Detection of GHR|AluI gene polymorphism and its association with body weight of Madura cattle in Indonesian Beef Cattle Research Station

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Abstract. Madura cattle is a native cattle that are genetically tolerant of tropical climates. Madura cattle are one of the beef cattle breeds in Indonesia. Madura cattle can be a superior breed, but improving growth trait in Madura cattle needs to be conducted. The early step that was needed was for detecting the genes which were involved in growth traits. The purpose of this research was for detecting GHR gene polymorphisms and its association with body weight of Madura cattle population in Indonesian Beef Cattle Research Station. This study used 110 DNA samples of Madura cattle that was collected from the experimental barn at Indonesian Beef Cattle Research Station. DNA samples were extracted by DNA extraction kit. Genotype of GHR gene was detected by PCR-RFLP method using AluI restriction enzymes. In this study, association genotype and body weight was analysed by univariate GLM method. The birth weight, weaning weight, yearling weight and 18 months weight of Madura cattle were 16.8±0.3 kg; 82.4±2.3 kg; 124.6±3.7 kg and 166.7±5.0 kg, respectively. The GHR genes of Madura cattle had 3 genotypes AA, AG and GG and genotype frequencies of GHR gene were 0.273 (AA), 0.373 (AG), 0.355 (GG), respectively. Whereas the allele frequencies of A was 0.459 and the allele frequencies of G was 0.541. Based on Chi-square (X2) analysis showed that the population sample was not disequilibrium. The result of association analysis was significant (P <0.05) on weaning weight, yearling weight and 18 month weight. It was concluded that the GHR gene has potential as a genetic marker for growth traits and can be used as MAS in Madura cattle in Indonesian Beef Cattle Research Station.

Keywords: GHR|AluI gene, polymorphism, body weight, association analysis, Madura cattle

1. Introduction

Madura cattle is a native cattle that are genetically tolerant of tropical climates and marginal environments and are resistant to disease attacks. Madura cattle have a large contribution as beef cattle, which is predicted to reach 24% of the demand for beef cattle originating from East Java. So far, Madura cattle breeding activities have been more focused on producing superior breeds through selection. One of the characteristics of production which has important economic value is growth trait.

Selection of growth traits can be carried out both conventionally and by molecular approaches. One of the genes associated with the growth trait is the GHR (Growth Hormone Receptor) gene. The GHR gene acts as a cell surface receptor that played a role as conductor GH on somatic cell growth. GH has
a role in growth and metabolism through interaction with a specific receptor that was called growth hormone receptor (GHR) on the surface of target cells [1] so that GHR has a role the biological metabolism of GH on target cells. The position of GHR gene is on chromosome 20 [2] and in exon 10, SNPs that change amino acid are found at 200 bp was Ala / Thr and 257 bp was Ser / Gly [3]. The SNPs at 257 bp in more research that had been reported were studied and detected by the PCR-RFLP method using the AluI restriction enzyme. Based on more the report of The GHR | AluI that Bos taurus [4][5][6] and Bos indicus [7] was found polymorphism, but Bos javanicus (Bali cattle) [5] was found monomorphism.

GHR | AluI gene was associated with final weight and meat quality traits had been reported in Bos taurus [1][4][8]. The research from Said et al. [9] found that weaning weight and daily gain is associated significantly with weaning weight and daily gain in Pasundan cattle. Based on this information, the GHR gene has the potential to be a genetic marker in local Indonesia beef cattle. The purpose of this study is for detecting the polymorphism of the GHR gene and its association with body weight of Madura cattle in Indonesian Beef Cattle Research Station (IBCRS)

2. Materials and methods

2.1. DNA sample

Samples for DNA use 110 heads Madura cattle calves in research station of Indonesian Beef Cattle Research Station, Grati Pasuruan. DNA was extracted from blood of Madura cattle that were collected from the jugular vein (3-5mL) using a vaccutainer tube containing K3 EDTA.DNA and the blood sample stored at -20°C. DNA extraction use DNA extraction kit (zymo merck). On the other hand, the data of birth, weaning, yearling and 18 month weight from 110 heads Madura cattle calves were collected.

2.2. PCR-RFLP analysis

The GHR | AluI gene were amplified using primer pairs designed by [1] (Genbank number AF140284.1). The primer information used was given in Table 1. PCR reaction was performed in a total volume of 20 µL. The PCR reaction contained 3 µl DNA (approximately 20 ng/µL of DNA), 0.4 µl of each primers, 10 µL PCR mix (MyTaq™ HS Red Mix (Bioline, USA)), and 6.2 µl double destilation water (DDW) to a final volume of 20 µl. the PCR condition was 95°C for 1 min (pre-denaturation), 95°C for 15 s (denaturation), 53.8°C for 15 s (anneling), 72°C for 10 s (extension) and 72°C for 5 min (final extention) and the PCR condition was followed by 35 cycles. 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 Documetation System (Syngene, UK). AluI restriction enzyme (New England Biolabs, USA) was used to digest the PCR products of GHR. 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 Documetation System (Infiity VX2).

Table 1. The primers used to amplify specific fragments of GHR gene in Madura cattle.

| Gene | Amplicon Size (bp) | Location | Primer Sequences (5'-3')       |
|------|-------------------|----------|--------------------------------|
| GHR  | 342               | Exon 10  | F:5'-GCT AAC TTC ATC GTG GAC AAC-3' |
|      |                    |          | R : 5'-CTA TGG CAT GAT TTT GTT GTT CAG-3' |

2.3 Data analysis

Genotype and alleic frequency were analyzed according to the formula Nei & Kumar [10]. Hardy-Weinberg (H-W) equilibrium was calculated according to the formula Kaps and Lamberson [11] and heterozigosity in the Madura cattle were analyzed according to Weir [12]. formula and the Polymorphism Information Content (PIC) value of GHR gene were analyzed according to [13] formula. Analisys of association on the genotype data of GHR gene with the birth weight, weaning weight, yearling weight and 18 month weight were performed by general linier model (GLM) model by IBM SPSS ver 20.0 software.
3. Results and discussion

3.1. Body weight performance of Madura cattle in IBCRS.
Descriptive analysis of body weight performance of Madura cattle in IBCRS is shown in Table 2 below:

Table 2. Body weight of Madura cattle in Indonesian Beef Cattle Research Station.

| Sex   | N  | BW ± SE | WW ± SE | YW ± SE | 18 MW ± SE |
|-------|----|---------|---------|---------|------------|
| Male  | 54 | 17.6 ± 0.4ª | 82.7 ± 3.2ª | 128.9 ± 5.4ª | 175.6 ± 7.2ª |
| Female| 56 | 16.1 ± 0.4ᵇ | 82.1 ± 3.2ª | 120.5 ± 5.1ª | 158.4 ± 6.7ª |
| Total | 110| 16.8 ± 0.3  | 82.4 ± 2.3  | 124.6 ± 3.7  | 166.7 ± 5.0  |

n = number of observation; BW : birth weight; WW : weaning weight; YW : yearling weight; 18 MW: 18 month weight; SE: standard error.
ªᵇ the different superscripts in same column showed significantly (P < .05).

According to Table 2, the result showed that the average of birth weight, weaning weight, yearling weight and 18 months weight of Madura cattle were 16.8 ± 0.3 kg ; 82.4 ± 2.3 kg; 124.6 ± 3.7 kg and 166.7 ± 5.0 kg, respectively. The results of this research showed that sex had a significant effect (P<0.05) on birth weight of Madura Cattle. The birth weight of male calves was higher than the birth weight of female calves were 17.6 ± 0.4 kg and 16.1 ± 0.4 kg, respectively. The birth weight of Madura cattle in this study was higher than the birth weight of male and female Madura cattle in Bangkalan Madura Regency were 15.42 kg and 13.60 kg, respectively [14] but lower than birth weight and weaning weight at Madura cattle on the island of Madura [15] [16]. According to Table 2, weaning weight, yearly weight and body weight at 18 months of Madura cattle was not affected by sex (P > .05), although in this period the growth is strongly influenced by the environment because the age of 7 months of calves has begun to wean and learn to consume finished feed until mature.

3.2. Genotype and allele frequencies, X2 tes, He, Ho, PIC and Ne values of GHR|AluI gene
The results of genotype and allele frequencies analysis of the GHR | AluI gene in Madura cattle in IBCRS can be seen in Table 2.

Table 3. Genotype and allele frequencies, X2 tes, He, Ho, PIC and Ne values of the GHR | AluI gene in Madura cattle.

| Gene | Sex | n  | Genotype Frequency | Allele Frequency | χ²test | He   | Ho   | PIC  | Ne  |
|------|-----|----|-------------------|-----------------|--------|------|------|------|-----|
|      |     |    | AA    | AG    | GG    | A    | G    | A    | G    |
| GHR  | Male| 54 | 0.352 | 0.333 | 0.315 | 0.519 | 0.481 | 5.967 | 0.499 | 0.504 | 0.375 | 1.997 |
|      | Female| 56 | 0.196 | 0.411 | 0.393 | 0.402 | 0.598 | 1.187 | 0.481 | 0.485 | 0.365 | 1.926 |
|      | Total| 110| 0.273 | 0.373 | 0.355 | 0.459 | 0.541 | 6.849 | 0.497 | 0.499 | 0.373 | 1.987 |

n : number of observation; χ² test : chi-square value; He : heterozigosity expected; Ho: heterozigosity observed; PIC: polymorphic information content; Ne: number of effective allele.

According to Table 3, The genotype of GHR | AluI genes of Madura cattle in IBCRS was found AA, AG and GG. The AG and GG genotypes frequency in the GHR | AluI genes of Madura cattle were found at almost the same frequency, were 37.3% and 35.5%, while genotype AA was found in lower frequencies than AG and GG was 27.3%. In male Madura cattle, the A allele is the common allele while in female Madura cattle the common allele is the G allele were 51.9% and 59.8%, respectively. Several previous research results also reported that in most Indonesia local beef cattle allele A is a common allele were Pasundan cattle with allele frequency A = 0.67 and B = 0.33 [17], PO cattle with allele frequency A = 0.788 and allele B = 0.212 [7], Bali cattle with an allele frequency of A = 0.991 and G =
0.009 [5] and in Simmental bull with an allele frequency of A = 0.716 and G = 0.248 [6]. Thus the GHR | AluI gene in most local Indonesia beef cattle are polymorphic as well as the Madura cattle in IBCRS.

The results of Hardy-Weinberg Equilibrium (HWE) analysis showed that the genotype frequency of the GHR | AluI gene in Madura cattle was disequilibrium condition, especially in the male calves population, while the female calves population was equilibrium condition. This condition described that the frequency of alleles and genes in the Madura cattle population is not constant from generation to generation, especially in males that have selection and have mating arrangements, causing gene frequencies to change. Statement from Vasconcellos et al. [18] that the disequilibrium condition was caused by inbreeding, selection, genetic drift and the population that was divided. Selection, mutation, migration, genetic drift and evolution was factors that could change the frequency of genes [19] [20].

The genetic diversity of Madura cattle is shown in Table 3. The genetic diversity of a gene can be evaluated by the heterozygosity value (Ho, He, Ne) and the level of informative marker (PIC). The results of this analysis showed that the expected heterozygosity (He) and observed heterozygosity (Ho) values in the GHR | AluI gene were 0.497 and 0.499, respectively. These condition that the Madura cattle had high genetic diversity. Based on [13] that the PIC value category was low (≤0.25), moderate (0.25 <PIC <0.5), and high (≥0.5). According to this category, the GHR | AluI gene has a PIC value of 0.373 and is in the moderate category. The PIC value in the moderate category indicates that the marker on the GHR | AluI gene is very informative so that it can be associated with growth traits in Madura cattle population in IBCRS to become marker assisted selection (MAS) candidates.

### 3.3. Association of GHR | AluI genes with body weight of Madura cattle in IBCRS

The analysis results of the genotype association of the GHR | AluI gene with birth weight, weaning weight, yearling weight and 18 month weight for Madura cattle are presented in Table 4 below:

| Genotype | n   | BW ± SE  | n   | WW ± SE  | N   | WY ± SE  | n   | 18 MW±SE |
|----------|-----|----------|-----|----------|-----|----------|-----|----------|
| Male (M) |     |          |     |          |     |          |     |          |
| AA       | 19  | 16.7±2.6 | 18  | 91.5±23.1| 18  | 144.3±34.6| 18  | 192.7±45.7|
| AG       | 18  | 18.5±3.8 | 18  | 75.2±20.1| 18  | 111.9±34.3| 18  | 156.8±56.3|
| GG       | 17  | 17.7±2.9 | 15  | 81.5±24.6| 15  | 130.9±44.0| 15  | 176.9±51.9|
| Total    | 54  | 17.6±3.2 | 51  | 82.8±23.2| 51  | 128.9±39.1| 51  | 175.4±52.7|
| Female (M) |     |          |     |          |     |          |     |          |
| AA       | 11  | 16.2±3.3 | 11  | 90.1±13.4| 11  | 137.7±18.4| 11  | 186.2±34.3|
| AG       | 23  | 16.3±2.1 | 22  | 80.3±23.6| 22  | 120.0±39.4| 22  | 163.2±45.6|
| GG       | 22  | 15.8±3.5 | 21  | 79.7±27.4| 21  | 112.1±37.3| 21  | 138.9±53.4|
| Total    | 56  | 16.1±2.9 | 54  | 82.1±23.6| 54  | 120.5±37.3| 54  | 158.4±49.5|
| M   +  F |     |          |     |          |     |          |     |          |
| AA       | 30  | 16.5±2.8a| 29  | 90.9±19.7b| 29  | 141.8±29.3b| 29  | 190.2±41.2b|
| AG       | 41  | 17.2±3.1a| 40  | 78.0±22.0a| 40  | 116.4±36.9a| 40  | 160.3±50.1a|
| GG       | 39  | 16.6±3.4a| 36  | 80.4±25.9ab| 36  | 119.9±42.5a| 36  | 154.7±55.4a|
| Total    | 110 | 16.8±3.1 | 105 | 82.4±23.3| 105 | 124.6±38.3| 105 | 166.7±51.5|

*ab the different superscripts in same column showed significantly (P < .05).*
association of the GHR gene on weaning weight and daily gain of Pasundan cattle. Curi et al. [21] who reported a significant association of GHR genes on daily gain and carcass weight of Nellore cattle (Bos indicus) and their crosses, but had no significant effect on reproductive performance in Friesian Holstein cattle [22]. In Bos taurus cattle, the GHR | AluI gene besides having a significant effect on birth weight is also polymorphic on final weight and several characteristics of meat quality [1] [4] [8]. Garret et al. [23] also reported that in Brangus bulls cattle the GHR gene had an effect on rib fat.

Based on Table 4, it can be seen that genotype AA in Madura cattle in IBCRS has the highest body weight performance at weaning weight, yearling weight and body weight at 18 months and then GG and AG genotypes. Based on the results of this study, genotype-based selection can be carried out on individuals with genotype AA with the selection criteria for weaning weight or yearling weights. Weaning weight and yearling weight are two growth traits that high economic value. Weaning weight that high value illustrates the mother's good mothering ability in raising calves and has good milk production [24]. In addition, the highest genetic correlation in Madura cattle in IBCRS was also obtained between weaning weight and yearly weight of 0.78, while the lowest genetic correlation was obtained at birth weight with a year weight of 0.27 [25].

This is also accordance with the heritability value obtained in these two traits, where the highest heritability is obtained at weaning weight and yearling weight were 0.85 ± 0.4 and 0.74 ± 0.4, respectively [26]. Thus, it can be stated that the GHR gene is very potential and can be used as a marker assisted selection (MAS) candidate of Madura cattle in IBCRS for exeleration genetic quality improvement and selection of superior breeds.

4. Conclusion
The GHR | AluI gene is polymorphic and was significantly associated with body weight of Madura cattle in Indonesia Beef Cattle Research Station (Grati, Pasuruan). GHR | AluI gene can become as a marker assisted selection (MAS) candidate in Madura cattle in Indonesia Beef Cattle Research Station (Grati, Pasuruan) to accelerate genetic quality improvement and selection of superior breeds.

5. Suggestion
Detection of GHR gene polymorphisms in Grati-Madura cattle population needs to be continued to the validation stage in a larger number of samples, so that it has a potential as marker assisted selection (MAS).

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