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# Polymorphisms in Calpastatin and mu-Calpain Genes Are Associated with Beef Iron Content

## Abstract

The objective of this study was to assess the association of markers in the *calpastatin* and *mu-calpain* loci with iron in beef cattle muscle. The population consisted of 259 cross-bred steers from Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford and Angus sires. Total iron and heme iron concentrations were measured. Markers in the *calpastatin* (referred to as *CAST*) and *mu-calpain* (referred to as *CAPN4751*) genes were used to assess their association with iron levels. The mean and standard error for iron and heme iron content in the population was  $35.6 \pm 1.3$   $\mu\text{g}$  and  $27.1 \pm 1.4$   $\mu\text{g}$  respectively. Significant associations ( $P < 0.01$ ) of markers were observed for both iron and heme iron content. For *CAST*, animals with the CC genotype had higher levels of iron and heme iron in longissimus dorsi muscle. For *CAPN4751*, individuals with the TT genotype had higher concentrations of iron and heme iron than did animals with the CC and CT genotypes. Genotypes known to be associated with tougher meat were associated with higher levels of iron concentration.

## Keywords

CAST, Cattle, genetic markers, tenderness

## Disciplines

Agriculture | Animal Sciences | Genetics | Meat Science

## Comments

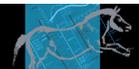
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## Polymorphisms in *calpastatin* and *mu-calpain* genes are associated with beef iron content<sup>1</sup>

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### Summary

The objective of this study was to assess the association of markers in the *calpastatin* and *mu-calpain* loci with iron in beef cattle muscle. The population consisted of 259 cross-bred steers from Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford and Angus sires. Total iron and heme iron concentrations were measured. Markers in the *calpastatin* (referred to as *CAST*) and *mu-calpain* (referred to as *CAPN4751*) genes were used to assess their association with iron levels. The mean and standard error for iron and heme iron content in the population was  $35.6 \pm 1.3 \mu\text{g}$  and  $27.1 \pm 1.4 \mu\text{g}$  respectively. Significant associations ( $P < 0.01$ ) of markers were observed for both iron and heme iron content. For *CAST*, animals with the CC genotype had higher levels of iron and heme iron in longissimus dorsi muscle. For *CAPN4751*, individuals with the TT genotype had higher concentrations of iron and heme iron than did animals with the CC and CT genotypes. Genotypes known to be associated with tougher meat were associated with higher levels of iron concentration.

**Keywords** *CAST*, Cattle, genetic markers, tenderness

Iron is deemed an important cofactor in multiple biological functions. Edison *et al.* (2008) indicate that iron has an important role in formation of hemoglobin and myoglobin. Single nucleotide polymorphisms in the *calpastatin* and *mu-calpain* genes have been associated with differences in meat tenderness (Casas *et al.* 2006). The association of genetic markers with iron concentration in muscle has been studied (Duan *et al.* 2011). The objective of this study was to assess the association of markers the *calpastatin* and *mu-calpain* loci with iron in beef cattle.

Experimental procedures were carried out according to guidelines by the Federation of Animal Science Societies, FASS (1999). The population consisted of 259 steers produced by inseminating Hereford, Angus or MARC III cows (¼ Hereford, ¼ Angus, ¼ Red Poll and ¼ Pinzgauer) with semen from Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford and Angus sires (Wheeler *et al.* 2010).

Animals were fed the same diet and serially slaughtered. Their average age at slaughter was 426 days.

Longissimus dorsi muscle samples were collected and dried (AOAC 2005). Muscle total iron concentration was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Spectro Analytical Instruments). Non-heme iron content was measured according to Rebouche *et al.* (2004). Heme iron concentration was calculated by subtracting non-heme from total iron concentration.

A single nucleotide polymorphism developed at the *calpastatin* gene was reported by Barendse (2002) and will be referred to as *CAST*. The marker used from the *mu-calpain* gene will be referred as *CAPN4751* (White *et al.* 2005).

DNA was extracted from white blood cells (Miller *et al.*, 1988). Genotyping was carried out using a MassArray system (Sequenom, Inc.).

The statistical model was carried out using the mixed procedure of SAS (SAS Institute, Inc.). It included the fixed effects of sire line, dam line, their interaction, year of birth, slaughter group within year of birth, the effect of individual markers and their interaction, and age at weaning as covariate. Sire nested within sire line was included as a random effect. A Bonferroni adjustment was carried out by multiplying *P*-values by a factor of 2.

The mean and standard error for iron and heme iron content in the population was  $35.6 \pm 1.3 \mu\text{g}/\text{kg}$  and

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**Table 1** Name of the single nucleotide polymorphism (SNP), its rs number, number of animals genotyped (*n*), the alleles of each SNP and the minor allele frequency (MAF).

SNP	Rs number	<i>n</i>	Alleles	MAF <sup>1</sup>
CAST	rs109677393	248	C/T	0.17 (C)
CAPN4751	rs17872050	253	T/C	0.37 (T)

<sup>1</sup>Minor allele in parenthesis.

**Table 2** Levels of significance, least squares means and standard errors of the genotype contrasts for the effect of CAST and CAPN4751 on total iron (Fe) and heme iron (HemeFe).

Marker	Fe <sup>1</sup>	HemeFe <sup>1</sup>
CAST		
Significance	0.0134	0.0072
Contrast		
CC – TT	29.1 ± 9.2	30.6 ± 9.0
CT – TT	0.1 ± 3.2	1.3 ± 3.1
CAPN4751		
Significance	0.0006	0.0016
Contrast		
TT – CC	16.4 ± 4.7	15.2 ± 4.7
CT – CC	1.2 ± 3.2	1.2 ± 3.2

<sup>1</sup>Values are expressed as µg/kg of wet weight of longissimus dorsi muscle.

27.1 ± 1.4 µg/kg respectively. Allele frequencies for CAST and CAPN4751 are shown in Table 1. Both markers are in Hardy–Weinberg equilibrium in the population ( $P > 0.05$ ). Animals included were a sample of those used by Casas *et al.* (2006) to assess the association of these markers with meat tenderness. Allele frequencies for CAST and CAPN4751 are similar to those reported by Casas *et al.* (2006), indicating that random sampling was successful and it is likely that results from the present study can be extrapolated to the entire population.

Significant associations ( $P < 0.05$ ) of CAST and CAPN4751 were observed for iron and heme iron content (Table 2). For CAST, animals with the CC genotype had higher levels of iron and heme iron. For CAPN4751, individuals with the TT genotype had higher concentrations of iron and heme iron. These genotypes are known to be associated with tougher meat (Casas *et al.* 2006). This could imply an association between iron levels and tenderness.

An apparent interaction ( $P < 0.01$ ) was observed because one animal with the CC genotype in both markers had the lowest average iron concentration. It must be emphasized that the effect of this combination of genotypes is underestimated, and results should be considered inconclusive. A similar interaction was observed by Casas *et al.* (2006).

Mateescu *et al.* (2013) estimated that iron levels in muscle are highly heritable (heritability = 0.54) in Angus.

Garmyn *et al.* (2011) reported a non-significant phenotypic correlation between iron levels in muscle and Warner–Bratzler Shear Force of  $r = -0.03$ . Mateescu *et al.* (2013) indicate that selection programs for increasing mineral content in beef are possible. It is uncertain if selection based on genomic information for meat tenderness would have an indirect effect on iron levels in muscle.

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