

Short Communication: Association of GH, IGF1R, and PIT1 genes polymorphism with average daily gain and body measurement in Pesisir cattle

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Abstract. Arnim, Yurnalis, Afriani T, Putra DE. 2017. Short Communication: Association of GH, IGF1R, and PIT1 genes polymorphism with average daily gain and body measurement in Pesisir cattle. Nusantara Bioscience 10: 221-225. Growth hormone (GH) and insulin-like growth factor I receptor (IGF1R), and Pit1 gene has important effect on growth, carcass, and meat quality traits in many species. The objectives of the present study were to study the associations of the GH/*AluI*, GH/*MboII*, IGF1R/*MspI* and PIT1/*HinfI* with growth and body measurement such as body length (BL), chest girth (CG), height withers (HW) in Pesisir cattle. A total 175 Pesisir cattle were used in this study. Weight gain, and body measurement were fitted using the General Linear Model (GLM) procedure of the SAS program. The results showed significant associations between the CC genotype of the GH/*MboII* polymorphism and higher weight gain and height withers ($p < 0.05$). The GH/*AluI* polymorphism did not have any effect on the weight gain and body measurement. Allele frequencies were found as A: 0.374 and B: 0.626 in GH/*MboII* polymorphism in GH gene. The effect of GH/*AluI* polymorphisms was not observed on average daily gain and body measurement in Pesisir cattle ($P > 0.05$). The effect of IGF1R/*MspI* polymorphism was not observed on average daily gain but show significant association between AA and AB genotype and height withers ($p < 0.005$). The PIT1/*HinfI* polymorphism was not associated with average daily gain and body measurement in Pesisir cattle. The results demonstrated that the GH/*MboII* and IGF1R/*MspI* polymorphisms could be used as a candidate gene for selection in Pesisir cattle.

Keywords: Average daily gain body length, chest girth, GH, height withers, IGF1R, Pesisir cattle, PIT1, RFLP

INTRODUCTION

Indonesia has many breeds of local cattle that have the potential to be developed, and one of them is Pesisir cattle. The Pesisir cattle breed is one of the existing indigenous cattle breeds in Indonesia that has been adapted to the relatively harsh environment, especially to hot and humid climate and low-quality feed. In the last 20 years, decreasing live weight occur in this cattle due to negative selection, where farmer maintains the small cattle and sold the big one to get the higher price (Yurnalis et al. 2013). The study into the use of genetic markers to select superior animals has been carried out on various animals. Recently, some genes concerning with economic characteristics of farm animals have been studied for marker assisted selection (MAS) (Aytekin and Boztepe 2013). Numerous genes are involved in the process of growth. The most important among them seem to be those participating in the somatotrophic axis - growth hormone (GH), insulin-like growth factor 1 (IGF1R) (Szewczuk 2016), Pit1 (Aytekin and Boztepe 2013).

The growth hormone (GH) gene has been intensively studied as a candidate genetic marker in cattle because it has important roles in regulating animal growth and production, and GH is believed to be effective in increasing average daily gain (Schlegel et al. 2006). So far, genetic polymorphism at candidate genes has been extensively

explored in a number of cattle breeds (Sodhi et al. 2007). The insulin growth factor 1 receptors (IGF1R) are glycoprotein membranes that mediate most of the biological actions of IGF-1 and IGF-2, which have important effects on livestock growth, carcass, and meat quality. Another very important hormone is leptin which is synthesized by adipocyte tissue. This hormone plays an important role in food intake and growth in livestock and has been reported that leptin increases GH levels in blood plasma (Wóik-Gładysz et al. 2010).

The growth hormone plays a crucial role in the postnatal growth and metabolism regulation. Additionally, GH affects indirectly by controlling the secretion of other hormones including IGF1, which interacts with insulin-like growth factor 1 receptors (IGF1R) in target tissues (Jones and Clemmons, 1995). The IGF1R is encoded by a single gene located on chromosome 21 (BTA21) and consists of 21 exons, separated by long introns (<http://www.ncbi.nlm.nih.gov/gene/281848>)

The finding of an association between polymorphism in the IGF1R gene and growth traits is in agreement with numerous reports in other species. The association of polymorphism in IGF1R gene on body weight were reported by many researcher, in beef cattle (De la Rosa Reyna et al. 2010), pig (Wang et al. 2005), yak (Liang et al. 2010), chicken (Lei et al. 2008) and Japanese quail (Moe et al. 2007). Moreover, the association of IGF1R gene

variation on average daily weight gain was also investigated and confirmed in many species such as Egyptian buffalo (El-Magd et al. 2013), beef cattle (De la Rosa Reyna et al. 2010), chicken (Lei et al. 2008) and Japanese quail (Moe et al. 2007), Sheep (Proskura and Szewczuk 2014).

PIT1 gene has been extensively studied in cattle, including Limousine Cattle (Dybus et al. 2003), Iranian cattle (Javanmard et al. 2005), *Brahman cattle* (Beauchemin et al. 2006), Zebu (Curi et al. 2006; Mukesh et al. 2008), Canchim Cattle (Carrijo et al. 2008; Grossi et al. 2015), Podolica cattle (Selvaggi et al. 2011; Selvaggi and Dario, 2011), Southern Anatolian red cattle (Oztabak et al. 2008), Hanwoo cattle (Han et al. 2010), Turkey cattle (Ozdemir, 2012), Qinchuan cattle (Zhang et al. 2009) and Piemontese cattle (Ribeca et al. 2014). Holstein Cattle (Trakovicka et al. 2014; Ebrahimi Hoseinzadeh et al. 2015), Brown Swiss Cattle (Aytekin and Boztepe 2013), Najdi Cattle (Beigi Nassiri et al. 2010), Slovak Spotted Cattle (Moravčíková et al. 2013), Chinese Cattle (Tang et al. 2012), Sahiwal Cattle (Chauhan et al. 2015).

The objectives of the present study were to study (i) the polymorphism of the GH/*AluI*, GH/*MboII* IGF1R/*MspI*, and PIT/*HinfI*, (ii) to determine the frequency of alleles and genotypes for these polymorphisms, and (iii) to estimate the associations between these polymorphisms and average daily gain and body measurement in Pesisir cattle.

MATERIALS AND METHODS

The blood sampels of these research contained 175 male Pesisir cattle of 1.5 years. The average of daily gain was measured by weighing the cattle for 3 months period. Genomic DNA from blood was extracted using Promega Wizard® Genomic DNA Purification Kit according to the manufacture protocol.

Genotyping

The primers and annealing temperatures used to amplify the regions including GH/*AluI*, GH/*MboII*, IGF1R/*MspI*, and PIT1/*HinfI* are shown in Table 1.

The polymerase chain reaction mixture (25 µL) contained the following components and conditions; genomic DNA 2 uL, 12.5 uL GoTag Green Master Mix (Promega, Madison, USA), 1.5 uL of each primer (10 pmol /uL) and 7.5 uL nuclease-free water. The mixture was subjected to PCR on an Eppendorf Mastercycler Gradient

(Hamburg, Germany) using the following program: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 45 sec at 94 °C; 45 s at annealing temperature; and 80 s at 72 °C followed by 10 min at 72 °C for final extension.

A 10 µL of PCR products of IGF1R gene were digested with 5 U of *MspI* (Promega, Madison, USA) restriction enzyme at 37 °C for 4 hour in waterbath and 10 µL of PCR product of GH gene were digested with 5 U *AluI* and *MboII* (Promega, Madison, USA) restriction enzymes. The digested PCR products were separated in 1.5% agarose gel (Thermo Scientific, Lithuania), stained with ethidium bromide, running in 1 × TBE at 150 V for 90 min for separation of the DNA fragments and viewed under UV light.

Statistical analysis:

The associations between the GH and IGF1R genotypes and average daily gain and body measurement were analysis using General Linear Model in the computer program SAS (SAS Institute 2002 Ver. 9). The following linear model was applied:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + G_k + \varepsilon_{ijkl}$$

Where: Y_{ijklm} : observed trait in $ijkl$ -th animal;

μ : mean of for popuLation; α_i : effect of sex; β_j effect of location and G_k : effect of genotypes (AA, AB, BB); ε_{ijkl} : random error.

Frequencies of genotype and allele were calcuLated directly. Hardy-Weinberg Equilibrium was calcuLated according to Warwick et al. (1990).

$$p^2 + 2pq + q^2 = 1$$

Where:

p^2 = frequency of -/-

$2pq$ = frequency of +/-

q^2 = frequency of +/+

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where:

χ^2 = Pearson's cumuLative test statistic, which asymptotically approaches a χ^2 distribution.

E = the expected (theoretical) frequency

O = the number of observations

Table 1. Oligonucleotide primers used in this study

Genes	Primer sequences (5' to 3')	Anealing temp. (°C)	Region	Length (bp)	Enzyme
GH	F: GGATGGCAGTGGAGGATGAT	58	Exon5	599	<i>AluI</i>
	R: AGGTCTGCTTGAGGATCTGC				<i>MboII</i>
IGF1R	F: TTCTTGCTGTTTCAATTGTTG	64	Intron12	164	<i>MspI</i>
	R: CTCGACTTGGGATCCATATTTT				
PIT1	F: ACTCGCTATTACACAATAGGAGAGCCT	60		260	<i>HinfI</i>
	R: TCCTGCCAACTCCTCACCTCCC				

RESULTS AND DISCUSSION

Distribution of genotype and allele frequencies

The growth hormone genes have been explored on the Indonesian native cattle (Putra et al. 2016; Yurnalis et al. 2017; Putra et al. 2018; Hartatik et al. 2018) associating with growth and economic traits performance. The allelic and genotypic frequencies of the GH/*AluI*, GH/*MboII*, PIT1/*HindfI* and IGF1/*MspI* polymorphisms were estimated by the genotyping of 175 individuals from Pesisir cattle using PCR-RFLP technique. As shown in Table 2, the results of the genotyping indicated that two genotypes of AA (79.43%) and BB (20.57%); CC (85.14%) and DD (14.86%) were observed in GH/*AluI* and GH/*MboII* genes respectively, suggesting that these genes are 0.79 A allele, 0.21 B allele, 0.85 C allele and 0.15 D allele in the population Pesisir cattle. On the other hand, three genotypes of AA (1.14% and 3.43%), AB (17.71% and 18.29%) and BB (81.15% and 78.28%) were observed in PIT1/*HindfI* and IGF1/*MspI* respectively.

The digestion of 599 bp fragment amplified of GH by *MboII* restriction enzymes resulted two fragments 567 and 32 (32 not visible) for DD allele, and one fragment 599 bp for CC genotype. The digestion of 260-bp fragment amplified of PIT1 by *HinfI* restriction enzyme resulted two fragments (51 and 113 bp) for BB allele and one uncut 164-bp-long fragment for AA allele and three fragments (164, 113, and 51) for AB allele. The CC genotype had the highest frequency (0.851) and DD genotype with frequency (0.149) while digestion with *AluI* restriction enzyme resulted three fragments for AA genotype and four

fragments for BB genotype, with AA the highest frequency (0.777; Table 3). The 164-bp fragment amplified of IGF1R to identify SNP contained a single *MspI* recognition site. The digestion resulted two fragments (51 and 113 bp) for BB allele and one uncut 164-bp-long fragment for AA allele and three fragments (164, 113, and 51) for AB allele. The BB genotype had the highest frequency (0.766), followed by the AB genotype (0.207) and AA genotype (0.027). The frequency of BB genotype in this research slightly higher than the research by Szweczek et al. (2013) in Angus cattle, who found 0.593 for frequency of BB genotype.

The result of the polymorphisms of the genes examined in the present study have been identified as the genes associated with particular traits of Indonesian native cattle. However, distribution of these polymorphisms in the zebu cattle remains unclear. Putra et al. (2016); Yurnalis et al. (2017); Putra et al. (2018); Hartatik et al. (2018) reported that genetic analyses of local breeds of Indonesian native cattle showed gene-flow of banteng (*Bos javanicus*), Southeast Asian wild cattle, into some of these breeds. In the present study, we found that the polymorphisms of GH/*AluI*, GH/*MboII*, PIT1/*HindfI* and IGF1/*MspI* genes reported in Indonesian native cattle were also observed in the population of Pesisir cattle. The insulin-like growth factor type 1 (IGF1), growth hormone (GH) and pituitary transcription factor (PIT1) are candidate genes for growth and reproduction traits because they participate on the hormonal system with a fundamental role in regulating animal's development (Grossi et al. 2015).

Table 2. Genotype distributions, allele frequencies and χ^2 test

Gene	N	Genotype distributions			Allele frequencies		Chi-square values for HWE test
GH/ <i>AluI</i>	175	A/A	A/B	B/B	A	B	175.00
		139	0	36	0.79	0.21	
GH/ <i>MboII</i>	175	C/C	C/D	D/D	C	D	175.00
		149	0	26	0.85	0.15	
PIT1/ <i>HindfI</i>	175	A/A	A/B	B/B	A	B	0.04
		2	31	142	0.10	0.90	
IGF1R/ <i>MspI</i>	175	A/A	A/B	B/B	A	B	4.95
		6	32	137	0.13	0.87	

Note: $\chi^2_{0.05;2} = 5.99$

Table 3. Average daily gain and body size of Pesisir cattle by 18 months of age

SNP	Genotyp	N	F	ADG	BL	CG	HW
GH/ <i>AluI</i>	AA	139	0.777	0.15185	90.987	108.563	91.040
	BB	36	0.233	0.18569	89.690	105.724	89.172
GH/ <i>MboII</i>	CC	149	0.851	0.20414	91.204	108.342	91.421
	DD	26	0.149	0.15088	87.952	105.905	86.286
Pit1/ <i>HindfI</i>	AA	2	0.011	0.21538	99.000	114.000	89.500
	AB	31	0.177	0.12990	89.987	106.769	91.040
	BB	142	0.811	0.14363	87.833	104.950	89.172
IGF1R/ <i>MspI</i>	AA	6	0.027	0.10784	94.000	110.500	94.000
	AB	32	0.207	0.24318	95.565	112.174	93.913
	BB	137	0.766	0.19616	90.899	107.357	89.542

Note: Values within columns with different letters differ significantly at $P \leq 0.05$. N= number of animals in the group; F= frequency of genotypes; ADG= average daily gain; BL= body length; CG= chest girth; HW= height withers

Association analyses

The association of the GH/*AluI*, GH/*MboII*, PIT1/*HindfI* and IGF1/*MspI* polymorphism with the growth traits did not show a significant effect on the majority of traits analyzed, as can be observed in Table 3. The association of GH, and IGF1R gene polymorphism with average daily gain and body measurement in the population was analyzed (Table 1). For new polymorphism GH/*MBoII* bull carrying CC genotype had high average daily gain (0.204 kg) and high height withers (91.421 cm) compare to DD genotype (0.151 kg) and (86.286 cm). With respect to body length (BL), chest girth (CG), generally, CC genotypes showed a similar tendency and they have higher values than the DD genotype. But, take account of P values, the genotypes at GH/*MboII* polymorphisms did not show a significant association with the body length and chest girth ($P>0.05$). The IGF1R/*MspI* polymorphism had a significant on HW ($P<0.05$) where AA genotype was associated with higher HW (94.00 cm) following AB genotype (93.913 cm) and BB genotype (89.542 cm). With respect to body length (BL), chest girth (CG), generally, AB genotypes also showed a similar tendency and they have higher values than the BB genotype. This results were in line with research by Szewczuk et al. (2013) in Angus cattle who found a significant association between this polymorphism with weight in 210 day. For GH/*AluI* and PIT/*HinfI* polymorphism had not observed any association with daily gain and body measurement.

In conclusion, the effect of GH/*AluI* polymorphisms was not observed on average daily gain and body measurement in Pesisir cattle. The effect of IGF1R/*MspI* polymorphism was not observed on average daily gain but show significant association between AA and AB genotype and height withers. The PIT1/*HinfI* polymorphism was not associated with average daily gain and body measurement in Pesisir cattle. the GH/*MboII* and IGF1R/*MspI* polymorphisms could be exploited as a candidate gene for selection of Pesisir cattle in this population.

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