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Genetic Polymorphism of Calpastatin (*CAST*) Gene in Pasundan Cattle

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ABSTRACT

The aim of this study was to determine genetic diversity of Calpastatin gene (*CAST*) in Pasundan cattle. Forty-four fresh blood samples were collected from UPTD BPPT Beef Cattle Ciamis West Java. Genomic DNA samples were extracted using High Salt method. A 523 bp fragment of Calpastatin gene was successfully amplified using PCR method. Genotyping of *CAST* gene was conducted by PCR-RFLP method using *Rsa*I restriction enzyme (GT^AC). Genotypes and alleles were analyzed using software Cervus 3.0.7. Parameters were observed i.e genotypes and alleles frequencies, heterozygosity observed (Ho) and expected (He), Hardy Weinberg Equilibrium (HWE), and Polymorphic Information Content (PIC). Result showed that three variant genotypes of GG, GC and CC were found and two variant alleles of G and C. Allele G was found higher (0.77) than allele C (0.23). Population of Pasundan cattle was found polymorphism and in the Hardy Weinberg Equilibrium. Polymorphic Information Content (PIC) value showed in a moderate (0.290) condition. Values of Heterozygosity observed and expected were 0.409 and 0.355 respectively. This research concludes that there is polymorphism of *CAST* gene in Pasundan cattle population and has genetic diversity. This result could be used as early genetic information in exploration of Pasundan cattle.

Key words: Beef cattle, Calpastatin gene, Pasundan cattle, Polymorphism, West Java

Introduction

Pasundan cattle is one of the local beef cattle that spread in West Java Province, *i.e.,* Ciamis, Majalengka, Tasikmalaya, Pangandaran, Sumedang, Purwakarta, Indramayu and Garut district (Arifin *et al*., 2015). Sutarno and Setyawan (2015), reported that Pasundan cattle is a crossbred cattle of *Bos javanicus* (Bali cattle) and *Bos indicus*. Pasundan cattle have dominant reddish-brown coat color. Their pelvis and leg (*tarsus* and *carpus*) colored white but not contrast with coat color. They also have back line in both of sex. The coat color of bulls changed to darken color or black after their sexual maturity at 12-18 months of age (Said *et al*., 2017). Based on the Decree of Agriculture Ministry No.1051/Ktsp/SR.120/10/2014, Pasundan cattle is adaptive cattle more than 10 generations of crossbreed *Bos sondaicus*/Banteng/Bali cattle with Java cattle, Madura cattle, and Sumba Ongole cattle. Pasundan cattle have mature body weight 200-250 kg (Baharun, 2015; Sulasmi, 2016) with carcass percentage reached 50% (Sutarno and Setyawan, 2015).

Meat quality of Pasundan cattle has water holding capacity (23-30%), cooking loss (25-45%), and tenderness (35-96 mm/10s/g) (Departement of Animal Husbandy West Java, 2013 *cit.* Said *et al*., 2017). Tenderness of meat is one of the most important factors that determining the consumer-eating satisfaction of meat (Jeleníková *et al*., 2008; Pinto *et al.,* 2010; Parra-Bracamonte *et al*., 2015). Meat tenderness was influenced with many factors such as species, age, sex, breed, nutrition, slaughter, post-mortem, chilling condition, ageing, pH (Koohmaraie, 1994; O'Connor *et al*., 1997; Koohmaraie and Geesink, 2006; Wheeler *et al*., 2010; Lomiwes *et al*., 2014).

Tenderization of meat in post mortem is influenced by the pH and calcium of the meat which involve activation or in activation of the proteolytic enzymes (du Toit and Oguttu, 2013). Cathepsins (cathepsin B and L), Calpain (m-calpain and μ- calpain) and Calpastatin are three enzymes that involved of tenderness process (O'Halloran *et al*., 1997; Goll *et al*., 2003). In post mortem, cathepsin enzymes degraded myosin, actin, troponin, and collagen. Capthesin reactived in low pH conditions while calpain in higher pH value. Calpain enzyme play more significant role than cathepsin enzyme in meat tenderness process (Varnam and Sutherland, 1996; du Toit and Oguttu, 2013). In pH value 6.3 condition, calpain enzyme is activated firstly to degrade myofibril. The other hand, calpastatin enzyme was

active. Calpastatin is a specific endogenous inhibitor of μ-calpain and m-calpain (Goll *et al*., 2003; Koohmaraie and Geesink, 2006). Kemp *et al.* (2010), reported that high level of calpastatin was associated with poor quality meat which caused by reducing of calpain activity thereby influencing of proteolysis for tender meat.

The bovine Calpastatin gene was mapped at BTA7 (Bishop *et al*., 1993). Previous studies found several SNPs in Bovine Calpastatin gene *i.e* AY008267. 282C>G (Schenkel *et al*., 2006), SNP 2959 (Li *et al*., 2010), SNP 2870 (Corva *et al*., 2007; Li *et al*., 2010), WSUCAST substitution of Cytosine with Thymine in exon 3 (base 263 of AY008267) (Garcia *et al*., 2006), CAST: c.155C>T (Barendse *et al*., 2007). Schenkel *et al.* (2006) reported that allele C of CAST gene associated with lean meat (LM) tenderness. Meanwhile, Li *et al*. (2010) found that SNP2959 and SNP 2870 had significant association with Warner-Bratzler Shear Force in Chinese Commercial cattle. Calvo *et al*. (2017) found three new SNPs in Calpastatin gene associated with beef tenderness. Those are BTA7: g.98533962C>G on UMD 3.0 in intron 5; g.98535683A>G in exon 7; and g.98545188T>A in intron 12.

Molecular marker of Calpastatin gene is important to be studied for the need of animal breeding program strategy through selection for improving of beef quality traits especially meat tenderness. It would impact on consumer's satisfaction of beef meat. Therefore, the objective of this study was to assess allele and genotype variations at the bovine calpastatin gene in Pasundan.

Materials and Methods

Samples and genomic DNA extraction

Forty-four (44) of Pasundan cattle were applied. Those samples belong to the UPTD Balai Pengembangan Perbibitan Ternak (BPPT) sapi Potong Ciamis, West Java, Indonesia. Three milliliters of fresh blood samples were collected from *vena jugularis* into vacuntainer containing anticoagulant (K_3EDTA). A high Salt method was used to extract genomic DNA (Montgomery and Sise, 1990). DNA was stored at -20^{o}C for the future analysis.

PCR-RFLP analysis

Genotyping of Calpastatin (*CAST*) gene was analyzed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A 523 bp fragment of CAST gene was amplified with a specific primer pair (F: 5' CCT CGA CTG CGT ACC AAT TCC GAA GTA AAG CCA AAG GAA CA 3' and R: 5' ATT TCT CTG ATG GTG GCT GCT CAC T 3') (Schenkel *et al*., 2006). The PCR amplification was performed in a total volume of 10 μl containing 5 μl PCR Master Mix (My Taq^{TM} Red Mix, Bioline), 1 μl (10 pmol/μl) of each primer, 2 μl free nuclease water and 1 μl DNA template. PCR mixture was run at Thermal cycler machine (Techne Plus, UK) with conditions of 94^{o}C for 2 min then followed with 40 cycles of 94^{o}C for 30 s, 60^{o}C for 45 s, 72^{o}C for 30 s, and final extension at 72^{o}C for 5 min. The PCR products were checked in 1% agarose gel (100 V, 1 hour) and staining with Ethidium bromide. Fragment of PCR product was visualized under UV light (MajorScience, USA) and documented by a digital camera.

PCR products were digested using *Rsa*I restriction enzyme for genotyping of each of samples using PCR-RFLP method. Five milliliters of PCR product was digested with 0.3 μl *Rsa*I (10U/μl) and incubated at 37^{o}C for 16 hours. Visualization of PCR-RFLP product was through 2% agarose gel (100 V, 1 hour) then stained with Ethidium bromide.

Data analysis

Frequency of genotypes and alleles, Heterozygosity (observed and expected) and Polymorphic Information Content (PIC) were calculated using software Cervus 3.0.7 (Kalinowski *et al*., 2007). However, Hardy-Weinberg Equilibrium (HWE) was calculated directly using formula (Noor, 2008; Ishak *et al*., 2011):

$$X^2 = \sum \frac{(Oi - Ei)^2}{Ei}$$

X^2 = *Chi – square*
O*i* = The number of observed genotypes i
E*i* = The number of expected genotypes i

Result and Discussion

Forty-four DNA samples of Pasundan cattle were successfully amplified for *CAST* gene using PCR technique. DNA Fragment of PCR product was found at 523 bp which is similar to previous study reported by Schenkel *et al*. (2006) (Figure 1).

Schenkel *et al*. (2006) reported that tranversion of G/C in CAST gene has been found at position 257 and recognized by *Rsa*I restriction enzyme (GT^AC). The digestion position was showed in Figure 2. Based on genotyping analysis of *CAST* gene in Pasundan cattle population, there were three variants of genotype *i.e* GG, GC and CC with two variant alleles of G and C (Figure 3). The GG genotype was determined with fragment DNA of 257 bp and 266 bp while CC genotype was 523 bp. Genotype GC has three DNA fragment (523 bp, 266 bp and 257 bp).

Genotype frequency of *CAST* gene showed that GG was dominant genotype (57%) than other and allele G was higher than allele C (Table 1). Contrast result was reported by Schenkel *et al*. (2006) and Kok *et al*. (2013) which allele C was dominant allele. Their studies used *Bos taurus* cattle *i.e* Simmental, Angus, Charolais, Limousin, and Grey Step Turkey. Different subspecies and breed cattle used from this present study resulted different pattern of *CAST* allele which Pasundan cattle is crossbred cattle of *Bos indicus* and *Bos sondaicus* (Agriculture Ministry, 2014; Sutarno and Setyawan, 2015).

Based on morphometric study, Pasundan cattle has a closer distance to Ongole Grade cattle while Cranium study showed closer distance to Madura cattle (Sulasmi, 2016).

Genetic diversity can be showed by heterozygosity value. This present study reported that heterozygosity observed was higher than heterozigosity expected (Table 1). This finding showed the genetic diversity of Pasundan cattle population. Chesnokov and Artemyeva (2015) stated that when Ho>He, population is a random mating while Ho<He showed inbreeding occurrence was in population. In this study, Pasundan cattle population was found in HWE.

Polymorphic was found in Pasundan cattle population which allele G and C was less than 99%. Polymorphic Information Content (PIC) value showed moderate (0.290) (Table 1). Botstein *et al*. (1980) classified PIC in three groups. Those are low (PIC<0.25), moderate (0.25<PIC<0.50) and high (PIC>0.50). PIC value indicated a quality of genetic marker. PIC

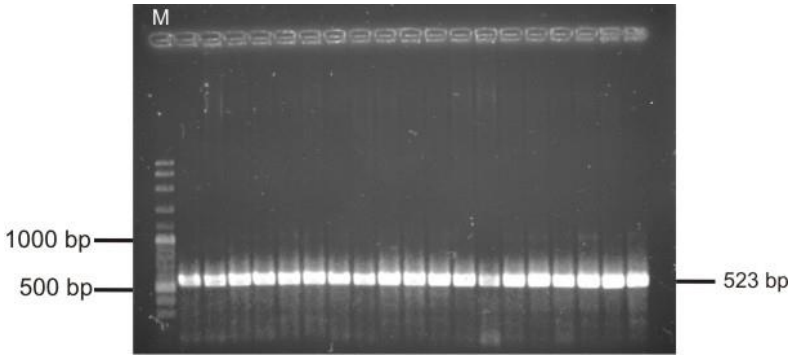

Figure 1. PCR product of *CAST* gene in Pasundan cattle.

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Restriction Enzyme Map:
1    GAAGTAAAGCCAAAGGAACACACAGAGGTAAGTAATCATTATTAGGACTTGATATCATAAGATGAAGCCTTTTTTTTTTT  80
81   CCCTTATTTTTGTGAAGGATAAAATTTTGAACTCTCATCTTTCAACACTTAAGTCCTACCTAGAATGGCAGTTATTTGTT  160
161  TTTCTGTTAAAACGGCACCTCTGTGTGGCATCAGCAGGTATTGCAATTTGCTTGTGTGATTCTTGCTGAATTTGGAAGGA  240
241  AGGAATTGCATTGTTTCAAATTTTGTACCCAAAGTGAAATTTGTCACATGTAAATCATACTAATTTAAATTCTCACAATT  320
                        RsaI (GT'AC)
321  GACTACATAAAACACAAGTGTTATGAATTGCTTTCTACTCCTCAGAGAAAAGTAGCAATATGTGTCATATTATTAACCCC  400
401  ATGGGGTGTATGCGTGTTTTCAGCCAAAAAGCCTACCCAAGCACTCATCAGATACAGGAAGCAAGCATGCTCCTAAGGAA  480
481  AAAGCCGTTTCCAAATCAAGTGAGCAGCCACCATCAGAGAAAT   523
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Figure 2. *Rsa*I restriction enzyme (GT^AC) position of *CAST* gene (GenBank Accesion No. AY008267).

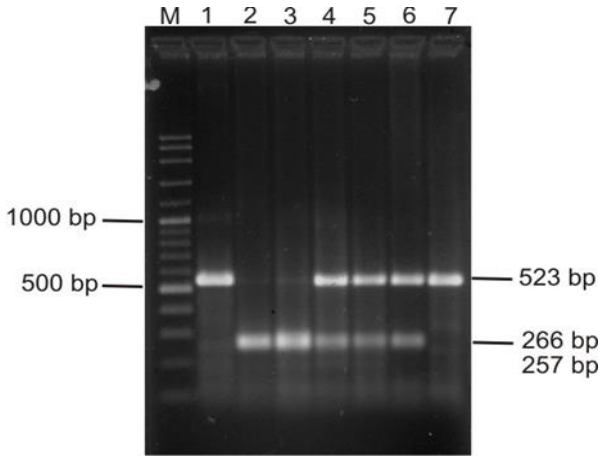

Figure 3. Genotype variants of *CAST* gene in Pasundan cattle. M: Marker 100 bp; 1: PCR product (523 bp); 2-3: genotype GG; 4-6: genotype GC; and 7: genotype CC.

Table 1. Genotypes, allele frequencies, HWE, Heterozygosity and PIC of Pasundan cattle population

Breed	Genotypes frequencies		Allele frequencies			HWE (X^2)	Ho	He	PIC
	GG	GC	GG	G	C				
N = 44 Pasundan	25 57%	18 41%	1 2%	0.77	0.23	0.351	0.409	0.355	0.290

Ho: Heterozygosity observed; He: Heterozygosity expected; PIC: Polymorphic Information Content
$X^2_{0,0;2}$: 5.99.

value in this present study (*CAST* gene of Pasundan cattle) has a moderate value. Therefore, it indicated that the marker could be associated with meat quality traits.

Based on Table 1, allele G was found dominant in Pasundan cattle population (*Bos indicus* x *Bos sondaicuss/javanicus*) and difference result from *Bos taurus* cattle. Previous studies of Schenkel *et al*., 2006; Li *et al*., 2010; Bressan *et al*., 2011; Calvo *et al*., 2017 reported that allele C was associated with meat tenderness, Lean Meat tenderness, and Warner Force Shear. Crouse *et al.* (1989) reported that shear meat value of crossbred cattle (75% *Bos indicus* x 25% *Bos taurus*) has greater value than *Bos taurus.* There were 14.7 lb and 9.7 lb respectively. Shear meat value reflected of tenderness meat. Another study, Wheeler *et al*. (1990) showed that lean firmness value of Hereford cattle (*Bos taurus*) has softer than Brahman cattle (*Bos indicus*). High level of Calpastatin was associated with poor quality of meat (Kemp *et al*., 2010). O'Connor *et al*. (1997), reported that lower calpastatin activity have little effect on beef tenderness.

Identification of genetic diversity of *CAST* gene in Pasundan cattle population could be used for improving of genetic quality through selection based on the molecular technology. The genetic information could be used to design of breeding program.

Conclusions

Polymorphism of *CAST* gene was found in Pasundan cattle population and frequency allele G is higher than allele C. Population of Pasundan cattle was in the Hardy-Weinberg Equilibrium while heterozygosity value has genetic diversity. Polymorphic Information Content (PIC) was in moderate condition which could be used as a marker for association study. This genetic information could be used by breeder to design their breeding program to develop genetic quality of Pasundan cattle.

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