

HERITABILITIES OF BODY SIZE BY GROWTH HORMONE (*GH-Msp1*) GENOTYPES USING PCR-RFLP IN ONGOLE GRADE CATTLE

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ABSTRAK

Tampilan genetik dalam istilah heritabilitas yang merupakan faktor penting pada sifat ekonomis ternak untuk pewarisan ukuran tubuh belum sepenuhnya dikaji pada ternak sapi Peranakan Ongole (PO). Tujuan penelitian ini adalah menentukan nilai-nilai heritabilitas bobot badan, lingkaran dada dan panjang badan pada sapi PO. Total 37 sampel darah diambil dari tetua induk betina dan 2 sampel darah dari tetua pejantan sapi Ongole. Semua sampel darah diuji gambaran keberadaan genotipe lokus hormon pertumbuhan (*GH*) menggunakan metode PCR-RFLP yang melibatkan enzim *Msp1* pada "agarose-gel" (1,2%) melalui elektroforesis. Data dianalisis menggunakan program statistik dalam Excel XP. Hasil analisis memperlihatkan bahwa rata-rata estimasi fenotipe populasi sapi PO untuk bobot hidup, lingkaran dada dan panjang badan masing-masing adalah $445,41 \pm 45,95$ kg; $175,35 \pm 4,11$ cm dan $139,70 \pm 5,73$ cm. Nilai dugaan heritabilitas bobot badan, lingkaran dada dan panjang badan masing-masing adalah 0,24; 0,003 and 0,41. Nilai heritabilitas ternak untuk bobot badan dan panjang badan dapat dikategorikan ke dalam nilai tampilan genetik sedang (moderate) sampai tinggi (high), sedangkan nilai heritabilitas lingkaran dada dapat dimasukkan dalam kategori heritabilitas rendah (low) untuk sifat ekonomis ternak.

Kata Kunci: Enzim GH-Msp1, sapi Peranakan Ongole, sifat genotipe, ukuran tubuh

ABSTRACT

Genotypic performance in term of heritability as the crucial factor of animal economical traits for body size inheritance had not been fully studied in Ongole-grade cattle. The objectives of this research were to define the heritability values of live weight, chest girth and body length in Ongole-crossbred cattle. Total of 37 blood samples were collected from parental cows and 2 blood samples from parental Ongole breed bulls. All blood samples were screened for the presence of growth hormone (*GH*) locus using PCR-RFLP method involving restricted enzyme *Msp1* on agarose-gel (1.2%). Data were analyzed using statistical program in Excel XP. Results showed that the phenotypic estimation average of Ongole grade cattle population of live weight, chest girth and body length were 445.41 ± 45.95 kg, 175.35 ± 4.11 cm, and 139.70 ± 5.73 cm, respectively. The heritability values of animal live weight, chest girth and body length in this study were 0.24, 0.003, and 0.41, respectively. These heritability values of animal live weight and body length would be categorized as moderate to high genotypic performance values, while the heritability of animal chest girth was included in low heritability standard of the animal economical trait performance.

Keywords: GH-Msp1 enzyme, Ongole-grade cattle, genotypic traits, body size

INTRODUCTION

The difference in the phenotypes of animals in the single locus-example is a function of the genotypic value. Parents do not pass their genotype on to their progeny but rather pass on only a random sample of one gene to each locus of the progeny. The goal of animal breeders is rapid genetic improvement, for which accurate prediction of breeding value is the most crucial factor. The breeder can rank the animals and cull those with the poorest evaluations while selecting those with the best evaluation as replacements. Accurate evaluation requires proper application of heritability and relationships to records of the either animal weight, chest girth or body length and its relatives (Van Vleck *et al.*, 1987).

In animal industry, growth traits of animals are always of primary concern during breeding for its determinant economical value. With the development of molecular biology and biotechnology, scientists are able to achieve more accurate and efficient selection goal by marker-assisted selection (MAS). In general, validating the genetic markers of growth traits is the initial and crucial step to establish a MAS system (Allan *et al.*, 2007).

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, the pattern of which plays an important role in pubertal, prenatal and postnatal longitudinal growth and development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (Ayuk and Sheppard, 2006). Effects of GH on growth were observed in several tissues, including bone, muscle and adipose tissue, so that GH gene, with its functional and positional potential, has been widely used for marker in several livestock species, including the cattle such as *Bos taurus* and *Bos indicus* (Beauchemin *et al.*, 2006). It has been reported that the restriction fragment length polymorphisms (RFLP) of GH were associated with body weight in Grati dairy cows (Maylinda, 2011).

The studies of GH gene *MspI* locus have been reported in Ongole grade cattle (Sutarno *et al.*, 2005), Brahman cattle (Beauchemin *et al.*, 2006), Indian Zebu cattle (Shodi *et al.*, 2007) and West coastal Sumatara cattle (Jakaria *et al.*, 2007; Jakaria *et al.*, 2009). Their studies indicated that *MspI*^{+/+} and *MspI*^{+/-} genotypes can be used as the

candidate genes in cattle selection for breeding program. The genotypic value is defined as the deviation of the phenotype from the average of the two homozygous phenotypes (Jain and Prabhakaran, 1992).

The difference between breeding values is additive gene and represent the term of heritability for certain animal economical trait such as animal live weight, chest girth and body length. Heritability (h^2) is defined as the ratio of the additive genetic variance to the phenotypic variance. Thus, h^2 is the proportion of the total variance that is due to differences among the breeding values of individuals in the population (Van Vleck *et al.*, 1987). However, the heritability of body size represented by body weight, chest girth and body length in individual Ongole grade cow, referred to its additive genetic merit of body size, has not been much studied. The objectives of this research were to define the genotypic performance referring to heritability of live weight, chest girth and body length from genotypic frequency of growth hormone (GH) *MspI* enzyme-restriction in Ongole grade cows population in North Sulawesi province of Indonesia.

MATERIALS AND METHODS

This study was carried out in the Northern Sulawesi Island of Indonesia using 37 cows (age ranging 4 to 5 years old) of Ongole grade cattle at Tumaratas Village as the artificial insemination (AI) service center of Minahasa regency, North Sulawesi province of Indonesia. All parental cows (G0) were reared under private areas belong to farmers with unknown ancestors. Progenies (G1) were born from those G0 mated by artificial insemination using germ plasmas (semen) of the two Ongole bulls called "Kirsta" and "Tunggul" from "The Artificial Insemination Bull Germ Plasma Center" in Singosari, East Java Province, Indonesia.

Prior to blood collection, body weights of animals (G0 and G1) were determined by using a digital weighing scale when animals were standing as described in (Ozkaya and Bozkurt, 2008). The total of 37 G0 consisted of 20 superior body weight animals (cow weights heavier than at least one fifth standard deviation above the mean) and 17 inferior body weight animals (cow weights lighter than one and half standard deviation below the mean) among cow (G0) population (n = 363 heads, with body weight average of 440.20 ±

58.03 kg) were included in this study as described in (Papatungan *et al.*, 2000).

The genotyping process was conducted at the Biotechnology Laboratory, Department of Biological Science, Faculty of Mathematics and Natural Science, Sam Ratulangi University, Manado. Genomic DNA from whole blood of Ongole grade cows, bulls and their calves were purified by standard protocol using proteinase K digestion as described by DNA extraction kit (AxyPrep Blood Genomic DNA Miniprep kit, AXYGEN Biosciences, Union city, CA, 94587, USA).

Following the genomic DNA isolation, the animals were genotyped for GH locus using PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) and 1.2% agarose gel electrophoresis (Sulandari and Zein, 2003). Amplification of the fragment of 327 bp at intron 3 (Gordon *et al.*, 1983) was done with PCR using the forward primer of 5'-CCCACGGGCAAGAATGAGGC-3'; and reverse primer of 5'-TGAGGAACTGCAGGGGCCCA-3' (Mitra *et al.*, 1995). The reaction mixture of PCR was performed by using 1x Taq pol 25 µl of master mix (Axygen Biosciences, CA, USA).

To digest this fragment, a protocol of RFLP with restriction enzyme *MspI* was used to

recognize the particular site of C↓CGG. The PCR product of GH gene was digested at 37 °C for 3 hours by *MspI* enzyme. Reaction consisted of 2 µL Buffer V2 10X, 7.5 µL H₂O, 0.5 µL Enzyme *MspI* (20 U/µL), and 10 µL PCR product. PCR protocols to amplify the fragment were by the initial denaturation temperature step at 94°C for 5 min for 1 cycle followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, elongation at 72°C for 30 s and a final extension at 72°C for 1 min. (Dybus, 2002). Following the end of PCR and RFLP process, the products were then subsequently electrophorated using 1.2% agarose gel to identify polymorphism of allele based on the length of the band as presented at Figure 1.

PCR-RFLP data were analyzed by allele frequency (Nei, 1987). The allele frequency was calculated by the methods as follows:

$$x_i = [(2n_{ii} + \sum n_{ij}) / 2N]$$

Where,

x_i = the *MspI*⁺ allele frequency,

n_{ii} = the number of cattle with the genotype of *MspI*^{+/+},

n_{ij} = the number of cattle with the genotype of *MspI*^{+/-},

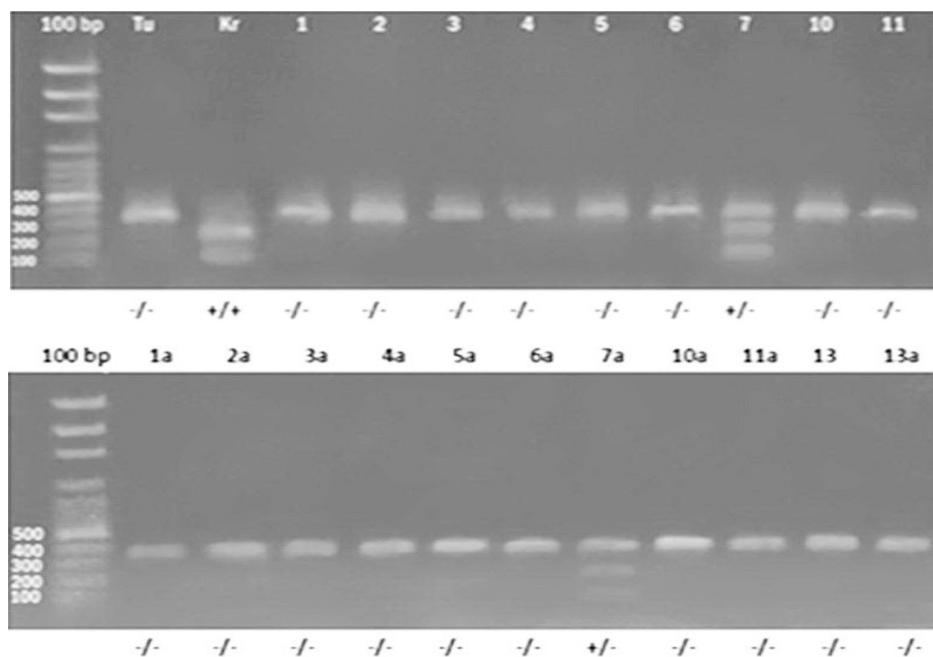


Figure 1. Genotyping Results of *MspI* Enzyme Restriction in GH Locus Detected by Agarose Gel Electrophoresis (Tu = Ongole bull called Tunggul; Kr = Ongole bull called Krista, source of sperm for Artificial Insemination (AI); 1 – 11 = samples of cows mated by AI; 1a-13a = samples of progenies produced by AI method using Tu sperms)

N = the total number of cattle tested.

DNA extraction and genotyping for GH and allele identification were done using the protocols in DNA Laboratory (Sulandari and Zein, 2003; Paputungan *et al.*, 2012) showing the indentified alleles presented in Table 1.

DNA extraction and genotyping for GH and allele identification were done using the protocols in DNA Laboratory as described by (Sulandari and Zein, 2003; Paputungan *et al.*, 2012) showing the indentified alleles and genotypes of the animals as presented in Table 1. The average of two homozygous phenotypes (P₁₁ and P₂₂), denoted by *m*, was calculated using formula (Van Vleck *et al.*, 1987) as follows:

$$m = \frac{1}{2} (P_{11} + P_{22}) \dots\dots\dots (1)$$

The genotypic values for each genotype of the cows in this study were calculated with the formula according to Van Vleck *et al.* (1987) as follows:

$$\text{Genotypic value of the } P_{11} (a) = P_{11} - m \dots (2)$$

$$\text{Genotypic value of the } P_{12} (d) = P_{12} - m \dots (3)$$

$$\text{Genotypic value of the } P_{22} (-a) = P_{22} - m \dots (4)$$

Because *m* was defined as the phenotypic mean for both homozygous genotypes of 405.03 kg (using formula 1), the genotypic values of each animal genotype were found for live weight as follows: *GH-MspI*^{+/+} (a) was 0.30 kg (using formula 2), *GH-MspI*^{+/-} (d) was 93.04 kg (using formula 3), and *GH-MspI*^{-/-} (- a) was - 0.30 kg (using formula 4). In addition, the phenotypic mean (m) for both homozygous genotypes of chest girth was 179.045 cm (using formula 1), the genotypic values were found as follows: *GH-MspI*^{+/+} (a) was 3.30 cm (using formula 2), *GH-MspI*^{+/-} (d) was 9.45 cm (using formula 3), and *GH-MspI*^{-/-} (- a) was - 3.30 cm (using formula 4). In the same case, the phenotypic mean (m) for both homozygous genotypes of body length was

Table 1. Band of the Fragment after *MspI* Enzyme Restriction

Length of DNA Band (bp)	Identified Allele	Genotype
224 103	Normal allele (<i>MspI</i> ⁺)*	<i>MspI</i> ^{+/+}
327 224 103	<i>MspI</i> ⁺ and <i>MspI</i> ⁻	<i>MspI</i> ^{+/-}
323	Mutant allele (<i>MspI</i> ⁻)**	<i>MspI</i> ^{-/-}

*Cut by *MspI* enzyme; **Uncut by *MspI* enzyme

137.54 cm (using formula 1), the genotypic values were found as follows: *GH-MspI*^{+/+} (a) was 3.88 cm (using formula 2), *GH-MspI*^{+/-} (d) was 8.17 cm (using formula 3), and *GH-MspI*^{-/-} (- a) was - 3.88 cm (using formula 4). These genotypic values were valuable in contribution for all phenotypic and genotypic parameters including population.

For a population in Hardy-Weinberg equilibrium, the phenotypic population mean (μ) was defined (Van Vleck *et al.*, 1987):

$$\mu = p^2P_{11} + 2pqP_{12} + q^2P_{22}$$

An alternative computing formula for obtaining the mean is based on substituting *m* + genotypic value for each phenotype. For a population in equilibrium, the mean is computed using formula (Van Vleck *et al.*, 1987) as follows:

$$\mu = p^2 (m + a) + 2pq (m + d) + q^2 (m - a)$$

Table 2. Average of Live Weight and Genotypic Value for Each Genotype GH Restriction Enzyme *MspI* in Ongole Grade Cows

Genotype of Cows	n	GF	Average of Phenotype			Genotypic Value (V _{ij})		
			LW, kg	CG, cm	BL, cm	LW, kg	CG, cm	BL, cm
<i>GH-MspI</i> ^{+/+}	8	p ²	P ₁₁ = 405.33	P ₁₁ = 166.75	P ₁₁ = 141.41	a = 0.30	-a = - 3.30	a = 3.88
<i>GH-MspI</i> ^{+/-}	14	2pq	P ₁₂ = 498.07	P ₁₂ = 179.50	P ₁₂ = 145.71	d = 93.04	d = 9.45	d = 8.17
<i>GH-MspI</i> ^{-/-}	15	q ²	P ₂₂ = 404.73	P ₂₂ = 173.34	P ₂₂ = 133.66	- a = - 0.30	a = 3.30	- a = - 3.88

n = number of cows; GF = Genotype frequency; LW = Live weight; CG = Chest girth; BL = Body length

$$= m(p^2 + 2pq + q^2) + a(p^2 - q^2) + 2pqd$$

Because $p^2 + 2pq + q^2 = 1$, and $(p^2 - q^2) = (p + q)(p - q) = (p - q)$, the mean is computed using formula (Van Vleck *et al.*, 1987) as follows:

$$\mu = m + [a(p - q) + 2pqd] \quad \dots\dots\dots (5)$$

Heritability is an extremely important population parameter that is used both for the estimation of breeding values for quantitative characteristics and for predicting the response expected from various selection schemes. The phenotypic variance (σ_p^2) was calculated using formula (Van Vleck *et al.*, 1987):

$$\sigma_p^2 = 2pqa^2 + (2pqd)^2 \quad \dots\dots\dots (6)$$

The Greek alpha (α) is used to represent specifically a part formula of $\{a + d(q - p)\}$ as found in Van Vleck *et al.* (1987). Thus, the Greek alpha (α) is defined as:

$$\alpha = [a + d(q - p)] \quad \dots\dots\dots (7)$$

Standard error of phenotypic variance is obtained from $\sqrt{\sigma_p^2}$ $\dots\dots\dots (8)$

The additive genetic variance (σ_A^2) for a single locus, is calculated using formula (Van Vleck *et al.*, 1987):

$$\sigma_A^2 = 2pqa^2 \quad \dots\dots\dots (9)$$

Heritability in the narrow sense (h^2) is defined as the ratio of the additive genetic variance to the phenotypic variance (Van Vleck *et al.*, 1987):

$$h^2 = \sigma_A^2 / \sigma_p^2 \quad \dots\dots\dots (10)$$

RESULTS AND DISCUSSION

The growth hormone (GH) genotypes using restricted enzyme of *MspI* for 37 cows were applied in this study. The PCR-RFLP data were used in establishing the observed homozygous *MspI*^{+/+} genotype, heterozygous *MspI*^{+/-} genotype and homozygous *MspI*^{-/-} genotype as showed in Table 1. The 37 genotyped parental cows showed that 15 cows were detected to have homozygous genotype of the *MspI*^{-/-} in GH locus, 14 cows were detected to have heterozygous genotype of the *MspI*^{+/-} in GH locus, and 8 cows were detected to have homozygous genotype of the *MspI*^{+/+} in GH locus.

The number of cows and the average of the phenotypic values of cow population for this study are presented in Table 2. Live weight was affected by the genotype at the locus of *GH-MspI*. The animal population was considered at the equilibrium with existing of gene frequency and phenotypic measurements as shown in Table 2. The *GH-MspI*⁺ represented allele affecting animal live weight. Cow genotype represented each

animal phenotype performance measured in the kg unit of cow body weight and cm unit of cow chest girth and body length. Genotype value was defined as deviation of phenotype from the mean of both homozygous phenotypes of P₁₁ and P₂₂ (shown at formula 1).

Using 37 samples of the cows in this study, the allele frequency *GH-MspI*⁺ (p) were 24/74 = 0.32. Because p = 0.32, the allele frequency of *GH-MspI*⁻ (q) = 0.68. Therefore, the population mean of the cows (μ) (using formula 5) were 445.41 kg. Those values indicated that live weight mean of the cow population in this study would be about 445.41 kg. The estimation average population of animal chest girth (using formula 5) in this study were 175.35 cm. Moreover, the estimation average population of animal body length (using formula 5) in this study were 139.70 cm.

Using 37 samples of the cows in this study, the allele frequency *GH-MspI*⁺ (p) were 24/74 = 0.32. Because p = 0.32, the allele frequency of *GH-MspI*⁻ (q) = 0.68. Thus, the genotype frequency of the animals in the population would be 0.10 for genotype *GH-MspI*^{+/+}, 0.44 for genotype of *GH-MspI*^{+/-}, and 0.46 for genotype of *GH-MspI*^{-/-}. Variation of observation for mean could be calculated in term of variance. In this study, the variance is denoted (σ_p^2) to represent phenotype variance. Using formula (7), the value of phenotype variance (σ_p^2) in this study were 2109.34. Thus, using formula (8), the standard error value of phenotype (σ_p) in this study were 45.93 kg. The estimation average population of animal live weight (using formula 5 and 8) in this study were 445.41 ± 45.95 kg.

Using formula (9), the additive genetic variance (σ_A^2) in a single locus was 496.90. Therefore, the heritability of cow live weight as calculated using formula (10) in this study was 0.24. This heritability value of cow live weight would be categorized as moderate value (Van Vleck *et al.*, 1987). For breeding program, the heritability (h^2) in narrow sense using the additive genetic variance (σ_A^2) was applicably more accurate in the improvement prediction for animal economically traits, including animal live weight, due to the representation and expression of the quantitative gene action involved. The accuracy of this heritability value of 0.24 based on individual records was about 55 percents (Legates and Warwick, 1990).

In chest girth measurement, the value of

phenotype variance (σ_p^2) in this study was 16.92 cm (using formula 7). Thus, using formula (8), the standard error value of phenotype (σ_p^2) in this study was 4.11 cm. The estimation average population of animal chest girth (using formula 5) in this study were 175.35 ± 4.11 cm. Using formula (9), the additive genetic variance (σ_a^2) in a single locus was 0.0045. Therefore, the heritability of cow chest girth as calculated using formula (10) in this study was 0.003. This heritability value of cow chest girth would be categorized as low value (Van Vleck *et al.*, 1987). For breeding program, the heritability (h^2) in narrow sense using the additive genetic variance was not applicably accurate in the improvement prediction for animal economically traits in term of animal chest girth, due to the expression of the quantitative gene action involved. The heritability value of less than 0.15 was categorized as low inheritance of the trait (Van Vleck *et al.*, 1987).

In body length measurement, the value of phenotype variance (σ_p^2) in this study was 32.89 cm (using formula 7). Thus, using formula (8), the standard error value of phenotype in this study were 5.73 cm. The estimation average populations of animal body length (using formula 5) in this study were 139.70 ± 5.73 cm. Using formula (9), the additive genetic variance (σ_a^2) in a single locus was 20.24. Therefore, the heritability of cow body length as calculated using formula (10) in this study was 0.41. This heritability value of cow body length would be categorized as high value (Van Vleck *et al.*, 1987). For breeding program, the heritability (h^2) in narrow sense using the additive genetic variance (σ_a^2) was applicably more accurate in the improvement prediction for animal economically traits in term of animal body length, due to also the expression of the quantitative gene action involved. The heritability value of more than 0.30 was categorized as high inheritance of the trait (Van Vleck *et al.*, 1987).

CONCLUSION

The phenotypic estimation average of Ongole grade cattle population of live weight, chest girth and body length were 445.41 ± 45.95 kg, 175.35 ± 4.11 cm, and 139.70 ± 5.73 cm, respectively. The genotypic performance in term of heritability values of animal live weight, chest girth and body length were 0.24, 0.003, and 0.41, respectively. These heritability values of animal live weight and body length would be categorized

as moderate to high genotypic performance values, while the heritability of animal chest girth was included in low heritability standard of the animal economical trait performance.

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