

GROWTH HORMONE GENE GENOTYPING BY Msp I RESTRICTION ENZYME AND PCR-RFLP METHODS IN ACEH CATTLE BREED AT INDRAPURI DISTRICT OF ACEH PROVINCE

W.P.B. Putra, T. Hartatik and Sumadi

Faculty of Animal Science, Gadjah Mada University,
Jl. Fauna No.3 Bulaksumur, Sleman, Yogyakarta 55281- Indonesia
Corresponding E-mail: banchet_putra18@yahoo.co.id

Received October 16, 2013; Accepted November 27, 2013

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi genotip gen growth hormone (GH) pada sapi Aceh yang diseleksi di Balai Pembibitan Ternak Unggul - Hijauan Pakan Ternak (BPTU-HPT) Sapi Aceh Indrapuri. Empat puluh satu ekor sapi Aceh terdiri dari 21 jantan dan 20 betina digunakan dalam penelitian ini. Ekstraksi DNA genom menggunakan darah sesuai metode Sambrook *et al.* (1989). Analisis Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) dan sekuensing digunakan untuk melihat situs restriksi MspI pada gen GH. Berdasarkan hasil sekuensing diketahui bahwa semua sampel sapi Aceh bersifat monomorfisme. Frekuensi genotip TT dan alel T masing-masing sebesar 1,00. Mutasi transisi C (cytosine) menjadi T (thymine) pada posisi 1548 bp menyebabkan situs restriksi MspI tidak ditemukan.

Kata kunci: Sapi Aceh, gen GH, PCR-RFLP, sekuensing, enzim restriksi MspI

ABSTRACT

The objective of this research was to identify growth hormone (GH) genes genotype in selected Aceh cattle at Indrapuri's Breeding and Forage Centre (IBFC) of Aceh Cattle. Fourty one cattle consisting of 21 male and 20 female cattle were used in this study. The genomic DNA was extracted from blood using Sambrook *et al.* (1989) methods. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) and mehod of sequencing was used to detect MspI site on GH gene. Based on sequencing results, it can be concluded that all cattle were monomorphism. The frequency of TT genotype were 1.00 and same as T allele frequency. The transition of C (cytosine) into T (thymine) on 1548 bp caused the lost of restriction site.

Keywords: Aceh cattle, GH gene, PCR-RFLP, sequencing, MspI restriction enzyme

INTRODUCTION

Aceh cattle is one of Indonesian beef cattle which is suitable to be bred in Indonesia. This type of cattle adapts well to Indonesian environment, tropical climate, and able to live feedstuff (Sari *et al.*, 2010). Even though Aceh cattle is able to adapt well, their productivity is still lower than other Indonesian local cattle. By improving the productivity of Indonesian local cattle, it is hoped that the breeders interest to breed local cattle will increase, so that the population of local cattle will increase and able to reduce Indonesian dependency on beef and cattle from other countries. The Indrapuri's Breeding and Forage Centre (IBFC) of Aceh cattle is a

breeding station for Aceh cattle that was established since 2002. All Aceh cattle in this station is selected especially for producing elite bull, cow and heifer.

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 of their biological significance on their quantitative traits of interest.

The study of GH gene *MspI* 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 were monomorphic and the frequency of TT genotype and T allele were 1,00 as same as Ongole cattle in India (Lagziel *et al.*, 2000). The research of GH gene *MspI* have never been reported in Aceh cattle. Based on description above it is necessary to carry out a research to identify the genotype of GH gene *MspI* in selected Aceh cattle at IBFC of Aceh cattle.

MATERIALS AND METHODS

Blood Sample

The total number of blood samples were taken from 40 cattle at the breeding station of Aceh cattle. The blood sample was taken using *venoject* (EDTA) 5 ml on jugular vein, and then was kept in refrigerator (4°C) for later laboratory analysis. The DNA isolation, extraction, amplification and digestion were all carried out in Laboratory of Animal Breeding, Faculty of Animal Sciences, Gadjah Mada University, Yogyakarta.

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 µl 10% SDS, 6 µl aquabidest and 7.5 µl proteinase-K. The DNA was precipitated using 71 µl 5 M NaCl and 600 µl 96% ethanol. The precipitate was washed three times by adding 1ml 70% ethanol, then was centrifuged with the speed 12,000 rpm for 5 minutes. Then the DNA precipitate was dissolved in 100 µl ddH₂O. The quality of the total genome was analyzed using 0.8% agarose gels electrophoresis (50 Volt, 15 minutes) in 1 x TBE and 10% ethidium bromide.

DNA Amplification

The DNA was amplified with Polymerase Chain Reaction (PCR). Each PCR reaction was made with the volume of 10 µl with the composition of 5 µl PCR master mix (KAPA, China); 0.5 µl primer forward and reverse; 0.5 µl DNA and 3.5 µl ddH₂O. The forward primer was 5'-CCCACGGGCAAGAATGAGGC-3' and reverse primer was 5'-GAGGAACTGCAGGGGCCA-3' (Mitra *et al.*, 1995). The position of primer forward and reverse in PCR product (327 bp) of GH gene are shown in Figure 1. The PCR protocols to amplify the fragment were done by the initial denaturation temperature step at 94 °C for 5 minutes for 1 cycle, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 50 seconds, elongation or extension at 72°C at 1 minute and a final extension at 72°C at 5 minutes (Zhou *et al.*, 2005). The PCR product were separated by horizontal electrophoresis (50 Volt, 15 minutes) in 1% agarose gels in 1 x TBE and 10 % ethidium bromide.

Genotyping for GH Gene

The GH gene were analyzed by using the Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) method. The PCR product of GH gene was

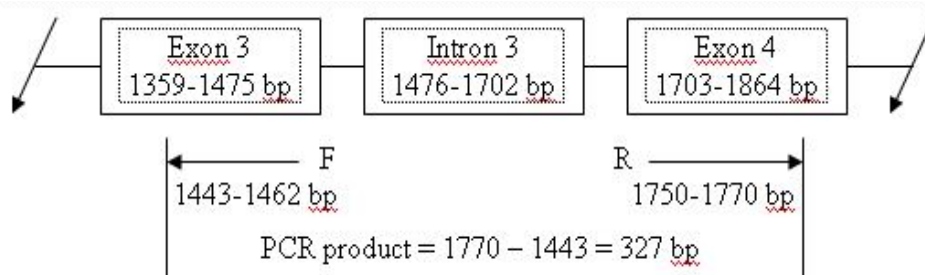


Figure 1. The Position of Forward and Reverse Primer in PCR Product of GH Gene

digested 37°C for 3 hours by *MspI* restriction enzyme. Reaction consisted of 1.2 µl Buffer V2 10x; 8.7 µl ddH₂O; 0.1 µl *MspI* restriction enzyme and 2 µl PCR product. Following the end of RFLP process, the products were then subsequently electrophorated (50 Volts, 30 minutes) using 1.5% agarose gel to identify polymorphism of allele. The *MspI* restriction enzyme recognized only the restriction site of four nucleotides for C↓CGG (Figure 2). The CC genotype consisted of two bands (105 bp and 223 bp), CT genotype consisted of three bands (105 bp, 223 bp, 328 bp), and TT genotype consisted of one band (328 bp).

Sequences of GH Gene Fragment

The PCR product of five cattle was used to sequence the GH gene fragment. Each reaction consisted of 30 µl PCR product; 10 µl forward and 10 µl reverse primer (10 pmol/µl) repaired for sequencing process by Macrogen-BioSM Indonesia. Sequences of GH *MspI* gene was used to find nucleotide mutation in that fragment. The sequences of GH *MspI* gene was carry out in individual homozygote CC and TT.

Data Analysis

PCR-RFLP data was 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 sequences was paralleled with the GH gene sequences from GenBank accessed code

M57764 by alignment software (BioEdit and ClustalW).

RESULTS AND DISCUSSION

Allele Frequency of GH *MspI* Gene

The DNA restriction fragments result was one genotype. Genotype of GH *MspI* gene showed 328 bp for TT genotype in all samples (Figure 3). Study of GH *MspI* 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 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 T allele and TT genotype frequency of this research and other breed cattle in Indonesia are presented in Table 1.

Sequencing for GH *MspI* Gene

The samples managed to be sequenced were five samples from IBFC of Aceh cattle. The failure of sequencing was caused by unsuccessful amplification, limited number of DNA, and too many peak duplication on sequence graphic. The analysis on the diversity of nucleotide sequence was conducted using alignment program (BioEdit and ClustalW) after the sequence of Aceh cattle DNA was compared to the sequence of GenBank (accessed code M57764).

The change from C to T nucleotide in GH *MspI* gene nucleotide sequence in Aceh cattle was found on position 1548 bp and 1695 bp. The transition of C into T on position 1548 changed the *MspI* restriction site. This result is similar to that of Musa *et al.* (2013) on Kenana cattle and

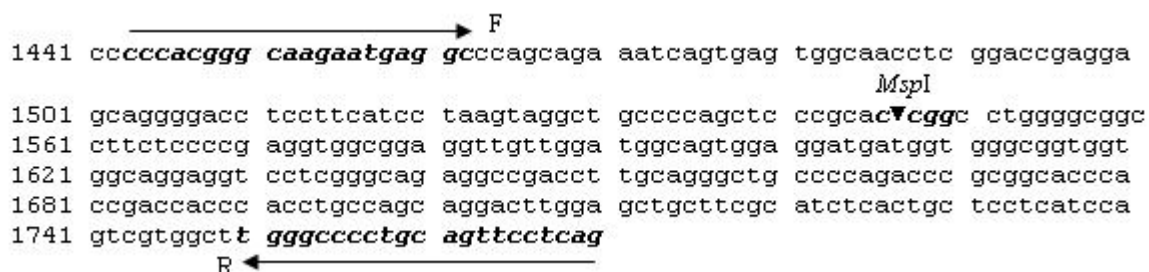


Figure 2. Position of Forward and Reverse Primer Based on GenBank (accessed code M57764) and Position of *MspI* Restriction Site

Table 1. The T allele Frequency in Aceh Cattle and Other Beef Cattle

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 <i>et al.</i> , 2009
Limousine	22	0.41	0.45	0.14	0.64	0.36	humpless	Jakaria <i>et al.</i> , 2009
Simmental	18	0.77	0.23	0.00	0.89	0.11	humpless	Jakaria <i>et al.</i> , 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 <i>et al.</i> , 2007
Ongole Grade	114	0.43	0.50	0.07	0.74	0.26	hump	Sutarno <i>et al.</i> , 2005
Madura	49	0.23	0.22	0.55	0.44	0.56	hump	Purwoko <i>et al.</i> , 2003
Aceh	41	0.00	0.00	1.00	0.00	1.00	hump	This research

N : the number of cattle tested

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)					Amono Acid Change	Type of mutation
	<i>Bos taurus</i> ¹	Vechur ²	Butana ³	Kenana ⁴	Aceh ⁵		
1541	-	T	T	T	T	CCC(<i>Pro</i>)>CTC(<i>Leu</i>)	Insertion
1548	C	T	T	T	T	CCG(<i>Pro</i>)>CTG(<i>Leu</i>)	Transition
1551	-	G	G	G	G	GCC(<i>Ala</i>)>GGG(<i>Gly</i>)	Insertion
1669	G	A	A	A	A	CAG(<i>Gln</i>)>CAA(<i>Gln</i>)	Transition
1670	A	G	G	G	G	ACC(<i>Thr</i>)>GCC(<i>Ala</i>)	Transition
1695	C	T	T	T	T	ACC(<i>Thr</i>)>ATC(<i>Ile</i>)	Transition
1697	T	T	T	-	T	TGC(<i>Cys</i>)>GCC(<i>Ala</i>)	Deletion

1 = GenBank (M57764); 2 = GenBank (JN232516); 3 = Genbank (EF592534); 4 = GenBank (EF592534); 5 = sequencing results; *Pro* = prolin; *Leu* = leusin; *Ala* = alanin; *Gly* = glisin; *Gln* : glutamin; *Thr* : threonin; *Ile* : isoleusin; *Cys* = sistein

Butana cattle. The transition mutation of T into C on 1547 position was found in Iranian native cattle such as Mazandarani, Golpaygani and Sarabi (Zakizadeh *et al.*, 2006). The length successful to sequence was from 1489 bp to 1772 bp (285 bp) on the third intron region. Based on this sequence results, it was concluded that several mutation found in this region. The variation of Single Nucleotide Polymorphism (SNP) which was identified in the third intron

region from GH gene on several cattle are shown in Table 2. Sari *et al.* (2011) reported 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 *Bos indicus* group (Vechur, Butana, Kenana).

CONCLUSIONS

The T allele was common allele in Aceh cattle at the breeding station of Aceh cattle. The GH *MspI* gene was monomorphic in Aceh cattle. The mutations occurred between C (*cytosine*) to T (*thymine*) on 1548 position. The GH *MspI* gene nucleotide sequence of Aceh cattle on third intron region was similar to *Bos indicus*, and was not similar to *Bos taurus* based on the GenBank sequence.

ACKNOWLEDGEMENTS

The authors would like to thank to all staff at the Breeding Station of Aceh cattle for their helping in sample collection. Thanks are due to all staff at Laboratory of Animal Breeding, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta for their help during the study and most particularly their skillful assistances in DNA analysis.

REFERENCES

- Dybus, A. 2002. Associations of growth hormone (GH) and prolactin (PRL) genes polymorphism with milk production traits in Polish Black and White cattle. *J. Anim. Sci.* 20(4): 203-212
- Falconer, D.S and T.F. Mackay. 1996. Introduction to Quantitative Genetics. 4th Ed. Department of Genetics. North Canada State University.
- Jakaria, D.D, R.R. Noor, and H. Martojo. 2007. Evaluasi Keragaman Genetik Gen Hormon Pertumbuhan (GH) pada Sapi Pesisir Sumatra Barat dengan Penciri PCR – RLFP. *J. Indonesian Trop. Anim. Agric.* 30 (2):1-10
- Jakaria, D.D., R.R. Noor, H. Martojo, D. Duryadi and B. Tappa. 2009. Identification of growth hormone (Gh) gene *MspI* and *AluI* loci polymorphism in beef cattle. Faculty of Animal Science, Bogor Agricultural University. Proceedings, the 1st International Seminar of Animal Industry, Bogor, Indonesia, November 23-24, 2009. P. 42-47.
- Lagziel, A., S. Danise, O. Hanotte, S. Dhara, V. Glazko, A. Broadhead, R. Davoli, V. Russo and M. Soller. 2000. Geographic and breed distribution of an *MspI* PCR-RFLP in the bovine growth hormone (bGH) gene. *Anim. Genet.* 31: 210-213.
- Maylinda, S. 2011. Genetic polymorphism of growth hormone locus and its associations with bodyweight in Grati dairy cows. *Int. J. Biotechnol. Mol. Biol. Res.* 2(7):117-120.
- Mitra, A.P Sciilee, C.R. Balakrisnan and F. Pirciiner. 1995. Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo. *J. Anim. Breed. Genet.* 112:71-74.
- Musa, L.M.A, M. Reissmann, I.A. Ishaq, S.A. Rahamtalla, G. Brockman and J.K. Peter. 2013. Characterization of the growth hormone gene (GH1) in Sudanese Kenana and Butana cattles. *J. Anim. Prod.* 3(2):28-34.
- Purwoko, A., Sutarno and N. Etikawati. 2003. DNA polymorphism at locus-2 growth hormone gene of Madura cattle growth. *Biodiversitas* 4 (1):7-11
- Sari, E.M., R.R. Noor, C. Sumantri and E. Trimargawati. 2010. Identification of genotype DNA microsatellite in association with performance of Indonesian Aceh cattle. *J. Genetic Eng. Biotechnol.* 8:43-51
- Sari, E.M., R.R. Noor, C. Sumantri, M. Yunus, J.L. Han and Muladno. 2011. Identification of Single Nucleotide Polymorphism on growth hormone gene in Aceh cattle. *Media Peternakan.* 36(1):21-25
- Sutarno, A. Junaidi and T. Baharudin. 2005. DNA polymorphism at locus-2 growth hormone gene of Madura cattle. *Biodiversitas.* 6(2):77-81
- Sutarno. 2010. Genetic variation among Indonesian native cattle breeds based on polymorphisms analysis in the growth hormone loci and mitochondrial DNA. *Biodiversitas.* 11(1): 1-5
- Zhou, G.L, H.G. Jin, C. Liu, S.L. Guo, Q. Zhu and Y.H. Wu. 2005. Association of genetic polymorphism in GH gene with milk production traits in Beijing Holstein cows. *J. Biocsi.* 30(5):595-598