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Frecuencia alélica del gen de la calpastatina en el ganado criollo limonero

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RESUMEN

Con el objeto de evaluar el polimorfismo del gen Calpastatina (CAST) en el ganado Criollo Limonero, fueron analizadas muestras sericas de 157 animales (44 machos y 113 hembras), la caracterización genética se realizó mediante la técnica PCR-RFLP, usando la enzima de restricción *XmnI*, las frecuencias alélicas y genotípicas se compararon mediante pruebas de ji-cuadrado. Los resultados mostraron frecuencias de 0,84; 0,03 y 0,13 para los genotipos AA, AB y BB, respectivamente. Las frecuencias alélicas fueron 0,85 y 0,15 para los alelos A y B, respectivamente. La prueba de ji-cuadrado no confirmó EHW. Es importante mencionar que el hallazgo en alta frecuencia del

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alelo A y su correlación con la terneza y calidad de la carne, resulta útil para establecer planes de selección asistida por marcadores, con la finalidad de incrementar tanto la producción de carne como la calidad de la misma, y darle así al Criollo Limonero una ventaja potencial.

PALABRAS CLAVE: Gen de calpastatina, polimorfismo genético, PCR-RFLP, ganado criollo, raza Limonero.

Allele Frequency of the Calpastatin Gene in Limonero Creole Cattle

ABSTRACT

In order to assess polymorphism of the calpastatin (CAST) gene in Limonero Creole cattle, blood samples were collected from 157 animals (44 male and 113 female). Genetic characterization was carried out by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) using the *XmnI* restriction enzyme. The allelic and genotypic frequencies were compared using chi-square tests. Results showed frequencies of 0.84, 0.03 and 0.13 for genotypes AA, AB and BB, respectively. Allelic frequencies were 0.85 and 0.15 for alleles A and B, respectively. Chi-square tests showed that the population was not in Hardy-Weinberg equilibrium. It is important to mention that finding the high frequency of the CAST gene A allele and its correlation with meat tenderness and quality, could be useful for establishing selection plans assisted by markers, in order to increase beef production and quality and, thereby, give the Limonero Creole breed a potential advantage.

KEY WORDS: Calpastatin gene, genetic polymorphism, PCR-RFLP, creole cattle, Limonero breed.

Introduction

The Venezuelan Limonero Creole cattle (*Bos taurus*) is Rojas *et al.*, (2009) a genetic resource oriented for milk production through selection The main adaptation of the breed's characteristics to tropical environment

is its consistent expression of heat tolerance, diseases and parasites tolerance in crossbred progeny of the Limonero Creole, Villasmil-Ontiveros, (2008a) consequently this genotype represents an option in crossbreeding programs

These animals belong to the 'Carrasquero' local station, assigned to the National Institute of Agriculture Research of Zulia (INIA-Zulia) and located in the northwest of Zulia State near the 'Limón' river that gives name to the breed Villasmil-Ontiveros, (2008b). Nowadays they are at risk of extinction with their population not exceeding 500 animals programs Villasmil-Ontiveros (2008a). This local population constitutes an interesting genetic resource for research that is not completely known and could be essential due to their potential in the bovine production system, since they could be a source of unique and valuable genes Uffo *et al*. (2006).

The calpastatin gene (CAST) codifies the calpastatin protein, responsible for the inactivation of the calpain enzymes and influences *post-mortem* tenderness of meat. CAST, which is an endogenous calpains' inhibitor, plays a central role in the regulation of calpains activity in the cell Goll *et al*. (2003), and it's considered to be one of the major modulators of the protein turnover. Therefore, CAST may affect proteolysis of myofibrils due to regulation of the activity of calpains and is responsible for initiation of *post-mortem* degradation of myofibrillar proteins Goll *et al*. (2003).

The bovine CAST gene has been mapped to BTA7 Bishop *et al*. (1993), with a relative position of 117.8 cM, Kappes *et al*. (1997). This gene has been sequenced from bovine skeletal muscle, with five different domains identified Killefer (1994) and Koohmaraie (1996).

In previous studies, two single nucleotide polymorphisms (SNPs) have been identified in the CAST gene, a G/C SNP in intron 5 Schenkel *et al*. (2006) and an A/G SNP in the 3' UTR region Barendese, (2002). Chung *et al*. (2001) found DNA polymorphism in the intron 6 using polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP) and XmnI as the restriction enzyme. Schenkel *et al*. (2006) found that a genetic marker at the calpastatin (CAST) locus in *Bos taurus* cattle from Canada was associated with shear force when steaks were aged for 21 days.

Tenderness is one of the most valuable attributes of beef eating quality Miller *et al*. (1995), and has been correlated genetically with calpastatin

activity by Warner-Bratzler shear force Casas *et al*. (2006). Genetic variations in the promoter region of the CAST gene suggest that the *locus* is highly polymorphic Juszczuc-Kubiac *et al*. (2009). It is clear that the genetic background of the animals makes a significant contribution to the variation in meat tenderness as tenderness varies among and within breeds.

Recently, genetic test for meat tenderness in bovine, utilizing genetic polymorphism, mainly calpastin (CAST) and or Calpain gene have been made available by two private companies, GeneSTAR Tenderness and Igenity Tender GENE test uses a G/A SNP in 3′ UTR region (base 2959 of AF159246) and G/C SNP in intron 5 of CAST (base 282 of AY008267), respectively Van Eenennaam *et al*. (2007).

The objective of this study was to assess the allelic and genotypic variation at the bovine CAST gene in the Limonero Creole cattle in Venezuela using PCR-RFLP.

1. Materials and methods

DNA was extracted from the blood of 157 male and female animals from the Limonero Creole herd of the INIA-Zulia. A 1500 bp fragment of the CAST gene using forward 5′-AGCAGCCACCATCAGAGAAA-3 and reverse 5′-TCAGCTGGTTCGGCAGAT-3′ primers (Chung *et al*., 2001).

The PCR reactions contained approximately 100 ng of genomic DNA, 2.5 μL 10X PCR buffer (670 mM Tris-HCl pH 8.8, 160 mM $(NH_4)_2SO_4$, 0.1% mM Tween 20), 1 μL $MgCl_2$, dNTP, 0.1 μg of each primer) and 2 U Taq DNA polymerase in a total volume of 20 μL.

Samples were amplified for 35 cycles (Mastercycler ep-gradient$^{(S)}$, Eppendorf) with the following program: denaturation step at 95°C for 1 min, annealing at 65°C for 30 sec and extension step at 74°C for 40 sec. Products of amplification were checked by electrophoresis in 1% agarose gel.

PCR-RFLP: The PCR products were digested by *XmnI* restriction endonuclease. Digestion was conducted at 37°C for 24 h and in 10 μL of specific Buffer, 0.3 μL (3 U) of restriction endonuclease and 6 μL of PCR products were separated in 2.0% electrophoresis agarose gel containing in ethidium bromide (0.4 μg mL^{-1}). Electrophoresis was performed in 1X TAE buffer (108 g Tris, 55 g boric acid and 40 mL of 0.5 M EDTA in 1,000 mL of 10X concentrated stock solution, pH 8.0) under 120 V for 45 min.

Based on the genotypes identified on gels, allele frequencies were calculated according to Weir, (1996). Hardy-Weinberg Equilibrium (HWE) was compared using chi-square tests.

2. Results and discussion

Two alleles (A,B) and three genotypes were observed using DNA restriction fragments for the CAST- *XmnI* polymorphism: 950 bp and 550 bp for AA genotype, 1500 bp, 950 bp and 550 bp for the AB and 1500 bp (no digestion) for the BB (figure 1).

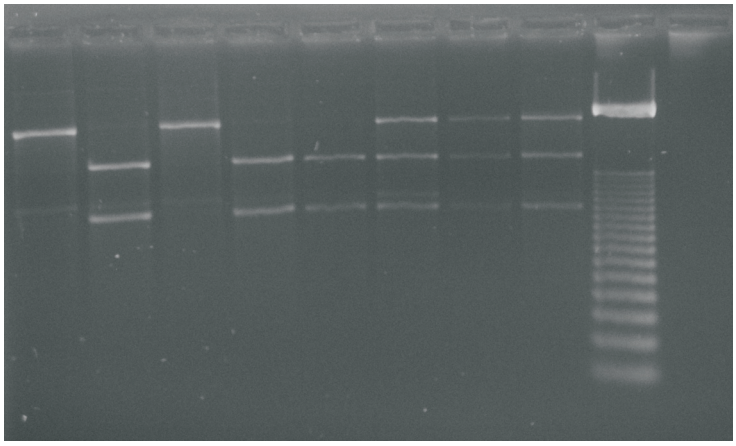

FIGURE 1. Electrophoresis of the CAST gene for *XmnI* restriction products of DNA samples from Limonero Creole bovine. Lane 1 undigested product, Lane 2, 4 and 5 are AA, Lane 3 is BB, Lane 6, 7 and 8 are AB, Lane 9 marker, Lane 10 negative control.

The frequency of alleles A and B in the population were 0.85 and 0.15, respectively, while the genotypic frequencies of AA, AB and BB were 0.84, 0.03 and 0.13, respectively (table 1). No Hardy-Weinberg equilibrium was observed ($P < 0.0001$).

TABLE 1

No.	Allelic Frequencies		Genotypic Frequencies		
	A	B	AA	AB	BB
157	0,85	0,15	(132) 0.84	(4) 0.03	(21) 0.13

These results obtained in Limonero Creole breed, will be compared with those observed in Angus and Bonsmara breed Wheeler *et al*. (1994); and Suguisawa, (2005), also, contrasted with those obtained in *Bos indicus* and other crossbred beef cattle Fortes *et al*. (2008) and ones reported in Nelore, and Caracu brazilian, Zaidan *et al*. (2009). Simmental animals showed greater frequency of allele A (frequency = 0.64) than all other continental breeds (frequency < 0.38) Fortes *et al*. (2008).

The frequency of the A allele, favorable for meat traits Barendese (2003), has been reported lower in *Bos indicus* than in *Bos taurus* × *Bos indicus* animals as expected, since, according to Wheeler *et al*. (1994), *Bos indicus* breeds produce less tender meat, when compared to *Bos taurus* and *Bos taurus* × *Bos indicus* animals.

Other studies have reported that the CAST gene where the AA genotype were correlated with lower Warner-Bratzler shear force measurements compared to the AB and BB genotypes Barendese (2002); Casas *et al*. (2006); Morris *et al*. (2006); Van Eenennaam *et al*. (2007). That is, animals with AA exhibit lower levels of calpastatin, causing greater μ-calpain activity during post-mortem meat tenderization Koohmaraie (1996). The A allele occurs with a frequency of 84-95% in *Bos taurus* cattle Morris *et al*. (2006).

Some earlier data presented and patented by Barendese (2002), suggested that genetic variation at the CAST *locus* contributes to variations in meat tenderness trait. They proved the potential genetic interaction between markers for two *loci* (CAPN1 and CAST) that are currently being used as the basis of commercial DNA tests for meat tenderness in beef cattle.

Conclusions

The Limonero Creole population, exhibiting a higher frequency of the CAST A allele, is potentially a valuable source for genetic improvement to beef tenderness in a dual purpose group in tropical environments and other cross *Bos taurus* × *Bos indicus* animals.

Thus, it may be concluded that CAST genotypes, when used as genetic markers in selection programs may moderately but significantly contribute to the improvement of meat quality production traits in cattle. A se-

lection based on markers, not only minimizes problems but also they are more reliable and animals can be selected at an early age for breeding programs

This molecular genetic information may be used by breeders to design the genetic selection programs for the development of this breed. This marker will be useful in the selection of bovines. The high frequency of the A allele observed in the studied population, allows this test to be applied with effectiveness, due to the association of this allele with meat tenderness.

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