p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, December 2019, 42(3):175-179
DOI: https://doi.org/10.5398/tasj.2019.42.3.175
Available online at http://journal.ipb.ac.id/index.php/tasj

Polymorphism and Association of 5′UTR CAPN1 Gene with Growth Traits in Bali Cattle by PCR-RFLP

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(Received 06-02-2019; Revised 22-05-2019; Accepted 27-05-2019)

ABSTRACT

The aim of this study was to identify the variation of 5′UTR CAPN1 gene and its association to growth traits in Bali cattle. DNA samples were obtained from 80 heads of Bali cattle originated from BPTU-HPT Denpasar. The average of Bali cattle age was 784 days (631 days-1098 days). Bali cattle were divided into 3 age groups namely, the first group (1.5 years to 2 years), the second group (2 years to 2.5 years), and the third group (2.5 years to 3 years). The observed growth traits were birth weight (kg), live weights (kg), average daily gain (kg), body length (cm), chest depth (cm), withers height (cm), hip height (cm), and heart girth (cm). Polymorphism identification of 5′UTR CAPN1 gene was conducted by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) with BglII as the restriction enzyme. Growth traits data association were analyzed using the General Linear Model (GLM) analysis. The 5′UTR CAPN1 gene|BglII was polymorphic in Bali cattle (GG, GT, and TT). Genotype frequencies for Bali cattle were 0.30 (GG), 0.66 (GT), and 0.04 (TT). The allele frequencies of G and T allele were 0.63 and 0.37, respectively. The G allele was the most frequent allele and GT genotype was the most frequent genotype among the cattle. The CAPN1|BglII had a significant effect ($p<0.05$) on growth traits in Bali cattle. Animal carrier of GG genotype had higher live weight and average daily gain than those with GT genotype, while the lowest values were associated with TT genotype.

Keywords: 5′UTR; Bali cattle; CAPN1 gene; PCR RFLP

INTRODUCTION

Selection for growth traits in Bali cattle is mostly conducted by traditional phenotypical selection methods. Today, molecular-based selection in cattle has been widely performed in other countries, including Indonesia. One of the genes that affect meat quality and growth traits is Calpain (CAPN1). Calpain is a group of neutral cysteine proteinase affected by calcium. Calpain 1 (CAPN1) is located on chromosome 29 (Pinto *et al*., 2010) consisting of 21 exons and 20 introns (Dear *et al*., 2000). In the distal region of bovine chromosome 29 that spans CAPN1, there is a substantial overlap of QTLs (http://www.animalgenome.org). Apart from beef tenderness, these QTLs also regulate growth (weaning weight, carcass weight) and feed efficiency. CAPN1 is considered to be the best candidate for tenderness QTL, however, there is little information about the genes underlying the other QTLs in the region (Pintos & Corva, 2011). CAPN1 gene has been reported to be associated with average daily gain, live weights, carcass weight, backfat thickness, m. longissimus thoracis et lumborum, pH, final weight, increased body weights in cattle (Ardicli, 2017; Miquel *et al*., 2009; Tait *et al*., 2014).

CAPN1 gene has a 5′ untranslated region (UTR) which has been recognized for controlling translation-initiation process (Gunawan *et al*., 2017), post-translation process, and the regulation of gene expression. Mignone *et al*. (2002) state that 5′UTR is one of mRNA elements contributing significantly to the translation efficiency of an mRNA. Certain features can suggest translational control of the messenger when a 5′UTR sequence is analyzed.

Numerous studies on 5′UTR fragment have also been reported in chicken (Bhattacharya *et al*., 2011; Korwin-Kossakowska *et al*., 2009), sheep (Sjakste *et al*., 2011; McKenzie *et al*., 2012; Sahu *et al*., 2016), pig (Shen *et al*., 2011; Brenig *et al*., 2015; Fang *et al*., 2011), and cattle (Öner *et al*. 2017; Manzoor *et al*., 2013; Sugimoto *et al*., 2012). There were 43 SNPs and three indels were found in the 5' UTR region in HSP70.1 gene in Turkish cattle breeds (Yerli Kara (YK), Boz ırk (BI), Yerli Güney Sarısı (YGS), Güney Doğu Anadolu Kırmızısı (GAK), Doğu Anadolu Kırmızısı (DAK)), and Holstein Friesian

(Siyah Alaca (SA). HSP70.1 is a member of the heat stress protein family, which is essential for life, production, and reproduction in cattle. The 5' UTR region of HSP70.1 gene was more variable in the Turkish native breeds than in the Holstein Friesian (**Öner** *et al*. 2017). Another study also stated that there was a significant finding of the incidence of the C → T polymorphism in 5'UTR CYP11B1 gene in Pakistan Sahiwal cattle breed (Manzoor *et al*., 2013). Khasanah *et al.* (2016) stated that myostatin (MSTN) promoter gene was polymorphic in Bali cattle and associated with intramuscular fat percentage. From the studies above, no study was conducted in Bali cattle. The results of the previous studies suggested that 5'UTR CAPN1 might also have a marked influenced on Bali cattle genetic performance. The aims of this study were to identify variation in 5'UTR CAPN1 gene and its association with growth traits in Bali cattle.

MATERIALS AND METHODS

Samples

This research was conducted in the laboratory of animal molecular genetics, Faculty of Animal Science, IPB University (Bogor Agricultural University). Blood samples were obtained from 80 heads of Bali cattle in BPTU-HPT Denpasar. The average of Bali cattle age was 784 days (631 days-1098 days). Bali cattle were divided into 3 age groups namely, the first group (1.5 years to 2 years), the second group (2 years to 2.5 years), and the third group (2.5 years to 3 years). The observed growth traits were birth weight (kg), live weights (kg), average daily gain (kg), body length (cm), chest depth (cm), withers height (cm), hip height (cm), and heart girth (cm).

DNA Isolation and PCR Amplification

Blood samples were collected from the jugular vein of the Bali cattle and kept in a vacutainer tube containing ethanol absolute as an anticoagulant. Extraction of DNA was carried out by a modified DNA Geneaid Kit. The primer was designed using data sequences from the National Center for Biotechnology Information (NCBI) with access number AH009246.3. Primer selection was performed by using Primer 3, Multiple Primary Analyzer, and Primary Stat. Forward primer was 5-CCC TTC CCA CCC AGA TAG G-'3 and reverse forward was 5'-CCT GGA GAC CGT GAG GAA C-'3. The primer was designed to amplify 5'UTR region of CAPN1 gene. For the PCR, 25 μL of PCR amplification mix contained 1 μL of DNA template contained 50 ng of DNA template, 12.5 μL of PROMEGA green master mix, 10.9 μL of nuclease-free water, 0.3 μL contained 5 pmol forward primer, and 0.3 μL contained 5 pmol reverse primer. The DNA amplification was conducted in predenaturation condition at 95°C for 5 minutes, annealing at 55°C for 20 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 min. The amplification process was carried out for 35 cycles. The expected amplified fragment size for CAPN1 gene was 478 bp.

Genotyping

Restriction enzyme was designed by NEBCutter v2.0 program. BglII was found as restriction enzyme with the site cut (A|GATCT). Polymorphism of 5'UTR CAPN1 was examined by PCR-RFLP method. A mixture containing *5* μL of PCR product, 0.9μL of nuclease-free water, 0.7 μL of BglII enzyme buffer, and 0.4 μL contained 4U/ μL of BglII enzyme was incubated at 65°C for 4 hours. The products were then electrophoresed in 2% agarose gel for 35 minutes and photographed with a gel imaging system (UV Transilluminator). GG genotype had 478 bp fragment, TT genotype had 354 bp and 124 bp fragments, and GT genotype had 478 bp, 354 bp, and 124 bp fragments.

Data Analysis

Genotype and allele frequency. The genotype and allele frequency were calculated using Popgene32 according to Nei & Kumar (2000) with the formula as follows: $\chi_{ii}= (n_{ii}/N)$ for genotype frequency and $\chi_i= (2n_{ii} + \Sigma n_{ij})/(2N)$ for allele frequency, where: χ_{ii} is frequency of ii genotype; χ_i is frequency of i allele; n_{ii} is number of individuals with ii genotype; n_{ij} is number of individuals with ij genotype; and N is number of samples.

Hardy-Weinberg equilibrium (HWE). Hardy-Weinberg equilibrium was determined using Popgene32 program, the Chi-Square equation (Hartl & Clark, 1997) as follows:

$\chi^2 = \sum \{(O-E)^2)/E\}$

where: χ^2 is HWE test; O is the observed number of genotypes; E is the expected number of genotypes. df = number of genotype probabilities – number of alleles.

CAPN1 gene association with growth traits. Growth traits data of Bali cattle were analyzed using General Linear Model (GLM) by SAS software. Further tests were carried out by Least Square Means. A mathematical model was formulated as follows:

$Y_{ij}= \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijkl}$

where: μ is overall mean for each trait; α_i is the effect of i^{th} genotype, i is 1,2,3; β_j is the effect of j^{th} sex, j is 1,2; γ_k is the effect of k^{th} age group, and k is 1,2,3; e_{ijkl} is random error.

RESULTS

Polymorphism of 5'UTR of CAPN1 Gene

The 5'UTR of CAPN1 gene of Bali cattle was successfully amplified using a pair of primer at an annealing temperature of 55°C for 20 s (Figure 1). The length of PCR product was 478 bp. Genotyping using BglII as the restriction enzyme resulted in three genotypes: GG (478 bp), TT (354 and 124 bp), and GT (478, 354, and 124 bp).

The 5'UTR CAPN1 gene was polymorphic in Bali cattle. The polymorphism of 5'UTR gene in Bali cattle

are presented in Figure 2. The genotype frequencies were 0.30 (GG), 0.66 (GT), and 0.04 (TT). The allele frequencies of G and T were 0.63 and 0.37 respectively. The G allele was the most frequent allele and GT genotype was the most frequent genotype among the cattle.

Association of CAPN1 Gene on Growth Traits

Association analysis showed that the 5'UTR CAPN1|BglII was significantly affected live weight and average daily gain in Bali cattle ($p<0.05$). Individuals with the GG genotypes had higher live weight and average daily gain than GT and TT genotypes ($p<0.05$). The association result is presented in Table 1.

DISCUSSION

Calpain (CAPN1) is a group of neutral cysteine proteinase affected by calcium. Calpain substrates consist of a variety of enzymes such as cytoskeletal proteins (Fedota *et al*., 2016) and epidermal growth factor receptors (Glading *et al*., 2000). The CAPN gene was investigated as a potential candidate gene for growth trait as reported in earlier studies of Mahrous *et al*., 2016; Hou *et al*., 2011; and Bosques *et al*., 2015.

Amplification of CAPN1 gene in 5'UTR fragment was successfully conducted using PCR for all samples. According to PCR-RFLP on 5'UTR CAPN1 gene using BglII as restriction enzyme, the region was polymorphic in Bali cattle. It is because the allele frequency obtained is more than 0.01 (Nei & Kumar, 2000). There were three genotypes (GG, GT, and TT) found in Bali cattle. The genotype frequencies were 0.30 (GG), 0.66 (GT), and 0.04 (TT). The allele frequencies of G and T were 0.63 and 0.37 respectively. The variation in 5'UTR CAPN1 gene in Bali cattle in BPTU-HPT Denpasar were not in Hardy-Weinberg equilibrium, χ^2 value was 13.92 ($p<0.05$). Gunawan *et al*. (2017) stated that a population was in Hardy-Weinberg equilibrium if the genotype frequencies of the dominant and recessive alleles were constant from generation to generation; there was no selection, mutation, migration, and genetic drift. Genotype-frequency imbalances of CAPN1 gene|BglII suggested that the population of Bali cattle in BPTU-HPT Denpasar had undergone selection.

The result of growth traits association with 5'UTR CAPN1|BglII showed that variations in 5'UTR CAPN1 gene significantly affected live weight and average daily gain in Bali cattle ($p<0.05$) (Table 1). Birth weight, body length, chest depth, withers height, hip height, and heart girth of Bali cattle with genotypes GG, GT, and TT had no significant difference based on Table 1. The average birth weight of Bali bulls was 18.9±1.4 kg (Prasojo *et al*., 2010) and 17.73±1.72 kg (Gunawan & Jakaria, 2011).

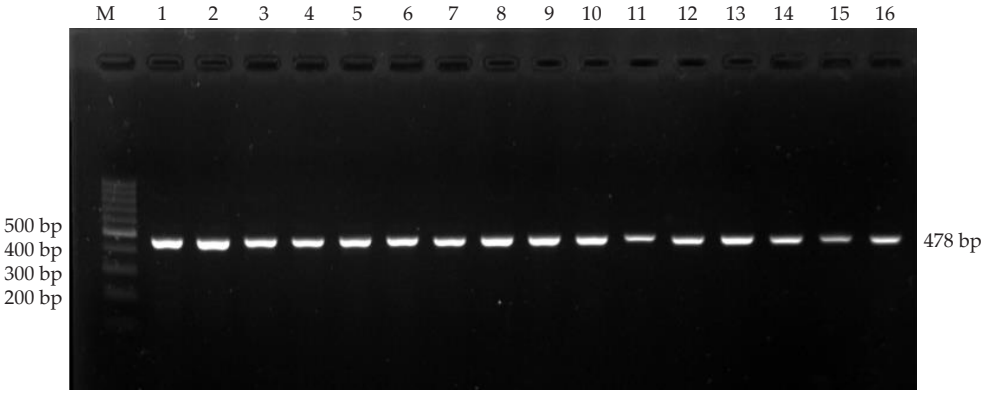

Figure 1. Amplification product of 5'UTR CAPN1 gene in Bali cattle in 1.5% agarose gel (w/v). Lane M= marker with 100 bp DNA.

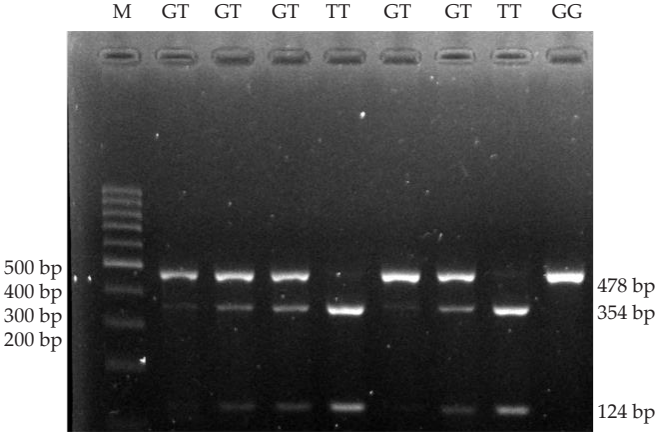

Figure 2. Amplification product of 5'UTR CAPN1 gene in Bali cattle in 2% agarose gel (w/v). Lane M= marker with 100 bp DNA.

Table 1. CAPN1 gene association with growth traits

Traits	Genotype		
	GG (n=24)	GT (n=53)	TT (n=3)
Birth weight (kg)	17.9±0.5	18.1±0.4	19.3±1.2
Live weight (kg)	211.4±7.1[a]	196.7±5.0[b]	160.0±17.3[c]
Average daily gain (kg)	0.248±0.010[a]	0.225±0.007[b]	0.177±0.024[c]
Body length (cm)	106.4±1.8	106.6±1.3	102.5±4.5
Chest depth (cm)	57.0±0.9	55.5±0.6	56.1±2.2
Withers height (cm)	110.7±1.1	111.9±0.8	108.9±2.7
Hip height (cm)	110.9±1.1	119.5±0.8	110.7±2.8
Heart girth (cm)	148.5±1.8	144.7±1.3	142.4±4.6

Note: means in the same row with different superscripts differ significantly ($p<0.05$).

The average birth weights of Bali cows were 18.9±1.4 kg (Prasojo *et al.*, 2010) and 17.55±1.70 kg (Gunawan & Jakaria, 2011).

Genotype GG was significant ($p<0.05$) for the highest live weight and average daily gain in Bali cattle. This shows that 5'UTR CAPN1|BglII can be used to select for live weight and average daily gain traits in Bali cattle. Miquel *et al.* (2009) found a significant association between CAPN1-316 marker with shear force, final weight, and average daily gain in Brangus and Angus steers. Pintos & Corva (2011) also reported a significant association between CAPN1-316 marker with birth weight, calving weight, and 18 months weight. They stated that to get high tenderness in meat, the cattle can be selected for low average daily gain and low final weight.

CONCLUSION

The 5'UTR CAPN1|BglII was polymorphic in Bali cattle and was not in Hardy-Weinberg equilibrium. Genotype GG was significant for the highest live weight and average daily gain in Bali cattle. Therefore, selection can be done by using 5'UTR CAPN1|BglII in Bali cattle for live weight and average daily gain traits.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

This research was financially supported by Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) program (1757/IT3.11/PN/2018). The authors would also like to give thank you to Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Bali (BPTU-HPT Bali) for providing blood sample of Bali cattle and growth trait data that were used in this study.

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