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Association of Calpastatin (CAST) Gene with Growth Traits and Carcass Characteristics in Bali Cattle

R. Putri , R. Priyanto, A. Gunawan, & Jakaria*
Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University
Jalan Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia
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ABSTRACT

Calpastatin (CAST) gene is well known as an inhibitor of muscle protein degradation and relates to muscle growth and meat tenderness. The objective of this study was to determine the association of CAST gene with growth traits and carcass characteristics in Bali cattle. A number of data from 35 Bali bulls were collected from BPTU Bali Cattle to obtain growth traits, carcass characteristics, and blood samples. Polymorphism of CAST gene in Bali bulls was analyzed by using PCR-RFLP and DNA sequencing. The association of CAST gene with growth traits and carcass characteristics were analyzed by using General Linear Model (GLM). The result showed that there were two genotypes (GG and AG) of CAST gene with allele frequencies of 0.857 and 0.143, respectively, for G and A. Notably, mutation A to G occurred in 253 bp CAST fragment gene in Bali Cattle. Genotypes GG and AG of CAST gene significantly affected (P<0.05) the back-fat thickness and *longissimus dorsi* without a significant effect on the growth traits. It could be concluded that CAST gene had a potency as a marker gene for carcass quality in Bali cattle.

Key words: Bali cattle, CAST gene, PCR-RFLP, polymorphism

ABSTRAK

Gen kalpastatin (CAST) dikenal sebagai penghambat degradasi protein otot yang berkaitan dengan pertumbuhan otot dan keempukan daging. Tujuan penelitian ini adalah untuk mempelajari asosiasi gen kalpastatin dengan sifat pertumbuhan dan karakteristik karkas pada sapi Bali. Sebanyak 35 ekor sapi Bali pejantan dikoleksi dari BPTU Sapi Bali di Provinsi Bali untuk mendapatkan data sifat pertumbuhan, karakteristik karkas, dan sampel darah. Keragaman gen CAST pada sapi Bali dianalisis menggunakan teknik PCR-RFLP dan DNA sekuensing. Asosiasi gen CAST dengan sifat pertumbuhan dan karakteristik karkas dianalisis dengan menggunakan General Linear Model (GLM). Hasil penelitian menunjukkan bahwa genotipe yang didapatkan dari hasil penelitian ini adalah genotipe GG dan AG dengan frekuensi alel G dan A masing-masing adalah 0,857 dan 0,143. Terdapat perubahan basa adenin (A) menjadi guanin (G) pada posisi pb 253 fragmen gen CAST pada sapi Bali. Genotipe GG dan AG gen CAST berpengaruh nyata (P<0,05) terhadap tebal lemak punggung dan *longissimus dorsi,* sedangkan sifat pertumbuhan tidak berbeda. Hal ini dapat disimpulkan bahwa gen CAST memiliki potensi sebagai penanda untuk karakteristik karkas pada sapi Bali.

Kata kunci: sapi Bali, gen CAST, PCR-RFLP, polimorfisme

INTRODUCTION

Selection in animal breeding is known as one of the breeding methods that can be used to increase the genetic quality of animals (Bourdon, 2000). Molecular technology can be used as a selection method to increase genetic quality based on marker-assisted selection (MAS). Genetic marker that can be used in molecular study based on DNA is Polymorphism Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). PCR-RFLP is one of genetic markers that use restriction enzyme in gene-polymorphism determination, especially in animal (Xiao *et al*., 2006).

One of Indonesia native cattle which have been recognized by FAO is Bali cattle (*Bos javanicus*) as the result of bull domestication (DGLS, 2003). Bali cattle has advantage that it is well adapted to tropical environmental stress and has a high fertility, especially when it

*Corresponding author:
E-mail: jakaria_karman@yahoo.co.id

is managed and fed with low quality diet (Purwantara *et al*., 2012). Therefore, Bali cattle are potential to be developed as beef cattle due to its good meat quality and low fat percentage (Bugiwati, 2007). Improvement of genetic quality of Bali cattle can be done on the economically-valuable traits such as growth traits and carcass characteristics. Growth traits and carcass characteristics have a high heritability (Baiduri *et al*., 2012; Kaswati *et al*., 2013; Putra *et al*., 2014), thus selection can be performed based on genotypes. The growth traits and carcass characteristics are controlled by many genes (Dunner *et al*., 2013). One of the genes that play a role in growth traits and carcass characteristics is calpastatin (CAST) gene (Schenkel *et al*., 2006).

Calpastatin (CAST) works by inhibiting protease enzyme to degrade muscle protein and relates to meat production, meat tenderness, and meat quality (Azari *et al*., 2012; Giusti *et al*., 2013). Calpastatin affects many processes, such as fusion regulation and myoblast migration, protein turn over, and muscle growth (Kempt *et al*., 2010; Schenkel *et al*., 2006). Muscle protein that plays an important role in growth of skeletal muscle is myofibril protein. Degradation occurred in myofibril proteins affects variation in loin-eye area (*Longisimuss dorsi*) (Kempt *et al*., 2010).

Several studies reported that CAST gene had an association with carcass and meat-quality characteristics such as *Longissimus* muscle area, marbling grade, fat, lean, fat grade, carcass weight, back-fat thickness, water content, cooking loss, pH, meat tenderness, and water holding capacity (Davis *et al*., 2008; Casas *et al*., 2006; Curi *et al*., 2009; Schenkel *et al*., 2006; Li *et al*., 2010; Kubiak *et al*., 2004). Most of the studies on CAST gene were carried out in *Bos taurus* and *Bos indicus* cattle. The objective of this study was to find out the association of CAST gene with growth and carcass traits in Bali cattle (*Bos javanicus*).

MATERIALS AND METHODS

Animal and Sampling

A total number of 35 Bali bulls (3, 4, and 6 years of ages) were collected to obtain data on growth traits (birth weight, finish weight, average daily gain, chest circumference, body length, and shoulder height), carcass characteristics (the thickness of back fat and *longissimus dorsi*), and blood samples. Bali bulls in this study were collected from BPTU Bali Cattle, Pulukan, Jembrana, Bali Province. Growth traits (birth weight, finish weight, average daily gain, chest circumference, body length, and shoulder height) were measured based on SNI (2008).

The measurements of thickness of back fat and *longissimus dorsi* were carried out on the 12th ribs, two third from medial to lateral side, by using Veterinary Ultrasound Scanner WED-3000V models (Gupta *et al*., 2013; Melendez & Marchello, 2014). The image results were analyzed by using Image-J NIH software (ImageJ®, NIH, USA).

SNP Identification and Genotyping

The blood was extracted by standard method from Green & Sambrook (2012). Calpastatin gene (exon 1C and 1D), forward: 5′ TGGGGCCCAATGAC GCCATCGATG 3′ and reverse: 5′ GGTGGAGCAGCACTTCTGATCACC 3′, were designed by Palmer *et al*. (1998). Polymerase-chain reaction was performed by using PCR-mix in total volume of 50 μL which consisted of 2 μL genomic DNA, 0.8 μL primer, 25 μL Go Taq master mix, and 22.2 μL distillation water. The PCR reaction was carried out with an initial pre-denaturation temperature of 95^{o}C (5 min), followed by 35 cycles of denaturation at 95^{o}C (10 s), annealing at 62^{o}C (20 s), and extension at 72^{o}C (30 s). The final extension was at 72^{o}C (5 min). The amplified PCR product was separated in 1.5% agarose gel containing ethidium bromide and then visualized by UV trans-illuminator.

The analysis of CAST gene polymorphism was conducted with PCR-RFLP (Polymorphism Chain Reaction-Restriction Fragment Length Polymorphism) method. The amplicon was digested with *AluI* restriction enzyme which has 5′-AGCT-3′ digestion site. The digestion reaction was carried out in 10 μL of mixture which consisted of 0.3 μL of *AluI* restriction enzyme, 5 μL of PCR product, 0.7 μL of buffer, and 1 μL of distilled water. The reaction mixture was incubated at 37^{o}C for 16 h, after that the restriction fragment was separated in 2% agarose gel containing *ethidium bromide* and then visualized by UV trans-illuminator.

Sequencing Analysis

Sequencing was performed for individual Bali bull representing different genotypes. Forward and reverse primer fragments were sequenced by using sequencer machine (ABI Prims 3100-Avant Genetic Analyzer) in 1st Base Selangor, Malaysia. The sequencing result was aligned by using MEGA version 4 software (Tamura *et al*., 2011).

Statistical Analysis

The genotypic (Xii) and allelic (Xi) frequencies from PCR-RFLP method were calculated according to Nei & Kumar (2000).

$$X_{ii} = n_{ii}/N \qquad X_i = (2N_{ii} + \Sigma n_{ij})/2N$$

where:
n_{ii} = the number of individual of *ii* genotype
n_{ij} = the number of individual of *ij* genotype
N = the total of individual samples.

The association analysis between CAST gene and growth traits (finish weight, average daily gain, chest circumference, body length, and shoulder height) and carcass quality (back fat thickness and *longissimus dorsi*) were analyzed by using the following General Linear Model (GLM) by ANCOVA (Kaps & Lamberson, 2004) with a mathematical model as follow:

$$Y_{ij} = \mu + \alpha_i + \beta X_{ij} + \epsilon_{ij}$$

where:
Y_{ij} = observed value
μ = overall mean
α_i = effect for genotype (GG and AG)
β = regression coefficient
X_{ij} = covariant (age)
ϵ_{ij} = random error

RESULTS AND DISCUSSION

Polymorphism of CAST Gene

Calpastatin gene amplification in Bali bull was contained in annealing at the optimal temperature of 60°C (20 s). The result of CAST amplification in Bali bull showed 624 base pairs (bp) PCR product (Figure 1). The amplification process of DNA fragment was determined by some factors, such as primer concentration, amplification temperature, denaturation duration, DNA samples, and $MgCl_2$ concentration (Williams 2005).

The genotyping result of CAST gene in Bali bull showed two genotypes (GG and AG) (Figure 2). GG genotype was found in 10 samples and AG genotype was found in 25 samples. The homozygous of GG genotype (no restriction in 253 bp position) produced one band of 474 bp, whereas the heterozygous of AG genotype produced three bands of 474 bp, 336 bp, and 138 bp (Figure 3). Those genotypes were different from genotypes reported by previous study. Kubiak *et al*. (2004) showed that there were three genotypes (GG, GC, and CC) in various breeds of cattle in the same fragment of CAST. The result showed there was an A (adenine) to G (guanine) mutation in 253 bp of the restriction site of CAST|*Alu*I (Figure 4). In this study, the genotype frequencies for GG and AG were 0.714 and 0.286, respectively. The allele frequencies for G and A were 0.857 and 0.143, respectively. This result showed that G allele was higher frequency than A allele, because there was no AA genotype that was found in this population. AA genotype was not found because of the uncontrolled

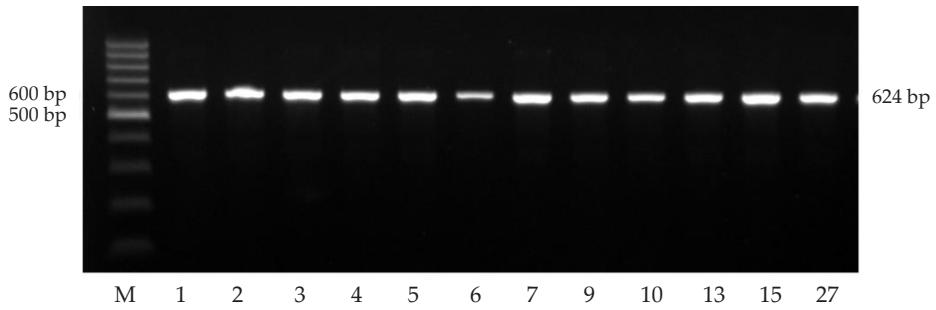

Figure 1. The result of CAST gene PCR fragments electrophoresis. Note: M= marker, 1-27 = number of animal)

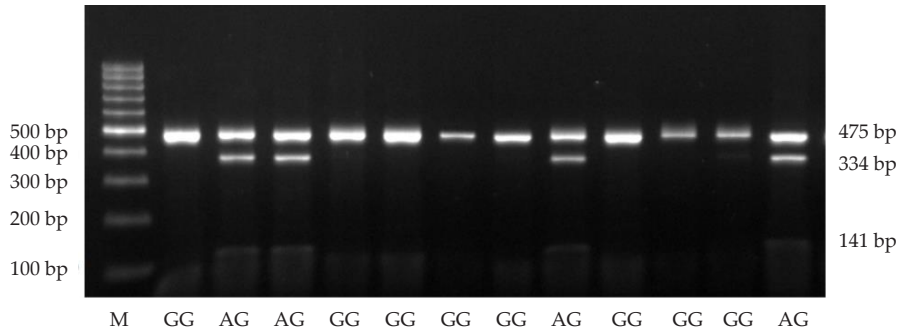

Figure 2. The result of CAST|*Alu*I fragment restriction on 2% agarose gel electrophoresis. Note: M= marker, GG, AG= genotype.

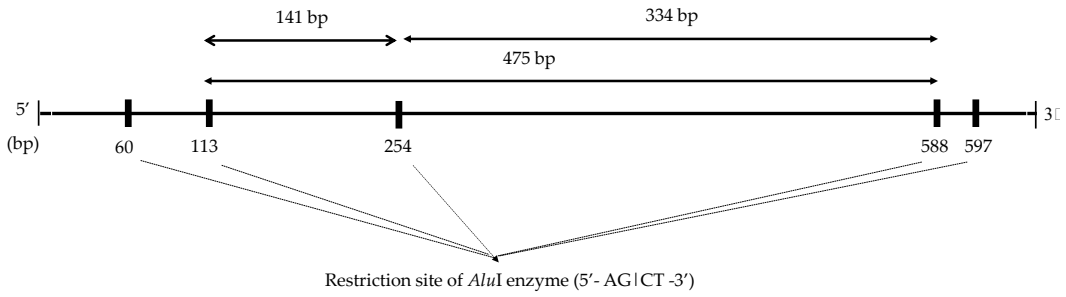

Figure 3. Restriction site of *Alu*I enzyme in Bali bull

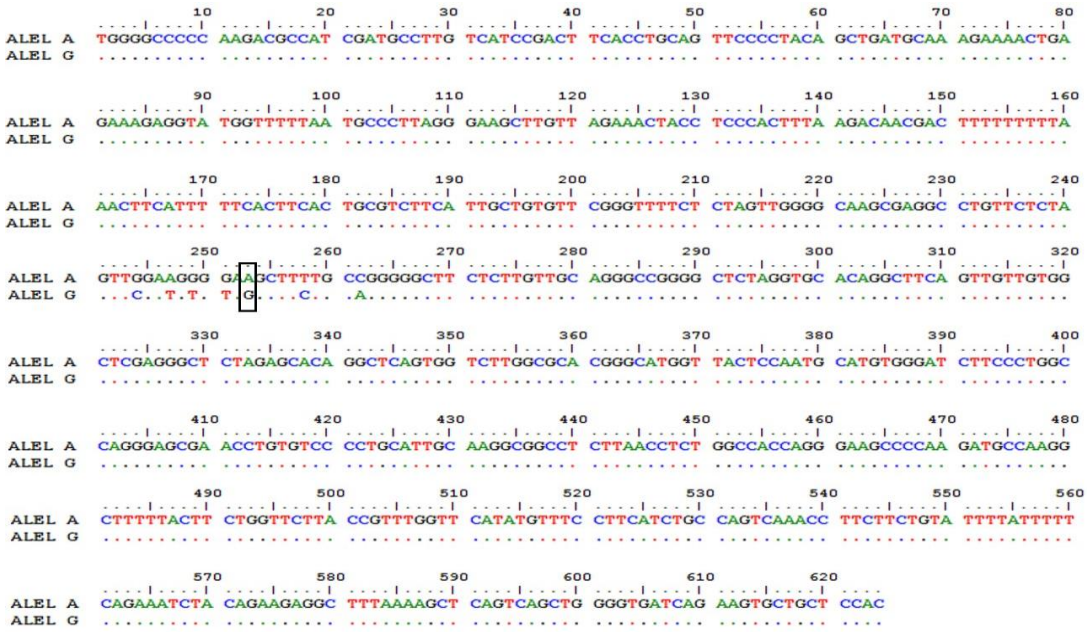

Figure 4. Mutation of A (adenine) to G (guanine) in 253 bp of the restriction site of CAST|*Alu*I in Bali bull

selection that was occurred in this population. However, the diversity of CAST|*AluI* in population of Bali bulls in this study was polymorphic that was represented by the allele frequencies that was lower than 0.99 or higher than 0.01 (Nei & Kumar, 2000).

Association Analysis

This study revealed an association of CAST with carcass characteristics (thickness of back fat and *longissimus dorsi*) (Table 1). However, the association of CAST was not found with growth traits (Table 1).

Figure 5 showed the result of ultrasound measurement of the thickness of back fat and *Longissimus dorsi*. The GG genotype had significantly higher back-fat thickness than that of AG. In contrast, the AG genotype had significantly higher muscle thickness of *longissimus dorsi*. The back-fat thickness is a parameter indicating carcass fattiness and meat yield (Gupta *et al*., 2013).The ranges of back-fat thickness for AG and GG in this study were 1.935±0.157 and 2.324±0.097 mm, respectively. The result showed that thickness of back fat in Bali bulls can be classified as an ideal carcass for traditional market. Halomoan *et al*. (2001) showed that the range of back-fat thickness for traditional market was from 1 up to 5 mm. There are several factors which influence the carcass composition, such as growth rate, nutrition, age, and body weight (Soeparno, 2005). The increase in back fat decreases meat proportion, or the decrease in back fat increases meat proportion (Irshad *et al*., 2013). The Bali bull with AG genotype showed a thinner back fat and tended to have a larger body frame. Therefore the Bali cattle with a larger body frame in this study might yield a higher proportion of meat.

Table 1. The association of CAST gene with growth traits and carcass quality in Bali bulls

Characteristic	Genotype	
	GG (n= 25)	AG (n= 10)
Birth weight (kg)*	17.800±0.200	17.480± 0.276
Finish weight (kg)	402.075±8.866	421.212±14.429
Shoulder height (cm)	127.164±0.985	128.390± 1.603
Chest circumference (cm)	181.807±1.568	184.782± 2.552
Body length (cm)	135.574±1.167	139.766± 1.900
Average daily gain (kg/d)	0.265±0.007	0.272± 0.012
Thickness of back fat (mm)	2.324±0.097[a]	1.935± 0.157[b]
Thickness of *Longissimus dorsi* (mm)	57.577±1.533[a]	63.818± 2.495[b]

Note: Means in the same row with different superscripts differ significantly (P<0.05); n= number of animal; *= analyzed by ANOVA.

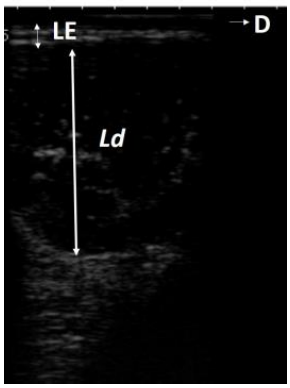

Figure 5. The result of ultrasound using Image-J NIH in Bali bull. Note: Ld= *Longissimus dorsi*, Le= back fat, D= dermis.

CONCLUSION

The CAST gene polymorphisms (GG and AG genotypes) had significant effect on *longissimus dorsi* and back-fat thickness traits, but it did not affect the growth traits. The CAST gene was a potential marker for *longissimus dorsi* and back-fat thickness in Bali cattle.

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