Single Nucleotide Polymorphism (SNP) 316 on Calpain Gene in Aceh Cattle

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Abstract. Genetic polymorphisms of μ-calpain, a calcium-dependent protease specifically expressed in muscle and related to meat tenderness, have been investigated in cattle worldwide, but not well documented in Indonesian beef cattle. This study was done to evaluate the frequency of SNP 316 in the μ-calpain gene in Aceh cattle, a local Indonesian beef cattle plays important role in the fulfillment of red meat for peoples in Aceh and its neighboring areas. For this purpose, genomic DNA was isolated from 29 sirloin meats samples collected from cull, female aceh cattle slaughtered at the Slaughter House of Banda Aceh. The cattle were purposively selected based on physical characteristics and hair colors referred to Indonesian law. For the SNP genotyping, PCR-RFLP methods were set up. The results showed all three genotypes namely CC, CG, and GG were found in Aceh cattle. The allele and genotype frequencies of SNP CAPN316 in the *CAPN* gene were: C – 0.14 and G – 0.86; CC – 3.4%, CG – 20.7% and GG – 75.9%. The observed and expected frequencies of CAPN SNP 316 in the cattle population examined were GG 22.0 and 21.6, CG 6.0 and 6.9, and CC 1.0 and 0.6.

Keywords: Aceh cattle, calpain, allele, genotype.

1 Introduction

Aceh cattle is Indonesian local beef cattle originated from the crossing of *Bos Indicus*, *Bos Javanicus* and *Bos Sondaicus* [1]. The cattle have good genetic potential as shown by average body weight 253±65 (male) and 148±37 kg (female), carcass percentage ranges from 49-51% [2], and total meat fat 6% [3], and marbling score is 1 [4]. Aceh cattle are superior to exotic cattle because they are adaptive to the warm, humid tropical climates [5][6], and have better resistance to infectious and parasitic diseases [2].

The significant role of Aceh cattle for the fulfillment of red meat in Aceh province and its neighboring provinces has been documented [2]. The role is now challenged by a significant decreased in population numbers, smaller body size and declined genetic quality due to inbreeding and slaughtering of productive females to fulfill market demand [7].

Efforts to improve genetic quality of Aceh cattle has been done by identifying phenotypic diversity of the cattle [2], determining its genetic relationship based on displacement-loop region of microsatellite DNA [1], and by establishing Indonesian National Standard criteria for Aceh cattle seedstocks (SNI 7651.3.2013) and by grading heifer seedstocks at the breeding center BPTU-HPT of Indrapuri [8]. Whilst results of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based molecular study showed novel mutation found in myostatin gene of Aceh cattle [9], no studies were done to explore diversity in the genes related to meat quality of the cattle. Among gene where nucleotide variations might influence meat tenderness, protein content and quality is *calpain* [10]. Calpain is a protease enzyme functions to degrade myofibril cell proteins and specifically expressed in muscles [11]. This study was done to identify single nucleotide polymorphism at the position of 316 of *the μ-calpain* gene (CAPN SNP 316) in Aceh cattle have different hair colors.

2 Materials and Methods

2.1 Ethical clearance

All protocols used in this study have been approved by the Veterinary Ethics Committee of Faculty of Veterinary Medicine of Universitas Syiah Kuala Number 28/KEPH/1I/2018.

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2.2 Sample collection

Fresh sirloin meats (250 gram each) of 29 Aceh cattle have different hair colors (red-brown/ brick, red, grayish-black, straight yellow and white) were purposively purchased in triplicate from the Slaughter-House of Banda Aceh. Meat samples were kept cool (4 °C), brought to the Laboratory of Research, Faculty of Veterinary Medicine of Universitas Syiah Kuala, and stored at -20 °C before the examination.

2.3 DNA Isolation

Genomic DNA Genomic DNA was extracted from fresh meats using PureLink™ Genomic DNA Mini Kits and protocol. DNA extracts were electrophorized on 1% agarose gel stained with SYBR™ Safe (Invitrogen) staining solution. DNA bands were visualized and documented by using a digital gel imager (BioRad, USA) [9].

2.4 Polymerase Chain Reaction

The SNP 316 CAPN fragment (709 bp) is PCR amplified using forward primer (5′→3′) CCAGGGCCAGATGGTGAA and reverse primer (5′→3′) CGTCGGGTGTCAGGTTGC. A 25 µl of PCR reaction containing 1 µl of each primer (final concentration of 50 pmol), 12.5 µl of GoTaq Green PCR master mix (Promega Corporation, Madison, WI), and 120 ng of DNA template was prepared and run using a BioRad thermal-cycler. The PCR conditions used were initial denaturation (1 cycle) at 95 °C for 5 minutes, followed by 35 cycles of three step PCR reactions consisting of denaturation at 95° for 45 seconds, annealing at 65 °C for 45 seconds and extension at 72 °C for 45 seconds, and ended with final extension at 72 °C for 5 minutes.

2.5 Restriction Fragment Length Polymorphism

The PCR products, 16 ng each, were digested with *Btg* I restriction enzyme (Invitrogen) for 4 hours at 37 °C. The reaction was prepared by mixing 5 µL of PCR product with 2.5 ml of 10X digestion buffer, 16.5 µL of nuclease-free water, and 1 IU of *Btg* I enzyme (Invitrogen). The products were electrophorized in 2% agarose gel at 80 Volt for 75 minutes, stained with ethidium bromide, and viewed using digital gel imager (BioRad).

2.6 Data Analysis

Based on DNA marker bands, the frequency of alleles, genotypes, and heterozygosity of expectations and observations will be determined. The degree of polymorphism will be calculated using the formula

$$PiCi = 1 - \sum p_{ij}^2 \text{ [12]}$$

A chi-square test was used to determine whether the allele distribution meets the Hardy-Weinberg balance [9].

3 Results and Discussion

The results showed that all three calpain genotypes namely CC, CG, and GG were found in Aceh cattle. The allele and genotype frequencies of SNP 316 in *CAPN* gene were: C – 0.14 and G – 0.86; CC – 3.0%, CG – 21.0% and GG – 76.0%. The observed and expected frequencies of CAPN SNP 316 in the cattle population examined were GG 22.0 and 21.6, CG 6.0 and 6.9, and CC 1.0 and 0.6.

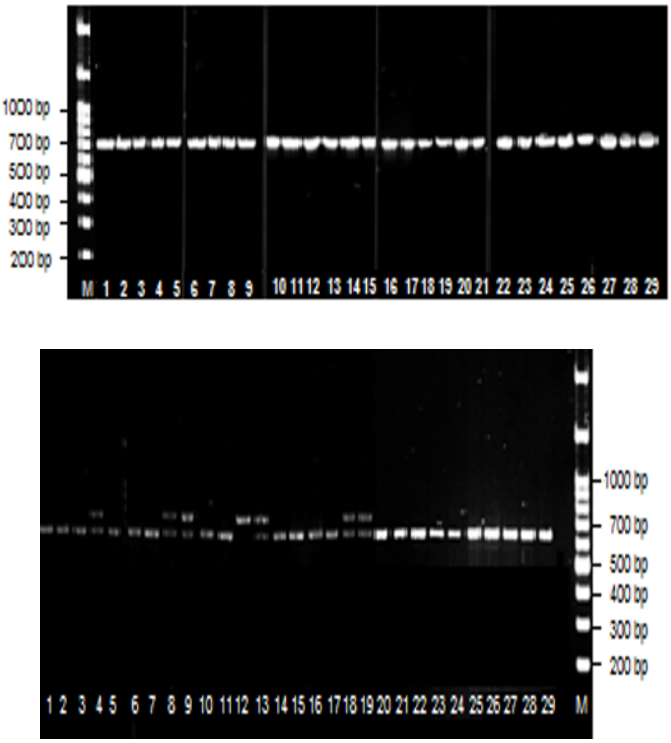

Fig. 1. Electrogram showing results of CAPN SNP 316 gene digestion using the Btg1 restriction enzyme. Line 12: CC genotype, line 4, 8, 9, 13, 18, 19: CG genotype, and besides line: GG genotype M = 100 bp DNA leader.

Table 1. Allele and genotype frequencies of CAPN gene in aceh cattle

Total genotif	Genotype frequency	Allele Frequency
GG = 22 ; CG = 6 ; CC = 1	Freq (GG) = 0.76; Freq (CG) = 0.21; Freq (CC) = 0.03.	Frequency (C) = 0.14 Frequency (G) = 0.86

Table 2. Allele and genotype frequencies of CAPN SNP 316 gene in aceh cattle

Heterozygosity		Polymorphism degree	Hardy-Weinberg Equilibrium (X^2)
Observed	Expected		
CC = 1	0.6	0.49	0.95
CG = 6	6.9		
GG = 22	21.5		

Polymorphism of CAPN is the most diversity studied about meat quality because the gene encodes the large subunit of µ-calpain, an enzyme related to the meat

tenderization process [13] and marbling [14]. The gene is located at chromosome 29 [15].

This study that is done to investigate SNP 316 of *the CAPN1* gene found that the gene is polymorphic at Aceh cattle populations as shown by the occurrence of GG, GC and CC genotypes that distributed following the Hardy Weinberg equilibrium (χ^2= 0.95). These results may reflect the actual allelic and genotypic frequencies for the corresponding CAPN locus in Aceh cattle populations. Distribution of the three genotypes in agreement with Hardy-Weinberg equilibrium was also reported in Simmental bulls [16], Brangus and Brahman bulls [17].

Whilst the results of some previous studies showed the absence of CAPN1 CC genotype in Simmental bulls [16, 18], Hereford and Limousin cattle [18], our study showed the presence of CAPN1 CC genotype in Aceh cattle. The low 0.14 frequency of C allele found in Aceh cattle supports reports found the low or absence of C allele in different cattle herds [17, 19, 20].

Previous studies showed that the SNP G316A of the *CAPN1* gene studied occurs in exon 9 (alleles C/G), resulted in the substitution of alanine by glycine in the amino acid 316 of the protein-domain II [17, 21]. This amino acid substitution has been showed to related to the final weight and average weight gain of Brangus cattle [21], and the meat quality of Brangus [21], Aberdeen Angus-sired beef cattle [22] and Nellore cattle [23]. CAPN1 G316A polymorphisms also influence the fattening performance of Simmental bulls [16]. Since these effect were not addressed yet in this study, the further experiment must be done to investigate effects of the occurrence of the three SS, SG and GG CAPN SNP 316 on meat and production performance of Aceh cattle by involving not only larger numbers of subjects, but also Aceh cattle bulls.

4 Conclusion

The occurrence of the GG, GC, and CC genotypes of CAPN SNP 316 in aceh cattle in appreciable frequencies showed the diversity of the *CAPN* gene that distributed following the Hardy-Weinberg equilibrium. Further study involving larger samples from both sexes needs to do in order to map the diversity in the Aceh cattle populations and to explore the effects of the polymorphisms on the production and reproduction of the cattle.

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