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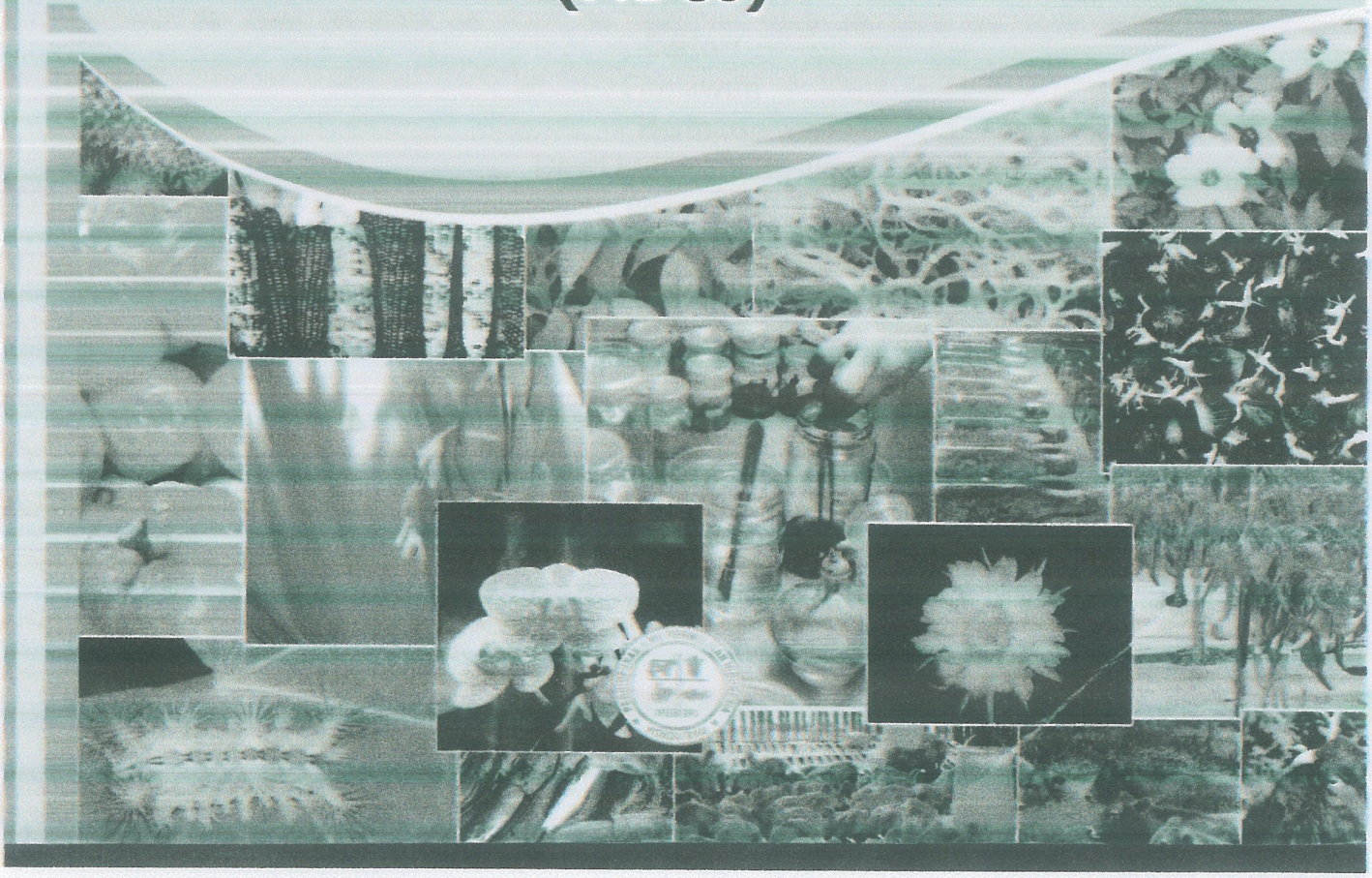

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SEMINAR NASIONAL PERHIMPUNAN PEMULIAAN INDONESIA (PERIPI) KOMDA RIAU

"Capaian Kegiatan-kegiatan Pemuliaan dalam Menyongsong Millennium Development Goals (MDGs)"

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DAFTAR ISI

NEW SINGLE NUCLEOTIDE POLYMORPHISMS DETECTED IN EXON 5 OF THE BOVINE GROWTH HORMONE GENE IN LOCAL CATTLE BREEDS IN WEST SUMATERA PROVINCE OF INDONESIA

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ABSTRACT

DNA isolated from 60 blood sample and PCR product of GH fragment (405 bp) were analyzed by direct sequencing. Three mutations were detected in position 2230, 2291, and 2450-51 with genotypes G→A, A→C, and AG→GT with frequency allele were 0.47, 0.80, and 0.50 respectively. Three insertions were detected: an insertion C in position 2379 and 2386, and insertion G in position 2393 with frequency allele were 0.06, 0.06, and 0.06 respectively. Three deletions detected in position 2406, 2412, and 2417 with frequency allele were 0.53, 0.76, and 0.57 respectively. These data provide evidence that GH gene is good polymorphic source and can be used for association with performance and investigate whether these polymorphic responsible for quantitative variation in growth.

Key words: *pesisir cattle, growth hormone gene, polymorphism, SNPs*

1. INTRODUCTION

The bovine growth hormone gene (bGH) is well known to play a significant role in metabolism regulation and mammary development (Jiang and Lucy, 2001), investigations concerning this gene are important when considering improvements in cattle production. The bGH in the bovine genome is approximately 1800 bp in size and is composed of five exons and four introns (Woychik *et al.* 1982; Vukasinovic *et al.*1999) and located on 19th chromosome in q26-qter band region (Hediger *et al.*, 1990). The complete DNA sequence has been determined (Gordon *et al.* 1983). This gene has been widely studied because of its effect on important biological function including growth, body composition and development of mammary cell, lactogenesis and proliferation of mammary cells (Horvat and Medrano, 1995; Nielsen *et al.*, 1995; Lagziel *et al.*, 1999).

The bGH gene is considered a promising marker candidate gene for improving growth, meat and milk production due to its role in galactopoietic metabolism and the growth process. Several polymorphic regions have been reported in different region of the bGH gene. Allelic variations in the structural or regulatory sequences contribute to the genetic characterization of population and could help to identify possible hybridization event in the past and they would have possible direct or indirect effects on milk production or growth performance (Zakizadeh *et al.*, 2006). Also, polymorphism in introns or flanking sequences have potential can be useful as genetic markers for genetic improvement of population (Mitra *et al.*, 1995; Falaki *et al.*, 1996). A considerable number of studies seeking an association between variants of this gene and productive traits has been reported (Høj *et al.*, 1993; Grochowska *et al.*, 2001; Lucy *et al.*, 1993; Lagziel *et al.*, 1996, 1999; Hasret *et al.*, 2009; Dybus, 2002; Tatsuda *et al.*, 2008).

Yao *et al.*, (1996) identify three SNP, first at position 1547, this mutation is due to a T to C transition, that creates a *MspI* restriction site, second a transversion of A to C at position 2291, this mutation is detectable by *DdeI* restriction enzyme, and third mutation T to C at position 1692 and position 2017. Kusamura de Mattos *et al.*, (2004) found that the bGH-*MspI*(-) allele was favorable for milk fat percentage ($P<0.05$) and association with fat production in both Red Danish and Norwegian Red dairy cattle (Høj *et al.*, 1993) and protein percentage in Israeli Holstein dairy cattle (Lagziel *et al.*, 1999).

Luci *et. al.*, (1993) and Yao *et al.*, (1996) detected a polymorphic site located in exon five, a transversion of C to G in position 2141, which substitutes Leu by Val in the protein product. This mutation is detectable by *Alu*I endonuclease (Zhang *et al.*, 1993). Grochowska *et al.*, (2001) reported that difference between the Leu/Leu and Val/Val genotypes for carcass and weight of meat in the carcass were significant ($p \leq 0.05$). Schele *et al.*, (1994) reported significant effect for LV genotype for carcass gain and meat value but reported no effect for milk breeding value. Associations between Leu/Val polymorphism and milk production traits of cows were found in first laction (Dybus, 2002). Cows with LL genotype had higher milk, fat and protein yield compared to LV individuals ($p \leq 0.01$). Hasret *et al.*, (2009) found *Alu*I polymorphism with VV genotype and *Msp*I polymorphism with -/- genotype had higher milk fat percentage. Schlee *et al.* (1994) reported that Simmental bulls with Leu/Leu and Leu/Val genotypes had significantly higher breeding values for carcass gain, and that those with the Val/Val genotype for the meat classification score. Beauchemin *et al.* (2006) reported that associations of DNA polymorphisms in bGH (including the Leu/Val polymorphism) relative to growth and carcass characteristics in growing Brahman steers were not clear. Tatsuda *et al.* (2008) reported three haplotypes A, B, and C that differ by amino acid mutation at position 127 and 172 in the fifth exon: (leucine127, threonine172), (valine127, threonine172) and (valine127, methionine172) respectively, they found high carcass weight and low beef marbling were associated with haplotype A and beef marbling was associated with haplotype C. Objectives of this study were to detect genetics variants in bGH gene as candidate gene in Local Cattle West Sumatera Province, Indonesia.

2. MATERIALS AND METHODS

Sixty blood sample (5 ml) 1.5 year old local cattle breeds (sapi Pesisir) in West Sumatera Province, were selected. Blood sample were collected by jungular vein puncture with a 14 gauge needle into a vacutainer containing 0.5 ml of 0.5 M EDTA. The blood samples were kept on ice and then store at -20 ^{o}C refrigerator. DNA was extracted from whole blood using Promega Wizard® Genomic DNA Purification Kit according to the manufacture protocol

The 404-bp bGH gene fragment, covering a part of the four intron and part of the adjacent five exon, was amplified using pure Tag Ready-To-Go PCR Beads from GE Healthcare. For each beads, add 2 µl (20 ng) genomic DNA, 2 µl (20 nM) of each primer, and 19 µl ddH_2O. The primer sequences were as follows: (forward): 5'-TAGGGGAGGGTGGAAAATGGA-3'; (reverse): 5'-GACACCTACTCAGACAATGC-3' (Yao *et al.*, 1996). PCR conditions were 5 min at 94°C, 60 s at 94°C, 90 s at 62°C, 80 s at 72°C, 40 cycles and 5 min at 72°C.

The resulting products were loaded on 1% agarose gel (1 x TBE and run at 100 V for 35 min for separation of the DNA fragments. The bands were stained with ethidium bromide prior to visualization by UV light. Following electrophoresis, DNA band were cut out from the gel and transferred into a 1.5ml micro centrifuge tube. DNA were purified using Ultraspine II according to the instruction of the supplier and DNA were store at 4°C or –20°C. Then the amplified 404 bp product was sequenced in Seqlab Laboratories Gottingen Germany.

3. RESULT AND DISCUSSION

The PCR amplified a 404 bp fragment from part intron 4 and exon 5, from electrophoresis it was seen the resulting fragmen is not specific (Figure 1), that need to purified, the position and length of the fragment were illustrated in Figure 2.

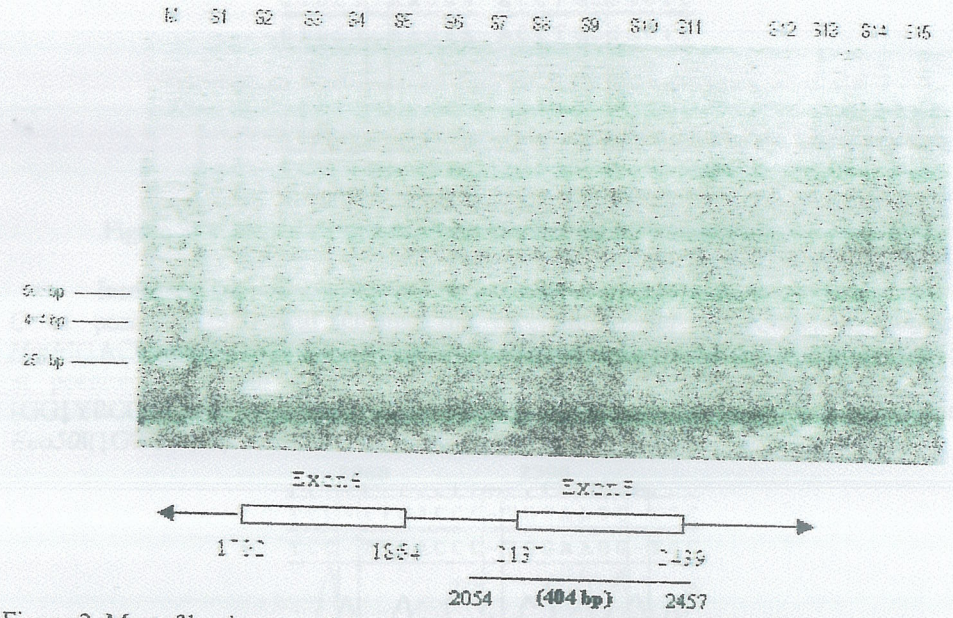

Figure 2. Map of bovine growth hormone gene with the position and length of the PCR amplified fragment from part intron 4 and exon 5 (Gordon *et al.*, 1983).

Using the sequence information of Gordon *et al.*, (1983) we mapped 2 mutations, 3 deletions, and 3 insertion (Table 1). Our analysis revealed two mutation: an C→T at position 2230 and A→C at position 2291, three insertion: insertion C at position 2279 and 2386, insertion G at position 2393, three deletion : deletion G at position 2406, deletion T at position 2412 and 2417.

New mutation C→T at position 2230 (Figure 1a) will change the codon CTC to CTT, these triplet codes for the same amino acid, leusine. This mutation will create the recognition site of the restriction enzyme *BsaF*I(↓CTTAAG) or enzyme *Bst98*I(C↓TTAAG). Therefore this polymorphism could also be identified as *BsaF*I-RFLP or *Bst98*I-RFLP.

The mutation A→C at position 2291 (Figure 3) that change the codon AGG to CGG have been reported earlier by Yao *et. al.* (1996), this mutation will change the recognition site of restriction enzymes *Dde*I (C↓TNAG) so it will not be cleaved when the mutation occurred.

Tabel 1. Eight polymorphisms in the bovine GH gene identified by Direct Sequencing

No.	Mutasi	Position	Frequency Allel
1	C → T	2230	0,47
2	A → C	2291	0,80
3	Insertion C	2379	0,06
4	Insertion C	2386	0,06
5	Insertion G	2393	0,06
6	Deletion G	2406	0,53
7	Deletion T	2412	0,76
8	Deletion T	2417	0,57

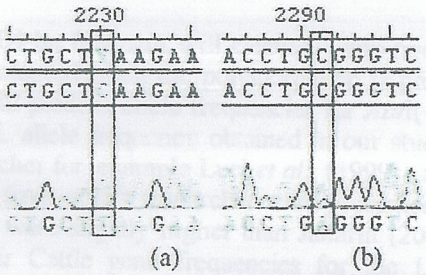

Figure 3. Mutation C→T and C→A at position 2230 and 2291

Insertion C at position 2379 (Figure 4) can only detected by sequencing. Insertion C at position 2386 will change the recognition site of restriction enzymes *HgiE*I(↓ACCNNNNNNGGT) so it could be identified as *HgiE*I-RFLP, and insertion G at position 2393 will change the recognition site of restriction enzymes *Ban*I (GG↓YRCC), or enzymes *ScrF*I(GGN↓NCC) or enzymes *Hgic*I(G↓GYRCC) or enzymes *Eco50*I(↓GGYRCC)

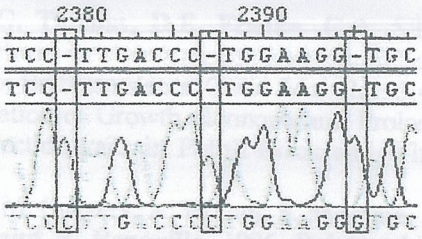

Figure 4. Insertion at position 2379, 2386, and 2393

Deletion G at position 2406 (Figure 2) will change the recognition site of the restriction enzyme *Bst4C*I(ACN↓GT) so could also be identified as *Bsat4C*I-RFLP, deletions T at position 2412 and deletion A at position 2417 (Figure 3) can only detected by sequencing.

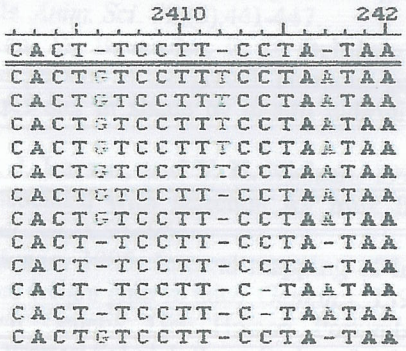

Figure 3. Deletion at position 2406, 2412, and 2417

From our sequences, no polymorphic was found at position 2141, these information revealed that 404 bp these fragment harbors a non polymorphic *Alu*I site. Polymorphism at position 2141 found by other researcher caused by point mutation C → G that change sequence CTG to GTG, this change converts the Leucine codon to the valine codon (Zang *et al.*, (1992, 1993), Luci *et al.*, (1993), and Yao *et al.*, (1996)). Polymorphism at position 2141 is also known as *Alu*I-RFLP since can be detected by

enzymes AluI. These 404 bp fragment will cleaved into two fragments upon digestion with *AluI* and will not cleaved when the polymorphism is present. When digestion with *AluI* these sequenced will produce allele frequencies for *AluI*(+) and *AluI*(-) are 1.00 and 0.00 respectively. The L allele frequency obtained in our study is agreement with those reported by other researcher for example Luci *et al.*, (1999), and de Mattos *et al.* (2004). The L allele frequency from others researcher were presented at Table 2. The L allele frequency in this study were slightly higher than Jakaria (2008) who using PCR-RFLP found GH *MspI* Pesisir Cattle gene frequencies for the L allele was 0.99. Higher frequencies in this research may be because of different methodology and lower sample size.

These data provide evidence that GH gene in Pesisir cattle is a good polymorphic source and can be used for association with performance and investigate whether this polymorphic might be responsible for quantitative variation in growth.

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