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Association of growth hormone (GH) gene polymorphism with growth and carcass in Sumba Ongole (SO) cattle

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ABSTRAK

Telah dilakukan suatu penelitian untuk mengidentifikasi keragaman gen *Growth Hormone* (GH) daerah intron 3 dan mengevaluasi asosiasinya terhadap beberapa parameter pertumbuhan dan produksi karkas pada sapi Sumba Ongole (SO). Sebanyak 267 sampel DNA sapi SO digunakan dalam analisa PCR-RFLP (*Polymerase Chain Reaction-Restriction Fragment Length Polymorphism*) dengan enzim restriksi *MspI* untuk mengidentifikasi beberapa genotipe di daerah intron 3. Data parameter pertumbuhan (n=44) yang diamati adalah bobot lahir (BW), bobot sapih umur 205 hari (WW_{205}), bobot umur 365 hari (YW_{365}) dan data produksi karkas (n=122). Terdapat dua alel gen GH yang ditemukan pada sapi SO dalam penelitian ini berdasarkan analisis PCR-RFLP, yaitu alel A (frekuensi=0,87) dan alel B (frekuensi=0,13) serta tiga genotipe (AA, AB, dan BB). Genotipe AA merupakan genotipe dengan frekuensi genotipe tertinggi (0,76) sedangkan genotipe BB memiliki nilai frekuensi genotipe terendah sebesar 0,02. Tingkat keragaman gen GH pada penelitian ini termasuk rendah berdasarkan nilai heterozigositas observasi (H_o) pada populasi sapi SO dalam penelitian yaitu sebesar 0,23 dan nilai PIC (*Polymorphism Information Content*) sebesar 0,20. Keragaman gen GH yang ditemukan dalam penelitian ini tidak memiliki asosiasi dengan parameter bobot lahir, bobot sapih, bobot umur satu tahun, dan persentase karkas (P>0,05) sehingga tidak dapat dijadikan sebagai penanda atau marker genetik dalam program pemuliaan sapi SO.

Kata kunci : asosiasi, growth hormone, pertumbuhan, karkas, Sumba Ongole

ABSTRACT

A study was conducted to identify the polymorphism in the intron 3 of the Growth Hormone (GH) gene and also to evaluate the association of the GH gene polymorphism with growth parameters and dressing percentage in the Sumba Ongole (SO) cattle. A total of 267 individual DNA samples were used in the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. The SO cattle growth parameters data (n=44) including birth weight (BW), weaning weight at 205 days of age (WW_{205}), yearling weight at 365 days of age (YW_{365}) and also dressing percentage (DP) (n=122) were investigated in this study. There were three genotypes (AA, AB, and BB) of the GH gene based on the PCR-RFLP analysis with allele frequency was 0.87 and 0.13 for A allele and B allele respectively. The highest genotype frequency in the SO cattle is AA (0.76) and the lowest is BB (0.02). The

Heterozygosity Observed (H_o) value in the SO cattle population is 0.23 and Polymorphism Information Content (PIC) value is 0.20. Therefore, the genetic diversity in the SO cattle based on the GH gene polymorphism is quite low. There is no association (P>0.05) in BW, WW_{205}, YW_{365}, and DP with genotypes of the GH gene. As the result, the GH gene in this study cannot be used as a genetic marker in the SO cattle breeding program.

Keywords :association, growth hormone, growth, carcass, Sumba Ongole

INTRODUCTION

The Sumba Ongole (SO) cattle is one of the Indonesian local breeds cattle. The existence of the SO cattle in Indonesia began since the Ongole cattle was imported from India in 1914 (Ministry of Agriculture of the Republic of Indonesia, 2014) and well adapted in Indonesian climate. The breeding program of the Ongole cattle was centralized in the Sumba island (East Nusa Tenggara Province), hereafter the Ongole cattle became known as Sumba Ongole cattle (Hardjosubroto, 2004). According to Agung *et al.* (2015), the SO cattle has excellent potential as beef cattle based on the ability to gain higher dressing percentage (>50%) compare with other local breeds cattle in Indonesia. Recently, Indonesian farmers can do the selection in the SO cattle based on the MPPA value, breeding value and also performance test (Said *et al.*, 2016a; Said *et al.*, 2016b) but it is need more genetic information to determine the population for the breeding program. However, the superior SO cattle can be generated through the breeding program with early detection system based on the genetic information using molecular technology. Nowadays, the genotype of specific genes that highly associated with the economic traits can be identified and used as genetic markers in the cattle breeding program.

Growth Hormone (GH) is a protein hormone that synthesized and secreted by the anterior pituitary gland part (Etherton and Bauman, 1998). This hormone encoded by the GH gene and affects the productivity in livestock (Pereira *et al.*, 2005; Beauchemin *et al.*, 2006). Furthermore, the GH is needed on tissue growth, fat metabolism, and normal body growth (Burton *et al*., 1994). In addition, the function of the GH is also play a role in the reproduction traits i.e. superovulation response, ovulation rate, fertility rate and embryo quality as reported by Sumantri *et al.* (2011). The importance of the function of GH gene make this gene as one of candidate genes for Marker Assisted Selection program (Tambasco *et al.*, 2003; Beauchemin *et al.*, 2006; Silveira *et al.*, 2008; Ribeca *et al.*, 2014).

The effectiveness of the utilization genetic markers in a breeding program depends on its polymorphism and its association with productivity parameters (e.g. birth weight, weaning weight, carcass weight, growth rate, etc.). Anwar *et al.* (2015) reported three genotypes of the GH gene in the SO cattle based on PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) and sequencing methods. Unfortunately, there was no information available yet about relationship of the GH gene with growth and dressing percentage in the SO cattle. It is important to determine the genotypes of the GH gene that associated with high growth parameters and high dressing percentage in the SO cattle to reach the breeding program objectives. This study was conducted to identify the GH gene polymorphism using PCR-RFLP method and also to evaluate its associations with growth parameters and dressing percentage in the SO cattle.

MATERIALS AND METHODS

Blood Samples and DNA Extraction

A total of 267 heads of SO cattle from the Sumba island, East Nusa Tenggara Province, Indonesia were used for blood sampling purpose. Blood samples (3-5 mL) were taken from coccygeal vein using *Venoject* and collected in *Vaccutainer* tubes containing anticoagulant. The blood samples were used in the DNA extraction process using the Genomic DNA Mini kit (Geneaid Biotech Ltd., Taiwan) following the producer's method.

Performance Data

The SO cattle growth parameters data (n=44) were obtained from individuals SO cattle belonged to PT. KAR Farm, Rumpin, Bogor including birth weight (BW), weaning weight at 205 days of age (WW_{205}), and yearling weight at 365 days of age (YW_{365}) data. Otherwise, the

dressing percentage (DP) data (n=122) were obtained from two slaughterhouses i.e. RPH Karawaci, Banten Province (owned by PT. KAR) and RPH East Sumba, East Nusa Tenggara Province (owned by local Government).

PCR-RFLP Analysis

One of methods that can be used in detecting the variation at the DNA level was PCR-RFLP, which is a further analysis method for the specific amplified DNA fragment. This method utilizes a specific restriction enzyme to provide information about the DNA fragment mutation based on the specific cutting sites. The primer in this study was based on Anwar *et al.* (2015). The PCR reagents composition were as follows: KAPA2G Ready Mix PCR Kit (Kapa Biosystems, Cape Town, South Africa), forward and reverse primers (200 ng/µL), DNA samples (5-50 ng/µL), and ddH_2O up to 12 µL final volume. The PCR program is set as follows : denaturation at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 45 seconds, extension at 72°C for 45 seconds; with a final extension at 72°C for 5 minutes on Mastercycler® gradient (Eppendorf, Hamburg, Germany). The restriction enzyme (*MspI*) was used to detect the GH gene variation in the RFLP method. The PCR and RFLP products was visualized using 1% agarose gel (PCR product) or 3% agarose gel (RFLP product) and followed by SyBr® staining and captured in *GBOX* documentation System (Syngene, UK).

Data Analysis

Allele Frequency. Individual cattle genotype in this study was determined based on the differences in the number and size of the bands that showed in the RFLP result. The frequency of the genotype and allele were calculated based on Nei and Kumar (2000) formula with the following statistical model: $\chi_{ii} = (n_{ii}/N)$ for genotype frequency and $\chi_i = (2n_{ii} + \Sigma n_{ij})/(2N)$ for allele frequency, where : χ_{ii} = frequency of ii^{th} genotype; χ_i = frequency of i^{th} allele; n_{ii} = number of individuals with ii genotype; n_{ij} = number of individuals with ij genotype; N = number of samples.

Heterozygosity. The GH gene data was processed using CONVERT ver. 1.3.1 (Glaubitz, 2004), CERVUS ver. 3.0.7 (Kalinowski *et al.*, 2007), and POPGENE ver. 1:32 (Yeh and Boyle, 1997) programs. The CONVERT program was used for conversion of the genotypes were observed for each individual samples to assure suitability for further analysis by POPGENE and CERVUS program. Data of the heterozygosity in the SO cattle population and the polymorphism information content (PIC) value were obtained from POPGENE ver. 1:32 and CERVUS ver. 3.0.7 analysis result.

Statistical Analysis. Analysis of variance (ANOVA) on the genotype data with the growth parameters (birth weight, weaning weight, yearling weight) and dressing percentage was performed using the general linear model (GLM) implemented in Minitab v.14 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Amplification and Polymorphism Identification

The GH gene fragment (about 1072 base pairs) in this study was successfully amplified. According to Anwar *et al.* (2015), the GH gene fragment in this study consist of a portion of the exon 2 up to exon 5. Identification of the GH gene variation in the SO cattle was performed by PCR-RFLP using *MspI* restriction enzyme (Biolabs Inc., New England) with restriction site is C‖CGG (temperature and incubation time following the producer's guidelines). Result of the GH gene polymorphism based on PCR-RFLP is shown in Figure 1.

PCR-RFLP method with *MspI* enzyme resulting three genotypes (AA, BB, and AB) in the SO cattle GH gene that similar with genotypes reported in Anwar *et al.* (2015). In addition, polymorphism in the GH gene in this study was caused by two mutations located in the intron region. Anwar *et al.* (2015) reported that there are two SNPs were found in the SO cattle GH gene i.e. a T>C substitution at position 1047 (intron 3) and cytosine insertion at position 1395 (intron 4). Furthermore, the GH gene alleles can be identified based on the g.1047T>C SNP that occurs only in the B allele samples. In consequence, the GH gene polymorphism in this study was intron regions polymorphism, especially intron 3. The mutations in intron 3 were also reported in other *Bos indicus* breeds e.g. Aceh cattle breed (Putra *et al.*, 2014) and Kenana and Butane cattle breed (Musa *et al.*, 2013).

Gene Frequency

Based on the GH gene polymorphism in this

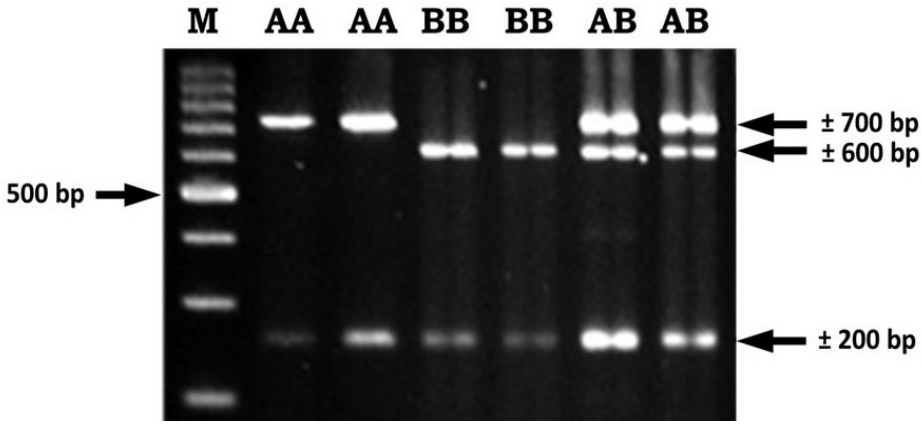

Figure 1. Visualisation of the GH Genotypes. M=DNA *Ladder* 100 bp; AA=Genotype AA; BB=Genotype BB; AB=Genotype AB.

study, the allele and genotype frequency were calculated and presented in Table 1. This is the first report for the GH gene and genotype frequencies in the Indonesian SO cattle.

The highest genotype frequency in the SO cattle is AA and the lowest is BB. Similarly, the A allele frequency was higher than B allele frequency in the SO cattle population. The observed heterozygosity (H_o) and the expected heterozygosity (H_e) values can be used to estimate the inbreeding value in a herd (Hartl and Clark, 1997). Generally, the He value is an indicator for the diversity of the population and can be used to assist the selection program for next generation (Moiloi *et al.*, 2004; Marson *et al.*, 2005).

The H_o value of the GH gene in this study is 0.23 that was equal with the H_e value which indicated that the genetic diversity in the SO cattle GH gene is quite low. The low heterozygosity values can be caused by several factors, including null alleles, assortative mating, the Wahlund effect, selection against heterozygotes, inbreeding, or a combination of all these factors (Cervini *et al.*, 2006). In addition, the low value of heterozygosity indicates that certain breeds are relatively well-conserved (Czerneková *et al.*, 2006). The low diversity of GH gene in the SO cattle in this study might also cause by the samples were used. The SO cattle samples that used in this study were obtained from the Sumba island in East Nusa Tenggara Province, Indonesia. The Sumba island was used as a breeding center for SO cattle in Indonesia since 1914 (Ministry of Agriculture of the Republic of Indonesia, 2014). Therefore, limitation number of sires and selection process may have happened in the SO cattle population. The PIC value (0.20) is also an indication of the low level of GH gene diversity in the SO cattle populations, it can be a parameter that emphasizing that the GH gene in the SO cattle is not informative for genetic diversity study.

Growth Parameters and Carcass Percentage

Descriptive statistics for the investigated traits in the SO cattle categorized based on the GH gene is presented in Table 2 and Table 3. The growth parameters were observed in 44 heads of SO cattle that belonged to PT. KAR farm, Bogor. They were fed with the same diet, according to the energy recommendations for calves, and they had free access to water. The BW and WW_{205} parameter seems to be higher in the BB genotype individuals than in the AA and AB genotype individuals (Table 2). Otherwise, the BB genotype individuals were low in yearling weight (YW_{205}) than AA and AB genotype individuals. Based on the statistical analysis, there is no association ($P>0.05$) in BW, WW_{205}, and YW_{365} with the GH gene. Moreover, no clear relationships ($P>0.05$) between DP and the GH gene. Therefore, the GH gene was found in this study cannot be used as a genetic marker in the SO cattle for breeding program based on the BW, WW_{205}, YW_{365} and DP parameters. In other *Bos indicus* breed e.g. Brahman cattle, Beauchemin *et al.* (2006) reported that polymorphism of the GH gene was also has no association with growth and dressing percentage. However, the GH gene polymorphism closely associated with growth and slaughter weight in *Bos indicus* crossbred (Pereira *et al.*, 2005; Curi *et al.*, 2006). Information about

Table 1. The GH Gene Allele and Genotype Frequency in the SO Cattle

Genotype Frequency			Allele Frequency		H_e	H_o	PIC
AA	AB	BB	A	B			
0.76	0.22	0.02	0.87	0.13	0.23	0.23	0.20

H_e: Expected Heterozygosity; H_o: Observed Heterozygosity; PIC: Polymorphism Information Content

Table 2. Descriptive Statistics for the SO Cattle Growth Parameters

Genotype	n	BW (kg)	WW_{205} (kg)	YW_{365} (kg)
		.....Mean ± SD.....		
AA	35	25.42 ± 6.66	73.24 ± 25.93	192.51 ± 46.78
AB	7	21.36 ± 5.23	77.90 ± 44.40	169.20 ± 28.40
BB	2	31.70 ± 5.23	81.10 ± 17.10	123.22 ± 5.97

BW=Birth Weight; WW_{205}=Weaning Weight at 205 days of age; YW_{365}=Yearling Weight at 365 days of age; SD=standard deviation.

Table 3. Descriptive Statistics for the SO Cattle Dressing Percentage

Genotype	DP^1 (%)		DP^2 (%)	
	n	Mean ± SD	n	Mean ± SD
AA	70	52.40 ± 2.14	34	52.63 ± 5.89
AB	11	51.73 ± 2.47	7	54.46 ± 6.15

[1]=From PT. KAR Farm, Bogor; [2]=From Sumba island, East Nusa Tenggara; DP=Dressing Percentage; SD=Standard Deviation

dressing percentage in the SO cattle obtained in this study was in agreement with Agung *et al.* (2015) that reported the potency of the SO cattle as the beef cattle breed considering the high dressing percentage (>50%) for both the SO cattle in their natural habitat in the Sumba island, and also the SO cattle in the Java island.

CONCLUSION

It can be concluded that the GH gene (intron 3) in the SO cattle population was polymorphic with two alleles (A and B), but not related with growth parameters (birth weight, weaning weight at 205 day of age, and yearling weight at 365 days of age) as well as with the dressing percentage.

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