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Short communication

## Meat tenderness genetic polymorphisms occurrence and distribution in five Zebu breeds in Mexico

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## ABSTRACT

**Background:** The Zebu cattle are represented by a diverse group of breeds in México. Traditionally these breeds have been associated with the tough beef characteristic. Validated genetic markers have the potential to be included in marker-assisted selection and management programs in order to improve traits such as beef tenderness. The incidence and distribution of Calpain and Calpastatin polymorphisms strongly associated with beef tenderness were estimated in registered cattle of five Zebu breeds in Mexico.

**Results:** A low and in some cases null frequency of favorable C allele of CAPN316 was determined in all breeds. Conversely, a more equilibrated frequency in CAPN4751 and CAST loci was observed.

**Conclusions:** Although the relatively low occurrence of favorable alleles in assessed loci may limit their use in selection programs, genotyping availability might be a practical and comprehensive tool for introgression programs by marker assisted selection and management as to improve meat tenderness of Zebu breeds.

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## 1. Introduction

The Zebu cattle are the second group of breeds most successful in terms of their worldwide distribution [1]. This cattle subspecies is widely used in tropical and subtropical regions because of their thermoregulatory ability under high temperatures and humidity and their genetic resistance to diseases and parasites [2].

A significant proportion of the cattle raised in tropical and subtropical regions took the advantage of *Bos indicus* breeds adaptability, with the resulting undesirable increase in toughness of the meat, as estimated in crossbred cattle with a 50% or higher Zebu inheritance [3]. Evidence indicates that a proportion greater than 25% of *B. indicus* inheritance may significantly affect meat tenderness [4]. Some studies have indicated that higher values of shear force, as a mechanical estimation of tenderness, in the meat from the Central and South regions of Mexico are related to the most frequent used Zebu-type animals [5].

Meat tenderness is one of the most important economic traits of beef cattle related to consumer acceptance and satisfaction. However, its improvement is limited by the difficulty and cost of measurement. Different strategies have been explored for genetic improvement of

this trait. Genetic markers have been extensively assessed, and some markers in Calpain and Calpastatin genes are documented and validated to be highly associated with meat shear force [6,7,8,9,10,11,12,13], explaining 20% of genetic variation of meat tenderness [8,14].

The objective of the present report is to assess the frequency of alleles of genetic markers associated with meat tenderness in five Zebu breeds of beef cattle in Mexico.

## 2. Experimental

Hair follicle samples of Brahman (n = 358), Gyr (n = 41), Guzarat (n = 21), Indubrazil (n = 25) and Sardo Negro (n = 109) purebred animals from different beef cattle farms registered in the Asociación Mexicana de Criadores de Cebú, were collected.

For this assessment, allele frequency of the micro-Calpain (CAPN) 316 (GenBank accession no. AF252504: CAPN316) 4751 (GenBank accession no. AF248054: CAPN4751) [15], and Calpastatin (CAST) T1 (GenBank accession no. AF159246: CAST-T1) loci [16] loci, were analyzed. Genotyping of the single nucleotide polymorphisms (SNP) was performed using the Sequenom MassARRAY® system (iPLEX GOLD; Sequenom, San Diego, CA, USA). Genotypic and allelic frequencies of all loci were estimated. Hardy–Weinberg equilibrium (HWE) was tested using GENEPOP ver. 4.2 [17].

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**Table 1**  
Genotype and allele frequencies of Calpain and Calpastatin polymorphisms in five Zebu breeds.

Locus	BRH	GYR	GZT	IBZ	SNG
CAPN316	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.005$
	GG	1.00	1.00	1.00	0.99
	GC	0.01	0.00	0.00	0.00
	CC	0.02	0.00	0.00	0.01
	G	0.97	1.00	1.00	1.00
CAPN4751	$P = 0.710$	$P = 1.000$	$P < 0.001$	$P = 1.000$	$P = 1.000$
	TT	0.84	0.83	0.95	0.92
	TC	0.15	0.17	0.05	0.08
	CC	0.01	0.00	0.00	0.00
	T	0.92	0.91	0.98	0.96
CAST-T1	$P = 1.000$	$P = 1.000$	$P = 1.000$	$P = 0.410$	$P = 0.848$
	CC	0.08	0.19	0.05	0.28
	CT	0.40	0.49	0.33	0.60
	TT	0.52	0.32	0.62	0.12
	C	0.28	0.44	0.21	0.58
T	0.72	0.56	0.79	0.42	

BRH: Brahman, GYR: Gyr, GZT: Guzerat, IBZ: Indubrazil, SNG: Sardo Negro.  $P$  values  $< 0.05$  are statistically significant for Hardy Weinberg deviation test.

### 3. Results and discussion

In Mexico the Zebu breeds are maintained as purebred animals but also as crossbred animals with European breeds in beef production systems and dual purpose systems as well [18]. A major contribution of Zebu breeds is to the composite breeds, such as Santa Gertrudis, Brangus, Beefmaster, Simbrah, Braford, and Charbray [1]. In Mexico, Brahman, Red Brahman, Nellore, Guzerat, Indubrazil, Gyr and Sardo Negro, are the most important representative Zebu breeds.

Results from the present study indicated low or null segregation of CAPN316 favorable genotypes in all studied Zebu breeds (Table 1); conversely, a more equilibrated occurrences of favorable C, and T alleles, were present for CAPN4751 and CAST-T1 loci, respectively (Table 1). The HWE test showed significant deviations in CAPN316 genotypes in all breeds ( $P \leq 0.005$ ). In contrast, Gyr was the only breed showing deviations from the HWE in the CAPN4751 locus. CAST-T1, exhibited no deviations from the HWE.

Some reports support the relative null and low frequencies of favorable allele segregating in Zebu, specifically in Brahman and Nellore breeds [9,10,15] (Table 2). There is only one study reporting CAPN316 and CAPN4751 frequencies in a Brahman cattle population in Mexico. Parra-Bracamonte et al. [15] reported relatively high

frequencies of favorable C alleles in both loci with a high occurrence in heterozygous genotypes. However, the results of the present study clearly indicate an almost null frequency of allele C in CAPN316 locus and a lower occurrence of favorable allele in CAPN4751 compared to that previous assessment clarifying the actual situation of Brahman populations from Mexico. For the other four breeds the authors did not find previous reports.

Theoretically, using validated SNPs for selection may represent the state of the art of genetic improvement for a trait that is very difficult or expensive to measure [8,19]. There is strong validating evidence of the usefulness of CAPN and CAST-T1 for the improvement of meat tenderness [7,8,9,10,16]. Additionally, Tait et al. [12,13] in recent research, evaluated the CAST-T1 effects on slice shear force in crossbred [12] and angus purebred cattle [13], and reported a significant improvement of shear force residual variance associated to the selection the favorable genotype, consequently preventing the risk of tough meat and strongly supporting the use of CAST-T1 polymorphism for marker assisted management or marketing of beef products.

Frequencies and distribution of favorable alleles for meat tenderness in the Zebu breeds considered in this study, at least for CAPN4751 and CAST-T1, suggest the possibility of using mating strategies to increase the frequency of the favorable alleles. For instance, in the Brahman breed, the most numerous sampled population, CAPN4751 and CAST-T1 haplotype combination showed an availability of 54% of animals with two favorable alleles and 6% of animals with three favorable alleles for meat tenderness. This information suggests the possibility to manage these available segregation frequencies to positively change meat toughness assisting current breeding objectives used in Brahman breed by selection of favorable allele carriers.

Despite the fact that the beef market in Mexico does not consider meat tenderness as an indicator for meat quality with no incentive for genetic improvement of this trait, since beef from Zebu cattle is considered tough, the implementation of strategies such as the one reported here may contribute to improve the perception of Zebu beef in some market niches and change the consumer perception. However, the application of measures such as Warner–Bratzler shear force in sire evaluation, as included for instance by the American Brahman Breeders Association in their breeding objectives [20], is comparatively more difficult in Mexico due to the absence of suitable infrastructure and other particularities of the beef cattle breeding sector. Therefore, the use of validated genetic markers may represent a more comprehensive strategy for the genetic improvement of meat tenderness in beef cattle, at least in the short term.

Economic returns for the implementation of introgression of the favorable alleles of the CAPN and CAST-T1 SNP markers have been

**Table 2**  
Literature reported genotype and allele frequencies of Calpain and Calpastatin polymorphisms in Zebu breeds.

Locus	Favorable allele	Breed	Genotype %			n	Frequency		Ref
			0	1	2		Favorable allele	Unfavorable allele	
CAPN316	C	Brahman	97	3	0	470	0.02	0.98	[7]
		Brahman	96	4	0	674	0.02	0.98	[8]
		Brahman	94	6	0	382	0.03	0.97	[10]
		Nellore	98	2	0	638	0.08	0.92	[11]
CAPN4751	C	Brahman	0	92	8	85	0.46	0.54	[15]
		Brahman	81	19	0	471	0.10	0.90	[7]
		Brahman	88	11	1	674	0.06	0.94	[8]
		Nellore	0	21	79	114	0.105	0.895	[9]
		Brahman	0	10	90	382	0.05	0.95	[10]
CAST-T1	T	Nellore	68	29	3	638	0.18	0.82	[11]
		Brahman	0	99	1	85	0.49	0.51	[15]
		Brahman	18	50	32	768	0.57	0.43	[8]
		Brahman	11	46	43	674	0.66	0.34	[8]
		Nellore	23	43	34	114	0.557	0.443	[9]
Brahman	11	47	42	382	0.66	0.34	[10]		
Brahman	6	44	50	444	0.72	0.28	[16]		

Ref: Reference. n: Sample size.

estimated by Weaber and Lusk [21]. Implementing a simulation assessment on the use of genetic markers for meat tenderness, Weaber and Lusk [21] indicated that a strategy in which bulls from upper 30% of genetic merit are selected each year would result in increased profitability of \$9.60/head for feeder cattle and \$1.23/head for fed cattle for 20 years. This significant economic benefit and the expected reduction of genotyping costs open the possibility of considering marker assisted selection or management as an actual option to improve meat tenderness.

In conclusion, the frequency of favorable alleles in Calpain genetic markers is low in the five Zebu breeds assessed in this study. Although the indirect improvement of meat tenderness by selection of tenderness segregating favorable alleles in Calpain loci would be limited, favorable alleles could be introgressed in target populations, making of CAPN4751 and CAST-T1 selection, a feasible alternative. Genotyping availability and cost reduction make this strategy a possibility to implement a marker assisted selection program and management in registered Zebu cattle.

### Conflict of interest

The authors declare no conflicts of interest.

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