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Association of single nucleotide polymorphisms in *CAPN1*, *CAST* and *MB* genes with meat color of Brahman and crossbreed cattle

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ABSTRACT

The objective of this research was to determine the association of SNPs in the candidate genes Calpain (*CAPN1*), Calpastatin (*CAST*) and Myoglobin (*MB*) with colorimetric parameters (L *, a *, b *, C *, hue) in a F1 population (n = 164) obtained from crossing *Bos taurus* × *Bos indicus* and *Bos indicus* × *Bos indicus*. SNPs were analyzed using PCR-RFLP and SSCP. Colorimetric measurements were performed in the muscles *Longissimus thoracis et lumborum* (*LTL*) and *Semitendinosus* (*ST*) at 7, 14 and 21 days *postmortem* applying the methodology CIE L* a* b*. The *CAST* gene showed a significant effect on the b* and hue* parameters in both muscles. *MB* gene showed significant association with all colorimetric parameters in both *LTL* and *ST* muscles, except with b* parameter. The *CAPN1* gene did not show any significant association. These results suggest an important role of genetics in meat color variation for cattle raised under the tropic conditions.

1. Introduction

Meat color is a quality trait that consumers use as an indicator of freshness and hygienic meat products. A brown discoloration of meat may indicate deterioration and it usually occurs in meat that has been stored for long periods. The customer's feeling and reaction to a cut of meat is influenced by the perceived color, and in this way, the customer could reject it without considering other possible traits, or accept it, considering other traits, such as tenderness, juiciness and flavor. Both, color and muscle structures are essential to establish a variety of meat categories (Castro Molina, 2013). Based on the color and structure of the meat, an extreme category could be pale, soft and exudative meat (PSE), which is frequently observed in pig meat, but also sometimes seen in the meat of other animal species. Another category is the dark, firm and dry meat (DFD) commonly known as dark meat that usually occurs in beef (Flores et al., 2008).

Meat color is influenced by the interaction of many factors that includes the animal genetics, pre and postmortem environmental conditions, the muscle chemistry and other factors related to meat processing, packaging, distribution, storage conditions, display at the market and finally the preparation for consumption (Mancini and Hunt, 2005).

The identification of genes that influence economically important traits, genetic mapping and the availability of the complete bovine genome sequence, have allowed the identification of variants or DNA polymorphisms associated with differences in the expression of a productive trait (Motter et al., 2009). The genetic background of the animals may significantly contribute to the overall color variation between breeds and within a breed, but the environment component may also contribute to color variation.

In cattle, the genetic influence that may affect meat color has been rarely studied, because of difficulties to obtain and measure the trait *in vivo* without altering the muscle structure. In addition, the potential effects of meat processing-related factors on the final meat color hamper homogenization and standardization of the measuring techniques (Behrends, 2004). Therefore, the importance of having a highly efficient alternative system for identifying molecular markers with additive effect on this trait would constitute a large support in the selection of beef cattle to produce better color meat and more constant over time. Several candidate genes have now been nominated with significant effects on meat quality traits. These include the neutral micromolar protease activated by calcium Calpain (*CAPN1*), which encodes a cysteine protease u-Calpain that degrades myofibrillar proteins in *postmortem* conditions. In the same process, Calpastatin (CAST) appears an inhibiting enzyme of the proteolytic activity of the Calpain. This enzyme complex plays an important role in meat tenderness and indirectly acts on other meat quality traits (W. Barendse et al., 2007; Casas et al., 2006).

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The Myoglobin gene (*MB*) has been mapped to BTA5 (De Donato et al., 2003) and it was selected as a good candidate, because at the molecular level of the meat owes its reddish hue to *MB* activity and its variants (Tang et al., 2005). But to date, there has been very little variability described in the bovine gene. Despite the importance of beef cattle industry in Colombia, the establishment of an animal breeding program for meat quality traits, such as color, is very limited and efforts in this area are aimed at a few researchers and farmers.

Hence the objectives were to identify the effect of single nucleotide polymorphisms (SNP) from the candidate genes Calpain 1 (*CAPN1*), Calpastatin (*CAST*) and Myoglobin (*MB*) on color changes in the muscles *Longissimus thoracis et lumborum* (*LTL*) and *Semitendinosus* (*ST*) of crossbred cattle *Bos indicus* × *Bos indicus* (*BI* × *BI*) and *Bos taurus* × *Bos indicus* (*BT* × *BI*) submitted to three aging times of 7, 14 and 21 days and in this way to contribute to knowledge about the influence of genetics on meat color of crossed beef cattle under conditions of the Colombian tropics.

2. Materials y methods

The present research was carried out with the authorization of Animal Welfare Committee and Bioethics at the Veterinary and Animal Sciences Faculty, National University of Colombia.

2.1. Population

Random matings were scheduled with 35 bulls *Bos indicus* (*BI*): n = 17 and *Bos Taurus* (*BT*): n = 18 mated to 352 Brahman females. The *BI* breeds were Brahman and Guzerat and *BT* breeds were Braunvieh, Limousin, Norman, Simmental, Blanco Orejinegro (BON) and Romosinuano. The straws of these bulls were kept in the ASOCEBU Germplasm Bank and females were inseminated using fixed time artificial insemination (FTAI).

The calves were born and raised in two farms located in the Aguachica municipality, south of Cesar department (Colombia); only males were used in this study. The progenies number per group were *BI* × *BI* = 71 and *BT* × *BI* = 93. Animals were bred and fattened under a rotational grazing system with mineral supplementation and weaning was performed at 8 months old. Once weaned, they were kept in the respective farm until the slaughter time; all males were castrated at 12 months old.

2.2. Muscle sampling and phenotype measurement

Slaughtering took place along the years 2010 and 2011, at the Colombian beef processing plant FRIOGAN located in Dorada town (Caldas department). These animals were slaughtered when they reached an age between 23 to 26 months and weighing about 500 kg. These two conditions allowed generating 5 different slaughter groups. Blood samples were taken before the animals were transported to the meat processing plant, fasting and slaughtered 24 h later. At the slaughter, the *LTL* and *ST* muscles from the left side of the carcass were collected and each muscle was divided into three pieces of 1 kg each and vacuum packed. The muscle samples were sent to the meat processing plant of the Institute of Science and Food Technology (ICTA) at the National University of Colombia, where each piece of muscle was subdivided into three 2.5 cm thick meat pieces, vacuum packed and properly identified. The collection of muscle pieces were randomly assigned into three maturation periods (7, 14, and 21 days), and stored at a temperature between 4 and 5 °C. As aging times were met the portions of each muscle were removed and prepared for obtaining color parameters (Maher et al., 2004).

The colorimetric measurements were carried out using the CIE L* a* b* methodology (CIE, 1976). This procedure is used to describe all the colors that can be perceived by the human eye, where three parameters represented by clarity of color or lightness, L* (L* = 0, or black color and L* = 100, or white color), a color between red and green (red-green) represented by a* (negative values indicate green, while positive values indicate red) and the color between yellow and blue (blue-yellow) represented by b* (negative values indicate blue and positive values indicate yellow). A Minolta colorimeter (Chroma Meter CR 300®, Minolta Camera Co. Ltd., Osaka, Japan) previously calibrated with a white plate supplied by the manufacturer was used to collect the measurements. The standard illuminant D65 and a standard observer 10° were also used. A total of 27 measurements for each parameter (L*, a*, b*) were collected from nine randomly selected positions in each piece of meat avoiding grain and accumulated fat tissue. Mathematical operations were used to calculate color intensity or C* (Chroma) by using the

Table 1
Genetic markers, allelic frequencies, genotyping method used and original citation of each marker.

	Allelic frequency									
Genetic marker	BI × BI group n = 79				BT × BI group n = 85				Genotyping technique	Bibliographic reference
CAPN530	G	0.8	A	0.2	G	0.7	A	0.3	RFLP	Page et al. (2002)
CAPN316	C	0.1	G	0.9	C	0.1	G	0.9	RFLP	Page et al. (2002)
CAPN4751	C	0.1	T	0.9	C	0.2	T	0.8	RFLP	White et al. (2005)
CAPN5331	A	0.3	T	0.7	A	0.4	T	0.6	RFLP	Casas et al. (2005)
CAST1	C	0.7	T	0.3	C	0.6	T	0.4	RFLP	Majidi et al. (2009)
CAST2959	G	0.4	A	0.6	G	0.3	A	0.7	RFLP	Barendse et al. (2007)
CAST2870	A	0.3	G	0.7	A	0.4	G	0.6	RFLP	Corva et al. (2007)
CAST282	C	0.7	G	0.3	C	0.6	G	0.4	RFLP	Schenkel et al. (2006)
MB1041	C	0.9	T	0.1	C	0.9	T	0.1	SSCP	Primers designed by the authors of this article
MB11754[a]	C	0.9	T	0.1	C	0.9	T	0.1	SSCP	
MB11756[a]	T	0.9	C	0.1	T	0.9	C	0.1	SSCP	
MB11759[a]	A	0.9	C	0.1	A	0.9	C	0.1	SSCP	
MB11760[a]	C	0.9	A	0.1	C	0.9	A	0.1	SSCP	
MB11763[a]	C	0.9	T	0.1	C	0.9	T	0.1	SSCP	
MB11764[a]	T	0.9	C	0.1	T	0.9	C	0.1	SSCP	
MB11059[b]	G	0.9	A	0.1	G	0.9	A	0.1	SSCP	
MB11062[b]	A	0.9	C	0.1	A	0.9	C	0.1	SSCP	
MB11069[b]	G	0.9	A	0.1	G	0.9	A	0.1	SSCP	
MB5870	C	0.2	T	0.8	C	0.2	T	0.8	SSCP	
MB11732	T	0.3	C	0.7	T	0.2	C	0.8	SSCP	

Subscript a = markers belonging to group MBG1 (6 different SNPs found in the same PCR fragment), subscript b = markers belonging to group MBG2 (3 different SNPs found in the same PCR fragment). All *MB* markers were designed by the research group.