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RESEARCH ARTICLE

Calpastatin (*CAST*) Gene Polymorphism in Indonesian PO Cattle

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ABSTRACT

In beef cattle production, meat quality is one of important characters which has to be considered. Calpastatin (CAST) has important function in meat quality. Calpastatin is the endogenous inhibitor of calpain proteases and it plays an important role in the development of muscle and in meat tenderness. This aim of this study was to identify polymorphism of *CAST* gene of PO cattle. Random blood samples were collected from 30 animals. DNA was extracted from blood by QIAamp DNA blood mini kit (Qiagen). A 600 bp gene segment was amplified by PCR (Polymerase Chain Reaction) using bovine specific primers. Restriction fragment length polymorphisms (RFLPs) in the amplified fragments were studied using *Hae*III restriction enzyme. In this population, AA, AB, AC and AD genotypes have been identified with the 53.33, 20, 13.33 and 13.33 % frequencies, respectively. A, B, C, and D alleles frequencies were 0.77, 0.1, 0.07 and 0.07, respectively. AA genotype is the dominant genotype and the A allele is the dominant allele. It can be concluded that *Hae*III locus of *CAST* gene of PO cattle were polymorphic.

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INTRODUCTION

Tenderness is an important character in beef cattle enterprise because it has a major impact on meat quality and consumer satisfaction (Allais *et al.,* 2011; Nowak, 2011; Kemp *et al.,* 2010; Schenkel, 2006). The calpain system is the principal contributor to postmortem proteolysis which is related to meat tenderness. Numerous studies have shown that the main role among the calpain system plays is m-calpain, µ-calpain and calpastatin (Nowak, 2011; Kemp *et al*, 2010; Camou *et al*, 2007). Calpain-calpastatin association can occur within cell depend on Ca^{2+} level (Melloni *et al.*, 2006). Calpains are protease family with regard that proteolytic calpain activity does contribute to meat tenderization. Calpastatin (CAST) contains 4 inhibitory domains, each of which can inhibit calpain activity. There are three regions which are binding into calpain in each domain (Kemp *et al.*, 2010). Calpastatin, which is an endogenous inhibitor (Ca^{+2} dependent cysteine proteinase) of calpain, plays a central role in regulation of calpain activity in cells (Tompa *et al.*, 2002; Mohammadi *et al.*, 2008). There is a negative correlation between calpastatin enzyme activity with tenderness, higher level of calpastatin enzyme activity postmortem are related to less meat tenderness (Woodward *et al.,* 2000).

Calpastatin encoded by gene is located on the seventh chromosome of cattle (Bishop *et al.*, 1993). Polymorphism of the *CAST* gene is closely associated with meat tenderness in cattle (Casas *et al*., 2005, Schenkel *et al*., 2006). The CAST genotype significantly influenced the average LM tenderness, homozygous genotype CC is more tenderness than heterozygous genotyeps CG and homozygous genotype GG (Schenkel *et al*., 2006). SNP2959 in the Chinnesse commercial cattle herds was significantly associated with Warner-Bratzler Shear Force (Li *et al*., 2010). Kuryl *et al* (2003) showed the polymorphism of *CAST* gene with three restriction enzymes (*Hinf*I, *MSP*I and *Rsa*I) in stamboek (Dutch Large white x Dutch Landrrace) pig breed.

PO cattle are one of indigenous bovine species in Indonesia. The fulfillment of the meat in Java is partially supplied by PO cattle. The objective of this study was to determine the occurrence polymorphism in the *CAST* gene of PO cattle using PCR-RFLP method.

METERIALS AND METHODS

Animals and DNA extraction

Blood samples were randomly collected from 30 PO cattle from East of Java. DNA was extracted from blood

by QIAamp DNA blood mini kit (Qiagen). Quality and quantity of DNA were measured by visual and spectrophotometer methods.

PCR primers and amplification

The bovine CAST sequence (GenBank : AY 834774) was used to design the PCR primer. The sequences of primers were forward primer, 5'-ATCCAGAAGACGGA AAGCCT-3' and reverse primer 5'-CTCACGATCCTCT TC TTTGG-3'.

The PCR was performed in a 20 ul reaction, containing 10 pmol of each primer, 50 ng of genomic DNA, 10 ul of PCR master mix (Intron Biotech). The PCR cycling conditions was an initial denaturation step of 94 ^{o}C (2 min); cycling reaction (31 cycles) of 94 ^{o}C (60 s), 58^{o}C (45 s) and 72^{o}C (60 s), final extension at 72^{o}C for 5 min. Electrophoresis of PCR products was performed in 2 % agarose gel containing ethidium bromide and visualized by a UV trans-illuminator.

Restriction Fragment Length Polymorphism (RFLP) analysis

The result amplicon of 600 bp was digested with *Hae*III restriction enzyme, the restriction site at GG*CC. The digestion reaction was carried out in 10 ul of mixture reaction which consist of 3 ul of PCR product, 1.2 ul Ne2 buffer, 0.5 ul free ionized water and 3.5 Unit *Hae*III restriction enzyme. The reaction mixture was incubated in 37^{o}C for 3 h. Electrophoresis of digested PCR products was performed in 7.5% non-denaturing polyacrylamide gels, stained by silver nitrate staining merhod. All the PCR-RFLP fragments resulted were used for analysis of polymorphism.

RESULTS

The analysis of *CAST* gene polymorphism was carried out using PCR-RFLP. The amplified *CAST* gene resulted in a DNA fragment with 600 bp (Fig. 1). The digestion of PCR product with restriction enzyme *Hae*III produced four alleles (A, B, C and D) and four genotypes (AA, AB, AC, and AD) (Fig. 2). This result shows that the polymorphisms were detected in *CAST* gene of PO cattle. AA genotype showed the three band pattern (300 bp, 150 bp, 50 bp). AB genotype showed the four band pattern (350 bp, 300 bp, 150 bp, 50 bp). AC genotype showed the four band pattern (350, 300 bp, 150 bp, 140 bp, 50 bp). AD genotype showed the six band pattern (500 bp, 400 bp, 300 bp, 150 bp, 140 bp, 50 bp).

In population of PO cattle, four genotypes (AA, AB, AC and AD) were detected. The homozygous genotype AA and heterozygous AB, AC and AD was detected in 16, 6, 4, and 4 cattle animals respectively. The frequencies of CAST alleles and genotypes are presented in Table 1.

DISCUSSION

Calpastatin gene is the most documented gene which associated to meat tenderness, and it has been considered as choice genes for selection of both quantity and quality of meat (Gabor *et al.*, 2009). In recent years, researches in DNA polymorphisms have been widely studied in the gene of cattle. A polymorphism was found to be associated to the meat tenderness (Schenkel *et al.*, 2006; Pinto *et al.*, 2010). Shackelford *et al.* (1994) reported that calpastatin activity gene is highly heritable and has pronounced effect in meat tenderness. In this study, distribution of allele frequencies of the PO cattle *CAST* gene showed that the frequency of allele A was higher than allele B, C and D (Table 1). The level polymorphisms that found in this study were higher than those reported by Schenkel *et al.* (2006) working with three different alleles in beef cattle. Another study using PCR-RFLP reported the polymorphisms of CAST-Msp1 in Holstein cattle with two types of genotypes (MM and MN) with the frequency of 46 %, 54 %, respectively (Yousefi *et al.*, 2012).

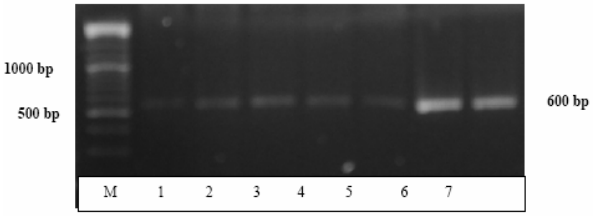

Fig. 1: Electrophoretic profile for the *CAST* gene fragments amplified by PCR in a 2 % agarose gel. M represented a marker with 100 bp ladder. Lane 1-7 represented a strand with 600 bp.

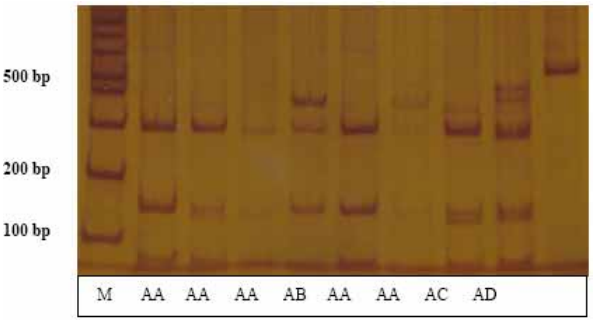

Fig. 2: Restriction pattern of 600 bp fragment of *CAST* gene after digesting with *Hae*III on 7.5% on non-denatured polyacrylamide gel after silver nitrate staining. M: DNA ladder 100 bp (Fermentas).

The frequency of the homozygous genotype AA was higher than heterozygous genotype AB, AC and AD in the studied PO cattle population (Table 1). Similar result by Gabor *et al.* (2012) reported in Slovak Simmental cattle that who observed homozygous genotypes was higher than heterozygous. Different result by Yousefi and Mojtaba (2012) reported in Holstein cattle that heterozygous genotypes were higher than homozygous genotypes.

Table 1: Distribution of CAST alleles and genotypes frequency

Gene	n	Genotype				Allele			
		AA	AB	AC	AD	A	B	C	D
CAST	30	16(53.33%)	6(20%)	4(13.33%)	4(13.33%)	0.77	0.1	0.07	0.07

n = number of animal

Further studies on *CAST* gene should be carried out in PO cattle and other Indonesian local beef cattle to detect novel associated of polymorphisms with meat quality traits.

Conclusions

This study concluded that there is polymorphism in the *Hae*III locus of the PO cattle *CAST* gene. Four genotypes observed include AA, AB, AC and AD with the genotype frequencies of 53.33%, 20%, 13.33% and 13.33%, respectively. An allele was the most common allele with frequency of 0.77 in population.

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