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The Favorable Allele of *CAPN1*-316 Genetic Marker is Absent in Bali and Sumbawa Cattle

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ABSTRACT: The *micromolar calcium-activated neutral protease 1* (*CAPN1*) gene encodes the μ-calpain enzyme, which plays a crucial role in meat tenderisation. Genetic diversity within the *CAPN1* gene, specifically a nucleotide substitution from G to C in exon nine resulting in a change from glycine to alanine at position 316 (*CAPN1*-316 marker), is known to significantly affect meat tenderness. This study aimed to assess the polymorphism of the *CAPN1*-316 locus in Bali and Sumbawa cattle. A total of 293 blood samples, 193 from Bali cattle and 100 from Sumbawa cattle were extracted and genotyped using PCR-RFLP with *Btg*I restriction enzyme (recognition sequence: 5'-C*CRYGG-3') applied to 706 bp PCR products. The results showed the presence of only one genotype (GG genotype) and one allele (G allele) in all DNA samples obtained from the Bali and Sumbawa cattle populations studied. In conclusion, the *CAPN1*-316 genetic marker showed a lack of diversity or monomorphism in Bali and Sumbawa cattle, making it unsuitable for further association studies in these breeds. Consequently, the CG/AG haplotype identified in Sumbawa cattle warrants further investigation and could serve as an alternative genetic marker, especially due to its monomorphism at the *CAPN1*-316 locus.

Keywords: *Calpain 1* (*CAPN1*); Polymerase chain reaction (PCR-RFLP); Single nucleotide polymorphism (SNP); Bali cattle; Sumbawa cattle

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INTRODUCTION

Various factors, including meat production capacity, fat composition, age, and diet, influence the quality of beef cattle. Equally important is the quality of the meat they produce. Meat quality is a crucial determinant of overall palatability. Its intrinsic characteristics, ranging from color and aroma to marbling, flavor, juiciness, tenderness, and texture, profoundly impact consumer perception (Miller *et al.*, 1995). Among these attributes, tenderness, juiciness, and flavor are vital requirements that significantly shape consumer preferences when selecting meat (Pethick *et al.*, 2011). Beyond sensory satisfaction, it is intriguing to observe that the peak of meat tenderness often encourages consumers to accept a higher price (Nowak, 2011). Hence, meat tenderness becomes a crucial aspect to investigate.

Meat tenderness, in particular, is determined by two primary determinants: antemortem and postmortem factors. Antemortem factors include genetics (breed and species), physiological variables, age, husbandry practices, sex, and stress levels. Postmortem factors include meat handling techniques, chilling and freezing methods, storage duration and temperature, and meat processing procedures, including cooking and meat tenderizers (Hou *et al.*, 2011; Maltin *et al.*, 2003). When studied, it is essential to recognize that genetic factors reveal a unique set of genes that contribute to the variability in meat tenderness for each breed or species.

Among the most extensively studied genes involved in meat tenderness is the *micromolar calcium-activated neutral protease 1* (*CAPN1*) gene. This gene has been precisely mapped to chromosome 29 and encodes the enzyme calpain (Smith *et al.*, 2000). Calpain, an endogenous proteolytic enzyme, plays a central role in the degradation of muscle cell proteins (myofibrils) immediately after the death of an animal (postmortem), thereby facilitating meat tenderization (Bhat *et al.*, 2018). Variations within the *CAPN1* gene determine its activity in regulating meat tenderisation (Page *et al.*, 2002, 2004).

Numerous studies have highlighted the significant effect of variations in the G/C nucleotides within exon 9 of the *CAPN1* gene on meat tenderness in different cattle breeds (Bonilla *et al.*, 2010; Corva *et al.*, 2007; Soria *et al.*, 2010). This non-synonymous nucleotide substitution converts the amino acid glycine to alanine (*CAPN1*-316), with the C allele proving advantageous due to its significant favorable influence on meat tenderness. However, it is worth noting that the frequency of the C allele in *Bos indicus* cattle is significantly lower (ranging from 0.00 to 0.19) than in *Bos taurus* cattle (ranging from 0.09 to 0.27) (Allais *et al.*, 2011; Soria *et al.*, 2010). This genetic difference contributes to the observed difference in meat tenderness between *B. indicus* and *B. taurus* cattle (Bressan *et al.*, 2011; White *et al.*, 2005). Consequently, genetic variation is emerging as a critical factor in beef cattle selection for improved tenderness.

The genetic diversity of *CAPN1* with SNP 316 (*CAPN1*-316 marker) in Indonesian local cattle has not been reported. Bali and Sumbawa cattle represent *B. javanicus* and *B. indicus* cattle, respectively, in Indonesia. Therefore, this study aims to elucidate the polymorphism of *CAPN1*-316 in Bali and Sumbawa cattle. his study will give preliminary information on genetic diversity in efforts to select cattle with desirable tender meat traits.

MATERIALS AND METHODS

Cattle and DNA Samples

Blood samples were obtained from a total of 293 cattle, comprising 193 Bali cattle (*Bos javanicus*) from 104 individuals in the Enrekang district and 73 from the Barru district in South Sulawesi province. Additionally, 16 Bali cattle raised by the Faculty of Animal Science, Hasanuddin University were used in this study. Furthermore, 100 Sumbawa cattle (*Bos indicus*) were collected from the Sumbawa regency in Nusa Tenggara Barat province.

For each animal, 3 mL of blood was collected from the jugular vein and placed in Vacutainer tubes containing K3EDTA as an anticoagulant. DNA extraction was performed using the Genomic DNA Mini Kit provided by Geneaid Biotech Ltd. (Taiwan). The resulting DNA samples were subsequently stored at -20°C until they underwent PCR analysis. This research was conducted following ethical guidelines and received approval from both the Research Ethics Clearance Committee at the Indonesian Institute of Sciences (LIPI) (approval number: 36/klirens/III/2021) and the Animal Care and Use Ethics Committee at the National Research and Innovation Agency (BRIN) (approval numbers: 035/KE.02/SK/8/2022 and 055/KE.02/SK/04/2023).

PCR Amplification

A specific 709 bp fragment containing the CAPN1-316 locus of the *CAPN1* gene was amplified. This fragment was obtained using a pair of primers designed by Corva et al., (2007), based on the GenBank accession number AF252504.1. However, However, upon verification on the NCBI website, it was determined that the accession number had been updated to AH009246.3. Detailed information about the designed primer pair is presented in Table 1.

Table 1. A pair of primers is used to amplify a specific fragment of the *CAPN1*-316 locus

Genetic Marker	GenBank*	Primer Sequence (5'-3')	Amplicon (bp)	Annealing (°C)	References
CAPN1-316	AH009246.3	F=CCAGGGCCAGATGGTGAA R=CGTCGGGTGTCAGGTTGC	709	62	Corva *et al.* (2007)

Note: *The GenBank accession number AF252504.1, which was used as a reference by Corva *et al.*, (2007), has been updated to the new accession number AH009246.3.

Amplification was performed using the Mastercycler Gradient machine (Eppendorf, Germany). Each amplification reaction consisted of a total volume of 10 μL containing 1.0 μL bovine DNA (at a concentration of 10-12 ng/μL), 4.0 μL MyTaq HS Red Mix, 2x (Bioline, USA), 0.2 μL primer F, 0.2 μL primer R and 4.6 μL nuclease-free water (Promega, USA). The PCR program started with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 25 seconds, annealing at 62°C for 25 seconds, and extension at 72°C for 25 seconds.

The amplification was completed with a final extension step at 72°C for 5 minutes. The PCR products were then verified by electrophoresis on a 1% agarose gel, followed by staining with GelRed dye (Biotium, USA) and incubation for 30-60 minutes. The resulting bands were visualized using a gel documentation system (Syngene, UK).

Genotyping

The genotyping method used in this study was restriction fragment length polymorphism (RFLP) using the *Btg*I restriction enzyme (NEB, USA), which recognizes the specific site 5'-C*CRYGG-3'. Each RFLP reaction mixture consisted of 2.0 μL PCR product, 0.2 μL restriction enzyme, 1.0 μL NE buffer, and 6.8 μL nuclease-free water (Promega, USA), giving a total volume of 10 μL per reaction. The reaction mixture was then incubated in a water bath at 60°C for 60 minutes, followed by inactivation at 80°C for 20 minutes. Genotype identification was achieved by electrophoresis of the RFLP products on a 3% agarose gel, and the resulting bands were visualized using a gel documentation system (Syngene, UK).

Data analysis

PCR-RFLP data were analyzed by calculating allele and genotype frequencies (Nei and Kumar, 2000). Genotype frequency, determined by the calculation of

the ratio of a specific genotype in each population, was calculated by the following formula:

$$x_{ii} = n_{ii}/N$$

Allele frequency was calculated as the ratio of a certain allele to the overall alleles at a certain locus in a population (Nei & Kumar, 2000). Allele frequency of *CAPN1* gene|*Btg*I was calculated by the following formula:

$$x_i = (2n_{ii} + \sum n_{ij})/2N$$

Where x_{ii} is the frequency of genotype A_{ii}, x_i is the frequency of allele A_i, n_{ii} is the number of genotype A_{ii}, n_{ij} is the number of genotype A_{ij}, and N is the total samples. A representative sample was sequenced in both the forward and reverse directions to characterize each pattern identified in this study comprehensively. The resulting sequences were then aligned to GenBank AH009246.3 using the Bioedit sequence alignment editor software (Hall, 1999). Sequence analysis determined the mutation positions and the specific *Btg*I enzyme restriction sites.

RESULTS AND DISCUSSION

CAPN1-316 Locus Amplification

This study successfully amplified the target *CAPN1* gene fragment to a size of 709 base pairs (bp) (Figure 1). This was consistent with the results of Corva *et al.*, (2007) using the same primer design. The gene fragment contained the SNP316 locus identified in Bali and Sumbawa cattle. The positions of the fragment and SNP316, based on GenBank AH009246.3, were shown in Figure 2. The forward primer (F) is designed to amplify part of exon 8, while the reverse primer (R) initiates from a segment of exon 10, resulting in a fragment size of 709 bp (Figure 3).

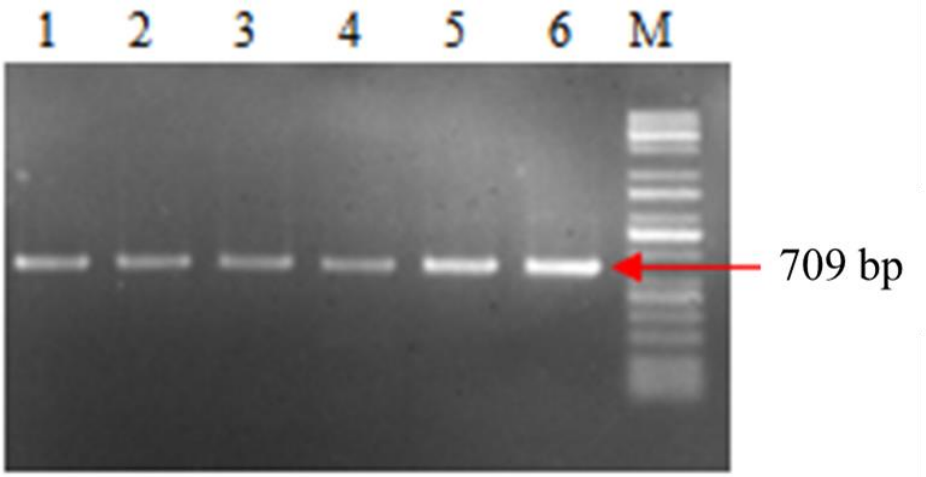

Figure 1. Visualization of the PCR product of the *CAPN1*-316 gene on a 1% (w/v) agarose gel. Lanes: 1-6 (PCR products of the *CAPN1*-316 gene), M: 100 bp DNA ladder.

The *CAPN1* gene is a genetic factor influencing post-mortem meat tenderisation in cattle (Ardicli *et al.*, 2017). The targeted SNP in this study was located in exon 9 at base 5709 (*g.5709G>C*), that has been deposited as rs17872000 in dbSNP of NCBI database, where the G allele encodes the amino acid glycine (GGC). The C allele encodes alanine (GCC) (p.Gly316Ala). This amino acid change occurs at position 316 and is therefore called *CAPN1*-316. It is also known as SNP316 or *CAPN*316, or G316A.

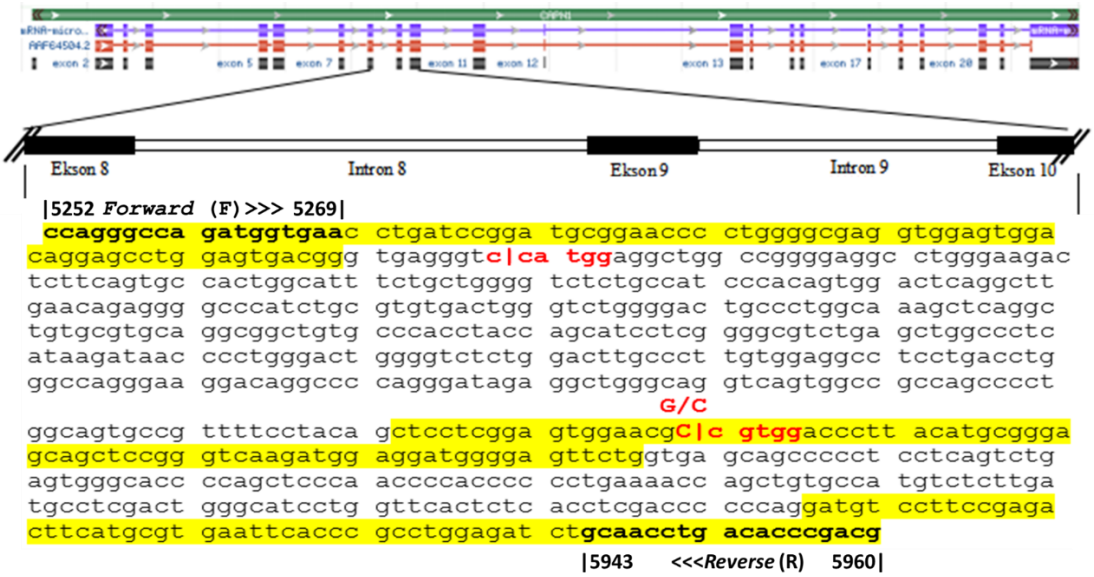

Figure 2. Schematic representation of the *CAPN1* gene fragment and the position of the target SNP 316 (*CAPN1*-316 marker) based on GenBank AH009246.3. The bold letters at the beginning and end are primer sequences; primer F amplifies from base position 5252, while primer R starts from base position 5960; the yellow-shaded sequences consecutively represent exon 8 (partial), exon 9, and exon 10 (partial); the bold red letter "C" and "G/C" marks the position of SNP316 (G>C) at base 5709; the red bold letters sequence "c|catgg" and "c|cgtgg" represents the *Btg*I restriction enzyme recognition site.

PCR-RFLP

The *Btg*I restriction enzyme was utilized in the PCR-RFLP procedure for genotyping. This enzyme recognizes the restriction site at 5'- C*CRYGG -3'. The genotyping results showed two banding patterns. The first pattern identified consisted of bands of 622 and 87 bp (GG genotype) and the second pattern observed shows a distinct banding profile of 709, 622, and 87 base pairs (bp) fragments (CG/AG haplotype). According to the alignment with GenBank AH009246.3, the first pattern occurred because the *Btg*I enzyme recognized its restriction site in intron 8, and a transition mutation from cytosine (C) to guanine (G) at base 5709 (in exon 9) prevented the *Btg*I enzyme from recognizing the restriction site, resulting in the two bands of 622 and 87 bp.

The identification results showed that all Bali and Sumbawa cattle samples had the GG genotype (100%) or only the presence of the G allele. The second pattern is called the CG/AG haplotype and denotes a combination of the CA genotype at SNP *g.5340A>C* (rs718259317) and the GG genotype at SNP *g.5709G>C* (rs17872000). The origin of this pattern can be traced to a specific adenine (A) to cytosine (C) mutation at base 5340, located within intron 8. This mutation, present in one allele, disrupts the recognition site for the *Btg*I enzyme, resulting in the formation of bands of 709, 622, and 87 bp.

This second pattern was identified in 11 Sumbawa cattle, representing approximately 11% of the studied population, whereas it was notably absent in Bali cattle. It has also been noted that Soria *et al.*, (2010) had previously reported this particular haplotype pattern, which was also found in Brangus cattle. The figure illustrating the different banding patterns observed in this study is shown in Figure 3. The CG/AG haplotype in Sumbawa cattle warrants further investigation and could serve as an alternative genetic marker, especially due to its monomorphism at the *CAPN1*-316 locus.

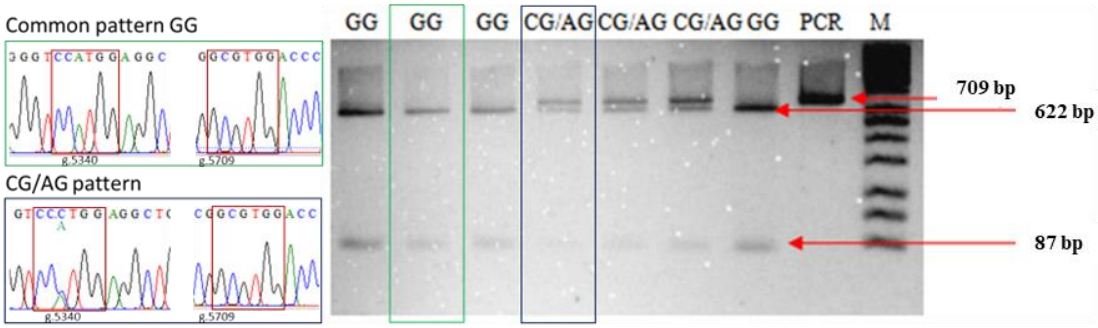

Figure 3. Visualization of two genetic variations from PCR-RFLP: green square, the GG genotype of SNP 316, and dark blue square the CG/AG haplotype resulting from a mutation at SNP *g.5340A>C* in Sumbawa cattle. The presence of a restriction site marked by red squares is identified using *Btg*I (5'- C*CRYGG -3') enzyme restriction. This visualization was achieved using a 3% (w/v) agarose gel. The rows in the figure correspond to different samples: GG (GG genotype), CG/AG (CG/AG haplotype), PCR (PCR product as a control), and M (100 bp DNA ladder).

The presence of the GG genotype in all observed Bali and Sumbawa cattle indicates that only the G allele, which is fixed in both populations. Therefore, there is no genetic variation in the *CAPN1*-316 marker in these two cattle populations, and it is considered monomorphic. Several previous studies showed that the G allele was common both in *B. indicus* and *B. taurus* populations (Table 2).

Several studies have shown that the C allele of the *CAPN1*-316 SNP is considered favorable as it is associated with tenderness. Corva *et al.*, (2007) reported that cattle with the CC genotype had 17% higher meat tenderness scores than cattle with the GG genotype. Similar results were reported by Curi *et al.*, (2010). Although they only found the CG and GG genotypes, they found that cattle with the CG genotype had a shear force value 0.36 kg lower than cattle with the GG genotype. This advantage was also confirmed by Avilés *et al.*, (2013) and Gill *et al.*, (2009) using both mechanical measurements (shear force) and sensory panel tests. This means the C allele carries a higher tenderness trait than the G allele. According to (Page *et al.*, 2002), the nucleotide change from G to C at SNP *CAPN1*-316 is predicted to alter the protein sequence in domain II as a proteolytic domain, thus triggering a change in the function of μ-calpain activity in the post-mortem myofibril degradation process, resulting in tenderer meat. Unfortunately, the C allele was rare in *B. indicus* cattle and was even absent in Sumbawa and Bali cattle in this study. This may be one of the genetic factors causing meat from *B. indicus* cattle to be less tender than that from *B. taurus* cattle (O'Connor *et al.*, 1997; Rodrigues *et al.*, 2017). One alternative approach to improving meat tenderness is to implement crossbreeding or composite cattle production. Consequently, although results may still demonstrate some variability, this method offers a potential solution. Page *et al.*, (2002) found significant improvements in tenderness when crossbreeding *B. taurus* cattle, with the CC allele had better tenderness results than the GG allele. Gill *et al.*, (2009) also reported similar results in crossbred cattle of the Aberdeen Angus, Aberdeen Angus cross, Simmental, and Limousin breeds. They reported that cattle with the CC genotype had better meat tenderness scores, with differences of 2.93 kPa in the tenderometer test and 0.37 units in the sensory panel test. Mazzucco *et al.*, (2010) also reported the superiority of the C allele in producing tenderer meat compared to the G allele in Brangus cattle ($P<0.05$).

Table 2. Genotype and allele frequency of *CAPN1*-316 locus in several cattle breed

Species	Breed	n	Genotype frequency			Allele frequency		References
			%CC (n)	%CG (n)	%GG (n)	C	G	
Bos taurus	Aberdeen Angus	440	5,0 (20)	35,0(152)	61,0 (268)	0,220	0,780	Gill *et al.*, 2009
	Angus	43	11,0 (5)	49.0 (21)	40,0 (17)	0,360	0,640	Li *et al.* 2013
	Hereford	233	0,0 (0)	2,0 (4)	98,0 (229)	0,010	0,990	Iglesias *et al.*, 2011
	Hereford	35	0,0 (0)	6,0 (2)	94,0 (33)	0,029	0,971	Li *et al.* 2013
	Charolais	1.084	0,4 (4)	16,4(178)	83,2 (902)	0,086	0,914	Allais *et al.*, 2011
	Charolais	109	4,0 (4)	21,0 (23)	75,0 (82)	0,142	0,856	Li *et al.* 2013
	Limousin	1.213	7,2 (87)	40,6(492)	52,3 (634)	0,275	0,725	Allais *et al.*, 2011
	Limousin	35	0,0 (0)	31,0 (11)	69,0 (24)	0,157	0,843	Li *et al.* 2013
	Blonde d'Aquitaine	967	0,3 (3)	8,0 (77)	91,7 (887)	0,043	0,957	Allais *et al.*, 2011
	Simmental	21	0,0 (0)	33,0 (7)	67,0 (14)	0,167	0,833	Li *et al.* 2013
	Simmental	81	0,0 (0)	14,8 (12)	85,2 (69)	0,074	0,926	Ardicli *et al.*, 2017
	Holstein	400	6,5 (26)	42,3(169)	51,2 (205)	0,276	0,724	Ardicli *et al.*, 2017
	Holstein-Friesian	296	6,1 (18)	46,3(137)	47,6 (141)	0,290	0,710	Ardicli *et al.*, 2019
	Retinta	89	58,4 (52)	11,3 (10)	30,3 (27)	0,640	0,360	Avilés *et al.*, 2013
Bos indicus	Brahman	470	0,0 (0)	2,6 (12)	97,4 (458)	0,013	0,987	Casas *et al.*, 2005
	Brahman	647	0,0 (0)	4,0 (27)	96,0 (647)	0,020	0,980	Van Eenennaam *et al.*, 2007
	Brahman	91	0,0 (0)	19,0 (17)	81,0 (74)	0,095	0,905	Iglesias *et al.*, 2011
	Nellore	114	0,0 (0)	1,8 (2)	98,2 (112)	0,009	0,991	Curi *et al.*, 2010
	Nellore	638	0,0 (0)	1,6 (10)	98,4 (628)	0,008	0,992	Pinto *et al.*, 2010
	Sumbawa	100	0,0 (0)	0,0 (0)	100,0 (100)	0,000	1,000	**This study**
Crossbreed	AX	174	18,0 (31)	56,0 (98)	25,0 (45)	0,460	0,540	Corva *et al.*, 2007
	HA	35	14,0 (5)	54,0 (19)	31,0 (11)	0,414	0,586	Corva *et al.* 2007
	HX	68	3,0 (2)	49,0 (33)	49,0 (33)	0,272	0,728	Corva *et al.* 2007
	LX	36	8,0 (3)	42,0 (15)	50,0 (18)	0,292	0,708	Corva *et al.* 2007
	Brangus	219	29,0 (63)	51,0(112)	20,0 (44)	0,545	0,455	Van Eenennaam *et al.* 2007
	Brangus	247	7,0 (18)	39,0 (97)	53,0 (132)	0,269	0,731	Mazucco *et al.* 2010
	Angus x Nellore	67	0,0 (0)	25,4 (17)	74,6 (50)	0,126	0,874	Curi *et al.* 2010
	Rubia Gallega x Nellore	44	0,0 (0)	0,0 (0)	100,0 (44)	0,000	1,000	Curi *et al.* 2010
	Chancim	41	0,0 (0)	17,1 (7)	82,9 (34)	0,085	0,915	Curi *et al.* 2010
	Brangus 3-way cross	19	0,0 (0)	42,1 (8)	57,9 (11)	0,210	0,790	Curi *et al.* 2010
	Braunvieh 3-way cross	15	0,0 (0)	13,3 (2)	86,6 (13)	0,067	0,933	Curi *et al.* 2010
	Braford	194	1,0 (3)	42,0 (82)	57,0 (111)	0,220	0,780	Iglesias *et al.* 2011
Bos javanicus	Bali	193	0,0 (0)	0,0 (0)	100,0 (193)	0,000	1,000	**This study**

Description: n = number of samples; AX: ≥ 75% Angus - ≤25% Hereford; AH: 50% Angus - 50% Hereford; HX: ≥75% Hereford - ≤25% Angus; LX: Limousin x Hereford-Angus.

In contrast, White *et al.*, (2005) did not find the *CAPN1*-316 marker informative in Brahman X Hereford crossbred cattle. These results suggest that the use of the *CAPN1*-316 marker is only applicable to crossbred cattle with dominant *B taurus* genetics. Page *et al.*, (2002) suggested using the *CAPN1*-316 SNP as a genetic marker in cattle selection to reduce the number of cattle producing tougher meat. However, this marker was only helpful for *B. taurus* cattle such as Angus and Belmont Red (Kostusiak *et al.*, 2023). Although the C allele is found in some cases, the CC genotype was not found in Hereford, Limousin, and Simmental cattle (Li *et al.*, 2013). In general, the *CAPN1*-316 marker cannot be used in *B. indicus* cattle (Allais *et al.*, 2011; Barendse *et al.*, 2007), because the C allele was found at low frequencies, as seen in

Brahman cattle (0.01-0.03) (Casas *et al.*, 2005; Johnston and Graser, 2010) and Nellore cattle (0.008-0.009) (Curi *et al.*, 2010; Pinto *et al.*, 2010). Although the C allele frequency in Brahman cattle studied by (Iglesias *et al.*, 2011) was higher than in other studies (0.095), the G allele was fixed with a Minor Allele Frequency (MAF) standard of ≥0.05. In this study, Sumbawa cattle (*B. indicus*) showed a similar allele distribution pattern with other *B. indicus* cattle. Therefore, this study supported the previous findings that SNP *CAPN1*-316 in *B. indicus* cattle was not informative and cannot be used for association analysis. This study also provides new information on Bali cattle (*B. javanicus*), which have the same allele distribution pattern as *B. indicus* cattle.

While many studies have demonstrated the superiority of the C allele for beef tenderness in cattle, using the *CAPN1*-316 marker in cattle breeding must consider the desired breeding objectives. Some studies have shown conflicting effects on different traits between the G and C alleles. Ardicli *et al.*, (2017) mentioned that the G allele had a favored effect ($p<0.001$) than the C allele on live weight, carcass weight, and longissimus et lumborum area in Friesian-Holstein cattle. Miquel *et al.*, (2009) reported that Angus and Brangus cattle with the CC genotype had tenderer meat but lower body weight and final weight gain than cattle with the GG genotype. Pintos and Corva, (2011) reported that in Angus-Argentine cattle, cattle with the CC genotype had a lower average birth weight (0.227 kg) and calf weight (1.767 kg) than cattle with the GG genotype. This may not always be due to the *CAPN1*-316 marker alone but may involve two or more other genes contributing to variations in specific traits (Pintos and Corva, 2011).

CONCLUSION

In this study, the *CAPN1*-316 gene was monomorphic or lacking diversity in Bali and Sumbawa cattle populations. The G allele and GG genotype were the only ones detected in this study. Consequently, the study results suggest that using the *CAPN1*-316 genetic marker for selection purposes is not feasible in the Bali and Sumbawa cattle populations due to the fixed presence of the G allele. Therefore, it is unsuitable for further association studies within these cattle breeds. In addition, the CG/AG haplotype found in Sumbawa cattle deserves further study and consideration as a potential alternative genetic marker due to its monomorphic at the *CAPN1*-316 locus.

REFERENCES

Allais, S., Journaux, L., Levéziel, H., Payet-Duprat, N., Raynaud, P., Hocquette, J.-F., Renand, G. (2011). Effects of polymorphisms in the calpastatin and μ-calpain genes on meat tenderness in 3 French beef breeds. *Journal of Animal Science*, *89*(1), 1–11. https://doi.org/10.2527/jas.2010-3063

Ardicli, S., Dincel, D., Samli, H., & Balci, F. (2017). Effects of polymorphisms at *LEP*, *CAST*, *CAPN1*, *GHR*, *FABP4* and *DGAT1* genes on fattening performance and carcass traits in Simmental bulls. *Archives Animal Breeding*, *60*(2), 61–70. https://doi.org/10.5194/aab-60-61-2017

Ardicli, S., Samli, H., Dincel, D., Soyudal, B., & Balci, F. (2017). Individual and combined effects of *CAPN1*, *CAST*, *LEP* and *GHR* gene polymorphisms on carcass characteristics and meat quality in Holstein bulls. *Archives Animal Breeding*, *60*(3), 303–313. https://doi.org/10.5194/aab-60-303-2017

Ardicli, S., Samli, H., Vatansever, B., Soyudal, B., Dincel, D., & Balci, F. (2019). Comprehensive assessment of candidate genes associated with fattening performance in Holstein–Friesian bulls. *Archives Animal Breeding*, *62*(1), 9–32. https://doi.org/10.5194/aab-62-9-2019

Avilés, C., Juárez, M., Peña, F., Domenech, V., Clemente, I., & Molina, A. (2013). Association of single nucleotide

polymorphisms in CAPN1 and CAST genes with beef tenderness from Spanish commercial feedlots. *Czech Journal of Animal Science*, *58*(10), 479–487. https://doi.org/10.17221/6997-CJAS

Barendse, W., Harrison, B. E., Rachel Hawken, Hawken, R. J., Ferguson, D., D. M. Ferguson, Bunch, R. J. (2007). Epistasis Between Calpain 1 and Its Inhibitor Calpastatin Within Breeds of Cattle. *Genetics*, *176*(4), 2601–2610. https://doi.org/10.1534/genetics.107.074328

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E.-D. A. (2018). Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness*, *7*(3), 196–204. https://doi.org/10.1016/j.fshw.2018.08.002

Bonilla, C. A., Rubio, M. S., Sifuentes, A. M., Parra-Bracamonte, G. M., Arellano, V. W., Méndez, M. R. D., Ortiz, R. (2010). Association of CAPN1 316, CAPN1 4751 and TG5 markers with bovine meat quality traits in Mexico. *Genetics and Molecular Research*, *9*(4), 2395–2405. https://doi.org/10.4238/vol9-4gmr959

Bressan, M. C., Rodrigues, E. C., Rossato, L. V., Ramos, E. M., & Gama, L. T. D. (2011). Physicochemical properties of meat from Bos taurus and Bos indicus. *Revista Brasileira de Zootecnia*, *40*(6), 1250–1259. https://doi.org/10.1590/S1516-35982011000600013

Casas, E., White, S. N., Riley, D. G., Smith, T. P. L., Brenneman, R. A., Olson, T. A., Chase, C. C. (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. *Journal of Animal Science*, *83*(1), 13–19. https://doi.org/10.2527/2005.83113x

Corva, P. M., Soria, L. A., Schor, A., Villarreal, E. L., Cenci, M. P., Motter, M. M., Naón, J. J. G. (2007). Association of CAPN1 and CAST gene polymorphisms with meat tenderness in Bos taurus beef cattle from Argentina. *Genetics and Molecular Biology*, *30*(4), 1064–1069. https://doi.org/10.1590/s1415-47572007000600006

Curi, R. A., Chardulo, L. A. L., Giusti, J., Silveira, A. C., Martins, C. L., & de Oliveira, H. N. (2010). Assessment of GH1, CAPN1 and CAST polymorphisms as markers of carcass and meat traits in Bos indicus and Bos taurus–Bos indicus cross beef cattle. *Meat Science*, *86*(4), 915–920. https://doi.org/10.1016/j.meatsci.2010.07.016

Gill, J., J. L. Gill, Bishop, S., Caroline McCorquodale, McCorquodale, C., Williams, J. L., & Wiener, P. (2009). Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*, *41*(1), 36–36. https://doi.org/10.1186/1297-9686-41-36

Hall, T. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*, *41*, 95–98.

Hou, G., Huang, M., Gao, X., Li, J., Gao, H., Ren, H., & Xu, S. (2011). Association of Calpain 1 (CAPN1) and HRSP12 allelic variants in beef cattle with carcass traits. *African Journal of Biotechnology*, *10*(63), 13714–13718. https://doi.org/10.5897/AJB11.338

Iglesias, P. P., Caffaro, M. E., Caffaro, M. E., Amadio, A. F., Mañotti, A. A., & Poli, M. A. (2011). CAPN1 markers in three Argentinean cattle breeds: Report of a new InDel polymorphism within intron 17. *Molecular Biology Reports*, *38*(3), 1645–1649. https://doi.org/10.1007/s11033-010-0275-z

Johnston, D. J., & Graser, H.-U. (2010). Estimated gene frequencies of

GeneSTAR markers and their size of effects on meat tenderness, marbling, and feed efficiency in temperate and tropical beef cattle breeds across a range of production systems1. *Journal of Animal Science*, *88*(6), 1917–1935. https://doi.org/10.2527/jas.2009-2305

Kostusiak, P., Slósarz, J., Gołębiewski, M., Grodkowski, G., & Puppel, K. (2023). Polymorphism of genes and their impact on beef quality. *Current Issues in Molecular Biology*, *45*(6), 4749–4762. https://doi.org/10.3390/cimb45060302

Li, X., Ekerljung, M., Lundström, K., & Lundén, A. (2013). Association of polymorphisms at DGAT1, leptin, SCD1, CAPN1 and CAST genes with color, marbling and water holding capacity in meat from beef cattle populations in Sweden. *Meat Science*, *94*(2), 153–158. https://doi.org/10.1016/j.meatsci.2013.01.010

Maltin, C., Balcerzak, D., Tilley, R., & Delday, M. (2003). Determinants of meat quality: Tenderness. *Proceedings of the Nutrition Society*, *62*(2), 337–347. https://doi.org/10.1079/PNS2003248

Mazzucco, J. P., Melucci, L. M., Villarreal, E. L., Mezzadra, C. A., Soria, L., Corva, P., Miquel, M. C. (2010). Effect of ageing and μ-calpain markers on meat quality from Brangus steers finished on pasture. *Meat Science*, *86*(3), 878–882. https://doi.org/10.1016/j.meatsci.2010.07.015

Miller, M. F., Huffman, K. L., Gilbert, S. Y., Hamman, L. L., & Ramsey, C. B. (1995). Retail consumer acceptance of beef tenderized with calcium chloride. *Journal of Animal Science*, *73*(8), 2308–2314. https://doi.org/10.2527/1995.7382308x

Miquel, M. C., Villarreal, E., Mezzadra, C., Melucci, L., Soria, L., Corva, P., & Schor, A. (2009). The association of CAPN1 316 marker genotypes with growth and meat quality traits of steers finished on pasture. *Genetics and Molecular Biology*, *32*(3), 491–496. https://doi.org/10.1590/S1415-47572009000300011

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Nowak, D. (2011). Enzymes in Tenderization of Meat – The System of Calpains and Other Systems. *Polish Journal of Food and Nutrition Sciences*, *61*(4), 231–237. https://doi.org/10.2478/v10222-011-0025-5

O'Connor, S. F., Tatum, J. D., Wulf, D. M., Green, R. D., & Smith, G. C. (1997). Genetic effects on beef tenderness in Bos indicus composite and Bos taurus cattle. *Journal of Animal Science*, *75*(7), 1822. https://doi.org/10.2527/1997.7571822x

Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., Smith, T. P. L. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle1,2. *Journal of Animal Science*, *80*(12), 3077–3085. https://doi.org/10.2527/2002.80123077x

Page, B. T., Casas, E., Quaas, R. L., Thallman, R. M., Wheeler, T. L., Shackelford, S. D., Smith, T. P. L. (2004). Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires1,2. *Journal of Animal Science*, *82*(12), 3474–3481. https://doi.org/10.2527/2004.82123474x

Pethick, D., Ball, A., Banks, R., & Hocquette, J.-F. (2011). Current and future issues facing red meat quality in a competitive market and how to manage continuous improvement. *Animal Production Science*, *51*, 13–18. https://doi.org/10.1071/AN10041

Pinto, L. F. B., José Bento Sterman Ferraz, Ferraz, J. B. S., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Silva, R. C. G. (2010). Association of SNPs on CAPN1 and CAST genes with

tenderness in Nellore cattle. *Genetics and Molecular Research*, *9*(3), 1431–1442. https://doi.org/10.4238/vol9-3gmr881

Pintos, D., & Corva, P. M. (2011). Association between molecular markers for beef tenderness and growth traits in Argentinian angus cattle. *Animal Genetics*, *42*(3), 329–332. https://doi.org/10.1111/j.1365-2052.2010.02160.x

Rodrigues, R. T. de S., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., Martins, T. da S. (2017). Differences in Beef Quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) Cattle through a Proteomic and Phosphoproteomic Approach. *PLOS ONE*, *12*(1), e0170294. https://doi.org/10.1371/journal.pone.0170294

Smith, T., Smith, T. P. L., T. P. L. Smith, Smith, T. P. L., Casas, E., Rexroad, C. E., Keele, J. W. (2000). Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. *Journal of Animal Science*, *78*(10), 2589–2594. https://doi.org/10.2527/2000.78102589x

Soria, L. A., Corva, P. M., Huguet, M. J., M. J Huguet, Miño, S., & Miquel, M. C. (2010). Bovine μ-calpain (CAPN1) gene polymorphisms in Brangus and Brahman bulls. *BAG. Journal of Basic and Applied Genetics*, *21*(1), 61–69.

Van Eenennaam, A. L., Li, J., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., Thomas, M. G. (2007). Validation of commercial DNA tests for quantitative beef quality traits1,2. *Journal of Animal Science*, *85*(4), 891–900. https://doi.org/10.2527/jas.2006-512

White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., Smith, T. P. L. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent1. *Journal of Animal Science*, *83*(9), 2001–2008. https://doi.org/10.2527/2005.8392001x