

## Association of SNP g.643G>A of MYF5 gene polymorphism with body weight and body measurements in Bali cattle

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### ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi SNP g.643G>A gen MYF5, kemudian mengasosiasikannya dengan bobot badan dan ukuran-ukuran tubuh pada sapi Bali. Sampel darah dikoleksi dari 80 sapi Bali di BPTU-HPT Denpasar Bali. Data fenotipe yang diamati meliputi; bobot lahir, bobot hidup, pertambahan bobot badan, panjang badan, dalam dada, tinggi pundak, tinggi pinggul, dan lingkaran dada. Keragaman gen MYF5 diidentifikasi menggunakan metode PCR-RFLP. Asosiasi genotip gen MYF5 dengan bobot badan dan ukuran tubuh dilakukan dengan menggunakan *General Linear Model* program SAS 9.4. Gen MYF5 | *MspI* bersifat polimorfik dengan tiga genotipe: AA, AG dan GG. Frekuensi genotipe AA, AG dan GG masing-masing adalah 0,04 (3), 0,30 (24) dan 0,65 (53). Frekuensi alel masing-masing 0,19 dan 0,81 untuk alel A dan G. Analisis frekuensi gen menunjukkan bahwa sapi Bali di BPTU-HPT Denpasar berada dalam keseimbangan Hardy-Weinberg. Asosiasi genotipe gen MYF5 | *MspI* dengan bobot badan dan ukuran tubuh tidak berbeda nyata. SNP g.643G>A belum dapat dijadikan penciri genetik sifat bobot badan dan ukuran tubuh pada sapi Bali.

*Kata kunci* : gen MYF5, PCR-RFLP, sapi Bali

### ABSTRACT

This study aimed to identify the SNP g.643G>A of MYF5 gene then associate it with body weight and body size measurements in Bali cattle. Blood samples were collected from 80 Bali cattle at BPTU-HPT Denpasar Bali. Data on phenotypic properties observed included; birth weight, live weight, average daily gain, body length, chest depth, withers height, hip height, and heart girth. Polymorphism of the MYF5 gene was identified using the PCR-RFLP method. Association of MYF5 genotypes with body weight and body size measurements was performed using General Linear Model by SAS 9.4 program. MYF5 | *MspI* gene was polymorphic with three genotypes: AA, AG, and GG. Genotype frequency of AA, AG and GG were 0.04 (3), 0.30 (24), and 0.65 (53), respectively. Allele frequencies were 0.19 and 0.81 for A and G alleles, respectively. Gene frequency analysis showed that Bali cattle at BPTU-HPT Denpasar was in Hardy-Weinberg Equilibrium. Association of MYF5 | *MspI* gene with body weight and body measurement were not significantly different. SNP g.643G>A could not be used as a genetic marker for the body weight and body size measurements in Bali cattle.

*Keywords* : Bali cattle, MYF5 gene, PCR-RFLP

## INTRODUCTION

Indonesia is a maritime country consists of many Islands and is rich in livestock genetic resources, one of them is Bali cattle (Mohamad *et al.*, 2009). Bali cattle (*Bos javanicus*) is one of the native Indonesian cattle produced by domestication of the bull (*Bibos banteng*) and has been recognized by Food and Agriculture Organization (FAO) as one of the world's cattle nations that lives in the Indonesian region (Martoyo, 2012). As a national animal genetic resources, Bali cattle should be maintained and utilized productively (Sutarno and Setyawan, 2016).

Bali cattle is adapted to the Indonesian climatic conditions and is able to be maintained intensively or extensively and still gives high production (Sutarno and Setyawan, 2016). This cattle has high conception rate (70-90%), high carcass percentage (45-57%) (Purwantara *et al.*, 2012). National meat requirements are mostly met by local Indonesian beef production. Indonesia's local cattle population in 2017 was estimated to be 16 599 247 heads and continues to grow by 3.59%. One of the local cattle that contributes to the fulfillment of national meat needs is Bali cattle. Even though it has superior potential, the use of Bali cattle genetic resources is not optimal, especially in terms of genetic quality. One approach to improve genetic quality and productivity is through a selection method (Supriyantono *et al.*, 2012).

Rapid development of DNA-based molecular technology has become one of the selection methods that uses genetic markers known as *Markers Assisted Selection* (MAS). Selection in bali cattle can be done on high economic traits. One of them is the of growth rate (Supriyantono *et al.*, 2012). The growth rate is controlled by many genes (Casas and Kehrl, 2016). One potential gene that plays a role in growth is the MYF5 gene (Chung and Kim, 2005; Nasr *et al.*, 2016).

The MYF5 gene in cattle is mapped on chromosome 5 which consists of 3 exons and 2 introns (Seong *et al.*, 2011). The MYF5 gene is a member of Myogenic Regulatory Factors (MRFs) which is expressed the earliest among members of other MRFs and one of the genes involved in the mechanism of skeletal muscle formation and the formation of lateral sclerotome derivatives (Hernández-Hernández *et al.*, 2017). MYF5 gene has an important role in myocyte determination

and differentiation (Li *et al.*, 2004) and also controls myogenesis and myoblast growth during prenatal embryo development (Guo *et al.*, 2015). Myogenesis also controls the expression of some myogenic regulatory factors (MYOD and MYF5) (de las Heras-Saldana *et al.*, 2019).

Several studies related to the SNPs identification of the MYF5 gene have been carried out widely in several livestock such as hanwoo cattle (Chung and Kim, 2005), Canada cattle (Li *et al.*, 2004) Jiaxian cattle, Qinchuan cattle, Luxi cattle, Nanyang cattle and Xianan cattle (Ujan *et al.*, 2011). This gene was found to be significantly associated with yearly body weight (Li *et al.*, 2004) and body gain (Chung and Kim, 2005). Some researches were conducted on MYF5 gene in bali cattle but are still few especially on MYF5 gene polymorphisms and its association with growth traits. Bali cattle is one of the livestock species used to provide meat in Indonesia. Therefore, identification of SNP g.643G>A of MYF5 | *MspI* in bali cattle has important role in increasing meat production in Indonesia. The present study aimed to identify MYF5 | *MspI* gene polymorphisms and its association with body weight traits and body size measurements in bali cattle.

## MATERIALS AND METHODS

### Animals and Samples

This research was conducted at BPTU-HPT Denpasar of Bali province and at the Molecular Genetic Laboratory of Animal Science, Department of Animal Production and Technology, IPB University (Bogor Agricultural University), Indonesia. Blood samples for DNA extraction were collected from 44 female and 36 male Bali cattle at jugular vein using venoject needle.

### DNA Extaction and Polymerase Chain Reaction-Rectriction Fragment Length Polymorphism (PCR-RFLP)

DNA extraction was performed using Geneaid DNA extraction kit procedure (Geneaid Biotech Ltd). PCR was performed for amplification of polymorphism region of MYF5 gene. A pair of primers used was designed by (Ujan *et al.*, 2011). These primers (*forward* and *reverse*) were 5'-ACGACCAACCCTAACC-3' and 5'-CCA ACTATCCACCAGTAACC-3', respectively. They were used to amplify a 285 base pairs fragment according to the beef cattle

genomic sequence in the GenBank database (accession number M95684.1). The PCR was performed under the following conditions, initial denaturation at 95 °C for 5 minutes and for 1 cycle. The second phase consisted of 35 cycles, each cycle consisting of denaturation process at 95 °C for 10 seconds, primer annealing at 51 °C for 20 seconds and DNA extension at 72 °C for 30 seconds. The final phase was the primer elongation or final extension at 72 °C for 5 minutes. The DNA amplification product of 285 bp was visualized by 1.5% agarose gel electrophoresis.

PCR products from polymorphic region of MYF5 gene (285 bp) were digested with *MspI* restriction enzyme selected according to the software ([http://tools.neb.com/NEB\\_cutter2/index.php](http://tools.neb.com/NEB_cutter2/index.php)) of the polymorphic site. PCR product and *MspI* restriction enzyme were incubated at 37 °C for 16 hours (Thermo Fisher Scientific, EU, Lithuania). The product of DNA fragments from PCR-RFLP were visualized using agarose gel electrophoresis with a concentration of 2%, 0.6 agarose powder was added to 30 ml of 0.5 x TBE, the mixture then heated to boiling and added to 1 uL florosafe electrophoresis was run on average voltage of 100 volts for 35 minutes. Gels were visualized under ultra-violet transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA).

## Data Analyses

### Genotype Frequency, Allele Frequency and Hardy-Weinberg Equilibrium

Genotype and allele frequencies, chi-square and Hardy-Weinberg Equilibrium (HWE) were

calculated using Popgene32 software program (Yeh *et al.*, 2000).

### Association of MYF5 Gene Polymorphisms with Body Weight and Body Size Measurements

The MYF5 gene polymorphisms and association with body weight traits and body size measurements was analyzed using General Linear Model (GLM) (SAS 9.4). The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijk}$$

Where:  $Y_{ijk}$  = number of observed;  $\mu$  = mean;  $\alpha_i$  = effect of i genotype;  $\beta_j$  = sexes effect;  $\gamma_k$  = effect of age: cattle were grouped according to their age (1.5 - 2 years; 2 - 2.5 years and 2.5 - 3.5 years);  $\epsilon_{ijk}$  = random errors. Least square means (LSM) was used to test the significance difference among genotypes.

## RESULTS AND DISCUSSION

### MYF5 Gene Polymorphism

The MYF5 gene Single Nucleotide Polymorphism (G>A) is located at the nucleotide position g.643, exon 1 of chromosome 5 with GenBank Accession Number: M95684.1. The 285 bp fragment of MYF5 (g.643G>A) gene was successfully amplified in Bali cattle using Applied Biosystems PCR machine under conditions of annealing temperature 51°C for 20 seconds as shown in (Figure 1).

The MYF5 fragment of 285 bp was digested with *MspI* restriction enzyme and resulted into three genotypes designed as AA (a single, uncut fragment of 285 bp), GG (two fragments of 115

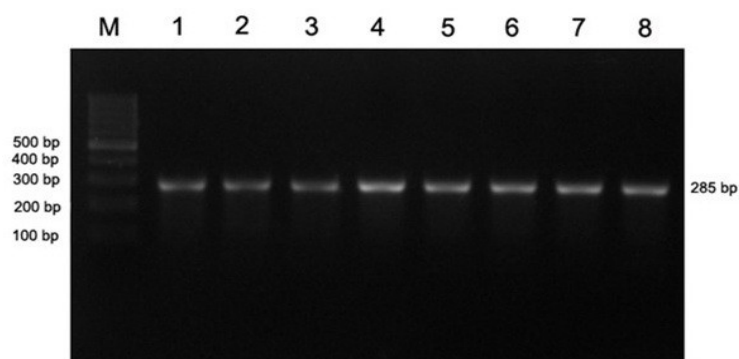


Figure 1. Amplification results of PCR for MYF5 gene in Bali cattle using 1.5% gel agarose; M=100 bp ladder size standard; Line 1-8= individual Bali cattle samples)

bp, and 170 bp), and GA (three fragments 115 bp, 170 bp, and 285 bp (Figure 2). MYF5 | *MspI* for Bali cattle at BPTU-HPT Denpasar is polymorphic. Polymorphism occurs when each allele frequency is greater than 0.01 (Allendorf and Luikart, 2007). The restriction enzyme recognizes DNA fragment and cuts it at specified position, while visualization of PCR-RFLP results was carried out using 2% agarose gel.

Genotype frequencies of AA, AG and GG are, respectively 0.04 (3), 0.30 (24) and 0.65 (53) and allele frequencies are presented in Table 1. Allele G was more frequent with 0.81 and 0.19 for allele A. Gene frequency analysis showed that Bali cattle population at BPTU-HPT Denpasar was in Hardy-Weinberg equilibrium as shown in Table 1. Chen *et al.* (2017) stated that Hardy-Weinberg equilibrium occurs in a larger population size, if the frequency of dominant and recessive genotypes is constant from generation to generation, there is no selection, mutation, migration, and genetic drift. Selection is one of

the factors that can quickly change the equilibrium in the population. The present study showed that there was no selection in Bali cattle reared at BPTU-HPT Denpasar.

#### Association of MYF5 Gene Polymorphism with Body Weight and Body Size Measurements of Bali Cattle

Association analysis of SNP g.643G>A MYF5 | *MspI* gene with body weight and body size measurements showed that the SNP studied was not significantly associated with body weight and body size measurements ( $P>0.05$ ). Results are presented in Table 2. Although mutation occurs in exon, SNP g.643G>A did not cause a significant effect because the mutation was synonymous. The SNP at g.643G>A position with CCG or CCA codon will translated to proline amino acid. This SNP is specific for Bali cattle, and it is not found in other Indonesian beef cattle.

Chung and Kim (2005) stated that mutation of A>G was found in the MYF5 intron 2 exon 3

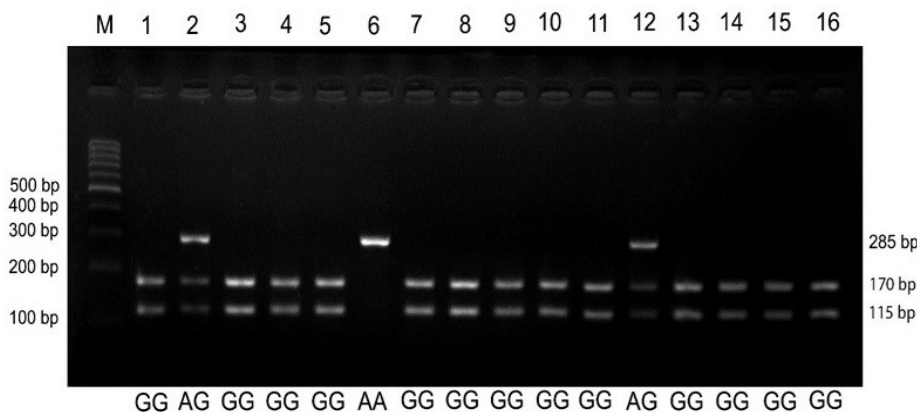


Figure 2. PCR-RFLP analysis of 285 bp fragment of MYF5 | *MspI* gene using 2% agarose gel. AA : AA genotype; AG : AG genotype; GG : GG genotype; M : DNA marker 100 bp.

Table 1. Genotype Frequency, Allele Frequency, and Hardy-Weinberg Equilibrium in MYF5 | *MspI* Gene Fragments in Bali Cattle

Populations	N	Genotype Frequency			Allele Frequency		Chi-Square ( $\chi^2$ )
		AA (n)	AG (n)	GG (n)	A	G	
Bali cattle	80	0.04 (3)	0.30 (24)	0.65 (53)	0.19	0.81	ns

N: number of individuals population,  $\chi^2$  table (0.05) = 5.99 for df 2, ns = not significant

Table 2. Association of MYF5 | *MspI* genes for Body Weight and Body Size Measurements in Bali Cattle

Phenotype	Genotype			P-value
	AA (n=3)	AG (n=24)	GG (n=53)	
Birth weight (kg)	19.0±3.6	18.7±2.3	18.7±2.2	ns
Live weight (kg)	168.0±37.6	188.8±38.7	190.0±34.3	ns
Average daily gain (kg)	0.21±0.05	0.22±0.06	0.24±0.05	ns
Body length (cm)	102.3±7.8	104.8±8.5	104.8±8.2	ns
Chest depth (cm)	55.7±4.0	55.6±4.5	55.2±4.1	ns
Withers height (cm)	107.3±3.0	111.8±5.0	110.5±4.4	ns
Hip height (cm)	106.3±1.5	111.1±5.8	109.9±4.1	ns
Heart girth (cm)	135.3±8.7	143.3±9.2	143.4±9.5	ns

n : number of individuals; ns: not significant

gene. This mutation was clearly associated with body weight of 12 months and daily body weight gain in Korean cattle. Seong *et al.* (2011) also reported that the MYF5 intron 2 gene in the SNP A1948G had a significant effect on the back fat thickness and a 6 month live weight in Hanwoo cattle. SNP of MYF5 gene that are associated with growth properties can be used as the genetic markers. In the future, the identification of MYF5 gene SNPs in cattle must be continued. It is expected that the use of genetic markers can be applied as an assisted selection marker to improve the genetic quality of Bali cattle.

### CONCLUSION

SNP g.643G>A on the MYF5 | *MspI* gene was polymorphic. The population of Bali cattle at BPTU-HPT Denpasar was in Hardy-Weinberg equilibrium. Association of the polymorphism of the MYF5 | *MspI* gene in Bali cattle did not show a significant difference on body weight and body size measurements of Bali cattle.

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