Effect of ageing and μ-calpain markers on meat quality from Brangus steers finished on pasture

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Abstract

Brangus steers (n=247) finished on pasture were used to evaluate the effects of post-mortem ageing and polymorphism CAPN1 316 and CAPN1 4751 markers on meat tenderness and objective colour measurements (CIEL*a*b*) of m. Longissimus dorsi. Ageing meat for 7 days decreased shear force (SF) by 13.7% and improved a* (8.4%) and b* (10%) compared to ageing for 1 day. No difference between 7 and 14 days of ageing was found for SF, a* and b*. However, L* increased markedly with ageing. Fitting both markers simultaneously, CAPN1 316 showed association with SF and L* and CAPN1 4751 with a* and b*. Fitting the markers individually, CAPN1 4751 affected all traits and CAPN1 316 showed association with SF and L*. Post-mortem ageing and the use of markers represent two independent and alternative tools that could be used for improving quality of meat from Brangus cattle.

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1. Introduction

Beef cattle production systems in Argentina are traditionally based on pasture. As a result of the expansion of the agriculture, beef cattle are being displaced to tropical and subtropical regions, and British breeds are being replaced by other genotypes, such as composites between Bos indicus and Bos taurus breeds. It is well known that meat from these cattle may show undesirable acceptability traits thus reducing its market value. Several researches have reported that shear force (SF) of meat increased as the percentage of B. indicus inheritance increased in crossbreeds (Crouse, Cundiff, Koch, Kooymaraie, & Seideman, 1989; Gallinger, Marcelia, Gonzalez, & Lasta, 1993; Gonzalez, Pazos, Salito, Garcia, & Lasta, 2003; Pringle, Williams, Lamb, Johnson, & West, 1997; Shackelford, Wheeler, & Kooymaraie, 1995). Post-mortem strategies such as ageing, electrical stimulation or alternate methods of carcass suspension (Kooymaraie, 1996; Riley et al., 2005; Thompson, 2002; White, O’Sullivan, Troy, & O’Neill, 2006) may be effective for improving tenderness. One of the most important proteolytic enzymes involved in post-mortem tenderization is the μ-calpain (Kooymaraie, 1996). This enzyme is expressed by the gene known as CAPN1 located in chromosome 29 (Smith, Casas, Rexroad, Kappes, & Keele, 2000). Single Nucleotide Polymorphisms (SNP) of this gene, such as CAPN1 316 and CAPN1 4751, have been reported to be associated with meat tenderness (Page et al., 2002, 2004; White et al., 2005). However, there are no reports on the effects of these markers on meat colour. Wulf, O’Connor, Tatum, and Smith (1997) reported a negative correlation between L* and b* with SF so, if an effect of CAPN1 316 and CAPN1 4751 is expected on SF an indirect effect of these SNP on colour would also be expected.

Most of the published experiments have been conducted with steers finished in feedlot, and there is limited scientific literature available on the palatability traits of the Brahman-derivative breeds finished on pasture. Therefore, the objective of this study was to evaluate the effects of post-mortem ageing treatment and of CAPN1 316 and CAPN1 4751 on meat tenderness and colour of Brangus steers finished on pasture.

2. Materials and methods

2.1. Animal resources and phenotypic information

Data from 247 Brangus steers coming from commercial herds located at the Central and Northern regions of Argentina and from two productive cycles were used. In the first cycle (n=60), the post-weaning phase was carried out in the Balcarce Experimental Station of the National Institute of Agricultural Technology (INTA) from April...
2004 to June 2005. Steers grazed a cultivated pasture of Lolium multiflorum, Dactylis glomerata, Bromus catharticus, Trifolium repens and Trifolium pratense having two high forage production seasons: fall and spring. In the second cycle (n=187), the post-weaning phase was carried out in the Experimental Farm of the University of Buenos Aires located in Carlos Casares between May 2005 and July 2006. Steers grazed a pasture of Medicago sativa, Trifolium pratense, Bromus catharticus, Dactylis glomerata and Phalaris bulboa, having a rather uniform forage production along the year with a slight decay in winter. All animals were weighed every month. Final body weight and ultrasound backfat thickness were recorded before slaughter.

Steers from both cycles were progressively sent to a private abattoir in Balcarce as they reached an ultrasound backfat thickness of at least 6 mm between the 12th and 13th ribs. There were three slaughter groups in the first cycle and six groups in the second. Average daily gain was 0.689 ± 0.015 kg/day for the first cycle and 0.541 ± 0.070 kg/day for the second. At the time of slaughter, mean live body weights were 414 ± 5.33 kg and 457 ± 3.56 kg, and backfat thickness was 6.01 ± 0.15 mm and 6.79 ± 0.12 mm for cycles 1 and 2, respectively. Animals were slaughtered following SENASA (National Service for Animal Health) regulations, after being kept for 24 h in paddocks deprived of feed but with full access to water.

2.2. Markers and genotyping

Two SNP, CAPN 316 (Page et al., 2002) and CAPN 4751 (White et al., 2005), previously associated with tenderness were evaluated.

ADN was isolated from 500 μl blood samples per animal. CAPN 316 is a cytosine/guanine (C/G) substitution in exon 9 of CAPN gene of chromosome BTA 29 (Page et al., 2002). CAPN 4751 is a cytosine/timine (C/T) substitution in intron 17 of the same gene. PCR/RFLP procedures were used to determine genotypes of both SNP. Primers were selected from published DNA sequences (GenBank accession numbers AF252504 and AF248054). For CAPN 316, a fragment of 709 bp was amplified with the primers CACGGCCCAGATGGTGAA (forward) and CCGGGCTGTCAGGTTGC (reverse) digested with BglII (New England Biolabs, Beverly, MA). For CAPN 4751, a fragment of 215 bp was amplified with the primers GAAGGGCTGGTGGGGAATGCTGGCAGAG (forward) and AGGCTGGGAGGGGTGTTCTCTTGAGTGCAGAG (reverse) digested with BsaI. Genotypes were determined on 1.6% agarose gel stained with ethidium bromide.

2.3. Meat sampling and physical determinations

After slaughter, the carcasses were suspended through the Achilles tendon and were chilled for 24 h. A joint including the Longissimus dorsi muscle (LD) between 11th and 13th ribs was removed from the left half carcass. Each block was deboned, sliced into three steaks and vacuum packaged. Steaks were randomly distributed between three ageing treatments: 1, 7 and 14 days. One day samples were immediately frozen and the rest were kept at 2 to 5 °C for 7 or 14 days, and then frozen and stored at −18 °C until they were thawed for study. Analytical determinations were performed at the University of Buenos Aires Meat Laboratory. Samples were thawed for 24 h at room temperature prior to cooking and all external fat and adjacent muscles were removed leaving only the LD. Thawed steaks of LD (about 2.5 cm thick) were placed in plastic bags, immersed in a water bath and heated for 50 min to an internal temperature of 70 °C. The cooked steaks were chilled under running cold water for 40 min; the bags were then drained and the cuts were gently blotted dry with a paper towel. Four 2.5 cm diameter cores were removed from each steak parallel to the muscle fibres and sheared at their mid-point using a 50 kg compression load cell and a Warner-Bratzler V-notch blade mounted on an Instron model 4442 testing machine (Canton, MA, USA) at a crosshead speed of 50 mm/min. The shear force (SF) was recorded as peak force and the value reported for each steak was the average value for all evaluated cores. Cooking loss was expressed as the percentage weight loss related to the initial weight of the steak.

Meat colour was measured on the exposed LD muscle in the L*, a*, and b* system (L*: lightness a*: redness; and b*: yellowness; CIE, 1976) using a Minolta colorimeter (Chroma Meter CR-300, Minolta Camera Co., Ltd., Osaka, Japan) previously calibrated against a white plate supplied by the manufacturer. The colorimeter has an 8 mm diameter measurement area and uses a light source of D65 and 0° standard observer. Determinations were done in 2.5 cm thick steaks after blooming for 1 h at 4 °C. The colour of the fat-free surface of each sample was assessed using the mean value of three colour determinations.

2.4. Statistical analysis

Two analyses were conducted. Analysis I considered the fixed effects of cycle, herd of origin within cycle, ageing time, molecular marker (CAPN 316 and CAPN 4751) and the interactions between each molecular marker and ageing time. In Analysis II the effect of each marker was individually analyzed with a model containing the same effects considered in Analysis I. In both analyses, cooking losses and the days that samples remained frozen were included as covariables. Pedigree information was available for only a few of the herd of origin so it was not included in the models. All statistical analyses were carried out using PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, 1998). An UNR (Unstructured using Correlations) covariance matrix was used to define the covariance structure of the error term, and the animal was used to define the effect whose levels identify observations belonging to the same subject. Observations from different subjects are independent. For statistically significant main effects (P<0.05), least squares means were reported and Bonferroni’s means separation test at P<0.05 was used to determine differences between them.

Partial correlations from the Error Sums of Squares and Cross Products Matrix (ESSCPM) of SF with muscle colour coordinates were calculated. The multivariate analysis of variance from which this Matrix was obtained included the same effects fitted for Analysis I.

3. Results and discussion

3.1. Ageing effect on SF

Least squares means of SF for the three ageing period are presented in Table 1. Extending the post-mortem ageing period from 1 to 7 days decreased significantly shear force values by 13.7% (P<0.05), although between 7 and 14 days no significant difference was detected (P>0.05). Gallinger et al. (1993) and Gonzalez et al. (2003) found a decrease in SF of 18.1% and 26.0%, respectively, between 1 and 7 days of ageing in Brangus cattle. However, our rates were lower than those reported by these authors. Johnson, Huffman, Williams, and Hargrove (1990) found that loin steaks from 3/4 Angus/1/4 Brahman decreased in shear values by 27%, and the steaks from the 1/2 Angus/1/2 Brahman and 1/4 Angus/3/4 Brahman decreased in shear value by only 9.4% and 16.7% during the 10 days post-mortem ageing period, respectively. These authors

### Table 1

<table>
<thead>
<tr>
<th>Traits</th>
<th>Ageing time (days)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>SF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73.20 ± 1.167a</td>
</tr>
<tr>
<td>L*</td>
<td>37.78 ± 0.213a</td>
</tr>
<tr>
<td>a*</td>
<td>19.98 ± 0.206a</td>
</tr>
<tr>
<td>b*</td>
<td>11.19 ± 0.136b</td>
</tr>
</tbody>
</table>

a,b: Means with different letters in the same row are significantly different (P<0.05).
suggested that this differential response to post-mortem ageing was due to the difference in tenderizing enzymes present in the muscle.

The tenderization through ageing involves several aspects that affect myofibrillar fragmentation, including animal characteristics (i.e. breed, age, stress, backfat thickness, and glycogen reserves), pH and pre-rigor conditioning, among others, and it is well documented that SF declines with longer ageing time (Shackelford, Koohmaraie, Miller, Crouse, & Reagan, 1991; Wheeler, Cundiff, Shackelford, & Koohmaraie, 2001). Despite that observed figures for SF were higher than the usual values observed in breeds like we used in this study, it is possible to consider that pre- and post-mortem conditions did not affect the obtained results, since all the animals were handled the same way with the previous slaughter. Likewise, samples taken to the laboratory to be analyzed for quality traits were treated alike. Besides, the pH of meat in this study, measured on the LD samples, was 5.55 ± 0.01 for cycle I and 5.53 ± 0.01 for cycle II (data not shown). These values were inside the range considered as normal for the bovine meat (Foegeding, Lanier, & Hultin, 1996), indicating that post-mortem pH decline proceeded normally.

3.2. Ageing effect on colour

Ageing was a significant effect on L*, a* and b* (Table 1). Ageing for 7 days was enough for improving a* (8.4%) and b* (10%) compared to the samples aged for 1 day. No difference between 7 and 14 days of ageing was found for a* and b* (P > 0.05) whereas L* increased markedly with ageing (P < 0.05). The increase in L* with ageing could be due to modifications of the protein structures giving a higher dispersion of light (Beriaín, Goñi, Indurain, Sarries, & Insauti, 2009). Similar results were observed by Boakye and Mittal (1996) for L*. These authors found a gradual increase of L* from day 0 to day 16, although for a* and b* they did not detect significant differences until 12 days of ageing. In contrast, Oliete, Moreno, Carballo, Mon serrat, and Sanchez (2006) and Beriaín et al. (2009) reported that the colour coordinates L* showed no significant effect of ageing, remaining constant until days 21 and 14, respectively, but they did observe that the values of a* and b* increased with ageing time, although differences were significant only when comparing 7, 14 or 21 days with 1 day of ageing. The decrease in activity of the oxygen-utilizing enzymes during storage might have allowed a greater blooming (Boakye & Mittal, 1996) because the oxygen is more available, and therefore a* value increased. The increase in b* could be due to the fact that meat surface has undergone some oxidation so there are larger amounts of metmyoglobin (Oliete et al., 2006). Feldhusen, Warnatz, Erdmann, and Wenzel (1995) concluded that physical measures of colour would increase during the first 5 days of vacuum storing, and that at longer periods of time such values would not almost be affected; these results were later confirmed by Insauti et al. (1999). The results of the present study for instrumental meat colour parameters were similar for L* and a* and higher for b* than those obtained in meat from Criollo Argentino and Braford steers finished on pasture in a subtropical region of Argentina (Orellana et al., 2009).

3.3. Allele frequencies of the SNP

The frequencies of the unfavorable alleles for tenderness, G for CAPN 316 and T for CAPN 4751, were 0.73 and 0.49, respectively, in this sample. In Brahman steers Smith, Thomas, Bidner, Paschal, and Franke (2009) found much higher frequencies for alleles G and T, 0.97 and 0.95, respectively. However, in a B. taurus–B. indicus crossbred population White et al. (2005) found frequencies for allele G and T similar to our results (0.78 and 0.36 for the CAPN 316 and CAPN 4751, respectively), and Van Eenennaam et al. (2007) found frequencies of 0.82 for G and 0.45 for T, in a Brangus population. Table 2 shows the distribution of animals according to genotype of markers.

### Table 2

**Distribution of animals according to genotype.**

<table>
<thead>
<tr>
<th></th>
<th>CAPN 316</th>
<th>CAPN 4751</th>
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<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>CC</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td>GG</td>
<td>14</td>
<td>62</td>
</tr>
</tbody>
</table>

3.4. Marker effect on SF

In Analysis I, SF was affected only by CAPN 316 (Table 3). CAPN 4751 did not show a significant effect on SF probably because TT was confounded with GG, except for 2 animals (Table 2).

Genotype GG for CAPN 316 was 5.37% higher in SF than CG (P < 0.05) but did not differ from CC (P > 0.05), thus animals with the CG genotypes produced more tender meat when compared to animals with the GG genotype. The unexpected lack of significance of the difference between both homozygous was probably due to the high standard error of the CC group. In a population that included B. indicus influenced crossbred, White et al. (2005) found that both markers had highly significant effects when fitted simultaneously in the analysis. However, in a population of B. taurus these authors found that CAPN 4751 continued to have a significant effect but CAPN 316 did not. Therefore they recommended to use a multiplex genotyping array incorporating both markers because it retains the strengths of both, and may be the most useful approach.

There were no significant interactions (P > 0.05) between each molecular marker and ageing treatment. However, some tendencies can be observed (Table 4): differences in SF tended to be larger between 1 and 7 days of ageing than those between 7 and 14 days for all genotypes of CAPN 316. CAPN 4751 means showed a tendency to be tougher as the number of T allele increased in the genotype when meat was aged for one day but means of genotypes did not follow a definite pattern when ageing time was 7 or 14 days.

The effect of CAPN 316 in Analysis II showed the same trend between genotypes than in Analysis I (Table 5). The exception was that the previously nonsignificant difference between SF adjusted means of CC and GG became significant (P < 0.05). Genotype GG showed 5.72% and 8.65% higher SF than CC and CG, respectively. Differences between genotypes were detected for the SNP CAPN 4751 for SF (Table 6). The presence of allele T increased SF in 4.02% and 6.44% in CT and TT, respectively (P < 0.05) relative to White et al. (2005) who reported a difference of 0.4 kg (3.92 N) in SF between CT and TT genotypes of CAPN 4751, pointing this out as an excellent marker for the functional variation affecting tenderness of Brahman meat.

Casas et al. (2005) and Van Eenennaam et al. (2007) reported that G allele of CAPN 316 is almost fixed in Brahman (allele frequencies of 99% and 98%, respectively). In the same way, White et al. (2005), Van

### Table 3

Least squares means and standard errors for shear force (SF), lightness (L*), redness (a*) and yellowness (b*) obtained in Analysis I.

<table>
<thead>
<tr>
<th></th>
<th>SF (N)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
</table>

**CAPN 316**

| G     | 64.84 ± 1.818<sup>a</sup> | 39.52 ± 0.332<sup>b</sup> | 21.21 ± 0.321<sup>c</sup> | 12.06 ± 0.211<sup>d</sup> |
| CG    | 65.68 ± 0.857<sup>a</sup>  | 39.30 ± 0.156<sup>b</sup> | 21.28 ± 0.151<sup>c</sup> | 12.04 ± 0.100<sup>d</sup> |
| GG    | 69.19 ± 0.677<sup>a</sup>  | 38.43 ± 0.123<sup>b</sup> | 21.13 ± 0.119<sup>c</sup> | 11.86 ± 0.079<sup>d</sup> |

**CAPN 4751**

| G     | 65.70 ± 0.875<sup>a</sup> | 38.92 ± 0.160<sup>b</sup> | 20.93 ± 0.154<sup>c</sup> | 11.79 ± 0.102<sup>d</sup> |
| CG    | 67.12 ± 0.914<sup>a</sup>  | 39.33 ± 0.167<sup>b</sup> | 21.44 ± 0.161<sup>c</sup> | 12.17 ± 0.106<sup>d</sup> |
| TT    | 66.90 ± 1.328<sup>a</sup>  | 39.03 ± 0.242<sup>b</sup> | 21.24 ± 0.234<sup>c</sup> | 12.00 ± 0.154<sup>d</sup> |

<sup>a</sup>Within genetic marker, means with different letters in the same column are significantly different (P < 0.05).

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Enennaam et al. (2007), and Smith et al. (2009) reported very high frequencies of T allele in Brahman (90%, 94% and 95%, respectively) so in Brangus it is probable that both alleles come from Brahman being both linked in chromosome 29 (Van Enenmann et al., 2007). B. taurus individuals have both alleles in similar proportions (Page et al., 2004; White et al., 2005). In case of selecting Brangus against both alleles, it is probable that the selected population would have animals with the chromosome 29 coming from Angus.

3.5. Marker effect on colour

In the analysis with both markers fitted simultaneously, effects of CAPN1 316 on L* and CAPN1 4751 on a* and b* (Table 3) were found. For CAPN1 316 genotype GG presented less brightness than GC and CC (2.41% and 3.24%, respectively, P < 0.05). Genotype TT was not significantly correlated (P > 0.16, r = 0.12 and r = 0.05; Table 4) with all three markers studied.

Table 5

<table>
<thead>
<tr>
<th>n</th>
<th>Traits</th>
<th>SF (N)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPN1 316</td>
<td>CC 18 63.93 ± 1.599a 39.37 ± 0.292a 20.93 ± 0.283a 11.86 ± 0.180b</td>
<td>11.86 ± 0.180b</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>CC 97 65.70 ± 0.733a 39.41 ± 0.134a 21.33 ± 0.130a 12.09 ± 0.086a</td>
<td>12.09 ± 0.086a</td>
<td></td>
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<tr>
<td></td>
<td>CC 132 69.46 ± 0.630a 38.49 ± 0.116a 21.22 ± 0.112a 11.93 ± 0.074a</td>
<td>11.93 ± 0.074a</td>
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</table>

4. Conclusions

The results obtained in the study indicate the possibility of improving tenderness and colour parameters of meat from breeds with B. indicus contribution assuring a period of 7 days of ageing. The use of breeding animals carrying favourable alleles for the SNP CAPN1 316 and CAPN1 4751 might improve tenderness of meat of a composite breed such as Brangus through mating strategies aiming to increase the frequencies of the favourable alleles in both SNP. However, in establishing selection programmes assisted by these markers, a strict control of productive traits is needed in order to avoid modifying convenient combinations of other genes influencing productive performance. Our results indicated that tenderness is associated with meat colour, but the association with the SNP studied in this paper was not consistent for a* and b* coordinates with previous results.

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