

Investigating new SNPs of CAST, CAPN and SCD genes in 5'UTR of Bali cattle

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Abstract. *Jakaria, Ulum MF, Lestari D, Akwila S, Sihite DEWT, Priyanto R, Muladno, Sumantri C. 2020. Investigating new SNPs of CAST, CAPN and SCD genes in 5'UTR of Bali cattle. Biodiversitas 21: 2971-2976.* The present investigation aims to explore specific SNPs of Bali cattle, focusing mainly on CAST, CAPN, and SCD genes in 5'UTR. DNA of Bali cattle (25 individuals) from Breeding Centre of Bali cattle in Bali Province, Indonesia, were extracted according to the protocol of GenAID. Amplification of the CAST, CAPN, and SCD genes were designed using the Primer3 program based on reference sequences (access numbers AH014526.2, AH009246.3, and AY241932). Furthermore, the PCR product was sequenced through forward primer sequencing. The data sequence results were analyzed using the Bioedit, MEGA7, Popgen 3.2, and DnaSP6 V6 programs. The results showed that SNPs were detected at g.921C>T and g.922T>G in 5'UTR for the CAST gene between the reference of GenBank AH014526.2 and Bali cattle, but it was monomorphic within the population. The high polymorphism of SNPs was found in g.232G>T for the CAPN gene and g.134A>del for the SCD gene. However, the SNP g.246C>del SCD gene was detected as monomorphic in this region. Moreover, this result shows that low nucleotide diversities (Pi) for CAST, CAPN, and SCD genes in 5'UTR of Bali cattle were 0.00422, 0.00632, and 0.00449, respectively. This finding was important for developing marker-assisted selection (MAS) to improve the meat quality of Bali cattle.

Keywords: Bali cattle, 5'UTR, meat quality, polymorphism

INTRODUCTION

Tenderness is a quantitative characteristic of meat, and it is modulated by two main factors, i.e., the environment and genetics. Genetically, some major genes are reported to account for meat tenderness, including calpastatin (CAST) and calpain (CAPN) (Corva et al. 2007); Shin et al. 2016), as well as the stearoyl Co-A desaturase (SCD) gene that alters the composition of fatty acids (Taniguchi et al. 2004). Marbling, tenderness, and the composition of the fatty acids are major indicators of meat quality (Nogalski et al. 2018). They not only determine the economic value but also serve as basic criteria in marker-assisted selection (MAS) to improve the genetic profile of beef cattle (Pintos and Corva, 2011). With regard to the improvement of meat quality, many research studies have been conducted to find single nucleotide polymorphisms (SNPs) in the CAST (Putri et al. 2015; Enriquez-Valencia et al. 2017), CAPN (Lu et al. 2013; Pratiwi et al. 2016) and SCD genes Wahid et al. 2010; (Oh et al. 2013; Alwiyah et al. 2016). Exploration and utilization of single nucleotide polymorphisms (SNPs) as genetic markers have been widely applied in livestock-based industries, including breeding programs (Lu et al. 2018).

In live muscles, the activity of calpastatin, an inhibitor of calpain (Koochmarai 1992), accounts for the alleviation of protein degradation, and at the same time, μ -calpain (Calpain 1) constitutes a main proteolytic enzyme that

plays a significant role in the meat tendering process (Casas et al. 2006), thus increasing the meat tenderness (Nowak 2011). Stearoyl CoA desaturase (SCD) is an enzyme that is controlled by the SCD gene, which is involved in catalyzing and converting saturated fatty acid (SFA) to monounsaturated fatty acid (MUFA) in mammalian adipocytes (Taniguchi et al. 2004; Oh et al. 2013). The CAST, CAPN, and SCD genes are appropriate candidates that exert major effects on meat quality (Casas 2006; Kaplanova et al. 2013; Kaplanova et al. 2013; Leal-Gutiérrez et al. 2018); their genetic structure was composed of a promoter, 5'UTR, an open reading frame (ORF) and 3'UTR (Shafee and Lowe 2017). In cattle, the CAST gene was found to be located on chromosome 7, consisting of 35 exons and 34 introns (Raynaud et al. 2005), while the CAPN gene existed in chromosome 29 and was composed of 21 exons and 20 introns. The SCD gene was present on chromosome 26, having 6 exons and 5 introns (Taniguchi et al. 2004).

In basic gene structure, the 5' untranslated region (5'UTR) is important for regulating translation to produce proteins (Shafee and Lowe 2017). It was reported to be a modulator of translation initiation, with a length of 53-218 nucleotides (Leppek et al. 2017). Specifically, 5'UTR existed prior to the start codon (ATG), in which the ribosome attached and transcription initiated (Mignone et al. 2002). This region also served in the modulation of gene expression (Araujo et al. 2012). Although 5'UTR has been

extensively researched in humans (Al-Zoubi et al. 2018; Lu et al. 2018), its investigation in livestock has scarcely been conducted. In current years, some researchers have initiated its exploration to collect more scientific evidence, including 5'UTR of the melanophilin (MLPH) gene in goats (Li et al. 2014), 5'UTR of the lactoferrin (LF) gene in dairy cows (Hajibemani et al. 2012), 5'UTR of the HSP70 gene in dairy cows (Öner et al. 2017) and 5'UTR of the EDG1 gene in Indonesian beef cattle, including Bali cattle (Sutikno et al. 2018). Based on previous research, there is a need to explore the 5'UTR region of the CAST, CAPN, and SCD genes, which are primarily associated with meat quality in Bali cattle (*Bos javanicus*), as an indigenous Indonesian cattle (Martoyo 2012). Therefore, the purpose of this study was to investigate the SNPs of the CAST, CAPN, SCD genes in 5'UTR of Bali cattle.

MATERIALS AND METHODS

Animals

A total of 25 Bali cattle (age of 18 - 21 months) were collected from the Breeding Centre of Bali cattle in Bali Province, Indonesia. The Bali cattle used in this study did not have family relationships between individuals based on the data recording. The cattle were reared under an extensive system in a paddock, on a concentrate and pasture-based feeding. The quantity of the feed was limited to 10% grass and 1% concentrate, based on body weight.

DNA extraction and gene amplification

Blood samples were taken through the jugular vein by a veterinarian. The blood samples were preserved in EDTA. The DNA extraction was performed by a DNA extraction Kit (GenAID protocol). Furthermore, the extracted DNA was stored in a freezer (-20°C). The primer used to amplify the CAST, CAPN, and SCD genes were designed using Primer3, Multiple Primary Analyzer, and Primary Stats programs according to reference sequences with access numbers AH014526.2, AH009246.3, and AY241932 (Table 1).

Amplification of the CAST, CAPN, and SCD genes was conducted by mixing 2 µL of DNA template, 25 µL PROMEGA Green Master Mix, 0.5 µL forward primer (25 pMol/µL), 0.5 µL reverse primer (25 pMol/µL) and 22 µL nuclease-free water, which yielded 50 µL of PCR volume. The PCR program was run as follows: predenaturation at 95°C for 5 minutes, denaturation at 95°C for 10 seconds,

annealing at 55-60°C for 20 seconds and extension at 72°C for 10 seconds (35 cycles)(Applied Biosystems, Foster City, CA, USA). The PCR product was analyzed by electrophoresis on 1.5% agarose gel and was evaluated using UV Transilluminator.

Sequencing and data analysis

The CAST, CAPN, and SCD genes from the PCR products were delivered to the 1st Base company in Selangor, Malaysia, for sequencing analysis. The sequencing used only the forward primer to detect the SNPs, especially in the region 5'UTR of the CAST, CAPN, and SCD genes. The CAST, CAPN, and SCD gene sequences were then analyzed using the BioEdit program (Hall 1999), while SNP identification was performed using the MEGA7 program (Tamura et al. 2013). The allele frequency was calculated using Popgen 3.2 (Yeh et al. 1997). Furthermore, analysis of the nucleotide diversity and nucleotide composition was conducted by the DnaSP v6 program (Rozas et al. 2017).

RESULTS AND DISCUSSION

Results

The results showed that PCR products were obtained at lengths of 422 bp, 478 bp, and 300 bp for the CAST, CAPN, and SCD genes, respectively, with annealing conditions at 55°C and 60°C (Figure 1). These genes were successfully sequenced in 8 cattle, 24 cattle and 16 cattle of the 25 cattle studied, respectively. The sequences of the CAST, CAPN and SCD genes in 5'UTR in Bali cattle revealed 6 SNPs, which were spread in different genes, as follows: 3 SNPs in the CAST gene, 1 SNP in the CAPN gene, and 2 SNPs in the SCD gene (Figures 2, 3 and 4), while the nucleotide diversity (Pi) was low (Table 2). The abundance of the G-C nucleotides in 5'UTR in the CAST, CAPN, and SCD genes was greater than that of the A-T nucleotides (Table 3).

This result suggested that the designed primer was suitable for amplifying the 5'UTR region for the CAST, CAPN, and SCD genes, which was by the length of the targeted fragment, although only several samples could be successfully sequenced. Based on the SNP detected, two SNPs demonstrated high polymorphisms in the population, i.e., SNP g.232G>T for the CAPN gene and SNP g.1890A>del for the SCD gene, while the remaining SNP was monomorphic to Bali cattle.

Table 1. Sequence of forward and reverse primers for the amplification of CAST, CAPN and SCD genes in 5'UTR of Bali cattle

Genes	GenBank reference sequence	Primer sequences (5'→3')	Targeted fragment	Product length (bp)
CAST	AH014526.2	Forward 5'-AGG GTG TGA GTT GCA AAC AG-3' Reverse 5'-AGA AGG CCA GGA AGG CTA AC-3'	5'UTR	422
CAPN	AH009246.3	Forward 5'-CCC TTC CCA CCC AGA TAG G-3' Reverse 5'-CCT GGA GAC CGT GAG GAA C-3'	5'UTR	478
SCD	AY241932	Forward 5'-AGC CTT TAA ATC CCC AGC AC-3' Reverse 5'-AGT GAG TCG CCC AGA AGC-3'	5'UTR	300

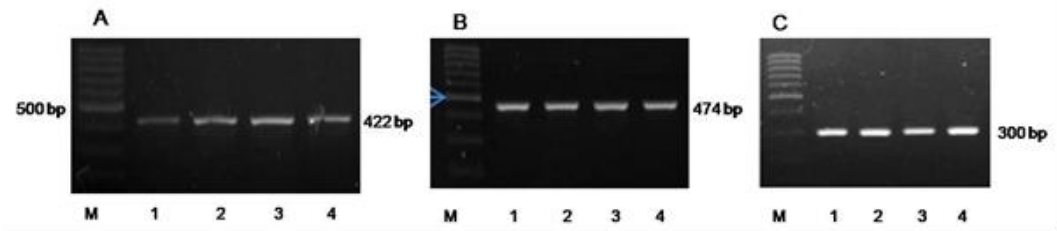


Figure 1. PCR products for the CAST (A), CAPN (B) and SCD gene (C) after electrophoretic analysis on 1.5% agarose gel (M: 100 bp DNA ladder, 1-4: samples)

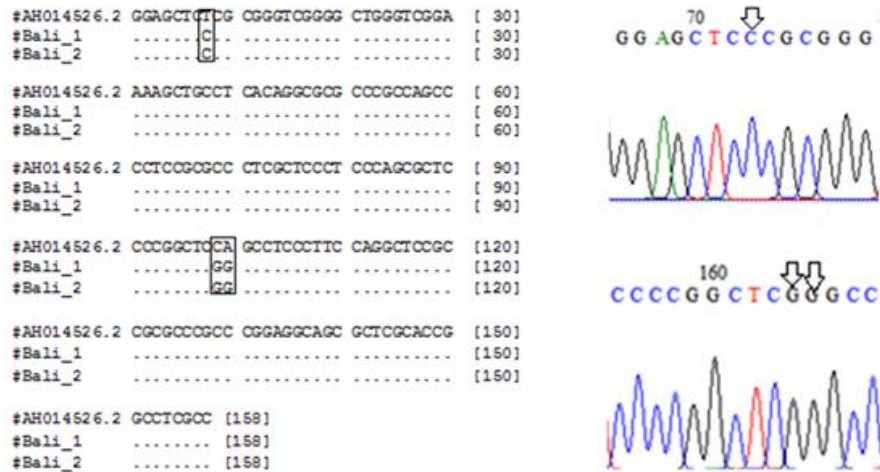


Figure 2. Locations of SNPs in 5'UTR in the CAST gene

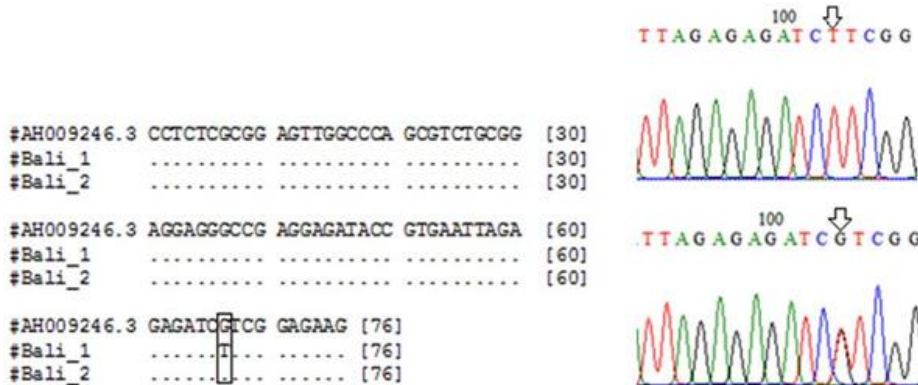


Figure 3. Location of SNP in 5'UTR in the CAPN gene

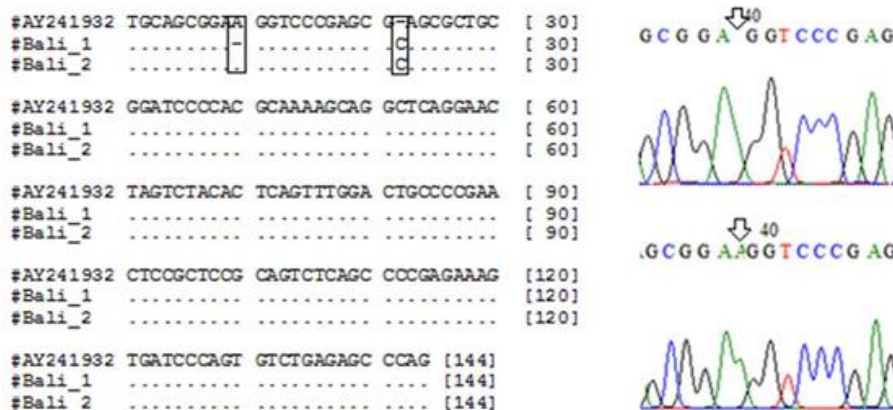


Figure 4. Locations of SNPs in 5'UTR in the SCD gene

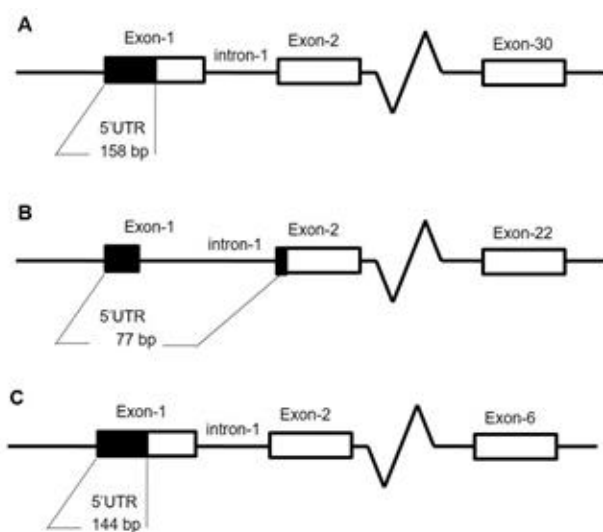
Table 2. SNP position and allele frequency for the CAST, CAPN and SCD gene in 5'UTR

Gene (n)	Sequence length in 5'UTR (bp)	SNP	Frequency		Nucleotide diversity (Pi)
			1	2	
CAST (8)	158	g.608T>C	0.00	1.00	0.00422
		g.699C>G	0.00	1.00	
		g.700A>G	0.00	1.00	
CAPN (24)	76	g.232G>T	0.68	0.32	0.00632
SCD (16)	144	g.1890A>del	0.19	0.81	0.00449
		g.1902C>del	1.00	0.00	

Note: n: means number of samples; 1 and 2 represent SNPs

Table 3. Composition (%) of nucleotides in the CAST, CAPN and SCD genes in 5'UTR

Gene	Sequence length in 5'UTR (bp)	T (U)	C	A	G	[A+T]	[C+G]
CAST	158	11.4	48.7	8.2	31.6	19.7	80.3
CAPN	76	17.1	22.4	22.4	38.2	39.5	60.5
SCD	144	15.3	34.0	22.9	27.8	38.2	61.8

**Figure 5** Location and length of the 5'UTR sequence: CAST (A), CAPN (B) and SCD (C), according to sequence references of access numbers AH014526.2, AH009246.3 and AY241932

Discussion

It is noteworthy that the 5'UTR region was essential in its gene structure, and it was closely located from the start codon (ATG) (Shafee and Lowe 2017). That location and the length of the sequence for the CAST, CAPN, and SCD genes in 5'UTR varied, i.e., 158 bp, 77 bp, and 144 bp, respectively (Figure 5). Furthermore, this study confirmed that the positions of these genes in 5'UTR were relatively similar to those reported by Leppek et al. (2017), in which the sequence length in 5'UTR ranged from 53 to 218, with the average number of nucleotides in cattle reaching 111 nucleotides.

In the composition of the A-T and G-C nucleotides, the CAST gene had the greatest amount of (G-C), reaching up to 80.3%. The abundance of G-C in 5'UTR was meaningful since this nucleotide served as the controller in translation as well as the site in which transcription began. For this reason, the G-C nucleotide is required in large quantities. The region of 5'UTR was also recognized as microRNA (Gu et al. 2014), which essentially acts in the translation modulation (Shafee and Lowe 2017) and translation initiation (Leppek et al. 2017). SNP g.232G>T and g.1890A>del found in 5'UTR in the CAPN and SCD genes were present with high polymorphism in Bali cattle. The mutation type in the SNP g.232G>T for the CAPN gene was a transversion (G→T), which refers to purine and pyrimidine, and at the same time, another SNP (SNP g.1890A>del in SCD gene) was an in/del (A→del).

The diversity of the CAPN and SCD genes in Bali cattle is highly essential since these genes are responsible for the profiles of meat tenderness and fatty acids; thus, further investigation into this area could contribute to raising the economic value of Bali cattle. Previously, the crucial role of the CAPN and SCD genes was reported by Avilés et al. (2013), which found that μ -calpain constitutes a major proteolytic enzyme that contributes to meat tenderness, while Taniguchi et al. (2004) and Oh et al. (2013) asserted that the SCD gene enabled the catalysis and conversion of SFA into MUFA.

Previous study reported that the CAST, CAPN, and SCD genes are known as genetic markers used for meat quality (Cassar-Malek and Picard 2016), especially for tenderness and marbling scores (Kök and Atalay 2018), as well as fatty acids (Mannen 2012; Sevane et al. 2013). Lozano et al. (2016) reported that SNP CAPN316 and CAPN475 significantly affected the meat tenderness, while SNP CAST-T1 did not affect the commercial beef cattle

and Sevane et al. (2013) reported that SNP g.10329T>C exon 5 significantly affected the fatty acids in European cattle breeds. Some research results were reported to be related to the SNPs CAST, CAPN and SCD gene exposures in Bali cattle, where an SNP was found in the exon 1 position of the CAST gene and significantly affected the back fat thickness and *longissimus dorsi* thickness (Putri et al. 2015), whereas in the CAPN gene, 8 SNPs were found in exon areas 5-6 and 3 SNPs, namely, SNP c.3669T>C, c.3854G>A and c.3899C>T, significantly influenced the rump thickness, rump fat thickness and marbling score in Bali cattle (Pratiwi et al. 2016). There were 8 SNPs of SCD genes found in Bali cattle: 3 SNPs were excised 5 and 5 SNPs in intron 5 and only 3 SNPs were polymorphic, namely, SNP g.10360G>A SNP g.10428C>T and SNP g.10487G>A); only one SNP, g.10428C>T, significantly affected the marbling score and the percentage of intramuscular fat (Alwiyah et al. 2016). However, the results of the genetic analysis conducted are usually in the coding region, and there is no information in the 5'UTR region, especially for the CAST, CAPN and SCD genes, that concerns meat quality traits, especially tenderness, marbling scores and fatty acid profiles, in Bali cattle. The 5'UTR region has been intensively characterized as in Lactoferrin (LF) gene diversity with the reproductive trait of dairy cattle (Hajibemani et al. 2012), and the HSP70 gene is associated with S/C in Pasundan cattle (Said and Putra, 2018), bovine PPARGC1A gene related to milk fat (Weikard et al. 2005), solute carrier family 44, member 5 (SLC44A5) gene related to birth weight in Holstein cattle (Sugimoto et al. 2012) and the friend leukemia integration 1 (FLI1) gene associated with the conception rate (CR) in Holstein cattle (Sugimoto et al. 2016).

The investigation of SNPs in 5'UTR, especially the SNPs g.232G>T and g.1890A>del in the CAPN and SCD genes, can be a key for future research; they are specific to Bali cattle with high polymorphism. SNPs that are found in beef cattle (*Bos taurus*) applied in Bali cattle (*Bos javanicus*) and displayed low diversity (monomorphic), as observed in the growth hormone receptor (GHR) gene (Zulkharnaim et al. 2010), growth hormone (GH) gene (Jakaria and Noor 2011), STAT5 gene (Paramitasari et al. 2015), and ADIPOQ and EDG1 genes (Sutikno et al. 2018), including 5'UTR region in the EDG1 gene. Developing SNPs as genetic markers for the CAPN and SCD genes of Bali cattle offers a promising method to increase the economic value of the cattle, primarily in producing high-quality meat, especially meat tenderness and fatty acid composition.

Based on our results, it can be concluded that two polymorphic SNPs were found in 5'UTR, i.e., SNP g.232G>T and g.1890A>del in the CAPN and SCD genes, which were specific to Bali cattle, while four monomorphic SNPs were also detected primarily in the CAST and SCD genes, including the SNPs g.608T>C, g.699C>G, g.700A>G and SNP g.1902C>del. These polymorphic SNPs in Bali cattle could be potential candidate genes for meat quality traits.

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