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Analysis of Tenderness and Marbling-related Polymorphisms in Beefmaster Cattle

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ABSTRACT

Tenderness and marbling are polygenic characteristics that are important parameters of meat quality. In this study, we analyzed the genetic predisposition for both parameters in Beefmaster cattle. Tenderness was determined by the presence of calpastatin enzyme (an inhibitor of the post-mortem softening process of meat) alleles inherited by each animal, marbling was judged by the thyroglobulin gene, which encodes a precursor of the thyroid hormone that is a major regulator of fat metabolism and storage. One hundred forty five blood, semen or follicle samples from Beefmaster cattle were analyzed to generate a gene allele profile associated with tenderness and marbling. Of the animals, 43.4% showed gene predisposition for marbling, gene predisposition for tenderness was observed in 62.1%. The findings are intended to be incorporated into the decision making process of breeding programs to increase the number of animals with superior meat quality. The use of these markers could guide investment in better cattle offspring as complementary indicators of meat quality.

Key words: Beefmaster, calpastatin, marbling, tenderness, thyroglobulin

INTRODUCTION

The principal consumer selection parameters for beef quality are tenderness and marbling. Tenderness is defined as the force required for shearing a cooked steak. This feature is one of the most important factors in consumer satisfaction. It is known that after slaughter, meat undergoes maturation, a process that involves its preservation for at least 14 days at 4°C (Curi *et al*., 2010). The calpain proteolytic system has been identified as the main factor responsible in meat softening (Motter *et al*., 2009) and it is countered by calpastatin (CAST), the main inhibitory enzyme of this system.

Research has been conducted to find genetic markers to distinguish allele variants of CAST and calpain genes, in order to identify animals with genetic potential for tenderness. In a study conducted in 2002, some Single Nucleotide Polymorphisms (SNP) were associated to CAST gene, identifying a substitution of Guanine to Adenine (G/A) at the 3' end non-translatable region of the CAST mRNA (rs109221039). It was established that the A allele was associated with highly tender meat (Barendse, 2002). This result was later confirmed using different purebred *Bos taurus* and

Bos indicus and their crossbreds; all of this as part of the Germplasm Evaluation Project of the USA Department of Agriculture (USDA) in Clay Center, Nebraska (Casas *et al*., 2006). Another working group identified the SNP AY_008267.1: g282C>G in intron 5, where the C allele showed an increase of the degree of post-mortem tenderness (Schenkel *et al*., 2006).

Since tenderness variability is mostly genetic, there are some differences within and among breeds. It is well known that those of Indian origin (Zebu) produce less tender meat than the European ones. The tenderness of different muscles decreases progressively as the percentage of Zebu genes increases (Crouse *et al*., 1989; Shackelford *et al*., 1995; Pringle *et al*., 1997). This lessening in tenderness is related to increased CAST activity in post-mortem zebu breeds. It has also been reported that CAST activity increases linearly with increasing percentage of Brahman (*Bos indicus*) genes in crossbred animals, reaching a peak in pure animals of this breed (Wheeler *et al*., 1990).

Marbling refers to the presence of fat between muscle fibers. Genetic predisposition for marbling is determined based on the genotyping of alleles of the Thyroglobulin Gene (TG). The product of this gene is a precursor of thyroid hormones that regulates fat metabolism and storage (Thaller *et al.,* 2003). A polymorphism in the 5' region of the allele 3 of this gene was associated with increased marbling and its utility has already been proved (Barendse, 1999). This SNP consists of a change from C to T at position 422 of the TG gene (Genbank X05380) (Shin and Chung, 2007) and it was observed that the T allele confers increased marbling predisposition (Anton *et al*., 2013).

The correlation of genetic variants with quantitative productive characteristics is called Quantitative Trait Loci (QTL). It is very useful in breeding programs due to its potential to identify individuals with a genetic predisposition to increase productivity and/or quality of the features recognized in the market. This type of breeding is called marker-assisted selection (Lopez-Zavala *et al*., 2007).

Mexico has a large livestock industry with significant variability of races, unfortunately it has been difficult to establish objective uniform criteria for breeding and production (Mejia *et al*., 2012). In order to promote the use of genetic profiling in breeding programs in Mexico, we describe herein the implementation of DNA test analyses for tenderness and marbling in collaboration with the Mexican Association of Beefmaster Cattle Breeders.

MATERIALS AND METHODS

This study was carried out with the support and agreement of the Local Cattle Breeders Association of Ciudad Mier, Tamaulipas, Mexico.

DNA extraction: A total of 145 samples from Beefmaster cattle (GPE Cycle 8, *B. taurus*×*B. indicus*) were collected from pens of the Local Cattle Breeders Association from Ciudad Mier, Tamaulipas, Mexico. Peripheral blood sampling was performed by puncturing the caudal vein of the animal and extracting 2 mL of blood from breed stallions. The samples were placed in 3 mL vacutainer tubes containing EDTA and were immediately introduced into a cooler at 4°C. They were then taken to the laboratory where DNA extraction was performed using the Wizard Genomic DNA Purification kit (Promega, Madison, WI), following the manufacturer's instructions. Semen samples consisted of straws that had been stored at -20°C. From these, only 100 μL were taken and processed with the Axyprep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences; Union City, CA) according to the manufacturer's specifications. Hair samples consisted of 5-15 follicles taken from the tail of the animal and these were processed with the aforementioned kit.

Gene test for tenderness: The DNA analysis was based on the identification of two variants of the CAST gene, located on chromosome 7. The SNP rs109221039 (G/A), found in the immediate 3' region of the gene determines the presence or absence of a restriction site for the enzyme *Dde*I. We used the PCR-RFLP approach, which couples PCR with RFLP analysis. This method consists of amplification of this gene region by PCR using the oligonucleotides: forward = 5'-CATTTGGAAAACGATGCCTCA-3' and reverse = 5'-CATGTGCCCAATGCACAGTA-3' and the subsequent digestion of the amplified product with the restriction enzyme *Dde*I. Hundred nanogram of total DNA used for a 15 μL PCR reaction. Finally, the digested fragments were visualized on 3.5% agarose gel to identify the gene variants.

Gene test for marbling: This test is based on the identification of two variants of the TG gene, which is located on chromosome 14 of the bovine karyotype. Specifically, the immediate 5' region of the gene was amplified. Genotyping of the aforementioned polymorphism C/T was carried out as in the tenderness assay except that the oligonucleotides were forward = 5'-GGGGATGACTACGAGTATGACTG-3' and reverse= 5'-GTGAAAATCTTGTGGAGGCTG TA-3' and the enzyme *Mob*I was used to reveal the RFLP genotype.

RESULTS

A population of 145 beef cattle specimens was analyzed for the presence of SNP indicators of meat marbling and tenderness. The favorable genotypes for both of these traits were the most frequent (Table 1). We calculated the allelic frequencies based on them and we showed a genetic equilibrium for the favorable alleles in tenderness but it was not the case for marbling.

Tenderness: A fragment of 143 bp was amplified from the CAST gene belonging to the 3' end-untranslated region of this gene's mRNA (Fig. 1A, B). The allele A causes the presence of a restriction site for the enzyme *Dde*I, which generates two fragments after digestion, one of 62 and the other of 81 bp. This renders a pattern for each of the possible genotypes: one related to a high degree of tenderness (A/A), where the enzyme cuts both alleles (Fig. 1C); a degree of regular tenderness (A/G), where the enzyme cuts only one allele (Fig. 1D) and a low degree of tenderness or unfavorable genotype (G/G), where the enzyme does not cut any allele (Fig. 1E), because the restriction site is absent in both of them.

From the 145 samples studied, 62.07% had a favorable genotype (A/A), 36.55% showed the heterozygous genotype (A/G) and the least desirable homozygous genotype (G/G) was represented in 1.38% of cases.

Marbling: The PCR amplification generates a product of 545 bp (Fig. 2A, B) with two cleavage sites for the enzyme *Mbo*I, whose digestion products are of 278, 191 and 72 bp in size. The C allele contains a third cleavage site, in which case digestion originates four fragments of 278, 174, 72 and 17 bp.

Table 1: Genotype frequencies and allelic frequencies of calpastatin and thyroglobulin genes in 145 specimens of beefmaster cattle

Genes	No.	Genotype	Frequencies	Allelic	Frequencies	HW
	90	AA	0.6207	A	0.803	NS
CAST	53	AG	0.3655	G	0.197	
	2	GG	0.0138			
	63	TT	0.4345	T	0.548	
TG	33	CT	0.2276	C	0.452	S
	49	CC	0.3379			

HW: Hardy-weinberg equilibrium, S: Significant p<0.01, NS: Not significant, p>0.05, CAST: Calpastatin, TG: Thyroglobulin

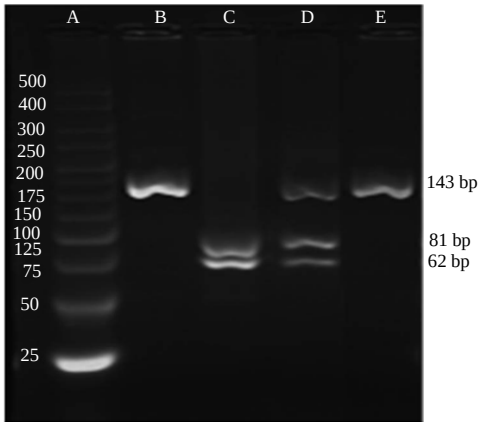

Fig. 1: Tenderness. Genotyping of SNP rs109221039 by PCR-RFLP, A: 25 bp marker, B: 143 bp non-digested fragment, C: A/A genotype, D: A/G genotype and E: G/G genotype

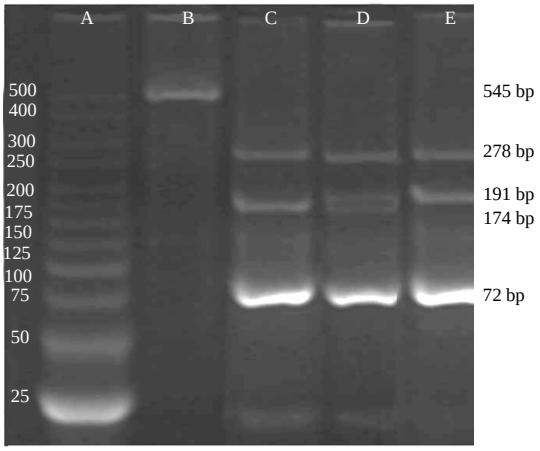

Fig. 2: Marbling. Genotyping of SNP C422T by PCR-RFLP, A: 25 bp marker, B: 545 bp non-digested fragment, C: C/C genotype, D: C/T genotype and E: T/T genotype

The unfavorable homozygous genotype (C/C, Fig. 2C) was observed in only 33.79% of the individuals analyzed. The analysis revealed the heterozygote genotype in 22.76% of all cases (C/T, Fig. 2D) in contrast to the desirable genotype (T/T, Fig. 2E) in 43.45% of these.

DISCUSSION

In the present study with Beefmaster cattle specimens, we report the analysis of polymorphic DNA markers which are commonly used to determine two of the most valued factors of meat quality in beef cattle: tenderness and marbling.

This cattle breed has been very popular among livestock producers, because it has a wide adaptability to different environments (SAGARPA., 2012), besides having a large resistance to different diseases and low mortality. Recently, Mexico's Agriculture and Livestock Ministry has reported an increasing interest from the Mexican Association of Beefmaster Cattle Breeders in the study of predisposition to quality meat in reference to marbling and tenderness.

For tenderness, a high frequency (0.803) of the allele A of the CAST gene was found in 145 Beefmaster individuals studied, contributing to the most desired genotype for post-mortem meat tenderization. These results agree with those obtained by Casas *et al*. (2006), where a higher frequency of the allele A was observed in *B. taurus* and crossbreeding of *B. taurus*×*B. indicus*, as is the Beefmaster cattle (Curi *et al*., 2009). This high frequency could indicate a strong contribution from *B. taurus* in the genetic background of crossbred cattle. However, different studies have reported that the presence of this SNP for tenderness and phenotypic correlation varies widely among breeds and types of growing environment (Parra-Bracamonte *et al*., 2011).

Searching to predict meat marbling, previous studies have shown that the TT genotype of the marker C422T in the TG gene presents frequencies from 6.7-18.9% in *B. taurus* and around of 1.5% in *B. indicus*. The present study revealed the presence of the desired TT genotype in 43.45% of individuals, showing a large increase compared to the individual percentage of the two purebreds of origin. This is of particular interest, since *B. taurus* is considered a breeding of higher marbling in meat (Bonilla *et al*., 2010). Bonilla *et al*. (2010) found a presence of 10% for the allele T in 26% of the specimens charolais analyzed, which on average increased the intramuscular fat mean from 4.4-6.6% (Parra-Bracamonte *et al*., 2011).

Little is known about SNP frequencies expected for marbling in the Beefmaster breed. Pannier *et al*. (2010) studied the presence of SNPs in three genes predisposing to marbling (DGAT1, FAB4 and TG) in different purebreds and reported that none of them correlates with phenotype in meat (Pannier *et al*., 2010). It would be desirable to implement a correlation study of the TG gene SNP marker thyroglobulin with the actual content of intramuscular fat in Beefmaster cattle breed which is so important in Mexico.

Molecular analysis technology can be used to select animals for specific favorable traits, especially those that are not measured routinely, as is tenderness and marbling in meat. Using stallions (animals with superior characteristics determined by genetic studies), inconsistency and variation in tenderness of herds could be reduced and eventually eliminated overtime.

The frequency of desirable genotypes in a herd will then depend on the discrimination of animals for breeding programs. The priority of a farmer should then be to develop a genetic profile of the animals in a herd according to its tenderness and marbling predisposition, selecting bulls and females with the desired features. Once this initial information is obtained, it will be possible to select those animals able to transmit to their offspring the favorable variants for tenderness and marbling. However, specific large population studies will be needed to establish an association of the levels of tenderness and marbling to the genotype of individuals.

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