**Association of single nucleotide polymorphisms in the µ-Calpain and Calpastatin gene with proteases activities in Retinta beef cattle: Preliminary results**

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**Introduction**

Tenderness is the most important factor related to consumers' acceptability of beef. Calpain, and it's inhibitor Calpastatin, are proven to have a *post-mortem* effect on tenderness by degrading key muscle proteins during ageing (Costello *et al.*, 2007). Genetic markers make possible to select breeding animals that carry the more positive Calpain and less of the inhibitor Calpastatin enzyme to create a positive effect on beef tenderness in the offspring (Koohmaraie, 1996). Retinto is an Iberian autochthonous breed, classified within the Red Convex Branch of cows in the Iberian Peninsula, and used for meat production. This breed is characterized by its adaptation to adverse environmental conditions and by its good maternal aptitude. That adaptation makes possible to rear Retinto in Southern Spain, where drought and shortage seasons are frequent. Retinto breed meat has been reported to get an improvement of tenderness after long ageing (Sañudo *et al.*, 1998), and the explanation to date was the high solubility of collagen and low connective tissue content. However, the Calpain-Calpastatin system has not been studied in this breed.

The aim of this study was to analyse the frequency of single nucleotide polymorphisms (SNP) found at the µ-Calpain (CAPN1) and Calpastatin (CAST) genes and the association with µ-Calpain, m-Calpain and Calpastatin enzymatic activities in Retinto beef.

**Materials and Methods**

For the present study, twenty three Retinto breed male calves were slaughtered at 14-16 months after birth. Samples from l*ongissimus dorsi* muscle were collected in the abattoir immediately after slaughter and frozen with liquid $N_2$. µ-Calpain, m-Calpain and Calpastatin enzymatic activities were analyzed following an adaptation of the method described by Uytterhaegen *et al.* (1992). Genomic DNA was isolated using a Dominion mbl DNA purification kit. Two DNA fragments belonging to CAPN1 gene and a DNA fragment of CAST gene were amplified by PCR using an Eppendorf EP Gradient Termocycler (Germany). The three pairs of primers were designed according to µ-Calpain and Calpastatin genes sequences (GenBank Accessions: AF 252504, AF248054 and AY008267). PCR products were sequenced at a commercial facility using an ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled with the application Sequencher v. 4. 1. 4.(Genes Codes Corporation) Nine SNPs were found and their allelic frequencies were computed.

**Results and Discussion**

Markers 316 and 530 of the µ-Calpain polypeptide which corresponds with 5709 and 4558 locus (sequences with AF 252504 and AF248054 number in the GenBank Accession) and marker 282 of the Calpastatin gene sequence (Nr. Genbank Accession AY008267) were studied together with six more SNP found in the same regions (non-published data).

Table 1. Retinto breed's SNP genotype frequencies in CAPN1 and CAST genes, and enzymatic activities

| | *Homozygote 1 (Hom1)* | | | | *Heterozygote* | | | | *Homozygote 2( Hom2)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | Frec (%) | µ-c | m-c | ct | Frec (%) | µ-c | m-c | Ct | Frec (%) | µ-c | m-c | ct |
| 5458 | 43.5 | 0.95 | 0.30 | 44.55 | 47.8 | 0.80 | 0.35 | 67.13 | 8.7 | 0.93 | 0.29 | 66.95 |
| 5680 | 40.9 | 0.94 | 0.29 | 44.06 | 50.0 | 0.80 | 0.35 | 67.12 | 9.1 | 0.93 | 0.29 | 66.95 |
| 5688 | 47.4 | 0.94 | 0.29 | 44.06 | 42.1 | 0.84 | 0.34 | 66.57 | 10.5 | 0.93 | 0.29 | 66.95 |
| 5709 | 47.4 | 0.94 | 0.29 | 44.06 | 42.1 | 0.84 | 0.34 | 66.57 | 10.5 | 0.93 | 0.29 | 66.95 |
| 5823 | 47.4 | 0.94 | 0.29 | 44.06 | 42.1 | 0.84 | 0.34 | 66.57 | 10.5 | 0.93 | 0.29 | 66.95 |
| 4506 | 59.0 | 0.86 | 0.34 | 53.89 | 31.8 | 0.86 | 0.33 | 53.54 | 9.1 | 0.97 | 0.25 | 71.23 |
| 4558 | 63.6 | 0.92 | 0.32 | 60.88 | 31.8 | 0.75 | 0.35 | 54.51 | 4.6 | 1.06 | 0.34 | 48.95 |
| 4685 | 57.9 | 0.92 | 0.32 | 58.96 | 31.6 | 0.80 | 0.34 | 60.69 | 10.5 | 0.88 | 0.28 | 47.68 |

Fre: Genotype frequency (%); µ-c: µ-Calpain activity; m-c: m-Calpain activity; ct: Calpastatin inhibition (%)

5458 Hom1:CC. Hom2:TT; 5860 Hom1:CC. Hom2:GG; 5709 Hom1:GG. Hom2:CC; 4558 Hom1:GG. Hom2:AA; 5688 Hom1:GG. Hom2:AA; 5823 Hom1:CC. Hom2:TT; 4506 Hom1:CC. Hom2:GG; 4685 Hom1:CC. Hom2:TT.

Although results showed differences in enzymatic activities, no statistical differences among most of the genotypes for each SNP and the enzymatic activities in the positions 5458, 5680, 5688, 5709 y 5823 were observed (Table 1). That can be due to the lack of linkage disequilibrium between the SNPs found in the µ-Calpain gene, since it is in a 500 pb region. The other three SNPs did not show differences, although

frequencies and enzymatic activities were not so similar. The study of the three SNPs reported by other authors had no clear results. Page *et al*, (2004) and White *et al*. (2005), analyzing others breeds (Angus, Charolais, Gelbvieh, Hereford, Limousin, Red Angus, Simmnental and Brahman), and C and G haplotypes (5706 and 4558), reported a link between those and a higher tenderness in meat and, therefore, higher enzymatic activity.

Figure 1 shows how CG haplotype had an enzymatic activity of 0.89 for µ-Calpain, 0.32 for m-Calpain and 71.61% of inhibition for Calpastatin. Schenkel *et al*. (2006) reported that the most favourable allele for CAST was C. Figure 2 shows the activities for each allele, and C allele had an activity of 0.87 for µ-Calpain, 0.33 for m-Calpain and a Calpastatin inhibition of 57.34%.

Further research must be done with a bigger number of Retinto breed animals to confirm the effect of the haplotypes on enzymatic activities, and its relation with tenderness assessed by sensory and texture analysis.

Figure 1. Calpain and Calpastatin enzymatic activity for haplotypes found in 5709 and 4558 positions in the gene of µ-Calpain in Retinto breed

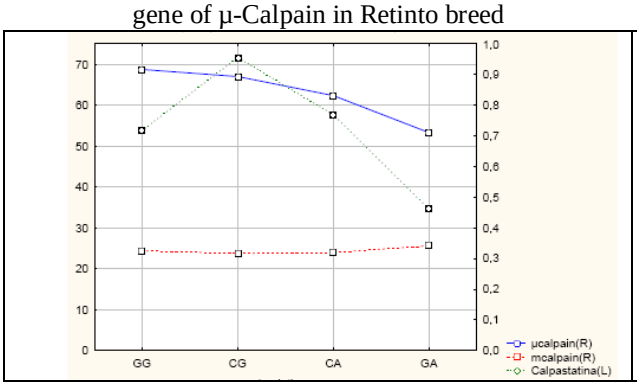
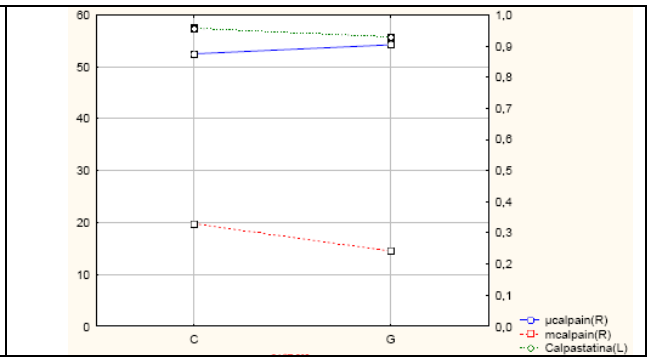



Figure 2. Calpain and Calpastatin enzymatic activity for SNP found in 282 positions in the gene of Calpastatin in Retinto breed




**Conclusions**

Results found for eight SNP in m Calpain gene in Retinto meat and µ-Calpain, m-Calpain and Calpastatin enzymatic activities were not conclusive. New studies must determine if any found SNP has an effect different to those reported for other breeds. With respects to 5680, 5688, 5823 5902 SNPs, from the first CAPN1 fragment, and the 4506 position SNP from the second fragment, found for first time in Retinto, no differences were observed. Enzymatic activities must be related, in future studies, to sensory and texture analyses.

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