Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Animal Science/ Original Article

Characterization of muscle and tenderness markers in meat from two local breeds in Panama

Abstract – The objective of this work was to characterize allelic variants related to musculature and meat quality of local Panamanian cattle breeds, using the Affymetrix axiom orcun SNP array. Cattle breeds used were Guaymi and Criollo Guabala. Blood samples of 5 mL were taken from 34 animals, out of which 15 were Guaymi and 19 were Guabala. From 10,000 SNP markers on an Affymetrix axiom orcun SNP array, DNA was evaluated in the search for polymorphisms of 17 SNP markers related to meat tenderness. The Guaymi breed showed a higher percentage of genetic variation and polymorphic loci than the Criollo Guabala breed. Some of the major variations were noticed in few genes of myostatin, calpastatin, and calpain. For the studied livestock breeds, the results found were of great importance for conservation and genetic improvement. Guaymi breed shows a greater genetic diversity than Guabala breed. Six genes show allelic variants related to meat quality and adaptability.

Index terms: bioinformatics, biotechnology, molecular markers, meat quality, livestock.

Caracterização de marcadores de musculatura e maciez da carne de duas raças bovinas do Panamá

Abstract – O objetivo deste trabalho foi caracterizar variantes alélicas relacionadas à musculatura e à qualidade da carne de raças bovinas locais panamenhas, por meio do uso dos marcadores *Affymetrix axiom orcun SNP array*. As raças bovinas utilizadas foram Guaymi e Criollo Guabala. Amostras de sangue de 5 mL foram coletadas de 34 animais, dos quais 15 eram Guaymi e 19 eram Guabala. A partir de 10.000 marcadores SNP de um *Affymetrix axiom orcun SNP array*, o DNA foi avaliado em busca de polimorfismos de 17 marcadores SNP relacionados à maciez da carne. A raça Guaymi apresentou maior percentagem de variação genética e *loci* polimórficos do que a raça Criollo Guabala. Algumas das principais variações observadas ocorreram em alguns genes de miostatina, calpastatina e calpaína. Os resultados encontrados nas raças de gado estudadas foram de grande importância para a conservação e o melhoramento genético. A raça Guaymi apresenta maior diversidade genética do que a Guabala. Seis genes apresentam variantes alélicas relacionadas à qualidade e à adaptabilidade da carne.

Termos de indexação: bioinformática, biotecnologia, marcadores moleculares, qualidade da carne, pecuária.

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Received
February 21, 2024

Accepted
October 08, 2024

How to cite
VILLALOBOS-CORTES, A.; RODRIGUEZ ESPINO, G.; FRANCO-SCHAFER, S. Characterization of muscle and tenderness markers in meat from two local breeds in Panama. **Pesquisa Agropecuária Brasileira**, v.60, e03685, 2025. DOI: https://doi.org/10.1590/S1678-3921.pab2025.v60.03685.

Introduction

Beef is regarded as a highly valuable food for human nutrition, as it is a significant source of protein, fat, vitamins, and minerals, playing a key role in human evolution (Zink & Lieberman, 2016; Scollan et al., 2017). Despite the presence of alternative protein sources, consumers exhibit a preference for beef, grounded in its flavor, texture, and product familiarity (Miller, 2020). It has been shown that consumers prefer farm-raised beef (72%) over vegetable-based substitutes (21%) and lab-produced meat (5%) (Van Loo et al., 2020).

Genomics is among the sciences that have contributed significantly to the advancements in the identification and characterization of genes associated with meat quality production. Allelic variants of quality-associated markers in the myostatin (*MSTN*) gene have been identified in breeds such as Hereford, Angus, Charolais, Simmental, Red Poll, and other composite breeds (Haruna et al., 2020). Similarly, allelic variants of markers in the calpastatin (*CAST*) and calpain (*CAPN*) genes have been reported and linked to tenderness in the meat of various cattle breeds (Enriquez-Valencia et al., 2017).

Myostatin (MSTN), or growth differentiation factor 8 (GDF8), is one of the primary regulators of skeletal muscle development. Animals with this deficiency exhibit an increase in skeletal muscle mass, a phenomenon known as double muscling. Some MSTN variants such as F94L (rs110065568), L64P (rs449270213), Q204 (rs110344317), E226 (g.6216204G>T), and E291 (g.6218432G>T) have been currently identified as strongly associated with meat quality traits (O'Rourke et al., 2013). In some breeds and genotypes, MSTN is expressed in terms of birth weight, ease of birthing, weaning weight. Such traits have been recommended to be included in genomic evaluation methods (Lee et al., 2019). Polymorphisms of this gene have been described in native breeds in Spain, Italy, and France. The Asturiana de los Valles, Rubia Gallega, Marchigiana, Piedmontese, Gasconne, and Parthenoise represent added value due to the reductions of production costs and calf mortality of animals that are heterozygous for alleles favorable to double muscling (Aiello et al., 2018).

Tenderness is one of the most important characteristics of meat. After slaughter, muscle undergoes various biophysical and chemical changes that transform muscle into meat. This process can be divided into three phases: the *pre-rigor mortis* phase, when collagen content primarily contributes to hardness; the *rigor mortis* phase, when further hardening occurs, due to muscle shortening; and the *post-rigor mortis* or tenderization phase, when muscles undergo a series of changes which result in a noticeable improvement in tenderness (Bhat et al., 2018). The calpain system comprises endogenous proteases (calpains) that are considered the main candidates for muscle protein degradation along with their inhibitor, calpastatin (Bhat et al., 2018).

Discovered in 1964, calpain (CAPN) is a large family of intracellular Ca2+-dependent cysteine proteases found in nearly all eukaryotic organisms and some bacteria. It is a protein responsible for *postmortem* proteolysis in meat (Enriquez-Valencia et al., 2017). Several SNPs in the *CAPN* gene have been identified as associated with meat quality traits (White et al., 2005) and are located in different chromosomal regions: calpain-3 in chromosome 10; calpain-13 in chromosome 11; calpain-5 in chromosome 15; calpain-8 in chromosome 16; and calpain-1 in chromosome 29, among others (Jiang et al., 2009).

Calpastatin (CAST) is an inhibitory protein that acts as a key regulator in muscle protein degradation during beef maturation (Lian et al., 2013). CAST helps maintain the texture and tenderness of meat, making it important for the final product quality. It is located in bovine chromosome 7 and is considered a candidate gene since it is known to act as a natural inhibitor of calpains in the meat proteolysis system (Koohmaraie & Geesink, 2006).

This study is part of the Innovative Management of Animal Genetic Resources (Image), a project sponsored by the Food and Agriculture Organization (FAO) (Image, 2024). The objective of IMAGE is to develop and provide free and publicly accessible multi-species single nucleotide polymorphism (SNP) arrays tailored for major farm animal species. These arrays are designed to genotype genetic collections at a target price of less than US$20 per sample.

The objective of this work was to characterize allelic variants related to musculature and meat quality of local Panamanian cattle breeds using an Affymetrix axiom orcun SNP array.

Materials and Methods

The polymorphism of 17 SNP markers associated with meat quality in Criollo Guabala and Guaymi cattle were evaluated and selected from an array of 10,000 SNP markers using a DNA sequencer from the company Affymetrix. The markers selected for the *MSTN* gene were 2:6213889, 2:6213980, 2:6214012, 2:6216138, 2:6216204, 2:6218432, and 2:6218499. Based on the Online Mendelian Inheritance in Animals (OMIA) catalogue, the markers selected for the genes are the following ones: for *CAST*, 7:98498047, 7:98534197, and 7:98566391; for *CAPN1*, 29:44069063, 29:44085769, and 29:44087629; for *CAPN3*, 10:37830642; for *CAPN5*, 15:57260972; for *CAPN8*, 16:2769204, and, finally, the marker for the *CAPN13* gene was the 11:69103108.

Samples of 5 mL of venous blood were taken from each animal and kept cold until their arrival at the laboratory. The samples of the Guabala breed were taken from the Guabala Livestock Conservation Center in Ollas Arriba de Capira, owned by the Agricultural Innovation Institute of Panama (IDIAP). The samples of the Guaymi breed were obtained from the Agricultural Innovation Center for Genetic Resources (CIARG/IDIAP) in Río Hato. The sex ratio of each sampled breed was 70% females and 30% males.

DNA extraction was performed using the commercial kit DNeasy Blood and Tissue from Qiagen. Each sample had an average concentration of 45 ng mL^{-1} and a volume of 50 uL, with a total amount of 2.5 ug DNA. The samples were sent to Affymetrix for analysis, complying with the Nagoya protocol. From the 10,000 selected SNPs, 8,416 met the company's recommended criteria with a conversion threshold of 0.6. All SNPs were aligned with the reference genome UMD 3.1.1. The results obtained in VCF format were validated and transformed to GDA format using the PGDSpider 2.1.1.5 software. Lately, the data were converted into text and Excel formats. To verify the SNP positions, the Integrative Genome Viewer v2.9.4.03 was initially used and paralleled with the Genome Data Viewer from the National Center for Biotechnology Information (NCBI), with the same reference genome UMD 3.1.1. Single nucleotide polymorphisms with a reference number (RefSNP) were used to locate them in the reference genome position ARS.UCD.1.2, using the Ensembl genome browser (Howe et al., 2021) and the European Variation Archive (EVA) (Cezard et al., 2021).

To evaluate genetic variability within each population, the following parameters were calculated: percentage of polymorphic loci; observed and expected heterozygosity (Ho, He); effective number of alleles (Ne); and deviations from the Hardy-Weinberg equilibrium (HW) by population, calculated using the exact significance test through the Markov chain method, adopting 1,000,000 chain length and 100,000 burn-in steps. Gene frequency, genotype frequency, and the values of Fis, Fst, and Fit were also calculated. The programs GENETIX v. 4.02, GenAlEx 6.501, and ARLEQUIN ver 3.5. were used. The Shannon diversity index was calculated using GenAlEx 6.501. The polymorphic variants were subjected to the Cattle QTLdb to identify possible associations with economically useful traits. The difference of means of genetic diversity for both Criollo Guabala and Guaymi cattle breeds was calculated using analysis of molecular variance in the software ARLEQUIN (Fst = 0.15), at 5% probability.

Results and discussion

The 17 gene variants related to muscle and meat tenderness of the Guaymi and Guabala breeds were identified (Table 1). The number of the gene variants found are described as follows: seven variants for myostatin (*MSTN*) – 2:6213889; 2:6213980; 2:6214012; 2:6216138; 2:6216204; 2:6218432; and 2:6218499; three for calpastatin (*CAST*) – 7:98498047; 7:98534197; and 7:98566391; three for calpain1 (*CAPN1*) – 29:44069063; 29:44085769; and 29:44087629; one for calpain 3 (*CAPN3*) – 10:37830642; one for calpain 5 (*CAPN5*) – 15:57260972; one for calpain 8 (*CAPN8*); 16:27692042; and one for calpain 13 (*CAPN13*), 11:69103108.

All 17 analyzed markers are 100% usable. A polymorphism rate of 41.2% (seven loci) for the Guabala breed, and 58.8% of polymorphic loci for the Guaymi breed (10 loci) were obtained. It is noteworthy that the seven *MSTN* variants turned out to be monomorphic in both breeds, including the *CAPN1* variants rs17872000 and rs17872050. The *CAPN8* variant rs109316815 was monomorphic only in Guabala breed.

Most of these allelic variants were identified with their respective reference sequences (RefSNP), except for four *MSTN*, which were identified by their positions in the reference genome.

The overall mean effective number of alleles (Ne) for polymorphic loci was 1.635, with a minimum value of 1.249 for the *CAPN8* variant rs109316815, and a maximum value of 1.993 for the *CAST* variant rs109354718 (Table 2). The Guabala breed showed a lower Ne value of 1.542 compared to the Guaymi, which had 1.727. The Ne value is important for the health and survival of a population, as a decrease in the effective number of alleles can lead to reduced genetic variability, potentially harming the population's adaptability to its environment (Solodneva et al., 2023).

In this case, the Guabala population exhibited lower genetic variability than the Guaymi population. This can be attributed to factors such as a higher rate of inbreeding and small herds throughout the country with a limited number of sires (Villalobos et al., 2021).

Upon calculating the Shannon index, an overall mean of 0.301 was observed (mean over populations and loci). The minimum value was 0.257 of the CAPN8 variant rs109316815, and the maximum value was 0.691 of the CAST variant rs109354718 (mean over populations and each locus) (Table 3). It is noteworthy that a significant difference ($p<0.05$) in genetic

Table 1. Gene variants associated with meat quality in Panamanian Creole cattle.

Gene	Name	Chromosome	Position	RefSNP[1]	Associated trait
MSTN	Myostatin	2	6,213,889	rs449270213	Musculature
MSTN	Myostatin	2	6,213,980	rs110065568	Musculature
MSTN	Myostatin	2	6,214,012	2:6214012	Musculature
MSTN	Myostatin	2	6,216,138	rs110344317	Musculature
MSTN	Myostatin	2	6,216,204	2:6216204	Musculature
MSTN	Myostatin	2	6,218,432	2:6218432	Musculature
MSTN	Myostatin	2	6,218,499	2:6218499	Musculature
CAST	Calpastatin	7	98,498,047	rs109804679	Tenderness
CAST	Calpastatin	7	98,534,197	rs109677393	Tenderness
CAST	Calpastatin	7	98,566,391	rs109354718	Tenderness
CAPN3	Calpain 3	10	37,830,642	rs109425380	Tenderness
CAPN13	Calpain 13	11	69,103,108	rs108960548	Tenderness
CAPN5	Calpain 5	15	57,260,972	rs41772701	Tenderness
CAPN8	Calpain 8	16	27,692,042	rs109316815	Tenderness
CAPN1	Calpain 1	29	44,069,063	rs17872000	Tenderness
CAPN1	Calpain 1	29	44,085,769	rs17871058	Tenderness
CAPN1	Calpain 1	29	44,087,629	rs17872050	Tenderness

(1)RefSNP, reference sequence.

Table 2. Mean of the effective number of alleles (Ne), Shannon index (I), observed heterozygosity (Ho), and expected heterozygosity (He) for Guaymi and Guabala breeds for alleles associated with meat quality.

Gene	RefSNP[1]	Guabala				Guaymi			
		Ne	I	Ho	He	Ne	I	Ho	He
CAST	rs109804679	1.557	0.543	0.467	0.370	1.362	0.436	0.316	0.273
CAST	rs109677393	1.991	0.691	0.533	0.515	1.870	0.658	0.526	0.478
CAST	rs109354718	1.991	0.691	0.533	0.515	1.994	0.692	0.632	0.512
CAPN3	rs109425380	1.991	0.691	0.400	0.515	1.362	0.436	0.316	0.273
CAPN13	rs108960548	1.724	0.611	0.600	0.434	1.819	0.642	0.368	0.462
CAPN5	rs41772701	1.301	0.393	0.267	0.239	1.699	0.602	0.474	0.422
CAPN8	rs109316815	1.000	0.000	0.000	0.000	1.498	0.515	0.316	0.341
CAPN1	rs17872000	1.000	0.000	0.000	0.000	1.978	0.688	0.474	0.508
CAPN1	rs17871058	1.867	0.657	0.467	0.480	1.870	0.658	0.421	0.478
CAPN1	rs17872050	1.000	0.000	0.000	0.000	1.819	0.642	0.474	0.462

(1)RefSNP, reference sequence.

diversity between both populations was found, for which the Shannon index of Guaymi was higher than that of Guabala (0.351 vs 0.252, respectively, obtained from the mean allelic patterns across populations). This difference means that both populations have distinct evolutionary histories confirming that they have different levels of genetic diversity, which agrees with previous studies using other markers (Ginja et al., 2019).

After analyzing the polymorphic loci, the following means for observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Fis) were obtained: 0.223, 0.208, and -0.085, respectively. The *CAPN8* variant rs109316815 stood out for showing the minimum values of Ho (0.158) and He (0.171); and the *CAST* allele rs109804679 stood out for showing the minimum Fis value (-0.246) (Table 3). The maximum values of Ho (0.582) and He (0.171) were observed in the *CAST* variant rs109354718 (Table 3).

In the Guabala and Guaymi breeds, the markers for *MSTN*, *CAPN1* variant rs17872000, and *CAPN1* variant rs17872050 did not show variability in Ho and He. In the Guabala breed, the markers for the *CAPN8* variant rs109316815 markers did not show variability in Ho and He as well. Three *CAST* variants (rs109804679, rs109677393, rs109354718) and *CAPN5* variant rs41772701 showed excess heterozygotes (Ho>He). In Guabala, *CAPN13* rs108960548 also showed excess heterozygotes (Ho>He), and *CAPN3* (rs109425380 and *CAPN1* rs17871058 showed deficit of heterozygotes (Ho<He), without reflecting deviations from HW equilibrium.

For the Guaymi breed, a greater number of markers with excess heterozygotes were found in the following gene variants: three ones of the *CAST* rs109804679, rs109677393, and rs109354718); one of the *CAPN3* rs108960548; one the *CAPN5* rs41772701; and one of the *CAPN1* rs17872050. Additionally, four allelic variants with a deficit of heterozygotes were identified for: *CAPN1* (rs17872000 and rs17871058); *CAPN8* rs109316815; and *CAPN13* rs108960548.

Several factors could explain the differences in the observed heterozygosity in these alleles. One factor is the evolutionary history of the Guabala and Guaymi populations, which may have experienced events of natural selection, genetic drift, or migration affecting allele distribution in the population. Another factor is that heterozygosity can be influenced by genetic and environmental factors. For instance, certain variants may be associated with specific physical or physiological characteristics that are more common in certain genotypes (Zhang et al., 2020; Arakawa et al., 2023) and they may influence the distribution of these alleles. Environmental factors, such as exposure to certain toxins or diet, could also affect the frequency of certain alleles in a population (Mwangi et al., 2019).

Table 3. Means across populations of Guaymi and Guabala breeds for each locus of effective number of alleles (Ne), Shannon index (I), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Fis) for alleles associated with meat quality; mean, minimum, and maximum values of each genetic variability parameter were calculated.

Gene	RefSNP(1)	Ne	I	Ho	He	Fis
CAST	rs109804679	1.46	0.49	0.391	0.322	-0.246
CAST	rs109677393	1.931	0.675	0.53	0.496	-0.101
CAST	rs109354718	1.993	0.691	0.582	0.514	-0.169
CAPN3	rs109425380	1.677	0.564	0.358	0.394	0.004
CAPN13	rs108960548	1.771	0.627	0.484	0.448	-0.124
CAPN5	rs41772701	1.5	0.497	0.37	0.331	-0.153
CAPN8	rs109316815	1.249	0.257	0.158	0.171	0.05
CAPN1	rs17872000	1.489	0.344	0.237	0.254	0.042
CAPN1	rs17871058	1.869	0.658	0.444	0.479	0.045
CAPN1	rs17872050	1.409	0.321	0.237	0.231	-0.052
Min		1.249	0.257	0.158	0.171	-0.246
Max		1.993	0.691	0.582	0.514	0.05
mean		1.635	0.512	0.379	0.364	-0.07

(1)RefSNP, reference sequence.

During the analysis of allelic frequencies in the Guabala and Guaymi populations, a monomorphic pattern was observed in all *MSTN* alleles (Table 4). However, three genetic variants were identified in both populations, from which two were nonsense variants, and the third was a missense variant. These variants, named g.6216204G>T (E226), g.6218432G>T (E291), and g.6218499G>A (C313Y) involve substitutions of the reference allele for its alternative allele. They are located at positions 2:6216204, 2:6218432, and 2:6218499 in the reference genome UMD 3.1.1. It is important to highlight that these variants were first described by Grobet et al. (1998) in European breeds. The E226 and E291 variants were reported in European breeds, such as the French Belgian-Blue, Charolais, Maine-Anjou, and the native Italian breed Marchigiana (Grobet et al., 1998; Meyermans et al., 2022). The C313Y variant was reported in the Gasconne, Parthenaise, and Piedmontese breeds (Konovalova et al., 2021).

Differences for the allelic and genotypic frequencies of the *CAST* gene variant rs109804679 were found between the Guabala and Guaymi breeds. In the Guabala breed, the allelic frequency for G and T was 0.766 and 0.233, respectively. In Guaymi, G was 0.842, and T was 0.157. The genotypic frequency showed a tendency towards homozygosity in both breeds; however, it was higher in Guaymi, than in Guabala (0.587) for the GG genotype (0.709). For the *CAST* gene, two variants were found In the Guabala breed, with frequencies higher than that expected over the reference allele T (0.466): a synonymous variant (rs109677393) with a frequency of 0.533, and an intronic variant (rs109354718) with a frequency of 0.533 for the alternate allele C. In Guaymi, a higher frequency of the alternate allele C (0.526) over the reference allele T (0.473) was observed in the variant rs109354718. The heterozygous CT genotypes had high frequencies in both breeds for the variants rs109677393 and rs109354718, with values of 0.498 and 0.465 in Guabala, and 0.498 and 0.499 in Guaymi, respectively. These differences for allelic and genotypic frequency may indicate a greater genetic diversity in the studied populations, which could confer a greater advantage for biodiversity, from the perspective of the conservation of domestic animal biodiversity towards genetic improvement.

In the *CAPN3* gene variant rs109425380 of the Guabala breed, a higher frequency of the reference allele T (0.533) was observed than that of its alternate C (0.466). In the Guaymi breed, the opposite was found, with a higher allelic frequency of C (0.842) than that of

Table 3. Allelic frequencies of polymorphic variants of genes associated with meat quality in Guaymi and Guabala breeds (Reference Genome UMD 3.1.1).

Gene	RefSNP(1)	Consequence	Guabala		Guaymi	
MSTN	rs449270213	Missense variant	1.000 (T)	0.000 (C)	1.000 (T)	0.000 (C)
MSTN	rs110065568	Missense variant	1.000 (C)	0.000 (A)	1.000 (C)	0.000 (A)
MSTN	2:6214012	Missense variant	1.000 (C)	0.000 (G)	1.000 (C)	0.000 (G)
MSTN	rs110344317	Nonsense variant	1.000 (T)	0.000 (C)	1.000 (T)	0.000 (C)
*MST*N	2:6216204	Nonsense variant	0.000 (T)	1.000 (G)	0.000 (T)	1.000 (G)
MSTN	2:6218432	Nonsense variant	0.000 (T)	1.000 (G)	0.000 (T)	1.000 (G)
MSTN	2:6218499	Missense variant	0.000 (A)	1.000 (G)	0.000 (A)	1.000 (G)
*CAS*T	rs109804679	Intronic variant	0.766 (G)	0.233 (T)	0.842 (G)	0.157 (T)
CAST	rs109677393	Synonymous variant	0.466 (T)	0.533 (C)	0.631 (T)	0.368 (C)
CAST	rs109354718	Intronic variant	0.466 (T)	0.533 (C)	0.473 (T)	0.526 (C)
CAPN3	rs109425380	Intronic variant	0.533 (T)	0.466 (C)	0.157 (T)	0.842 (C)
CAPN13	rs108960548	Intronic variant	0.700 (G)	0.300 (A)	0.342 (G)	0.658 (A)
CAPN5	rs41772701	Intronic variant	0.866 (G)	0.133 (A)	0.710 (G)	0.289 (A)
CAPN8	rs109316815	Intronic variant	1.000 (C)	0.000 (A)	0.789 (C)	0.211 (A)
CAPN1	rs17872000	Missense variant	1.000 (G)	0.000 (C)	0.447 (G)	0.552 (C)
*CAPN*1	rs17871058	Intronic variant	0.366 (C)	0.633 (T)	0.368 (C)	0.631 (T)
CAPN1	rs17872050	Intronic variant	0.000 (C)	1.000 (T)	0.342 (C)	0.658 (T)

(1)RefSNP, reference sequence.

the allele T (0.157). This finding suggests a divergence in natural selection between both populations, evidenced by the differences in the various agroecosystems where they were found in Panamá. Furthermore, differences for genotypic frequencies between both populations were found. In the Guabala breed, a higher frequency of the CT genotype (0.498) and a lower frequency of CC (0.284) and TT (0.218) were observed than those of the Guaymi breed, with a low frequency of the heterozygous genotype CT (0.266) and a high frequency of the TT genotype (0.702). This could suggest the presence of different selection factors in both populations, leading to a divergence in allelic and genotypic frequency of the variant rs109425380. The *CAPN3* gene has been related to meat quality of the loin area between Brahman and Angus breeds in the calpain system (Robinson et al., 2012).

The variant rs108960548 of the *CAPN13* gene was identified with values of 0.700 (G) and 0.300 (A), for the Guabala breed, and 0.342 (G) and 0.658 (A), for the Guaymi breed. As can be observed, the reference allele G in the Guaymi breed showed a lower frequency than that in Guabala. This is also seen in the GG genotype with a value of 0.117 for this breed. However, the frequency of heterozygotes AG (0.450) is slightly higher than the frequency of heterozygotes in Guabala AG (0.420). Studies for the Nellore breed have shown that calpain 13 is a candidate gene associated with meat quality parameters such as water holding capacity, a crucial aspect in meat tenderness (Tizioto et al., 2013).

The allelic frequency of the allele rs41772701 of *CAPN5* was 0.866 (G), and 0.133 (A), for Guabala breed, and 0.710 (G) and 0.289 (A), for Guaymi breed. These allelic frequency values are higher than the ones reported in Mexico by Trujano-Chavez et al. (2021) for the Braunvieh breed, which were 0.400 (A) and 0.600 (G). *CAPN5* gene seems to be related to meat quality traits in French breeds such as Blonde d'Aquitaine, Charolais, and Limousine (Ramayo-Caldas et al., 2016). Analyzing the genotypic frequencies of the allele rs41772701 of *CAPN5*, Guabala showed a greater proportion of homozygous AA (0.751) than that in Guaymi (0.505), while the Guaymi population has the highest proportion of heterozygotes AG (0.411). This proportion of heterozygotes is similar to what Trujano-Chavez et al. (2021) reported in Mexican Braunvieh cattle aimed at improving meat quality.

Alleles of the variant with monomorphic patterns were identified in the *CAPN8* gene variant rs109316815 of the Guabala breed, with allele A (1.000) being present in the evaluated group. The genotypic frequency of *CAPN8* in the Guaymi breed showed a high frequency of AA with a value of 0.623, which higher than the frequency of CA with a value of 0.332. The *CAPN8* gene has been identified and associated with feed efficiency and residual feed intake in the Nellore breed (Olivieri et al., 2016). Calpain 8 is a calpain isoform that has been studied mainly in humans and goats (Ncube et al., 2022).

Changes in the allelic frequency of two *CAPN1* gene variants in both breeds were found; in the variant rs17871058 of the Guabala and Guaymi breeds, an allele T frequency of 0.633 and 0.631, respectively, was observed. In the variant rs17872050, the frequency of allele T was 1.000 and 0.658 in the same breeds, respectively. Regarding the above, similar results were observed by Curi et al. (2009). High allelic frequencies of T were found in breeds such as Nellore and crosses of Rubia Gallega x Nellore, while high frequencies of the allele C were found in the Canchim breed. The allele C has been reported to be associated with meat tenderness (Robinson et al., 2012). The analysis of the genotypic frequencies showed a low frequency (12%) of homozygotes (CC) in the Guaymi breed, which coincides with what was reported by Lenis et al. (2018) in the Harton del Valle breed of Colombia. A high proportion of heterozygotes CT (45%) was also observed, which would be very advantageous, maintaining this allele diversity in future crossbreeding and genetic improvement programs.

Although no breeding programs have been implemented specifically for meat or milk production, certain zoometric indices indicate a significant potential for meat production. Notable indices include the dactyl-costal transverse pelvic index, longitudinal pelvic index, and relative thoracic depth index observed in Guaymi and Guabala cattle (Villalobos-Cortes et al., 2021). Currently, meat of these breeds is being commercially marketed by the Panamanian Creole Cattle Producers Association (ACCRIPA), resulting in substantial economic benefits (ACCRIPA, 2024).

Conclusions

1. The Guaymi breed shows a greater genetic diversity than the Guabala breed, showing a high potential for breeding programs.
2. Seventeen polymorphic variants associated with meat quality were identified, mainly for the myostatin, calpain, and calpastatin genes.
3. Six genes show allelic variants related to meat quality and adaptability.
4. Differences for allelic and genotypic frequencies between the Guaymi and Guabala breeds suggest these populations have undergone distinct selection and evolutionary processes, affecting the genetic distribution within each breed.
5. The lower genetic diversity observed in the Guabala breed indicates a high risk of inbreeding and reduced adaptive capacity, claiming for specific genetic conservation strategies to prevent biodiversity loss.

Acknowledgments

To Wagenigen University for their support in receiving and analyzing the DNA samples, in particular to Dr. Richard Crooijmans for his assistance with the reception of the samples; to Instituto de Innovación Agropecuaria de Panamá (IDIAP), to Secretaría Nacional de Ciencia, Technología e Innovación de Panamá (SENACYT), to Sistema Nacional de Investigación (SNI), and to Red Ibero-Americana sobre los Recursos Zoogenómicos y su Resiliencia (REZGEN-IBA, 123RT0139), for their support to this research.

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