphism, PO-Grati cattle

INTRODUCTION

Growth traits is one of the most important economic traits in beef cattle production. Body weight, body size measurements and weight gains are several types of growth traits. Growth traits have relationship between growth traits itself or with carcass and meat component (Meyer, 1995; Albertí *et al*., 2008; Caetano *et al*., 2013; Coyne *et al*., 2019), so it is widely used as a selection criterion in beef cattle breeding program. Body weight measurement is the most common type of growth traits used in the selection program, which are taken from birth and throughout an animal's life (Buzanskas *et al*., 2014).

The growth trait was controlled by many genes and their expression was the accumulation effects of genetic, environmental and interaction (Putra *et al*., 2014). Several genes that were thought to be the main genes in influencing growth traits were *growth hormone* (GH) and *growth receptor* (GHR) genes. The mechanism of action of these two genes has interrelated activities in influencing cell growth and metabolism in vertebrates. The GH gene in influencing growth and metabolism will interact with a specific receptor called the growth hormone receptor (GHR) on the surface of the targeted cell (Di Stasio *et al*., 2005).

Growth hormone (GH) has proven to be the major regulator of postnatal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production, and aging by modulating the expression of many genes (Ge *et al*., 2003). The growth hormone receptor (GHR) is a member of the class I cytokine receptor family with a single transmembrane domain (Waters and Brooks, 2015; Chia, 2014). GH receptor (GHR) are active in the central nervous system (CNS) and are crucial in regulating several aspects of metabolism (Cady *et al*., 2017). Therefore, the essential biological function of GHR, makes the GHR gene proposed as a potential candidate gene for several economically desirable traits including growth traits. The bovine *GHR* gene has been assigned located on bovine chromosome 20 (Moody *et al*., 1995).

The polymorphism of the bovine *GHR* gene is relatively high. Waters *et al*., (2011) have conducted bovine *GHR* gene resequensing and found a total of 39 SNP entire gene using five different breeds. One of these SNP is g.257A>G (in exon 10) which causes a change in the amino acid serine to glycine (Ge *et al*., 2000). This SNP can be identified using PCR-RFLP with *Alu*I restiction enzyme (GHR|*Alu*I). The GHR|*Alu*I gene was found to be polymorphic in bovine species (Di Stasio *et al*., 2005; Olenski *et al*., 2010; Zulkarnaim *et al*., 2010; Deepika and Salar, 2013; Deng *et al*., 2015). This polymorphism has been reported to be associated with the final weight and some meat quality traits in some Bos taurus cattle (Di Stasio *et al*., 2005; Reardon *et al*., 2010). However, has no association with various body weight parameters including calf birth weight (CBW) in Aberdeen Angus cattle (Fedota *et al*., 2017),as well as with CBW in Grati-PO cattle (Hartati *et al*., 2019). However, the associ-

ation has not been reported yet for other body weight parameters in Grati-PO cattle. Therefore, the present study was aimed to analyze the association between GHR|*Alu*I gene with body weight parameters in Grati-PO cattle.

MATERIALS AND METHODS

Performans Data of Grati-PO Cattle

Grati-PO cattle performance data were collected from 304 animals in the experimental enclosure at the Beef Cattle Research Station including birth, weaning, yearling and 18 months weight were accordance with recording data in breeding activities. Grati-PO cattle blood samples were collected from the jugular vein (3-5mL) using a vaccutainer tube containing K3 EDTA. DNA was extracted using a Quick-DNA mini prep kit (Zymo Research, USA) and then stored at -20^0 C for futher use.

PCR-RFLP Analysis

The method used to detect DNA variations was PCR-RFLP. The primers used for the *GHR* gene refers to Di Stasio *et al.* (2005). The primary sequences of forward and reverse are shown in Table 1. PCR reaction was performed in a total volume of 20 µL. A total 3 µl DNA containing of approximately (10-100) ng/µL of DNA, 0.2 µl of each primer, 10 µL PCR mix of MyTaqTM HS Red Mix (Bioline, USA), and 6.2 µl ddH$_2$O to a final volume of 20 µl. The PCR conditions were pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing for 15 s at 53.8°C, extension at 72°C for 10 s, and a final extension at 72°C for 5 min. PCR products were electrophoresed on 1.5% agarose gels, stained with GelRed®10,000X in water (Biotium, USA) and visualized under a G-BOX Gel Documentation System (Syngene, UK). The PCR products of GHR were digested with *Alu*I restriction enzyme (New England Biolabs, USA). The digested fragments were electrophorezed on 2% agarose gels, stained with GelRed®10,000X in water (Biotium, USA) and visualized under a G-BOX Gel Documentation System (Infiity VX2).

The sequencing analysis was performed in this study using two sample of PCR product (20 µl) to confirm the findings.The sequencing analysis was performed through commercial laboratory service (PT Genetika Science).

Statistical Analysis

Polymorphism indexes such as genotype frequency and allele frequency were calculated according to Nei and Kumar (2000). Expected heterozygosity (He) and observed heterozygosity (Ho) were calculated according to Weir (1991). Polymorphism Information Content (PIC) was calculated according to Botstein *et al.*, (1980) by direct counting. The genotypic frequency distribution for its deviation to HardyWeinberg Equilibrium (HWE) was analyzed by chi-square test was calculated according to Kaps and Lamberson (2004). The number of effective allele was calculated according to formula Nei and Tajima (1981).

Association between GHR|*Alu*I genotype and body weight parameters in Grati-PO cattle population were analyzed applying a randomized block design model as follows :

$$Y_{ijk} = \mu + \sigma_i + \beta_j + e_{ijk}$$

Table 1. GenBank Accession Number, Amplicon Size (bp), Location of Polymorphic Site and Primer Sequences GHR |AluI Gene of Grati-PO Cattle

Gene	GenBank Accession number	Amplicon size (bp)	Location of polymorphic site	Primer Sequences (5'-3')
GHR|AluI	AF140284.1	342	Exon 10	F:5'-GCT AAC TTC ATC GTG GAC AAC-3' R : 5'-CTA TGG CAT GAT TTT GTT CAG-3'

Di Stasio *et al.* (2005)

Where,

Y_{ijk} = dependent variable (BW, WW, YW or 18 MW)

μ = overall mean

σ_i = the effect of ith sex (male and female)

β_j = fixed effect of the jth genotype (AA, AG, GG)

e_{ijk} = random residual effect

RESULTS AND DISCUSSION

Body Weight Performance of Grati-PO Cattle

Descriptive analysis of the body weight performance of Grati-PO cattle in the GHR|*Alu*I gene validation material was shown in Table 2. In this study, the mean of birth weight, weaning weight, yearling weight and 18 months weight of Grati-PO cattle were 25.4 ± 0.2 kg; 113.8 ± 2.3 kg; 159.9 ± 2.7 kg and 213.7 ± 3.6 kg, respectively. The results of statistical analysis show that sex has a significant effect (P<0.05) on birth weight of Grati-PO cattle. The birth weight male calves was higher than female calves were 26.0 ± 0.3 kg and 24.8 ± 0.2 kg, respectively. The performance of Grati-PO cattle birth weight in this study was not different from the birth weight in the previous study that was 25.58 ± 3.31 kg (Hartati *et al.*, 2015). The birth weight of Grati-PO cattle in this study was higher than the birth weight of PO cattle in Napis village, Tambakarejo, Bojonegoro district that was 11.07 ± 3.92 kg (Nonok and Darmawan, 2014). However, the birth weight of PO cattle in this study was much lower than those of PO Kebumen which reached 31.88 ± 3.78 kg (Maharani *et al.* 2018) or compared to the same *Bos indicus* cattle in other country, such as Brahman cattle in Columbia which reach to 33.06 ± 3.60 kg (Martínez *et al.*, 2017), and Nellore cattle in Brazil which reach to 32.30 ± 3.80 kg (Chud *et al.*, 2014).

In this study showed insignificant results between sex on weaning weight and yearling weight in Grati-PO cattle although during this period, growth was strongly influenced by the environment because at the age of 7 months the calves have begun to wean and learn to consume finished feed until mature. Meanwhile, at 18 months weight there was a very significantly (P<0.05) between sex, where the body weight of the 18 months PO male cattle : as higher than the female PO cattle (225.2 ± 5.8 kg and 203.2 ± 4.2 kg, respectively). This probably because the age of yearling to 18 months was the optimal growth period for beef cattle, male and female cattle growth was influenced by sex steroid hormones. The estrogen hormone can inhibit bone growth, especially at the ends of the bones, so that female growth was stunted after puberty begins, whereas male after puberty will continue to grow for a longer period which was stimulated by androgen hormones (testosterone) especially bone growth and other parts, so that **:** males were always heavier than females at the same age (Djagra *et al.*, 2002)

GHR|*Alu*I Gene Polymorphism

Visualization of GHR restriction with *Alu*I enzyme, sequence of *GHR* gene and chromatogram of *GHR* gene are presented in Figure 1, 2 and 3.

In Figure 3 was shown the chromatogram sequence of the GHR gene from individual PO cattle with genotypes AA and GG genotypes. In the GG genotype, there was a substitution of A to G at exon 10 causing a change in the amino acid serine (**A**GC) to glycine (**G**GC). Morihito *et al.* (2017) stated that the mutation in DNA in organisms was substitutions, deletion or addition of one or more parts of the DNA molecule.

The genotype frequency, allele and heterozygosity of the GHR|*Alu*I gene in Grati-PO cattle were shown in Table 3. The genotype frequency in Grati-PO cattle were detected to have three genotypes, namely AA, AG and GG (Figure 1 and Figure 2). The AA and AG Genotypes of the GHR|*Alu*I gene in Grati-PO cattle were found at almost the same frequency, that were 42.1% and 44.4%, while the GG genotype was found in the lowest frequency that was 13.5%. The A allele was a common allele found in Grati-PO cattle with a frequency of 0.643 meanwhile the **:** the G allele was 0.357.
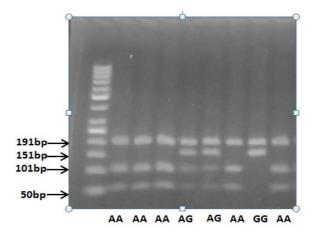
Figure 1. Visualization of *GHR* restriction with *Alu*I restriction enzyme

Figure 2. Sequence alignment of the AA and GG genotype sequenced samples with Genbank AF140284.1 of bovine *GHR* gene. The g.257A>G are targeted SNP of bovine GHR gene in present study. The grey highlighted sequences are flanking primers (forward and reverse). The restriction site of *Alu*I (5' AG^CT 3') are shown in the yellow highlighted sequences. The three novel SNPs found between the PO cattle sequences (present study) compared with Genbank (AF140284.1) are indicated by red letters.

The previous research on several local Indonesian beef cattle, the A allele was also a common allele, such as on Grati-Bali Cattle 0.579 (Hartati, *et al.*, 2021), Kebumen cattle 0.58 (Soewandi *et al.*, 2021), Bali cattle 0.991 and Pesisir cattle 0.615 (Zulkharnaim *et al*., 2010). Likewise with other exotic cattle such as Simmental cattle, the most common genotype

Table 2. Performance of Grati-PO cattle

Sex	N	BW ± SE	WW ± SE	YW ± SE	18 MW ± SE
Male	146	26,0 ± 0,3[a]	113,2 ± 3,2	162,1 ± 4,1	225,2 ± 5,8[a]
Female	158	24,8 ± 0,2[b]	114,3 ± 3,2	157,9 ± 3,5	203,2 ± 4,2[b]
Total	304	25,4 ± 0,2	113,8 ± 2,3	159,9 ± 2,7	213,7 ± 3,6

N : number of observation; BW : birth weight; WW : weaning weight; YW : yearling weight; 18 MW : 18 month weight; SE: standard error; means in the same row with different superscripts differ significantly (P<0.05).

Table 3. Genotype Frequency, Allele and Heterozygosity GHR|AluI Gene of Grati-PO Cattle.

Gene	Sex	N	Genotype Frequency			Allele Frequency		χ^2test	He	Ho	PIC	Ne
			AA	AG	GG	A	G					
GHR	Male	146	0.418	0.459	0.123	0.647	0.353	0.004	0.457	0.458	0.352	1.840
	Female	158	0.424	0.430	0.146	0.639	0.361	0.707	0.461	0.463	0.355	1.856
	Total	304	0.421	0.444	0.135	0.643	0.357	0.323	0.459	0.460	0.354	1.849

N : number of observation; X^2 test : chi-square value; He : heterozigosity expected; Ho: heterozigosity observed; PIC: polymorphic information content; Ne: number of effective allele.

was the AA genotype (64.19%) and the A allele was the common alel (0.720) (Ardicli *et al*., 2017). In contrast, the G allele was the common allele found in Simmental and Limousin cattle raised at Singosari National Artificial Insemination Center BBIB Singosari Malang were 0.714 and 0.735, respectively (Zulkharnaim *et al.,* 2010).

The results of statistical analysis showed that the genotype frequency of the GHR|*Alu*I gene in Grati-PO cattle was still in Hardy-Weinberg Equilibrium (HWE). This explains that the frequency of alleles and genotype in the Grati-PO cattle population will remain constant

from generation to generation. The PO cattle population was large enough to have the chance to mate randomly, which causes the gene frequency to remain the same. The factors that can change the frequency of genes in a population were selection, mutation, migration, genetic drift and evolution (Falconer and Mackay, 1996).

The genetic diversity of a gene can be evaluated by the heterozygosity value (Ho, He, Ne) and polymorphism information content (PIC) value. The statistical analysis result showed that the expected heterozygosity (He) and the observed heterozygosity (Ho) values in the GHR|*Alu*I gene were 0.459 and 0.460. This
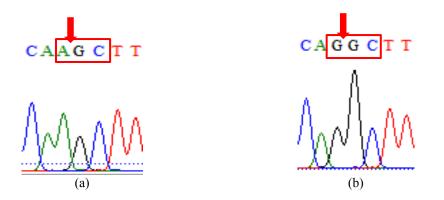

Figure 3. Chromatogram sequences of *GHR* gene in individual of (a) PO with AA genotype (b) PO with GG genotype. Substitution of A to G at exon 10 causing a change in the amino acid serine (**A**GC) to glycine (**G**GC).

Table 4. Association between GHR|AluI Gene Polymorphism and Body Weight in Grati-PO Cattle

Genotype	n	BW $\pm$SE	n	WW $\pm$ SE	n	WY $\pm$ SE	n	18 MW$\pm$SE
Male (M)								
AA	61	25.8±0.4	60	108.0±4.5	60	147.6±5.2	58	202.1±7.1
AG	67	26.1±0.4	67	117.8±5.2	67	174.4±6.5	66	242.6±9.1
GG	18	26.4±1.0	17	113.4±7.5	17	164.9±11.8	17	236.3±17.7
Female (F)								
AA	67	25.3±0.4	67	104.4±3.5	67	146.9±4.9	67	179.8±4.0
AG	68	24.6±0.3	64	122.4±5.7	64	170.3±5.3	64	224.9±7.3
GG	23	23.9±0.7	23	120.6±9.9	23	155.4±9.8	23	211.0±11.6
M and F								
AA	128	25.5±0.3	127	106.1±2.8[a]	127	147.2±3.5[a]	125	190.2±4.1[a]
AG	135	25.3±0.3	131	120.0±3.8[b]	131	172.4±4.2[b]	130	233.9±5.9[b]
GG	41	25.0±0.6	40	117.5±6.5[ab]	40	159.4±7.5[ab]	40	221.8±10.1[b]

n = number of observation; BW : birth weight; WW : weaning weight; YW : yearling weight; 18 MW : 18 month weight; SE: standard error; means in the same row with different superscripts differ significantly (P<0.05).

result shows that Grati-PO cattle have a high enough value on genetic diversity. The Ne valu in this study was 1.849 which means that there were differences in allele frequencies in the GHR|AluI gene for Grati-PO cattle. Determination of the informative level of a marker can be seen from the PIC value with with three categories, namely the low category (≤0.25), the moderate category (0.25<PIC<0.5), and high category (≥0.5). The PIC value was 0.354 and in the moderate category. The PIC value of Grati-PO cattle in this study was included in the moderate category which indicated that the markers on the GHR|AluI gene were very informative so that it can be associated with body weight in the Grati-PO cattle population.

Association of GHR | AluI Gene with Body Weight in Grati-PO Cattle

The analysis result of the association of GHR|AluI genotypes with body weight of Grati-PO cattle are presented in Table 4. The statistical analysis results showed that there was no interaction between sex and genotype even though the effect of sex was significantly different on birth weight and body weight at 18 months (Table 2). The genotype of the GHR|AluI gene was significantly (P<0.05) associated to weaning weight, yearling weight and 18 months weight in Grati-

PO cattle but not significant (P>0.05) with birth weight of Grati-PO cattle. The *GHR* gene was also reported to be significantly associated with weaning weight and daily weight gain in Pasundan cattle (Said *et al*., 2016), weight gain and carcass weight in Nellore cattle (*Bos indicus*) and it cross breed (Curi *et al*., 2006) but it has no effect on reproductive performance in Holstein-Friesian cattle (Hadi *et al*., 2015). In *Bos taurus* cattle, the GHR|AluI was found to be associated with birth weight, final wieght and several characteristics of meat quality (Di Stasio *et al*., 2005; Reardon *et al*., 2010).

This research found that the AG genotype in Grati-PO cattle has the highest body weight performance both at weaning weight, yearling weight and 18 months weight. Based on the results of this study, genotype-based selection can be carried out on individuals with the AG genotype with the selection criteria for weaning weight or yearling weight. Weaning weight and yearling weight were two growth characteristics that has high economic value. The high weaning weight illustrates the mother's good mothering ability in raising calves and having good milk production (Falconer and Mackay 1996). In addition, the heritability value obtained for the two traits where the highest heritability was obtained at the weaning and yearling weight of 0.47±0.15

and 0.63±0.17, respectively (Hartati *et al.*, 2015). Thus it could be proposed that the *GHR* gene is very potential and could be used as a genetic marker on genetic improvement and selection for superior of Grati-PO cattle .

CONCLUSION

The GHR|*Alu*I gene was found to be moderately polymorphic in Grati-PO cattle. There was no association between GHR|*Alu*I gene genotype and birth weight, but it was significant with weaning weight, yearling weight and 18 month weight. Therefore, GHR|*Alu*I gene may be a potential genetic marker for those three body weight parameters in Grati-PO cattle. However, this association needs to be validated to the subsequent offspring so that it can optimistically be used as a genetic marker in improving body weight performance in the breeding program for Grati-PO cattle.

ACKNOWLEDGEMENTS

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