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The Association of Single Nucleotide Polymorphism (SNP) g.281G > A of *CAST* Gene with Meat Quality of Boerka Goat

Research Article

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ABSTRACT

Calpastatin gene has been known as a candidate gene for meat quality in cattle, sheep, and chicken. The purpose of this study was to identify *CAST* gene polymorphisms and its association with meat traits in Boerka goat. The data of pH, cooking loss (CL), warner-bratzler shear force (WBSF), water holding capacity (WHC), cholesterol, water, ash, fat, and protein contains were recorded. Sequencing of 21 samples revealed five polymorphisms of *CAST* gene in intron 12 within Boerka goat, namely g.146C > A, g.224A > G, g.281G > A, g.737C > T, and g.431G > A. Only single nucleotide polymorphism (SNP) g.281G > A was used for genotyping. The genotype and allele frequency based on g.281G > A showed 14.29% (GG genotype) and 85.71% (GA genotype) followed with 57% G allele and 43% A allele. The chi-square test showed deviation from HWE ($P<0.05$) in Boerka goat. The SNP g.281G > A revealed having significantly effect to CL. The GA animals had lower CL percentage compared to the GG animals. In conclusion, the SNP selected may be used for identify meat having low CL in Boerka goat.

KEY WORDS Boerka goat, calpastatin, polymorphism.

INTRODUCTION

Goat is one of the most adaptable animals with different environmental conditions (Bahrampour and Mohammadi, 2017). In Indonesia, the growth rate of goat meat production is considered quite low which was 3.8% per year (Tarigan *et al.* 2018). So far, goat genetic improvement scheme in Indonesia has involved the crossbreeding trials and conventional breeding methods. Boerka goat is a newly introduced meat-type goat, which developed by crossbreeding of the Boer buck and Kacang does. Boerka has 33-48% higher growth performance and greater carcass characteristics (carcass weight and length, comparable pH and protein contain, and lower fat contain) compared to Kacang goat (Ginting and Mahmilia, 2008).

Carcass and meat traits are one of the major concern of profitability which is controlled by many genes. Calpastatin (*CAST*) gene is a potential candidate gene for meat traits. *CAST* gene is located in chromosome 7 of goat sized 134 Kb length (14434087...14568155 based on GenBank Acc. No NC_030814.1) consist of 34 exonic regions. *CAST* gene encoding a specific inhibitor of the calpain, affecting the decrease rate of myofibrillar protein degradation during post-mortem (Koohmaraie *et al.* 1995; Goll *et al.* 2003; Corva *et al.* 2007; Singh *et al.* 2012). Correlation between the depravity of myofibrillar proteins in the muscle and calpain system has proven gives a strong impact on the variation of meat characteristics (Asadi and Khederzadeh, 2015). The associations of *CAST* gene variation and meat traits have been reported in cattle, sheep, pig, and chicken

(Corva *et al.* 2007; Ropka-Molik *et al.* 2014; Asadi and Khederzadeh, 2015). In Holstein bulls, Ardicli *et al.* (2017) reported that the *CAST* S20T has statistically significant with live weight, inner chest depth, and b* meat colour value.

Later, the polymorphism study of *CAST* gene in goat has been reported in Beetal (Khan *et al.* 2012), Khalkhali (Jahromi *et al.* 2015), Raini and Tali (Bahrampour and Mohammadi, 2017), Baladi, Barki, and Zaraibi goats (Othman *et al.* 2016).

Evaluations of genetic polymorphism and its relation to meat characteristics could be used as a tool for predicting animal meat quality. Therefore, breeders can improve favorable meat characteristics (Jahromi *et al.* 2015). Marker assisted selection (MAS) method considered to be efficient improving the accuracy and selection in animal stock (Koohmaraie *et al.* 1995). By using the molecular marker, genotyping animals helps to classify carcasses before slaughter (Lonergan *et al.* 1995). To date there has been no report about meat traits improvement using a molecular genetic approach in Boerka goat. Therefore, this study aimed to explore the genetic variation within the *CAST* gene and its effect in meat quality of Boerka goat.

MATERIALS AND METHODS

Resource populations

In total, 21 male Boerka goats were investigated in this study. All studied animals were fed and raised under the same environmental conditions in Goat Research Center in Deli Serdang, North Sumatera, Indonesia. The animals were kept in flock consict of 10 to 15 goats and slaughtered at 15 months of age. After dressing, the *L. dorsi* muscle region were labeled for meat traits measurement.

Sample collection and DNA isolation

Approximately, 3 mL of blood samples from the jugular vein were collected using ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (BD Bioscience, Germany). Blood samples were transported to the laboratory and kept in -4 ˚C before the next treatment. The genomic DNA was isolated using a commercial gSYNC DNA Extraction Kit (Geneaid, Taiwan) according to the manufacturer's standard procedure. The isolation product was visualized by electrophoresis in 1% agarose gel mediated in a UV transilluminator.

PCR amplification

A fragment of the caprine *CAST* gene was amplified by the polymerase chain reaction technique using SEDI G Thermal Cycler (Wealtec Corp, USA). The used primers were according to the method presented by Othman *et al.* (2016).

The 25 µL final reaction volume consists of 9.5 µL ddH_2O, 12.5 µL MyTaq™ HS Red Mix (Bioline, UK), 0.5 µL of each primer, and 2 µL of genomic DNA. Amplification was performed with the following conditions, initial denaturation 95 ˚C 5 min, followed by 35 cycles of denaturation 95 ˚C 1 min, annealing 62 ˚C 1 min, extension 72 ˚C 2 min, and the final extension for 72 ˚C 10 min. The result of amplification was verified on 2% agarose gel (1st BASE, Singapore), added with 100 bp DNA-ladder (New England Biolabs, United States) as a molecular weight marker to confirm the length of PCR product (approximately 620 bp). The gel was visualized on a UV transilluminator.

SNPs identification

The PCR products were sent to Central University Laboratory of Universitas Gadjah Mada for DNA sequencing. Raw sequence data were edited using BioEdit software (Hall, 1999). Sequence alignments were analyzed with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to identify single nucleotide polymorphism (SNPs) of *CAST* gene and to genotype the samples. Manual examination of electropherogram was used to confirm the polymorphic site found by sequence comparison.

Meat quality test

The pH of the 21 meat samples was calculated using pH meter with two replication for each sample. The pH meter was calibrated using standard buffers before each session. The cooking loss (CL) was calculated as the difference between the weight before and after cooking and expressed as a percentage of the initial weight (Honikel, 1998). The Warner-Bratzler shear force (WBSF) was measured as described by Honikel (1998). The water holding capacity (WHC) was determined according to the method described by Strydom *et al.* (2016) with the filter paper press. The proximate analysis was used to measure the water, ash, fat, and protein contain.

Statistical analysis

The genotype and allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He), and Chi-square values (X^2) for Hardy-Weinberg equilibrium (HWE) were calculated using Pop-Gene 1.32 program (Yeh *et al.* 1997). The association of SNP g.281G > A of *CAST* gene genotypes with meat traits were analyzed using SPSS (2011) with the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:
µ: average of the population.
T_i: effect of K-individual genotype.

ε_{ij}: effect of random error (Maharani *et al.* 2018).

The $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Meat quality of Boerka goat

The meat quality was measured based on its physical and chemical parameter. The average value for pH, WBSF (kg/cm^2), CL (%), and WHC (% mg H_2O) of Boerka goats meat were 5.85 ± 0.15, 6.07 ± 1.81, 47.30 ± 3.63, and 36.59 ± 3.09, respectively. Based on the chemical parameter, the Boerka meat contains 77% water, 1.15% ash, 0.66% fat, 18.68% protein, and 65.27 mg/100 g cholesterol.

SNPs identification

A DNA fragment (620 bp) within the *CAST* gene has successfully amplified (Figure 1). The amplified-fragment has covered the sequences of exon and intron regions. The sequence alignments from 21 samples revealed no polymorphism detected in the exonic region, whereas five polymorphisms was found in non-coding region (intron 12), namely g.146C > A, g.224A > G, g.281G > A, g.737C > T, and g.431G > A. Manual inspection to the electropherogram showed clear peaks for each genotype in each SNPs (Figure 2).

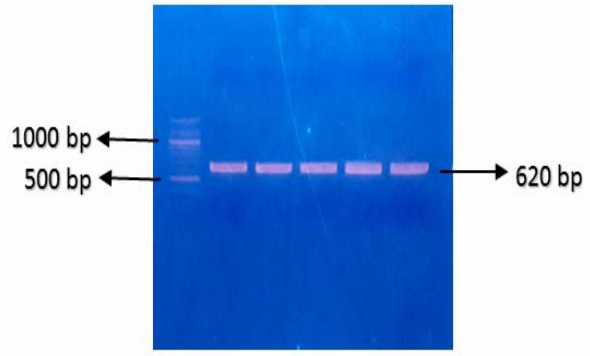

Figure 1 PCR amplification product of *CAST* gene

Genotype and allele frequency

The number of homozygous CC, AA, CC, and GG animal for SNP g.146C > A, g.224A > G, g.737C > T, and g.431G > A respectively, were more than 90%. There was only one animal detected to have heterozygous genotype. In contrast, based on the SNP g.281G > A the number of heterozygous GA animal (n=18) was higher than homozygous GG animal (n=3). Hence, genotype and allele frequencies analysis and the association study with meat quality only calculated by using the SNP g.281G > A.

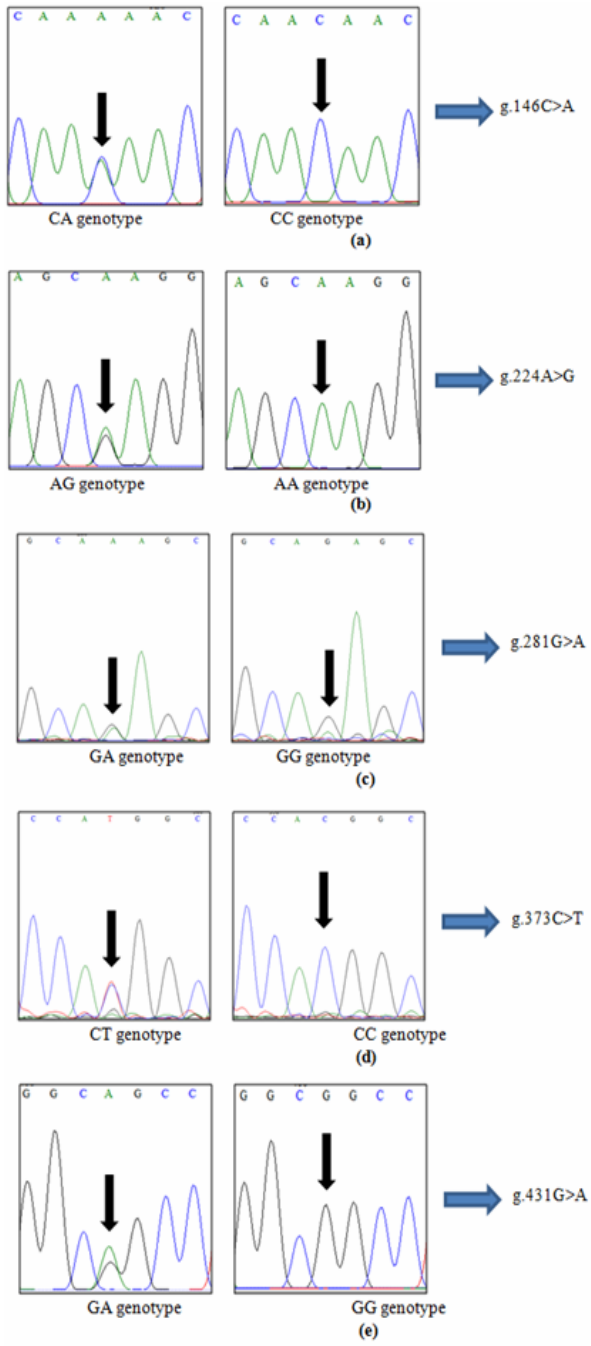

Figure 2 Manual inspection on electrophoregrams showed the genotype of SNPs (a) g.146C > A, (b) g.224A > G and (c) g.281G > A, (d) g.373C > T, and (e) g.431G > A

The allele and genotype frequencies, chi-square test, and expected homozygosity and heterozygosity values are presented in Table 1. As a result, the homozygous AA animal was absent in this Boerka population. The frequency of observed heterozygosity was higher than the homozygosity value. The G allele (0.57) was more dominant than A allele (0.43).

Table 1 The allele and genotype frequencies, chi-square test value, and expected homozygosity and heterozygosity, values for single nucleotide polymorphism (SNP) g.281G > A within *CAST* gene in Boerka goat

Locus	Genotype frequencies		Allele frequencies		χ^2	Expected	
g.281G > A	GG	GA	G	A	11.09	Ho	He
	0.14	0.86	0.5714	0.4286		0.4983	0.5017

Table 2 Least square means of physical and chemical meat quality that carry the GG and GA genotypes based on single nucleotide polymorphism (SNP) g.281G > A

Traits		Genotype	
		GG	GA
Physical	pH	5.92	5.78
	Tenderness	6.43	5.22
	Cooking loss	51.24[a]	43.36[b]
	Water holding capacity	36.57	36.62
Chemical	Cholesterol	64.01	65.48
	Water contains	76.56	77.07
	Ash contains	1.18	1.15
	Fat contains	0.81	0.64
	Protein contains	18.77	18.67

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The chi-square tests showed that the g.281G > A in Boerka goat were deviated from HWE (P<0.05).

The effect of SNP g.281G > A to meat quality

This study found that animals with the homozygous GG genotype had greater CL percentage than those with the GA genotype (P<0.05). However, the pH, WBSF, WHC, cholesterol, protein, fat, water, and ash contains were not affected by the SNP g.281G > A as is shown in Table 2.

The pH level of Boerka meat was similar to the findings of pH level in Anglo-Nubian cross-bred (Silva *et al.* 2015) and Boer goats (Pophiwa *et al.* 2017; Brand *et al.* 2018). The WBSF found in this study was lower than that reported by Ortega *et al.* (2016) in Serrana goat but higher than Abuelfatah *et al.* (2016) and Pophiwa *et al.* (2017) in Boer goat. Moller (1981) stated that the WBSF are measured from the connective tissue and myofibril that contribute to meat tenderness. There is a general agreement that the threshold for WBSF is below 4.6 kgf to be categorized as tender (Silva *et al.* 2015).

The CL value reported in this study was higher than previous studies reported by Silva *et al.* (2015), Brand *et al.* (2018), Basinger *et al.* (2019), and Sacca *et al.* (2019) in cross-bred Kiko × Boer, cross-bred Anglo-Nubian, Boer, and Alpine goats, respectively. The WHC and CL are closely linked to the juiciness of the meat (Schönfeldt *et al.* 1993).

The CL has a correlation with WBSF (Suryati *et al.* 2008). The higher CL percentage results to higher WBSF value. Ranken (2000) and Widiati *et al.* (2002) indicated that cooking process leads to muscle depression and myofibril tension.

The fat and protein contains in Boerka meat considered to be lower than the data of Madruga *et al.* (2009), Tomovic *et al.* (2016) and Brand *et al.* (2018). In contrast, the water content of Boerka meat was higher than previously reported.

The *CAST* locus indicated to be highly polymorphic in goat, and the level of polymorphisms was higher than in other ruminants (Zhou and Hickford, 2008). In this study, no exonic polymorphism was found. However, five SNPs were identified in intron 12. Similarly, nine novel SNPs have been found in an intron (5, 7, and 8) of caprine *CAST* gene (Sharma *et al.* 2013). In contrast, no SNPs were discovered in intron 12 of seven Indian goat breeds. Zhou and Hickford (2008) reported one missense mutation in exon 6 of the caprine *CAST* gene resulting in amino acid change Ser to Arg in the L domain of the protein.

The frequency of GA genotype was higher than GG genotype with no AA genotype showed in the studied population. The G allele (0.57) was higher compared to the A allele (0.43).

Khan *et al.* (2012) reported that only MM genotype was observed in Beetal goat in Pakistan using *CAST|MspI* method. The Boer goat studied in Javanmard *et al.* (2010) showed higher frequencies of B allele (0.54) than A allele (0.46) for the *CAST|XmnI* locus. The Boerka population was in disequilibrium from Hardy-Weinberg. The result was similar to the study stated by Javanmard *et al.* (2010) in Boer goat. The deviation from Hardy-Weinberg may be caused by the limited sample size and the crossbreeding program between Boer and Kacang goats.

Similarly, in small population/sample size, genotype frequencies are deviate from Hardy-Weinberg even though the population is under random mating (Duenk *et al.* 2017).

Moreover, the result from Esfandyari *et al.* (2015) showed that the observed heterozygosity of the crossbred population was 0.49 on average, which was higher than was found in the pure lines, i.e 0.33 and 0.34 on average for breed A and B, respectively. Falconer and Mackay (1996) also stated that the difference in the level of allelic heterozygosity between 2 pure breeds and their crosses is directly related to the level of heterozygosity with respect to the breed of origin of genes. Hence, it can be achieved that the increase of observed heterozygous animal number will affect the alleles frequencies which could alter the HWE calculation.

The association analysis revealed that SNP g.281G > A was associated with CL, but showed no effect in other meat traits. These findings were different from the previous study. Li *et al.* (2016) reported that polymorphism within *CAST* gene has a significant effect on the intramuscular fat content and density of muscle fibre, but no effect in pH, muscle color, and WHC. The variation in the *CAST* gene has affected the muscle fibre density and diameter in chicken (Liu *et al.* 2008). In swine, Ropka-Molik *et al.* (2014) revealed that the meat colour, pH, WHC, and texture parameters were influenced by the genotype variance of *CAST* gene. Yassen *et al.* (2016) studied the *CAST*|*MspI* locus in Cyprus goats and conceded that the MM genotype has higher total collagen in LD muscle compared to the MN genotype. The study of calpastatin by Fortest (2007) found that AA animals have more tender meat in Zebu and crosses cattle. Ciobanu *et al.* (2004) observed *CAST* gene in pig and found that one *CAST* haplotype was significantly related with higher juiciness and tenderness. Other variations were associated with differences in phosphorylation of *CAST* by a protein kinase. Nakaya *et al.* (2007) stated that nucleotide variance could be considered directly responsible in the intronic region for phenotypic changes as non - coding RNAs (micro RNAs) participate in diverse biological processes, such as transcriptional and post - transcriptional gene expression control (Nakaya *et al.* 2007).

CONCLUSION

Five polymorphisms were found in intron 12, namely g.146C > A, g.224A > G, g.281G > A, g.737C > T, and g.431G > A. Only SNP g.281G > A was used for genotyping. The association study of SNP g.281G > A revealed a significant effect only to CL. The heterozygous (GA) animal has a lower CL than the homozygous (GG) animal. Hence, it can be concluded that the SNP g.281G > A may be used to identifying meat having low CL in Boerka goat. In respect of the low sample size, further study should be perform to make the final decision in animal breeding design.

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