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Full Length Research Paper

Diversity of growth hormone gene and its relation with average daily gain in Simmental cattle in West Sumatera Province, Indonesia

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This study was aimed to analyse the genetic polymorphism of Growth Hormone (GH) polymorphism of Simmental cattle using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and its relation to average daily gain. The research was conducted in the Padang Mangatas Breeding Centre, Limapuluh Kota district, West Sumatera Province and Biotechnology Laboratory of Faculty of Animal Husbandry, Andalas University. The research used 100 Simmental calves. DNA were isolation from blood sample using DNA purification Kit from Pomega. The PCR procedure was used to amplify 591-bp of bGH exon 1 (GH1) and 694-bp exon 2 (GH2). The PCR product were digested by restriction enzymes *Msp*I and *Alu*I. Digestion of 591-bp GH gen PCR product with enzyme restriction *Msp*I reveal allele A(+) and B(-) with frequency 0.875 and 0.125 respectively and digestion with restriction enzyme *Alu*I revealed allele C(+) and D(-) with frequency 0.95 and 0.05 respectively and. Digestion of 694-bp PCR product by *Msp*I represent allele P(+) and Q(-) with frequency 0.88 and 0.12 respectively and digestion with *Alu*I enzyme represent allele R(+) and S(-) with frequency 0.94 and 0.06 respectivly. The observed heterozygosity, effective allele numbers and polymorphism information content of GH1/MspI, GH2/MspI, GH1/*Alu*I, and GH2-*Alu*I were 0.11/0.1948, 0.04/0.1889, 0.00/0.0927, and 0.00/0,1096 respectively. Using GLM, there was no relation between these polymorphic and avarage daily gain of calve.

Key words: Simmental cattle, growth hormone (GH) gene, polymorphism, avarage daily gain growth.

INTRODUCTION

Growth traits are extremely important to animal husbandry. With the development of molecular biology and biotechnology, more accurate and efficient selection goal can be achieved through marker assisted selection (MAS). Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner (Ayuk and Sheppard, 2006), the pattern of which

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Table 1. Primers used for PCR analysis of GH gene.

Fragment	Primer sequence	Location	*Size* (bp)
GH1F	5'CCAGTGGTCCTTGCATAAATGT-3'	554/1144	591
GH1R	5'CTCGGGAGCTTACAAACTCTTT-3'		
GH2F	5'-ATGTCCTTGTCCGGCCTG-3'	1048/1741	694
GH2R	5'-CTGGATGAGGAGCAGTGAGAT-3'		

Table 2. Characteristics of restriction enzyme MspI and AluI.

Enzyme	Sequences identified	Intersection	t°C Incubation
*Msp*I	5'...C↓C G G...3'	976,1059,1084,1375,1438,1547	37°
*Alu*I	5'..A G↓C T… 3'	81,415,701,709,729,803,928,1105, 1137,1327,1538,1712	37°

plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction, as well as protein, lipid, and carbohydrate metabolism (Akers, 2006; Ayuk and Sheppard, 2006; Thidar et al., 2008; Musa et al., 2013). Effects of GH on growth are observed in several tissues, including bone, muscle and adipose tissue. Therefore is a great interest in using GH gene as a promising candidate for selection purposes in breeding program of animals (Grochowska et al., 2002). GH gene in cattle is located on chromosome 19 consisting an arranged 191 amino acids (Hediger et al., 1990; Schlee et al., 1994), it has 2856 bp of nucleotide and consists of five exons and four introns (Woychik et al., 1982; Gordon et al., 1983; Vukasinovic et al., 1999). The sequence variations in the *GH* gene are well documented (Lucy et al., 1993; Yao et al., 1996; Hetch and Geldermann, 1996; Lagziel et al., 2000; Lagziel et al., 1996; Ferraz et al., 2006; Yurnalis et al., 2013).

Relationships between several polymorphic sites in *GH1* and milk production traits have been much investigated, particularly, the Leu/Val polymorphism (at residue 127) in exon five and polymorphic *Msp*I restriction site (TC/G insertion/transition) in the third intron. In beef production, Oka et al. (2007) reported that the carcass weight of the Leu/Leu the Val/Val group, while Barendse et al. (2006) found the Val variant to be associated with lower marbling. It has been reported that the restriction fragment length polymorphisms (RFLPs) of GH-*Taq*I were associated with body weight at 7 and 13 months of age in Belgian White Blue bulls (Sneyers et al., 1994). Significant effects were found for bGH genotype on yearling weight, with positive effects associated with the LV (leucine/valine) genotype in the Canchim beef cattle (Pereira et al., 2005). Regarding the effects of the polymorphic *Msp*I restriction site, Hoj et al. (1993), Lagziel et al. (1996), Lee et al. (1993) and Falaki et al. (1996) found that the allele lacked a functional *Msp*I site (*Msp*I[-]) to be associated with higher fat and protein yield and percentage in different dairy cattle breeds. In contrast, Yao et al. (1996) found the *Msp*I [-] allele to be associated with a statistically significant decrease in milk, fat and protein yield. The present study was carried out to detect allelic variants of the GH gene in relation to growth traits in Simmental cattle breeds.

MATERIALS AND METHODS

Blood samples and DNA extraction

Blood samples were collected from 100 Simmental cattle from Padang Mangatas Breeding centre, Lima Puluh Kota distric, West Sumatera Province, Indonesia. DNA was extracted using DNA purification Kit from Promega, following manufacturer instructions. Two regions of the GH gene (591 and 694 bp) were amplified from bovine genomic DNA using two primer pairs that designed using online primer3 program base on GH gene sequence from GenBank access number M57764.1 (Table 1).

Both PCR reactions were performed in a 25 µl mixture containing 2 ul of 10 pmol each primers, 12.5 ul master mix from thermo scientific, 6.5 ul nuclease free water, and 2 ul of 50 ng genomic DNA as template. The PCR cycling conditions included an initial denaturation step of 94°C for 5 min followed by 94°C for 1 min, 58°C for 1 min and elongation at 72°C for 1 min. After 35 cycles, a final extension was given at 72°C for 5 min. Samples were held at 4°C until further use. To check fragment integrity PCR products were electrophoresed at 150 V in a 1.5% agarose gel containing 0.5 µg ethidium bromide/mL along with a DNA molecular size marker. The gels were visualized and documented with the Gel documentation system (Gel doc 1000, Bio-Rad, USA).

The 591 and 694 bp amplicon was treated using *Msp*I and *Alu*I restriction enzyme to identify polymorphisms at the GH gene. A volume of 20 mL of PCR product was digested with 5 U *Msp*I and *Alu*I enzyme and the digested product was separated through ethidium bromide staining in 2% of agarose gel (Table 2).

Genotypic frequencies of different PCR-RFLP patterns were estimated from the combinations of various alleles generated based on presence or absence of one or more restriction sites. Allelic frequencies were calculated from genotypic frequencies using standard methods. The mean expected heterozygosity and deviations from Hardy-Weinberg equilibrium were calculated. Chi-square test was carried out to evaluate allelic and genotypic frequency differences across the investigated cattle breeds.

Analysis of the data is used as follows.

Table 3. Mean and standard deviation of Post weaning live weight gain simmental heifers and steers in Animal Breeding Center and forage Padang Mengatas.

No.	Source	Number	Post weaning live weight gain (kg)
1	Steers	71	0.36± 0.15
2	Heifers	29	0.24± 0.11
Population		100	0.33± 0.15

Frequency of genotype

Genotype frequency was calculated based on the number of genotypes divided by the number of samples (N) with the equation as follows:

$$F_1 = \frac{\sum X_i}{N}$$

Xi = the observed genotype and N = the number of animals analyzed.

Frequency of allele

Alel frequency of GH gene is obtained from PCR analysis that was calculated by sum of all alels divided by twice the number of samples (2N):

$$F_1 = \frac{\sum X_i}{2N}$$

Xi = the observed allele and N = the number of animals analyzed.

Diversity of genetic (genetic variability)

Diversity of genetic was obtained by estimating the frequency of heterozygosity observations (Ho) and heterozygosity expectations (He) and calculated by using formula (Nei, 1987; Weir, 1996) as follows:

$$H_o = \sum_{i \neq j} \frac{N_{1\,ij}}{N}$$

H_o = the frequency of observations heterozygosity; N_{1ij} = number of individuals heterozygous at the locus to-I, and N = number of individual analyzed.

$$H_e = 1 - \sum_{1=i}^{n} P_{1i}^2$$

H_e = frequency of heterozygosity expectations; P_{1ij} = frequency of allele to-i on the locus to-1^t, and n = number of allel on locus to-i^t.

Polymorphic informative content (PIC)

Information level for an alel is calculated using a value approach of Polymorphic Informative Content (PIC) (Botstein et al., 1980). PIC value can also be used to determine whether there is a polymorphic alel aside from being based on the value of heterozigisitas.

$$PIC = 1 - \sum_{I=1}^{N} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_1^2 P_j^2$$

Pi = alel frequency to-I; n = number of allele on each marker.

Hardy - Weinberg equilibrium

Estimation of heterozygosity values is useful to get an idea of the genetic diversity of a livestock population (Marson et al., 2005). The balance of gene in Simmental cattle population (Hardy - Weinberg equilibrium) was tested using chi-square (χ^2) test (Hart and Clark, 1997) as follow:

$$\chi^2 = \sum \frac{(O_{IJ} - E_{IJ}).^2}{E_{ij}}$$

χ^2 = Chi-Squere test; O_{ij} = number of genotype observed to-i in group to-j; E_{ij} = number of genotype expectation i to group-j

The correlation of genotype fragment GH gene with post-weaning growth

Analysis of the correlation genotype of fragment GH gene with post-weaning growth of Simmental cattle in BPTU HPT Padang Mengatas was done by using *General Linear Model* as follow:

$$Y_{ij} = \mu + G_i + Hi + E_{ij}$$

Yij = Value Observation due to genetic influences to-I; μ = Mean common; Gi = Effect of sex I;
Hj = Effect of genotype to-I; Eijk = Effect of error experiment.

RESULTS AND DISCUSSION

Post weaning live weight gain

Mean and standard deviation of Post weaning live weight gain Simmental heifers and steers during the interval from 177 to 582 days of age are presented in Table 3.

Interactions between heifers and steers were significant sources of variation ($t < 0.05$), indicating heifers of the different sex studied responded not similarly. These results reflect the bull has higher than the cow. These results is lower than Suhada (2008) that claimed Post weaning live weight gain Simmental is 0.42 ± 0.10 kg. Sawyer et al. (1991) post weaning live weight gain Simmental heifer in southwest Australia could be 1.07 kg/day. Speer (2016) average of weight gains of

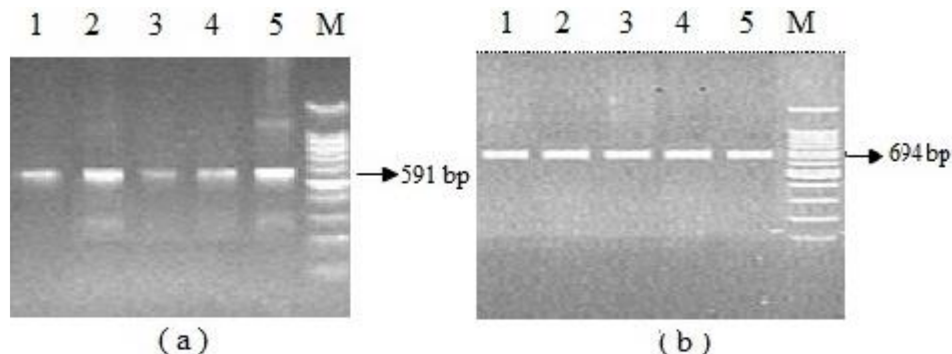

Figure 1. (a) Electrophoresis resulted of PCR product bGH with bGH-1; (b) Electrophoresis resulted of PCR product bGH with bGH-2. M: Marker (DNA ladder 100 bp), 1, 2, …etc = Number of sample.

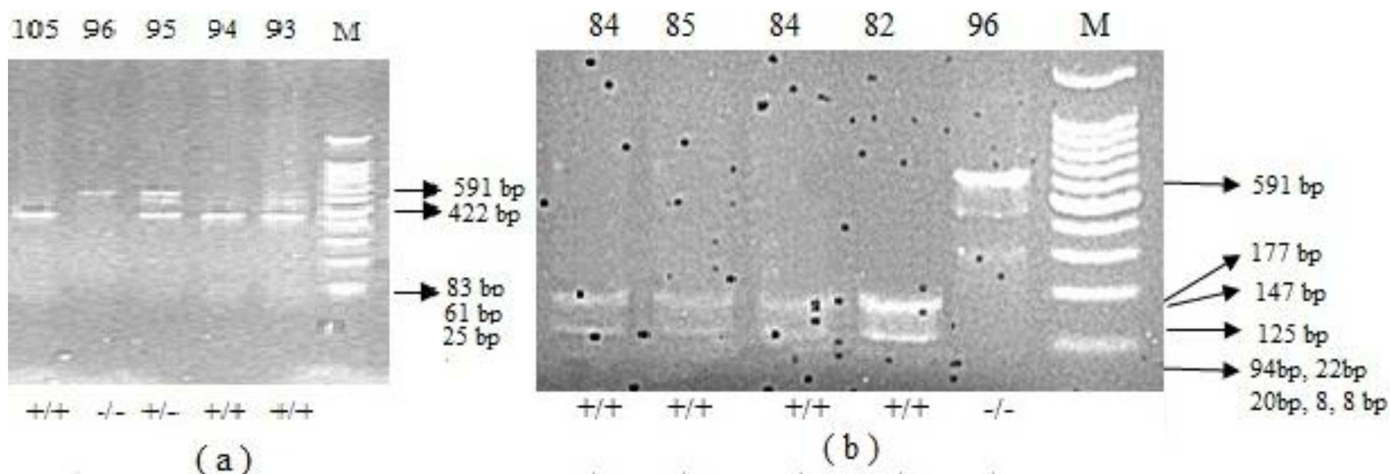

Figure 2. Different genotypes resulted from bGH-1 and bGH-2 with *Msp*I and *Alu*I endonuclease enzym restriction (a) b GH-1 *Msp*I (b) bGH-1 *Alu*I. M: marker (DNA ladder 100 bp), 93, 94,…etc = number of sample. +/+,+/-,-/- = genotype.

Simmental which treatments in feddlot for 1.51 kg/day.

These differences can be expected because of cattle adaptation capability to climate changing of Indonesia which is not optimal yet. Livestock production appearance can be affected by some factors, genetic, feed, management, eradication and prevention of disease and environment factors. Of the many possible influences operating to affect weaning weight the nutritional status of the calf is undoubtedly a most important one. The variation in weaning weight may be accounted for by differences in the milk production of the dams.

Genotyping genotype and allele frequency

The amplified bGH-1 resulted in a DNA fragment with 591 bp and bGH-2 resulted in a DNA fragment with 694 bp (Figure 1). Different genotypes resulted from bGH-1 and bGH-2 with MspI and AluI endonuclease enzym restriction (Figures 2 and 3).

bGH-1 MspI polymorphisms

In homozygous animals either a unique band (591 bp, -/- genotype), or four-band (422, 83.61 and 25 bp, +/+ genotype) patterns were observed. Heterozygous animals gave a five-band (591, 422, 83, 61 and 25 bp, +/- genotype), or four-band (422, 83, 61 and 25 bp, +/+ genotype) patterns were observed) pattern (Figure 2). Considering the 71 sterrs and 29 heifers analysed, the overall genotype frequencies were 0.82 for (+/+), 0.11 for (+/-) and 0.07 for (-/-). Gene frequencies of alleles (+) and (-) were 0.875 and 0.125 respectively (Table 4).

bGH-1 AluI polymorphisms

In homozygous animals either a unique band (591 bp, -/- genotype) or eight-band (177, 147, 125, 94, 22, 20, 8 and 8 bp, +/+ genotype) patterns were observed (Figure 2). Considering the 71 sterrs and 29 heifers analysed, the

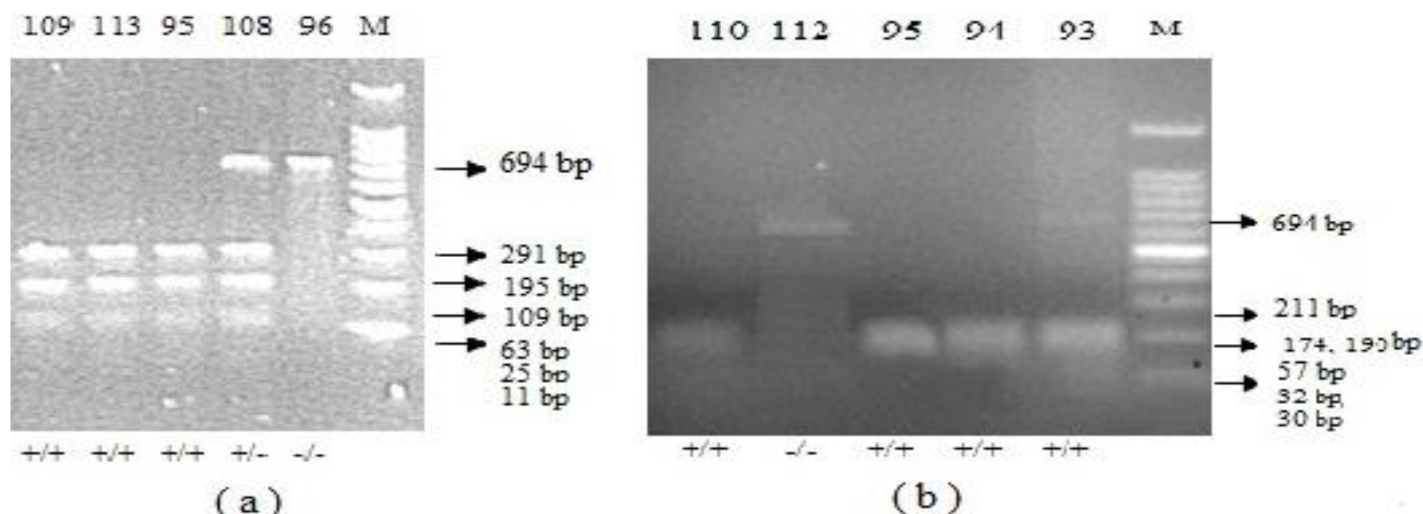

Figure 3. Different genotypes resulted from bGH-1 and bGH-2 with *Msp*I and *Alu*I endonuclease enzym restriction (a) b GH-2 *Msp*I (b) bGH-2 *Alu*I. M: Marker (DNA ladder 100 bp), 93, 94,…etc = number of sample (+/+,+/-,-/-) = genotype.

Table 4. Distribution of genotype frequencies and Allele frequencies (%) of RFLP polymorphism at the *Msp*I and *Alu*I loci in the bGH gene of Simmental.

Primer	Enzyme	Number of sample	Genotype frequency			Allele frequency	
			(+/+)	(+/-)	(-/-)	(+)	(-)
GH1	*Msp*I	100	0.82	0.11	0.07	0.875	0.125
	*Alu*I	100	0.95	0	0,05	0.95	0.05
GH2	*Msp*I	100	0.86	0,04	0.10	0.88	0.12
	*Alu*I	100	0.94	0	0.06	0.94	0.06

overall genotype frequencies were 0.95 for (+/+) and 0.05 for (-/-). Gene frequencies of alleles (+) and (-) were 0.95 and 0.05 respectively (Table 4).

bGH-2 MspI polymorphisms

In homozygous animals either a unique band (694 bp, -/- genotype), or six-band (291, 195, 109, 63, 25 and 11 bp, +/+ genotype) patterns were observed. Heterozygous animals gave a seven-band (694, 291, 195, 109, 63, 25 and 11 bp, +/+ genotype) patterns were observed (Figure 3). Considering the 71 sterrs and 29 heifers analysed, the overall genotype frequencies were 0.86 for (+/+), 0.04 for (+/-) and 0.10 for (-/-). Gene frequencies of alleles (+) and (-) were 0.88 and 0.12 respectively (Table 4).

bGH-2 AluI polymorphisms

In homozygous animals either a unique band (694 bp, -/- genotype) or six-band (211, 190, 174, 57, 32 and 30 bp, +/+ genotype) patterns were observed (Figure 3). Considering the 71 sterrs and 29 heifers analysed, the overall genotype frequencies were 0.95 for (+/+) and 0.05 for (-/-). Gene frequencies of alleles (+) and (-) were 0.94 and 0.06 respectively (Table 4).

Table 4 suggests that the genotype and allele frequency range of the MspI and AluI for Simmental cattle breeds of Aniamal Breeding Centre and Forage Indonesia. The Simmental cattle have genotype frequency (++) higher than other genotype frequency and allele frequency (+) of Simmental cattle is higher than alel frequency (-). This condition described that population of Simmental have alleles that are polymorphic, where in one population has more than one allele. An allele is said to be polymorphic if one alelnya less than 99% (Nei and Kumar, 2000). The frequency of allele (-) in this breed is lower than some of the reported Allele frequencies of GH variant (-) were: 0.32 in Bavarian Simmental bulls by Schlee et al. (1994), 0.44 in Slovak Simmental bulls by Chrenek et al. (1998).

Hardy-Weinberg equilibrium

The animals considered in this study have deviated from a Hardy-Weinberg equilibrium (Table 5), the overall chi-

Table 5. Equilibrium testing of GH1 and GH2 gene are restricted with the enzyme MspI and AluI.

Primer	Enzyme	Number	$(\chi^2)_{test}$
GH1	*MspI*	100	24.667**
	AluI	100	90.811**
GH2	*MspI*	100	65.706**
	AluI	100	90.92**

Table 6. The observations heterozygosity value (Ho) and expectations heterozygosity (He) GH gene are restricted with the enzyme MspI and AluI.

Gene fragment		Number sample (n)	$H_{observed}$ (Ho)	$H_{expected}$ (He)
GH1	*Msp*I	100	0.11	0.2188
	*Alu*I	100	0.00	0.0950
GH2	*Msp*I	100	0.04	0.2112
	*Alu*I	100	0.00	0.1128

square value for GH-1*Msp*I and GH-1 *Alu*I were 24,667 and 90,811 respectively. GH-2 *Msp*I and GH-2 *Alu*I were 24,667 and 90,811, respectively. This genetically unbalanced condition could be caused the main purpose of keeping Animal Breeding Center and Forage Padang Mengatas is livestock breeds production and the livestock kept in imported from Australia which has been selected beforehand.

Heterozygosity value

The observed heterozygosity was found to be less than expected in growth hormone gene locus in the whole investigated group of animals as well as in Simmental cattle (Table 6). The expectation value of heterozygosity (He) was bigger than the observations of heterozygosity (Ho). If the expectations of heterozygosity values bigger than the value of the observation of heterozygosity (Ho <He) identifies that the sample population had a degree of endogamy (marriage within the group) as a result of an intensive selection process (Machado et al., 2003).

The relationship of polymorphism GH gene with post weaning live weight gain

The relationship of growth hormone genotypes on growth traits are of great interest for their breeders. The results analysis of relationship polymorphism GH gene with post weaning live weight gain Simmental heifers and steers in Animal Breeding Center and Forage Padang Mengatas was observed as non-significant (P > 0.05). These matters showed the variation in each group is homogeneous. This caused by the nature of production is a trait that is controlled by many genes (polygenes) and environmental influences are very large (Warwick et al., 1983; Falconer and Macay, 1996).

In conclusion, it may be stated that growth hormone gene is low polymorphic and non-significant relationship between the genotypes polymorphic GH gene *Ms*pI and *Alu*I with post weaning live weight gain in the Simmental Cattle. The present study is the first report on GH genotyping of Simmental in Indonesia and has to be considered as a preliminary study. A larger number of observations are needed to establish or deny the existence of an association between GH genotypes and quantitative traits in those breeds.

Conflicts of Interests

The authors have not declared any conflict of interests.

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This study contains some data from the author's dissertation work.

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