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Full length Research Paper

Genetic polymorphism of growth hormone locus and its association with bodyweight in Grati dairy cows

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The aim of this research was to study genetic polymorphism in growth hormone (GH) locus and its association with body weight of 43 Grati dairy cows ranging from 2 to 4 years old. Polymorphism of GH locus was ascribed using a PCR-RFLP method involving restricted enzyme Msp1 on 2% agarose gel. All data were subjeced to statistical analysis based on one way classification model using a statistical software package of Genstat 200 version 2. The results showed that frequencies of normal allel ($Msp1^+$) and mutant allel (Msp^-) were 0.34 and 0.66 respectively with 0.22 polymorphism was found in this locus. There was significant relationship between $Msp1^+/Msp1^+$, $Msp1^+/Msp1^-$ and $Msp1^-/Msp1^-$ genotypes and body weight ($P < 0.05$) but $Msp1^+/Msp1^+$ and $Msp1^+/Msp1^-$ genotypes had a stronger correlation to the higher body weight than $Msp1^-/Msp1^-$ genotype.

Key words: Grati cows, polymorphism, growth hormone, body weight.

INTRODUCTION

Grati cattle (GC) is regarded as indigenous breed that was a result of crossing between Friesian Holstein (FH) and Ongole crossbred cattle in Pasuruan regency of East Java since 19th century (Huitema, 1986). They have been adapted to relatively harsh environment especially to hot and humid climate and low-quality feed to produce milk and power to plough a farm land prior to planting. The cow yields milk between 2,500 to 4,500 kg/lactation which is far below the potential of FH dairy cows. Nevertheless, GC plays a significant role in the income generation for smallholder dairy farms in the region. Recently, selection for better performance of such important indigenous breed has received more attention especially with the advancement of genetically molecular biotechnology. Cheong et al. (2006) and (Kish, 2008) stated that growth hormone (GH) plays a vital role in post-natal growth and general metabolism including for lactation. Thus it is not surprising if GH has been the most intensive object of studies in ruminant animals to associate mutation of GH with the productive traits (Lara et al., 2002; Zhou et al., 2005; Khatami et al., 2005; Ferraz et al., 2006; Pawar et al., 2007; Mouzavisadeh et al., 2009). Yardibi et al. (2009) reported a significant effect of GH hormone gene polymorphism on milk yield of Eastern and South Anatolian Red breed cows. More recently, Komisarek et al. (2011) carried out a study on 209 dairy cows in Poland and found a significant decrease in milk production upon GHR-F279Y and shorter calving interval upon GHL127V polymorphism, respectively. Growth is a complex trait, which is controlled by multiple genes.

During the peak lactation milk is often synthesised at the expense of tissue catabolism, especially when the intake of feed energy is inadequate and a decline in body weight results. Under field conditions smallholder dairy farmers usually use body weight for selection criterion due to the lack of proper milk recording. This study aims at investigation of GH locus polymorphism in Grati cows and its association with body weight that potentially can be used as a marker assisted selection.

MATERIALS AND METHODS

Animals

43 Grati dairy cows that were kept at a dairy stall belonging to Suka Makmur Agricultural Farming Cooperative at Grati district, Pasuruan regency of East Java were obeserved during 2004 to 2005. The cows aged between 27 and 30 months old and varied between 1 and 3 lactation periods. All cows received a diet consisting of elephant grass (*Pennisetum purpureum*) and local concentrates.

Table 1. Band pattern of the fragment after being digested by Msp1 enzyme.

Length of DNA of particular band (bp)	Identified as allele	Genotype
224 105	Normal allele ($Msp1^+$) *)	Msp1+ /Msp1+
329	Mutant allele ($Msp1^-$) **)	Msp1+ /Msp1-
224 105 329	($Msp1^+$) and ($Msp1^-$)	Msp1- /Msp1-

Cut by enzyme ; **) uncut by enzyme.

The bodyweight was estimated from the chest girth (CG) prior to blood collection using a Schoorl formula:

$BW_{kg} = (CG_{cm} + 22)^2/100$ (Sudono et al., 2003)

The reason for estimating body weight by chest girth measurement was poorly due to the unavailability of weighing scale in the site of study, and based on the many reports that chest girth is the best predictor for body weight (ILRI, 2011)

Genotyping for GH

Approximately 10 ml of blood was taken from jugular vein of each cow using venojet spuit and mixed with EDTA 10% in the Falcon tube and stored at -25°C. Following the genomic DNA isolation, the cows were genotyped for GH locus using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and 2% agarose gel electrophoresis (Sulandari and Zein, 2003). Amplification of fragment of 329 bp at intron 3' (Dybus, 2002) was done with PCR using primer F: 5'– CCCACGGGCAAGAATGAGGC - 3' dan R:5' – TGAGGAACTGCAGGGGCCCA – 3'. To digest this fragment, a protocol of RFLP (restricted fragment length polymorphism) with restriction enzyme Msp1 was used to recognize the particular site of CC↓GG. The protocol for PCR analysis is stated in Table 1. PCR protocols to amp lily that fragment were by predenaturation at 94°C for 5 min for 1 cycle, denatauration at 94°C for 40 s, annealing at 60°C for 40 s and elongation at 72°C for 30 s for 30 cycles (Dybus, 2002).

Alleles identification

Following the end of PCR and RFLP processes, the products were then subsequently electrophorated using 2% agarose gel to identify polymorphism of alleles based on the length of the band (Table 1).

Statistical analysis

One-way analysis of variance was carried out to compare polymorphism level using a statistical software Genstat 2000 version 2. Polymorphism level was calculated using formula of Budak et al. (2003) that is:

$PIC_i = 1 = \sum p^2_{ij}$

where PIC_i is polymorphic information content at i-th locus, p_{ij} is the frequency of j-th allele and i-th locus.

RESULTS AND DISCUSSION

Growth hormone gene polymorphism

The number cows that met the criteria for this study was only 43. This mainly due to the large variation in farm size and husbandry practise between smallholder dairy farms in the village under study. Figure 1 indicates the number of dairy cows showing polymorphism at GH locus bearing $Msp1^+/Msp1^+$, $Msp1^+/Msp1^-$ and $Msp1^-/Msp1^-$ genotype were 7, 15 and 21, respectively (Table 2). It was found that the frequency of $Msp1^+$ and $Msp1^-$ alleles was 0.34 and 0.66. The level of polymorphism at that locus was 0.22 indicating that Grati dairy cows are polymorphic as has been generally accepted that the minimum value of polymorphism is 1% (Hyperdictionary, 2000; Dorak, 2006). This finding suggests that in Grati dairy cows there was high variability in the GH locus and offers opportunity to use GH genotype for selection criteria. From the previous study of Jakaria et al. (2007) on 132 indigenous cattle breed (Pesisir cattle) in West Sumatra, the MspI-GH gene showed significant relationship with bodyweight as estimated by chest girth measurement. Based on the Chi Square test (Table 2), it was found that alleles frequency were under genetic equilibrium.

Similar findings were reported in dairy cattle by Pawar et al. (2007) that polymorphism occurred in exon 5 of GH1 gene. Allelic frequency in genetic equilibrium means allele frequency will never change resulting in alleles and genotypic frequency stability.

Relationship of GH locus polymorphism with body weight

Table 3 shows the significant association ($p < 0.05$) between genotypes and body weight. However, the $Msp1^-/Msp1^-$ genotype gave less influence on body weight than those of $Msp1^+/Msp1^+$ and $Msp1^+/Msp1^-$ genotypes. Another locus in growth factor such as somatomedine or IGF1 gene has also been reported plays an important role in growth performance of beef

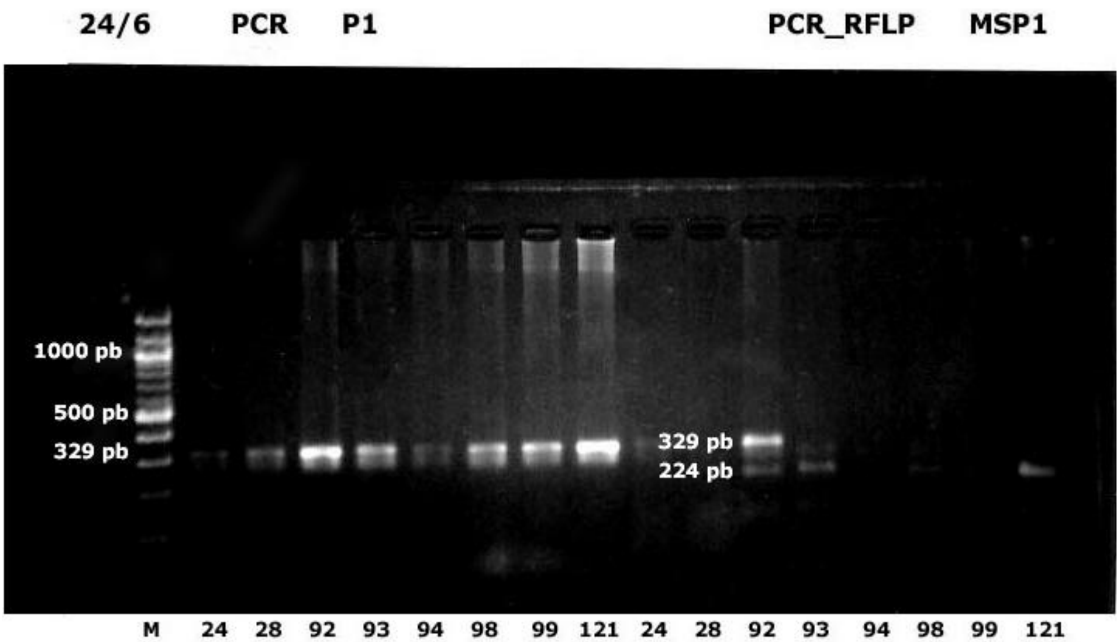

Figure 1. Eelectrophoresis result of PCR-RFLP products.

Table 2. Frequency of alleles Msp1+ and Msp1 at GH1 locus.

Allele frequency	Genotypic frequency
(Msp1[+]) = 0.34 *)	Msp1+ /Msp1+ = 7
(Msp1[-]) = 0.66 *)	Msp1+ /Msp1- = 15
	Msp1- /Msp1- = 21

*) The values denote that ppopulation were in genetic equilibrium based on the Chi square test ($p < 0.05$).

Table 3. PCR-RFLP product and mean of body weight of dairy cows in each genotype.

Genotype	N (**)	Mean of body weight (kg)
Msp1+ /Msp1+	7	448.8 ± 48.60^b
Msp1+ /Msp1-	15	447.7 ± 45.77^b
Msp1- /Msp1-	21	408.8 ± 50.83^a
Total	43	428.9 ± 51.58

*) Body weight was estimated using Schoorl formula, **) number of dairy cow. Values bearing unlike superscript in the same column differ significantly ($p < 0.05$).

cattle (Reyna et al., 2010). This notion supports the previous findings in other species of animals that GH gene can be promoted as candidate gene to improve animal performance such as body weight and carcass weight in poultry (Thakur et al., 2006) and Angus cattle (Zhao et al., 2004). As has been known that the growth of animals is under the hormonal control of GH, growth hormone receptor (GHR) and insulin-like growth factor I (IGF-I). Polymorphism that occurs in the regulatory region (for example promoter region) and coding region (exons) of the genes responsible for those three hormones will influence the expression of the genes and the function of protein during the translation process (Gee et al., 1999). There is strong indication that in all animals the level of blood GH reflects the GH genotype. Current knowledge in dairy biology indicates that genetically superior animals differ from lesser animals mainly in their regulation of nutrient utilization and that growth hormone (GH) release

(Bauman, 1992). The report of Sørensen et al. (2002) demonstrated that induction of growth hormone releasing hormone (GHRH) in Danish Jersey calves affected the GH Leu/Leu genotype to increase the level of GH in blood as compared with their counterparts with GH Val/Val genotype. Such an effect was only intermidiate when applied to GH Leu/Val genotype.

Conclusion

It was concluded that dairy cow population at Grati is polymorphic at locus GH with 22% polimorphism level. $Msp1^{+}/Msp1^{+}$ and $Msp1^{+}/Msp1^{-}$ genotypes can be used as the candidate genes in dairy cow selection to improve body weight.

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