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Growth hormone genotyping by *Msp*I restriction enzyme and PCR-RFLP method in Aceh cattle breed at Indrapuri District, Aceh Province, Indonesia

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ABSTRACT

Putra WPB, Hartatik T, Sumadi. 2014. Growth hormone genotyping by Msp*I restriction enzyme and PCR-RFLP method in Aceh cattle breed at Indrapuri District, Aceh Province, Indonesia. Biodiversitas 15: 1-5.* The objective of this research was to identify growth hormone (GH) gene in Aceh cattle at Indrapuri's Breeding and Forage Centre (IBFC) of Aceh Cattle. Forty one cattle consisting of 21 male and 20 female cattle were used in this study. Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP) and sequencing method was used to detect *Msp*I site on GH gene. Based on the sequencing, it can be concluded that all cattle were monomorphic. The frequency of TT genotype and T allele were 1.00 relatively. The transition of C (*cytosine*) into T (*thymine*) on 1549 position caused the lost of restriction site. The insertion of T and G (*guanine*) on 1542 and 1552 position caused the length of GH gene were 329 bp in Aceh cattle.

Key words: Aceh cattle, GH gene, PCR-RFLP, sequencing, *Msp*I restriction enzyme

INTRODUCTION

Aceh cattle were one of Indonesian beef cattle suitable to be bred in Indonesia. This type of cattle adapts well to Indonesian environment, tropical climate, and is able to live on local water and food (Sari et al. 2010). Aceh cattle are able to adapt well, but its productivity is still lower than that of the imported one. By improving the productivity of Indonesian local cattle, it is hoped that the breeders' (especially IBFC of Aceh cattle) interest inbreeding local cattle will increase, so that the population of local cattle is increasing and is able to reduce Indonesian dependency on beef and cattle from other countries.

The GH gene is a single peptide of molecular weight equal to 22-kD secreted from *pituitary* gland in circadian and pulsatile manner, the pattern of which plays important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (Dybus et al. 2002). GH gene with its functional and positional potential has been widely used for marker in several livestock species including the Indonesian local cattle (Jakaria et al.. 2009; Sutarno 2010). Molecular genetic markers in animal breeding programs could make selection precise and efficient. Some of these markers are called candidate gene, e.g. the growth hormone genes, which are usually selected because there are biological significance on their quantitative traits of interest.

The studies of GH gene *Msp*I locus of Indonesian beef cattle have been reported in Ongole crossbred cattle (Sutarno et al. 2005; Sutarno 2010), Madura cattle (Purwoko et al. 2003), Pesisir cattle (Jakaria et al. 2007), Bali cattle (Jakaria et al. 2009) and Grati dairy cows (Maylinda 2011). Their studies indicated that polymorphism was found on Ongole crossbred cattle, Madura cattle, Pesisir cattle and Grati dairy cows. Bali cattle was monomorphic and the frequency of TT genotype and T allele were 1.00 the same as Ongole cattle in India (Lagziel et al. 2000). The research of GH gene using *Msp*I restriction enzyme in Aceh cattle has never been reported. Based on description above it is necessary to carry out a research to identify the genotype of GH gene *Msp*I in selected Aceh cattle at IBFC of Aceh cattle.

MATERIALS AND METHODS

Blood sample

The genomic DNA was extracted from blood using Sambrook et al. (1989). The blood samples were taken from 41 cattle at IBFC of Aceh cattle. The blood sample from Aceh cattle was taken using *venoject* (Vacutainer®, USA) 5 ml on jugular vein, and then it was kept in the refrigerator (4 ^{0}C) for later laboratory analysis. The DNA isolation, extraction, amplification and digestion were all carried out in Laboratory of Animal Breeding, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta.

Figure 1. The profile of locally Aceh cattle breed (photo: Bahrul Mahdi)

Genome DNA extraction

Genome DNA extraction was carried out using Sambrook et al. (1989) method which was modified using buffer lysis cell on each sample containing of 15 L 10% SDS (Sodium Dodecyl Sulphate), 6 L ddH_2O and 7.5 L proteinase-K. The DNA was precipitated using 71 L 5 M NaCl and 600 L 96 % ethanol. The precipitate was washed three times by adding 1ml 70 % ethanol, centrifuged with the speed 12,000 rpm for 5 minutes. Then the DNA precipitate was dissolved in 100 L ddH_2O. The quality of the total genome was analyzed using by 0.8 % agarose gels electrophoresis in 1 x TBE (Tris-Boric-EDTA). Agarose gels were made by weighing 0.10 g agarose powder and placed into 12.5 ml buffer 1x TBE. Loading samples were done by dropping 1 L DNA genome, 1 L loading dye (Fermentas) and 5 L ddH_2O into each well of agarose gel to run the electrophoresis processed. The products of electrophoresis were immersed in the 0.1 L ethidium bromide (EtBr) at 50 Volt for 15 minutes

DNA amplification

The DNA was amplified with Polymerase Chain Reaction (PCR). Each PCR reaction was made with the volume of 10 L with the composition of 5 L PCR master mix (KAPA2G Fast ReadyMix PCR Kits, USA); 0.5 L primer forward and reverse; 0.5 L DNA and 3.5 L ddH_2O. The forward primer (GH*Msp*I-F) was 5'-CCCACGGGCAAGAATGAGGC-3' and reverse primer (GH*Msp*I-R) was 5'-TGAGGAACTGCAGGGGCCCA-3' (Mitra et al. 1995). The position of both primers for GH gene is showed in Figure 1. The PCR protocols to amplify the fragment were done by the initial denaturation temperature steps at 94 ^{0}C for 5 minutes for 1 cycle, followed by 30 cycles of denaturation at 94 ^{0}C for 1 minute, annealing at 60 ^{0}C for 50 seconds, elongation or extension at 72 ^{0}C at 1 minute and a final extension at 72 ^{0}C at 5 minutes (Zhou et al. 2005). The PCR products were then subsequently electrophorezed using 1 % agarose gels in buffer 1x TBE. Agarose gels were made by weighing 0.10 g agarose powder and placed into 12.5 ml buffer 1x TBE. Loading samples were done by dropping 1 L PCR product, 1 L loading dye and 5 L ddH_2O into each well of agarose gel and into control well of DNA ladder (ΦX174 DNA/BsuRI/HaeIII) to run the electrophoresis process. The products of electrophoresis were immersed in the 0.3 L EtBr at 50 Volt for 15 minutes to identify the length of the band. The picture of DNA band products were visually taken on the UV transiluminator (UVP TEM-40, USA) using camera and compared with DNA ladder (marker) for allele and genotype identified.

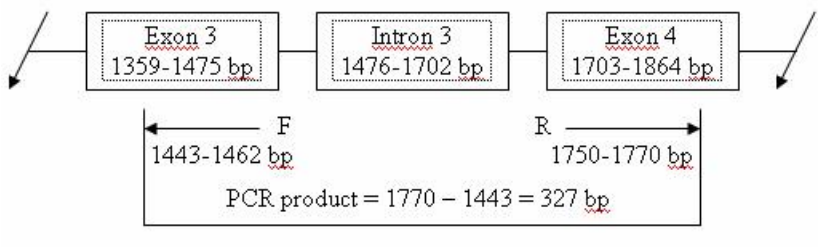

Figure 2. The position of Forward and Reverse primer in PCR product of GH gene

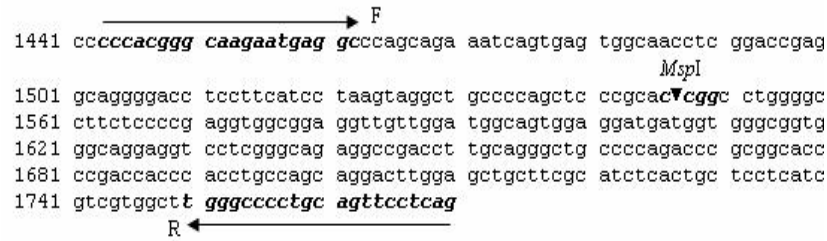

Figure 3. Position of Forward and Reverse primer based on GenBank (accessed code M57764) and position of *Msp*I restriction site

Genotyping for GH gene

The GH gene fragments were digested by the PCR-RFLP method with *Msp*I restriction enzyme. The PCR product of GH gene was digested 37 ^{0}C for 3 hours by *Msp*I restriction enzyme. Reactants consisted of 1.2 L Buffer 10x; 8.7 L ddH_2O; 0.1 L *Msp*I restriction enzyme and 2 L PCR product. A 1.5 % agarose gels were made by weighing 0.20 g agarose powder and placed into 13 ml buffer 1x TBE. Loading samples were done by dropping 10 L PCR product of digested, DNA mixed with 2.5 L loading dye into each well of agarose gel and into control well of marker to run the electrophoresis processed. The products of electrophoresis were immersed in the 0.2 L EtBr at 50 Volt for 15 minutes to identify polymorphism of alleles based on the length of the band. The picture of DNA band products was visually taken on the UV transiluminator using camera and compared with marker for allele and genotyped identification. The *Msp*I restriction enzyme recognized only the restriction site of four nucleotides for C↓CGG (Figure 2). The CC genotype consisted of two bands (104 bp and 223 bp), CT genotype consisted of three bands (104 bp, 223 bp, 327 bp) and TT genotype consisted of one band (327 bp).

Sequencing of GH gene fragment

The PCR products of five cattle were used for sequence analyses. Each reactant consisted of 30 L PCR product; 10 L forward and 10 L reverse primer (10 pmol/UI) repaired for sequencing process by Macrogen-BioSM Indonesia. Sequences of GH *Msp*I gene were used to find nucleotide mutation in that's fragments.

Data analysis

PCR-RFLP data were analyzed by allele frequency (Falconer and Mackay 1996). The allele frequency was calculated by counting method as:

$$p = \frac{2(CC)+(CT)}{2N} \text{ and } q = \frac{2(TT)+(CT)}{2N}$$

Where p is the C allele frequency, q is the T allele frequency and N is the total number of cattle tested. The sequencing results were paralleled with the GH gene sequences from GenBank accessed code M57764, JN232516, EF592533 and EF592534 by alignment software (BioEdit and ClustalW).

RESULTS AND DISCUSSION

Allele frequency of GH *Msp*I gene

The DNA restriction of fragments resulted in one genotype. Sequencing and genotyping of GH *Msp*I gene showed 329 bp for TT genotype in all samples (Figure 3). Study of GH *Msp*I gene in several worlds' cattle reported that the frequency of T allele was higher in *Bos indicus* (hump) cattle group and lower in the *Bos taurus* (humpless) cattle group (Lagziel et al. 2000). The same research results were also obtained that the frequency of T allele 1,00 in Bali cattle (Jakaria et al. 2009) and Ongole cattle (Lagziel et al. 2000). The comparison of T allele and TT genotype frequency which used same primer (Mitra et al. 1995) to other breed cattle in Indonesia is shown in Table 1.

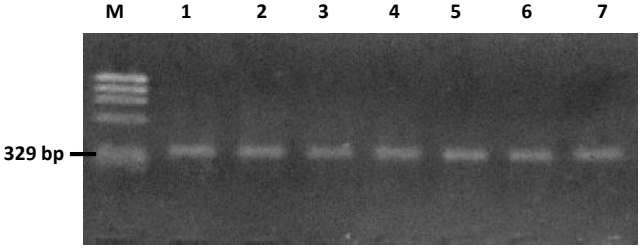

Figure 4. Genotyping result of GH *Msp*I gene detected by 1.5 % agarose gel. M : marker (ΦX174 DNA/BsuRI/HaeIII), 1 : PCR product (329 bp), 2-7 : TT genotype (329 bp)

Sequencing for GH *Msp*I Gene

The samples which could be sequenced were five samples from IBFC of Aceh cattle. The failure of sequence was caused by unsuccessful amplification, limited number of DNA, and too many peak duplication on sequence graphic. The analysis on the diversity of nucleotide

Table 1. The T allele frequency of GH gene in Indonesian beef cattle breeds

Cattle breeds	N	Frequency					Breed type	Authors
		Genotype			Allele			
		CC	CT	TT	C	T		
Bali	47	0.00	0.00	1.00	0.00	1.00	humpless	Jakaria et al. (2009)
Limousine	22	0.41	0.45	0.14	0.64	0.36	humpless	Jakaria et al. (2009)
Simmental	18	0.77	0.23	0.00	0.89	0.11	humpless	Jakaria et al. (2009)
Grati	43	0.16	0.35	0.49	0.34	0.66	humpless	Maylinda (2011)
Pesisir	133	0.05	0.30	0.65	0.20	0.80	hump	Jakaria et al. (2007)
PO	114	0.43	0.50	0.07	0.74	0.26	hump	Sutarno et al. (2005)
Madura	49	0.23	0.22	0.55	0.44	0.56	hump	Purwoko et al. (2003)
Aceh	41	0.00	0.00	1.00	0.00	1.00	hump	Research result

Note: N : individual number.

Table 2. The variation of Single Nucleotide Polymorphism (SNP) which has been identified in the third intron region from GH gene on several cattle

Position	Single Nucleotide Polymorphism (SNP)					Amino acid changes	Type of mutation
	Bos taurus [1]	Vechur [2]	Butana [3]	Kenana [4]	Aceh [5]		
1542	-	T	T	T	T	CCC(*Pro*) > CTC(*Leu*)	Insertion
1549	C	T	T	T	T	CCG(*Pro*) > CTG(*Leu*)	Transition
1552	-	G	G	G	G	GCC(*Ala*) > GGG(*Gly*)	Insertion
1615	G	G	G	-	G	GGC(*Gly*) > GGG(*Gly*)	Deletion
1669	G	A	A	A	A	CAG(*Gln*) > CAA(*Gln*)	Transition
1670	A	G	G	G	G	ACC(*Thr*) > GCC(*Ala*)	Transition
1695	C	T	T	T	T	ACC(*Thr*) > ATC(*Ile*)	Transition
1697	T	T	T	-	T	TGC(*Cys*) > GCC(*Ala*)	Deletion

Note: 1 = GenBank (M57764); 2 = GenBank (JN232516); 3 = GenBank (EF592534); 4 = GenBank (EF592534); 5 = sequencing results; *Pro* = proline; *Leu* = leucine; *Ala* = alanine; *Gly* = glycine; Gln = glutamine; *Thr* = threonine; *Ile* = isoleucine; *Cys* = cysteine

sequence was conducted using alignment software. The sequence of Aceh cattle DNA was parallel with the sample comparison sequence from GenBank accessed code M57764, JN232516, EF592533 and EF592534.

The result of GH *Msp*I gene nucleotide sequence in Aceh cattle, the change from C to T nucleotide was found on position 1549 bp and 1695 bp. The transition of C into T on position 1549 changed the *Msp*I restriction site. This result is similar to that of Musa et al. (2013) on Kenana cattle and Butana cattle. Transition mutation of T into C on 1549 position was found in Iranian native cattle such as Mazandarani, Golpaygani and Sarabi (Zakizadeh et al. 2006). Based on this sequence results it was concluded that several mutation found in this region. The variation of Single Nucleotide Polymorphism (SNP) which has been identified in the third intron region from GH gene on several cattle showed in Table 2. Sari et al. (2011) reported that one new mutation on fifth exon on 2230 bp in which C nucleotide turned into T nucleotide, and this was called silent mutation (CTC/*Leu* > CTT/*Leu*). Based on the sequencing result, it was concluded that the sequence of Aceh cattle on third intron was similar to that of *Bos indicus* groups (Vechur, Butana and Kenana).

CONCLUSION

Based on our research results, it can be concluded that the T allele was common allele in Aceh cattle at IBFC of Aceh cattle. The GH *Msp*I gene was monomorphic in Aceh cattle. The mutations occurred between C (*cytosine*) to T (*thymine*) on 1549 position changed *Msp*I restriction site. The GH *Msp*I gene nucleotide sequence of Aceh cattle on third intron region was similar to that of *Bos indicus* based on the GenBank sequence.

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