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Research article

Association of polymorphisms in CAPN1 and CAST genes with the meat tenderness of Creole cattle

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ABSTRACT: Single nucleotide polymorphisms are variations of a single nucleotide base pair and can be associated to phenotypic characteristics. This study aimed to determine the association of CAPN1 and CAST gene polymorphisms with the tenderness of Creole cattle meat from the Amazonas region, Peru. The texture profile (adhesiveness, cohesiveness, Warner-Bratzler shear force, elasticity, gumminess, and chewiness) of 100 animals was determined in 100 g of *Longissimus dorsi* et *lumborum muscle*. Allelic frequencies, genotypic frequencies, and Hardy-Weinberg equilibrium (HWE) of calpain (CAPN-316, CAPN-530) and calpastatin (CAST-2959) gene polymorphisms were studied. Allelic and genotypic frequencies were calculated, as well as the Hardy-Weinberg equilibrium with the Chi-square test. The texture profile of each group of samples corresponding to a polymorphism was compared with the Duncan's test and the t-test for independent samples ($p < 0.05$). Genotypic frequencies were 78 % GG and 22 % CC for CAPN-316; 68 % GG, 5 % GA, and 27 % AA for CAPN-530; and 74 % AA, 18 % AG, and 8 % GG for CAST-2959. The CAPN-316, CAPN-530, and CAST-2959 polymorphisms were not in Hardy-Weinberg equilibrium. The CC genotype of CAPN-316 marker influences meat tenderness on day 21 of meat aging. In contrast, the GG genotype of CAST-2959 marker affects meat tenderness at days 14 and 21 of meat aging concerning the other genotypes.

Keywords: *Bos taurus*, SNPs, CAPN-316 and CAST-2959, Warner-Bratzler, meat aging

Introduction

Creole cattle accounts for more than 60 % of the bovine population in Peru, according to the last Peruvian National Agricultural Census report (INEI, 2012), widely distributed in the national territory, providing protein to ensure food security. Creole cattle have survival advantages because the species is adapted to the agro-climatic diversity from Peru (Arbizu et al., 2022; Estrada et al., 2022). Moreover, the animals are resistant to diseases, longevous, and capable of surviving with low nutritional requirements (Arbizu et al., 2022; Encina et al., 2021).

Gene expression associated to meat tenderness is detectable in the postmortem stage and calpastatin (CAST) (Ajayi et al., 2018; Dang et al., 2020). These proteins are a superfamily of neutral proteases that regulate a wide range of calcium-dependent processes (Croall and Demartino, 1991; Campbell and Davies, 2012). Calpain is an enzyme encoded by the CAPN1 gene and is responsible for meat aging (Chung and Davis, 2012), whereas the CAST gene encodes calpastatin and inhibits the proteolytic activity of calpains (Cong et al., 1998; Raynaud et al., 2005; Barendse et al., 2007). Single nucleotide polymorphisms (SNPs), such as CAPN-316 and CAPN-530, have been associated to meat tenderness (Corva et al., 2007; Motter et al., 2009; Allais et al., 2011). The CAPN1 gene is located on chromosome 29 of the bovine genome, formed by 22 exons and 21 introns, with a length of 2255 bp (Page et al., 2002; Juszczuk-Kubiak et al., 2004), whereas the CAST gene is located on chromosome 7 of the bovine genome, formed by 35 exons with a length of 4551 bp (Raynaud et al., 2005; Cong et al., 1998).

Tenderness is a complex attribute of meat quality, which the shear force can instrumentally determine, classifying meat as tender or tougher (Li et al., 2013). Meat tenderness depends on intrinsic factors, such as breed, sex, age, and genotype (Chriki et al., 2013), as well as on extrinsic factors, such as feeding, slaughter type and operations, postmortem treatment, among others (Malheiros et al., 2019; Tesson et al., 2020). Applying traditional genetic selection to improve certain meat traits is difficult because only postmortem phenotypic data is obtained (Reardon et al., 2010). However, selection for meat tenderness based on molecular markers associated to meat quality traits can be an effective alternative to traditional selection (Enriquez-Valencia et al., 2017). Thus, this research aimed to determine the association of CAPN1 and CAST gene polymorphisms to meat tenderness of Creole cattle from the Amazonas region, Perú.

Materials and Methods

Ethics statement

The research was carried out under the guidelines of the Resolution of the University Council No.

647-2019-UNTRM/CU regarding experimental animal studies and under the guidelines of the Animal Protection and Welfare Law - No. 30407 of the Peruvian government.

Animals

The experiment was conducted from Oct 2020 to Feb 2021 and 100 Creole animals (58 males and 42 females) were used. All animals were obtained from stake-rearing systems and were grazed on meadows with forage species: *Trifolium repens* L., *Taraxacum officinale* Weber ex F.H.Wigg., *Lolium multiflorum* L, *Dactylis glomerata* L, *Pennisetum clandestinum* Hochst. ex Chiov., *Setaria sphacelata* (Schumach.) Stapf and Hubbard ex Moss, *Trifolium pratense* L, *Brachiaria brizantha* Stapf, *Rumex obtusifolius* L, *Paspalum penicillatum* Hook f, *Sporobolus indicus* (L.) R. Br., *Philoglossa mimuloides* (Hieron.) H. Rob. & Cuatrec., and *Paspalum bonplandianum* Flugge. The animals were slaughtered at 4.6 ± 1.4 years of age, with an average live weight of 357.9 ± 28.05 kg in Chachapoyas, Amazonas region, Peru (6°13′33″ S, 77°52′07″ W, 2373 m altitude). One hundred grams of fillets of the *Longissimus dorsi* et *lumborum* (LDL) muscle were sampled between ribs five and seven of the carcasses.

Meat texture profile

The samples were divided into four equal parts to determine meat tenderness, vacuum packed, and aged at 2 °C for 0, 7, 14, and 21 days. After this period, the meat was cooked in a water bath (RAYPA, BOD-12) at 71 °C for 50 min (Chung et al., 2014; Corva et al., 2007) and then stored for 12 h at 2 °C in a refrigerator (Bosch, KAN58A40J). Each meat sample was subdivided into five equal parts, cylindrical in shape, making a longitudinal cut in the muscle fiber direction (White et al., 2005). Each piece was subjected to the shear force measurement by the Warner-Bratzler method and values of adhesiveness, cohesiveness, gumminess, and chewiness were calculated based on the force/distance data collected during the comprehension test of both cycles according to Botinestean et al. (2016) in a TexturePro CT V1.8 Build 31 texture meter (Brookfield Engineering Labs. Inc.). The method consisted of measuring the force required to pass a blunt blade perpendicular to the muscle fibers of the cooked meat sample (White et al., 2005), applying a cutting speed of 20 cm min^{-1} and a capacity of 20 kg (Castro et al., 2016; Malheiros et al., 2018; Riley et al., 2003). Finally, values of meat tenderness were represented by the arithmetic mean obtained from the five sub-samples.

Sequencing, allelic, and genotypic frequencies

DNA was extracted from LDL muscle tissue to determine genotypes using the QIAamp DNA Mini Kit-50 (QIAGEN), according to the manufacturer's instructions. DNA amplification was performed by conventional PCR in a thermocycler (Applied Biosystems SimpliAmp, A24812, Thermo Fisher Scientific) with the OneTaq® DNA Polymerase commercial kit (New England Biolab, Inc.), according to the manufacturer's recommendations. Single nucleotide polymorphisms (SNPs) of the CAPN1 gene (CAPN-316 and CAPN-530) and CAST-2959 of the Calpastatin gene were amplified. The markers were commercially synthesized (Macrogen) and the details are described in Table 1. Sequencing was performed using the commercial BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystem) on the Genetic Analyzer 3500 sequencer (Applied Biosystems).

Statistical analysis

Allelic and genotypic frequencies were calculated, as well as the Hardy-Weinberg equilibrium with the Chi-square test. For that, the equation $p^2 + 2pq + q^2$ was used, where p and q are the allele frequencies and p^2, q^2 and 2pq are the genotype frequencies of the homozygotes and heterozygotes, respectively.

Software Geneious Prime® 2022.0., Build 2021-11-16 10:57, Java Version 11.0.12+7 (64 bit) was used to identify SNPs, applying parameters, such as minimum coverage of ten, minimum Phred score quality of 20, minimum variant frequency of 5 %, and exact test.

The phenotypic variables (adhesiveness, cohesiveness, Warner-Bratzler shear force, elasticity, gumminess, and chewiness) were subjected to the analysis of variance ($p < 0.05$). The multiple comparisons of the means of genotypes were carried out with the Duncan test (more than three groups, CAPN-530 and CAST-2959) and the *t*-test (two groups, CAPN-316) ($p < 0.05$). All statistical analyses were performed in the

Table 1 – CAPN1 and CAST primers used for DNA amplification of Creole meat.

Marker	Sequence	Fragment size (bp)[1]	Specie	Reference
CAPN-316	F5'-GGGCCAGATGGTGAACCTGA-3' R5'-TTGCGGAACCTCTGGCTCTT-3'	669	*B. taurus* *B. indicus*	Page et al. (2002)
CAPN-530	F5'-GAGCCCAACAAGGAAGGT-3' R5'-AATACAGCCCAATGATGAGG-3'	497	*B. taurus* *B. indicus*	Page et al. (2002)
CAST-2959	F5'-CATTTGGAAAACGATGCCTCAC-3' R5'-CTACGATTAGCAGCTCAAGAGGAG-3'	133	*B. indicus* *B. taurus*	Barendse (2002)

[1]Base pairs.

statistical package SPSS v. 26. The model applied was Yij = μ + Pi + Sj + eij; where: Yij = Phenotypic variable, μ = Population mean, Pi = Effect of the i-th genotype of the marker, Sj = Effect of j-th maturation time, Eij = Random error.

Results

Allele frequencies

In the sequenced 628 bp of the CAPN1 gene, 18 polymorphic sites were found (Table 2). In this CAPN-316 SNP, the genotype with the highest frequency was GG (78 %) compared to the CC genotype (22 %) in a homozygous condition and no heterozygous genotypes were found. According to the 482 bp analysis of the CAPN1 gene, the SNP CAPN-530 had a genotypic frequency of 68 %, 5 %, and 27 % for GG, GA, and AA, respectively. In addition, allele A in the SNP CAPN-530 is favorable for meat production with more significant meat tenderness (Table 2). In SNP CAPN-530, allele A is associated to meat tenderness and we found that the Creole meat studied had 30 % allele A in this study. The CAST-2959 genotypic frequencies found in Creole cattle from Amazonas were 74 %, 18 %, and 8 % for AA, AG, and GG, respectively (Table 2). Allele A and G frequency were 83 % and 17 %, respectively.

Hardy-Weinberg equilibrium (HWE)

In the CAPN1 gene studied in the Amazonas Creole cattle, 17 regions were not in HWE, including the CAPN-316 marker (previously reported SNP), and two were in equilibrium (Table 2). In addition, eight polymorphic

Table 2 – Single nucleotide polymorphisms, allelic frequencies, and HWE in Creole cattle.

Name	Reference Sequence	Variant Nucleotide	SP	EP	Coverage	Polymorphism Type	Variant Frequency	Change	Variant *p*-value	HWE
		s					%			
CAPN-316										
CAPN.316.5901	C	A	5901	5901	100	transversion	99.00	C -> A	9.90E-197	ns
CAPN.316.5823	T	C	5823	5823	100	transition	99.00	T -> C	9.90E-197	ns
CAPN.316.5788	C	T	5788	5788	100	transition	18.00	C -> T	1.40E-17	*
CAPN.316.5764	C	T	5764	5764	100	transition	5.00	C -> T	0.0034	*
CAPN.316.5709[a]	C	G	5709	5709	100	transversion	78.00	C -> G	5.90E-135	*
CAPN.316.5688	A	G	5688	5688	100	transition	70.00	A -> G	2.20E-115	*
CAPN.316.5680	G	C	5680	5680	100	transversion	71.00	G -> C	9.30E-118	*
CAPN.316.5537	C	T	5537	5537	100	transition	23.00	C -> T	1.20E-24	*
CAPN.316.5534	C	G	5534	5534	100	transversion	13.00	C -> G	3.20E-11	*
CAPN.316.5529	C	T	5529	5529	100	transition	10.00	C -> T	7.60E-08	*
CAPN.316.5463	C	T	5463	5463	100	transition	20.00	C -> T	2.50E-20	*
CAPN.316.5458	T	C	5458	5458	100	transition	75.00	T -> C	1.90E-127	*
CAPN.316.5447	C	T	5447	5447	100	transition	8.00	C -> T	0.0000082	*
CAPN.316.5404	C	T	5404	5404	100	transition	20.00	C -> T	2.50E-20	*
CAPN.316.5402	C	T	5402	5402	100	transition	17.00	C -> T	3.00E-16	*
CAPN.316.5392	C	T	5392	5392	100	transition	19.00	C -> T	6.10E-19	*
CAPN.316.5383	C	A	5383	5383	100	transversion	10.00	C -> A	7.60E-08	*
CAPN.316.5372	C	T	5372	5372	100	transition	18.00	C -> T	1.40E-17	**
CAPN.316.5257	G	C	5257	5257	80	transversion	7.50	G -> C	0.00016	*
CAPN-530										
CAPN.530.4877	G	A	4877	4877	11	transition	9.10	G -> A	0.10000	ns
CAPN.530.4876	A	C	4876	4876	12	transversion	8.30	A -> C	0.11000	ns
CAPN.530.4874	T	C	4874	4874	97	transition	17.50	T -> C	1.80E-16	*
CAPN.530.4685	C	T	4685	4685	100	transition	19.00	C -> T	6.10E-19	**
CAPN.530.4627	G	A	4627	4627	100	transition	8.00	G -> A	0.0000082	**
CAPN.530.4558[a]	G	A	4558	4558	100	transition	29.50	G -> A	2.00E-31	**
CAPN.530.4506	C	G	4506	4506	100	transversion	27.00	C -> G	9.50E-31	**
CAPN.530.4380	G	A	4380	4380	79	transition	5.10	G -> A	0.17000	ns
CAST-2959										
CAST.2959.3021	T	C	3021	3021	14	transition	50.00	T -> C	3.20E-11	*
CAST.2959.2987	T	C	2987	2987	100	transition	38.00	T -> C	3.10E-49	**
CAST.2959.2959[a]	A	G	2959	2959	100	transition	17.00	A -> G	0.0000018	**
CAST.2959.2894	G	T	2894	2894	100	transversion	99.00	G -> T	9.90E-197	ns

[a]Previously reported; SP = Start position; EP = End position; HWE = Hardy-Weinberg Equilibrium; *Chi-square tabulated at 95 % confidence level and 5 % significance ($p < 0.05$); **Chi2 tabulated at 99 % confidence level and 1 % significance ($p < 0.01$). ns = not significant.

regions of the CAPN1 gene were found, three of which were in HWE ($p > 0.05$), and five were not in HWE ($p < 0.01$). Four SNPs were identified in the sequenced region of the CAST gene, including the previously reported CAST-2959 (Table 2). The SNP CAST-2959 was not in HWE ($p < 0.01$). Likewise, significant deviations from the theoretical Hardy-Weinberg ratios were observed in two other positions ($p < 0.01$).

Texture profile

The texture profile did not vary at day 0 (adhesiveness, $p > 0.05$; cohesiveness, $p > 0.05$; Warner-Bratzler shear force, $p > 0.05$; elasticity, $p > 0.05$; gumminess, $p > 0.05$; chewiness, $p > 0.05$ and at day seven (adhesiveness, $p > 0.05$; cohesiveness, $p > 0.05$; Warner-Bratzler shear force, $p > 0.05$; elasticity, $p > 0.05$; gumminess, $p > 0.05$; chewiness, $p > 0.05$) for the CAPN-316 polymorphism genotypes. However, the genotypes were significant for adhesiveness ($p < 0.01$) on day 14 and Warner-Bratzler shear force ($p < 0.05$) on day 21 of aging (Table 3).

Adhesiveness was not affected by CAPN-530 marker genotypes (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$), cohesiveness (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$), Warner-Bratzler shear force (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$), elasticity (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$), gumminess (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$), and chewiness (day 0, $p > 0.05$; day 7, $p > 0.05$; day 14, $p > 0.05$; day 21, $p > 0.05$) (Table 3).

The texture profile analysis according to the CAST-2959 marker genotypes showed significance for the Warner-Bratzler shear force at 14 ($p < 0.05$) and 21 ($p < 0.05$) days of aging (Table 3). Meat with GG genotype presented higher shear force than AA or AG genotypes.

Discussion

Allele frequencies

We found 18 polymorphisms in the sequence region of the CAPN1 gene. The SNP CAPN-316 reported previously (Page et al., 2002) is located at nucleotide base 5709. In CAPN-316 SNP, we found 78 % for GG genotype and 22 % for CC. These findings, in terms of proportions, are higher than the frequencies reported by Trujano-Chavez et al. (2021). The authors evaluated CAPN1 markers in meat in Mexican Braunvieh cattle and reported a frequency of 50 % for GG genotype and 50 % for CC genotype. Similarly, Rincon et al. (2015) reported a higher frequency for the GG genotype (74 %) compared to CC (3 %) and CG (23 %) in Mexican Charolais cattle. Moreover, 88 % were found for the GG genotype, 12 % for heterozygotes, and 0 % for CC in Creole Limonero cattle (Torres-Rodríguez et al., 2015). On the other hand, although the frequency of CC genotype (allele associated

Table 3 – Meat texture profile by aging time, according to CAPN1 and CAST genotypes of Creole cattle[1].

Aging time	Genotype	CAPN-316			CAPN-530				CAST-2959			
		GG	CC	*p*-value	GG	GA	AA	*p*-value	AA	AG	GG	*p*-value
	N	78	22		68	5	27		74	18	8	
0 days	Adhesiveness (mJ)	141.4 ± 13.8	178.8 ± 28.9	0.218	144.3 ± 13.8	250.1 ± 115.8	144.5 ± 22.7	0.257	160.8 ± 16.1	117.5 ± 16.4	119.0 ± 26.2	0.45
	Cohesiveness	0.97 ± 0.03	0.89 ± 0.06	0.182	0.96 ± 0.03	1.14 ± 0.02	0.89 ± 0.04	0.081	0.95 ± 0.03	1.03 ± 0.07	0.82 ± 0.92	0.115
	WBSF (N)	100.7 ± 4.6	97.4 ± 9.3	0.742	98.8 ± 5.1	78.3 ± 5.5	106.9 ± 7.9	0.421	96.8 ± 4.8	109.1 ± 8.5	109.2 ± 17.6	0.534
	Elasticity (mm)	24.6 ± 0.4	25.6 ± 0.7	0.21	24.8 ± 0.4	25.5 ± 1.8	24.9 ± 0.6	0.943	25.1 ± 0.4	24.4 ± 0.9	23.5 ± 1.2	0.412
	Gumminess (N)	89.2 ± 4.3	104.2 ± 9.8	0.123	92.9 ± 5.2	85.9 ± 6.7	92.8 ± 6.6	0.942	92.0 ± 4.9	96.9 ± 8.7	88.0 ± 12.6	0.864
	Chewiness (mJ)	2237.3 ± 116.9	2665.9 ± 242.6	0.096	2342.6 ± 141.0	2230.7 ± 274.2	2322.4 ± 169.9	0.972	2340.4 ± 131.6	2411.0 ± 228.2	2071.2 ± 198.8	0.754
7 days	Adhesiveness (mJ)	217.7 ± 22.9	230.6 ± 51.4	0.801	241.8 ± 27.3	203.9 ± 103.0	170.2 ± 30.4	0.339	233.0 ± 25.8	195.6 ± 44.5	161.6 ± 43.3	0.59
	Cohesiveness	1.3 ± 0.1	1.1 ± 0.03	0.288	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	0.838	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.03	0.807
	WBSF (N)	96.5 ± 4.0	88.7 ± 5.3	0.343	93.4 ± 3.6	77.7 ± 13.9	101.3 ± 8.0	0.431	90.6 ± 3.8	104.0 ± 9.3	112.9 ± 8.5	0.129
	Elasticity (mm)	25.8 ± 0.6	25.3 ± 1.2	0.728	26.0 ± 0.6	26.4 ± 4.2	24.6 ± 1.2	0.491	26.1 ± 0.6	23.8 ± 1.4	26.1 ± 0.6	0.266
	Gumminess (N)	94.2 ± 3.4	86.1 ± 6.5	0.322	93.5 ± 4.1	80.2 ± 12.5	92.0 ± 6.8	0.723	91.1 ± 3.6	89.4 ± 10.2	111.3 ± 11.7	0.265
	Chewiness (mJ)	2496.6 ± 128.4	2300.8 ± 268.4	0.487	2501.6 ± 140.4	2289.6 ± 633.3	2363.0 ± 222.1	0.803	2438.4 ± 128.6	2299.4 ± 336.5	2940.4 ± 346.8	0.415
14 days	Adhesiveness (mJ)	145.0 ± 16.2 b	265.8 ± 50.4 a	0.003**	191.4 ± 22.6	223.7 ± 86.2	111.8 ± 23.2	0.128	179.5 ± 21.2	172.4 ± 40.6	96.5 ± 15.2	0.536
	Cohesiveness	1.1 ± 0.03	1.1 ± 0.06	0.457	1.1 ± 0.03	1.0 ± 0.1	1.1 ± 0.1	0.805	1.1 ± 0.02	1.0 ± 0.1	1.0 ± 0.1	0.086
	WBSF (N)	74.1 ± 3.1	66.5 ± 5.8	0.252	71.1 ± 3.2	73.6 ± 12.2	75.3 ± 5.9	0.735	68.4 ± 2.7b	77.0 ± 8.2b	86.9 ± 2.7a	0.029*
	Elasticity (mm)	25.0 ± 0.6	23.3 ± 1.6	0.235	24.8 ± 0.7	24.7 ± 3.7	24.4 ± 1.1	0.96	24.8 ± 0.7	23.5 ± 1.0	25.7 ± 1.9	0.619
	Gumminess (N)	73.7 ± 2.9	66.0 ± 6.1	0.228	70.2 ± 3.3	72.2 ± 8.9	76.4 ± 4.8	0.598	70.4 ± 2.7	78.0 ± 8.5	73.5 ± 10.6	0.552
	Chewiness (mJ)	1863.6 ± 91.5	1624.4 ± 238.0	0.265	1810.2 ± 110.0	1901.5 ± 477.9	1796.2 ± 158.6	0.956	1792.6 ± 99.3	1807.6 ± 236.1	1989.1 ± 349.5	0.829
21 days	Adhesiveness (mJ)	134.2 ± 15.5	188.3 ± 39.6	0.214	156.2 ± 19.3	135.4 ± 60.6	122.5 ± 24.9	0.659	146.5 ± 17.3	177.2 ± 41.9	72.0 ± 5.4	0.283
	Cohesiveness	1.2 ± 0.07	1.3 ± 0.09	0.728	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.665	1.3 ± 0.05	1.2 ± 0.3	1.3 ± 0.1	0.846
	WBSF (N)	51.0 ± 2.1a	43.1 ± 3.2b	0.045*	50.3 ± 2.4	49.4 ± 8.4	46.8 ± 2.6	0.653	46.9 ± 1.9b	48.8 ± 2.8b	59.4 ± 5.7a	0.030*
	Elasticity (mm)	24.0 ± 0.7	25.1 ± 1.5	0.556	24.3 ± 0.8	23.0 ± 3.1	24.3 ± 1.1	0.844	24.6 ± 0.8	22.3 ± 1.4	25.9 ± 0.5	0.28
	Gumminess (N)	52.7 ± 2.0	48.6 ± 3.3	0.304	51.1 ± 2.0	56.4 ± 8.4	52.5 ± 3.4	0.76	51.2 ± 1.8	49.3 ± 5.2	62.2 ± 6.3	0.185
	Chewiness (mJ)	1334.4 ± 60.8	1261.2 ± 134.9	0.624	1296.5 ± 66.0	1405.1 ± 347.6	1357.0 ± 108.9	0.824	1285.1 ± 64.9	1321.0 ± 134.5	1619.2 ± 171.5	0.277

[1]The values presented are mean ± standard error of the mean; [a,b]Different letters in each row and for each marker indicate differences of effects; *Significant at $p < 0.05$; **Highly significant at $p < 0.01$.

to meat tenderness) was lower compared to the GG genotype, the frequency of genotype associated to meat tenderness was higher than in meat-producing cattle breeds, such as the Hereford, Limousin, or Simmental. In these breeds, the CC genotype was not found (Li et al., 2013), and even in Angus cattle, a frequency of 11 % was reported for the CC genotype (Pinto et al., 2010). However, in Creole cattle from Peru, subsequent studies should be reflected in the analysis of certain regions of the genome, such as Quantitative Trait Locus or QTL. The identification of QTLs is a viable way to identify genes of interest, in this case, meat quality traits that are controlled by one or several genes that act at the same time to express certain quantitative traits (Bruscadin et al., 2022; Muniz et al., 2022).

In SNP CAPN-530, allele A is associated to meat tenderness. In Mexican Braunvieh cattle, the loci of CAPN1 genotypic traits were 25 %, 50 %, and 25 % for the GG, AG, and AA genotypes, respectively (Trujano-Chavez et al., 2021). Furthermore, the results reported are inconsistent with the results found by Leal-Gutiérrez et al. (2015), except for the GG genotype. The authors found that F1 populations from White Brahman females with Limousin, Normando, Braunvieh, Simental, BON, Romosinuano (*B. taurus*), Guzerat, Red Brahman, and White Brahman (*B. indicus*) males had an SNP CAPN-530 genotypic frequency of 60 %, 40 %, and 0 % for GG, GA, and AA, respectively. In our study, a frequency of 30 % was found for allele A in Creole cattle. Allele A frequency is higher than 20 % in F1 cattle (crossbreeding white Brahman female with Limousin, Normando, Braunvieh, Simental, BON, Romosinuano, Guzerat, Red Brahman, and White Brahman males) (Leal-Gutiérrez et al., 2015). Our results for the presence of nucleotide A in the SNP CAPN-530 allow its future validation as a possible marker associated to meat quality phenotype in Creole cattle from the Amazonas region in Peru, thus contributing to their genetic improvement.

In SNP CAST-2959, allele A is associated to meat tenderness. This study, found that 83 % of Creole cattle presented this allele. Allelic frequencies of 69 % were observed for allele A and 31 % for allele G in Creole Limonero cattle (Torres-Rodríguez et al., 2015). In other taurine breeds descent, such as Hereford (Van Eenennaam et al., 2007), Angus, Quincahm, Luxi and their crosses (Li et al., 2010), and *B. indicus* such as Brahman (Cafe et al., 2010), allele A frequencies around 60 % were observed. Allele A of the CAST-2959 marker was associated to meat tenderness (Li et al., 2010). In our study, allele A frequencies exceed the values reported in the literature, indicating that Creole cattle of the Amazonas region in Peru may have high-quality meat, with lower shear force, a trait of meat tenderness. Likewise, the population of Creole bovine in the Amazonas region in Peru may have meat quality potential, as their genetic structure has traits associated to meat quality indicators, as shown in the literature.

Hardy-Weinberg equilibrium (HWE)

In the CAPN-316 marker, 17 regions were not in HWE in Amazonas Creole cattle. Ríncon et al. (2015) found a similar result in Mexican Charolais cattle. The authors reported that the CAPN-316 marker was not in HWE, especially in cattle raised in Sonora, northwestern Mexico and in the northeastern region of the state of Nuevo León. The non-equilibrium of the polymorphisms could be attributed to intense selection pressure, a small number of heterozygous animals, migration of animals from other regions, and a genetic drift (Ríncon et al., 2015). This result could also be attributed to the introduction of European breeds in local herds, such as Holstein, Simmental, Brown Swiss, and Jersey to obtain an F1 that ensures higher profits to producers. Furthermore, producers may be phenotypically selecting male cattle for breeding stock without considering inbreeding (Encina et al., 2021). In our study, the SNP CAPN-530 was not in HWE (Table 2). The non-theoretical Hardy-Weinberg in CAST-2959 could be due to the absence of heterozygous cattle in the population evaluated.

Texture profile

In CAPN-316, our findings show that meat with homozygous genotype CC is tenderer than with genotype GG. Corva et al. (2007) corroborate this result, as the authors reported lower shear force values in CC compared to GG genotypes in Angus-Hereford and Limousin-Hereford-Angus crossbred cattle. In Charolais cattle, allele G of CAPN-316 was associated to meat toughness when evaluating the Warner-Bratzler shear force. Moreover, allele G of CAPN-316 was also associated to higher meat tenderness scores on day 14 of aging (Allais et al., 2011). To date, it is known that association of CAPN-316 with meat tenderness is due to allele C, which presents a stronger linkage disequilibrium with the tenderness allele (Corva et al., 2007; Single and Thomson, 2016).

Regarding CAPN-530 marker genotypes, *B. taurus* cross-ancestry cattle (Angus, Hereford, and Limousin) were not reported with AA genotypes. However, AG genotypes affected the shear force in GG genotypes (Corva et al., 2007). In our research, no effect was observed, like Carvalho et al. (2017), as the authors did not report the effects of the AA or AG genotypes on meat tenderness of Nellore meat matured at 7, 14, and 21 days. Our results are also consistent with the reports by White et al. (2005). The authors found no effects between the marker CAPN-530 and shear force in *B. taurus* and *B. indicus* crosses. In addition, other studies reported significant associations of the CAPN-530 marker with shear force (Page et al., 2002; Chung et al., 2014). However, although these associations previously reported were not replicated in our research, it is necessary to indicate that the validations of the markers depend on the specific nature of the cattle population

studied. Genetic improvement without adequate control could influence the effect size of polymorphism (Buss et al., 2023).

Meat with GG genotype presented higher shear force than AA or AG genotypes when the SNP CAST-2959 was evaluated. In F1 from meat-producing breeds (Angus, Hereford, Limousin-Luxi cross, Charolais-Fuzhou cross, Simmental-Jinnan cross, Simmental-Mongol cross, Luxi, Jinnan, and Quinchuan), an effect like that found in our study was reported. In our study, GG genotype animals (50.33 N) have significantly higher Warner-Bratzler shear force on day seven of aging than cattle with genotype GA (39.34 N) or AA (39.04 N) (Li et al., 2010). The shear force between genotypes shows differences in the meat of Jersey, Limousin, Angus, and Herefords crossbreds. Meat with AG genotype has greater shear force than meat with AA (Morris et al., 2006). Similar to crossbred cattle populations of *B. taurus*, *B. taurus* with *B. indicus*, differences were found between this marker, the Warner-Bratzler shear force, and tenderness measured on day 14 by a group of panelists (Casas et al., 2006). Shear force by Warner-Bratzler on Nellore meat, 1/2 Angus-1/2 Nellore, Canchim (5/8 *B. taurus* 3/8 *B. indicus*), three-way Brangus crosses (9/16 *B. taurus* and 7/16 *B. indicus*), and three-way Braunvieh crosses (3/4 *B. taurus* and 1/4 *B. indicus*) had differences at day 14. In addition, AA genotypes (33.94 N) had lower shear force compared to AG genotypes (38.06 N) (Curi et al., 2009). In our study, the shear force was similar on days 0, 7, and 14, perhaps due to the non-degradation of the calpastatin, since calpastatin is almost completely degraded after seven days postmortem, lowering its activity to 20 % or 30 % of the original level of calpain inhibition (Boehm et al., 1998; Rhee et al., 2006). Therefore, calpastatin concentration in the first postmortem hours is an essential factor that affects meat tenderness (Sensky et al., 2001). In our study, we did not determine the calpastatin concentration in the meat of Creole cattle; therefore, future studies could be carried out to corroborate the inhibitory activity of calpastatin concerning calpains. In addition, based on current reports, genotypes of the CAST gene are associated to meat tenderness. Unfortunately, the location of polymorphisms related to meat tenderness is at the 3'untranslated end, and it is unknown if they are functional or if they could be in linkage disequilibrium with polymorphisms that are not yet known (Corva et al., 2007). Thus, further studies are required to identify the reasons that justify the differences found during the postmortem proteolytic process to allow the identification of the genotype related to each polymorphism, gene expression, and enzymatic activity (Corva et al., 2007).

We conclude that CAPN-316 (G with 78 and C with 22), CAPN-530 (G with 70.5, A with 29.5), and CAST-2959 (A with 83 and G with 17) allelic and genotypic frequencies were not in HWE, similar to other reports. It was confirmed that CC genotype (43.1 N) of the CAPN-316 marker influences meat tenderness on day 21 of aging, while GG genotype (51 N) and GG genotype of the CAST-2959 marker influence meat tenderness on days 14 and 21 of aging. The sequenced regions of the CAPN1 and CAST genes are polymorphic and possibly improve meat quality in the Creole cattle from the Amazonas region in Peru; however, further studies are needed since polymorphisms with low frequencies were found in the population studied.

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