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Genetic and management factors affecting beef quality in grazing Hereford steers

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ABSTRACT

Attributes contributing to differences in beef quality of 206 Hereford steers finished on pasture were assessed. Beef quality traits evaluated were: Warner–Bratzler meat tenderness and muscle and fat color at one and seven days after slaughter and trained sensory panel traits (tenderness, juiciness, flavor, and marbling) at seven days. Molecular markers were CAPN1 316 and an SNP in exon 2 on the leptin gene (E2FB). Average daily live weight gain, ultrasound monthly backfat thickness gain and rib-eye area gain were estimated. Molecular markers effects on meat quality traits were analyzed by mixed models. Association of meat quality with post weaning growth traits was analyzed by canonical correlations. Muscle color and marbling were affected by CAPN1 316 and E2FB and Warner–Bratzler meat tenderness by the former. The results confirm that marker assisted selection for tenderness is advisable only when beef aging is a common practice. The most important sources of variation in tenderness and color of meat remained unaccounted for.

1. Introduction

One of the objectives of beef producers is to offer a product that fulfills the requirement of high quality by consumers. There are several factors affecting the final quality of beef. Genetic variation in quality attributes, among and within breeds, has been well documented (Marshall, 1999). Aside from the genetic background of the animals other non genetic factors, especially feeding regime, highly influence meat quality. Pre-slaughter handling and slaughter and processing procedures (Belk, Scanga, Smith, & Grandin, 2002) also play a very important role in defining the final quality of carcasses. Most reported experiments evaluating the effect of these factors on meat quality apply to feedlot cattle (Eilers, Tatum, Morgan, & Smith, 1996; Jiang et al., 2010; Monsón, Sañudo, & Sierra, 2005; Wheeler, Savell, Cross, Lunt, & Smith, 1990). However, there could be a differential response of beef quality traits to those same factors in more extensive systems. A good example are grazing systems, which are recognized for producing beef with less fat and with beneficial properties for human health, when compared to more intensive production systems (Wood et al., 2003).

Beef color and marbling mostly define the preferences of beef buyers. Meat color, including fat color, is usually associated with freshness and quality, but it has also been associated with tenderness (Wulf, O'Connor, Tatum, & Smith, 1997). Furthermore, color could be the main single factor used by consumers to determine whether they will purchase a meat cut (Kropf, 1980). On the other hand, tenderness, juiciness, connective tissue content and flavor of meat are quality attributes evaluated at the time of consumption which is between 1 and 5 days after slaughter in Argentina. Even though being subjective sensations, sensory panels and objective measurements provide objective information to establish the influence of the different factors. According to Caine, Aalhus, Best, Dugan, and Jeremiah (2003), the mean correlations of WBSF with sensory assessment of beef tenderness are in the range of −0.75 to −0.77, but the variability across experiments is high.

The discovery of molecular markers, accounting for a significant proportion of additive genetic variance in economic traits, provided an additional tool to animal breeders. Traits like tenderness and meat color, which are difficult to measure under commercial conditions or previous to slaughter, have become of special interest for researchers due to the possibility of identifying molecular markers that would be used as an aid to selection (Van der Werf & Kinghorn, 1999). In live animals, calpains participate in the protein breakdown, and after slaughter are responsible for the maturation process. Single

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nucleotide polymorphisms (SNPs) on the μ-calpain gene (CAPN1) have been associated with meat tenderness (Page et al., 2004), but also with different growth traits (Miquel et al., 2009). Leptin is involved in the regulation of energy balance. Plasma concentration and molecular markers on the gene have also been associated with several growth and carcass traits that could have an influence on beef quality (Altmann & Von Borell, 2007; Schenkel et al., 2005). However, Johnston and Graser (2010) observed that markers should be evaluated in the populations that they are intended to be used. The objective of this research was to identify attributes of the animal that contribute to differences in beef quality for Hereford cattle finished on pastures.

2. Materials and methods

2.1. Animals and phenotypic information

Animal handling and experimental procedures were in accordance with the Handbook of Procedures for Animal Welfare of the National Service of Animal Health (*Servicio Nacional de Sanidad Animal*, SENASA) of Argentina. The study was conducted on 206 Hereford steers that were fattened on perennial, fertilized pastures. The experiment started in April, 2006 when the steers were 8 to 10 months old, and it ended in October, 2008. All the steers were kept and fed in the same field throughout the whole period of the trial. Pasture was a mix of different types of legumes and grasses, including alfalfa, white clover, perennial ray grass, fescue and orchard grass. The steers were weighed monthly, and scanned by utrasound for backfat thickness and rib-eye area over the 12th–13th rib interval, every 3 months. The experiment was planned in order to slaughter the steers by the end of fall (June) 2008, with at least 6 mm of backfat thickness. Several unexpected complications, including a severe drought, made it impossible to reach that target end point. Therefore, from May 2008 on the frequency of ultrasound measurements was increased (monthly) in order to slaughter steers as soon as they reached the specified backfat thickness. By October 2008 it was decided to finish the experiment, sending to slaughter only those steers with known sire that carried the least frequent marker genotypes and were closest to the target backfat thickness. In this way, slaughter took place on five different occasions between March and October 2008. In total, 162 steers of known age, with complete carcass evaluation and sired by 15 bulls were slaughtered (Table 1).

2.2. Molecular analyses

One SNP on the CAPN1 gene (CAPN1 316) and another on the Leptin gene (E2FB) were analyzed. These two SNPs were the first commercial markers for beef quality traits (tenderness and body fatness, respectively). The CAPN1 316 is a G/C SNP in exon 9 of CAPN1 gene (Page et al., 2002) and E2FB is a C/T transition in exon 2 of the Leptin gene (Buchanan et al., 2002). DNA was extracted from 300 μl of blood using Illustra GFX™ Genomic Blood DNA Purification Kit (GE Healthcare, Buckinghamshire, UK). Marker CAPN1 316 was analyzed by PCR-RFLP method using the following primers: 5′-ccagggccagatgg tgaa-3′ (forward) and 5′-cgtcgggtgtcaggttgc-3′(reverse) and BtgI restriction enzyme. Marker E2FB was genotyped with tetra-primer ARMS-PCR method (Ye, Dhillon, Ke, Collins, & Day, 2001) using two sets of primer pairs (outer and inner primers). The outer primer pair was: 5′-gacgatgtgccacgtgtggtttcttctgt-3′ (forward) and 5′-cggttctacct cgtctcccagtccctcc-3′ (reverse). The inner primer pair was: 5′-tgtcttac gtggaggctgtgcccagct-3′ (forward) and 5′-agggttttggtgtcatcctggacctttc g-3′ (reverse).

Among the 15 bulls identified as sires of steers, there were 1 CC, 2 CG and 12 GG, according to their CAPN1 316 genotypes; and 2 CC, 7 CT and 6 TT sires according to the E2FB marker.

Genotypic frequencies of the initial sample of steers (n = 206) for CAPN1 316 were 1, 13 and 86% for CC, CG and GG, respectively. Genotypic frequencies for E2FB were 7, 40 and 53% for CC, CT and TT, respectively.

For the sample of slaughtered steers (n = 162) genotypic frequencies were 1 CC, 16 CG and 83 GG (CAPN1 316 marker) and 9 CC, 42 CT and 49 TT (E2FB marker).

2.3. Meat sampling and physical determinations

Steers were slaughtered at a private abattoir after resting for 24 h in paddocks with available water, according to SENASA regulations. At slaughter, left carcass sides were electro stimulated applying 21 V and 0.25 A during 5 s and placed in a chiller at 1–5 °C for 24 h. Carcass pH and temperature were measured at 1, 3, 6, 9 and 24 h post-mortem in the *longissimus* muscle, at a point located over the interval between ribs 12th and 13th. A block of steaks corresponding to the 8th to 13th ribs was removed from each left half carcass. The block was divided into two pieces that were vacuum-packed. The fraction between 8th and 10th ribs was frozen at -18 ± 1 °C (1 day aging treatment) and the rest was aged for 7 days at 3 ± 1 °C (7 day aging treatment), and then frozen at -18 ± 1 °C. Prior to being thawed, each block was subsampled using an electric saw in steaks of 2.5 cm width, vacuum packaged and kept at −18 °C.

The following analytical determinations in meat samples were performed at the *Instituto de Tecnología de Alimentos*, *Instituto Nacional de Tecnología Agropecuaria* (INTA) in Castelar, Buenos Aires.

2.4. Warner–Bratzler shear force (WBSF)

WBSF was measured in steaks corresponding to the 10th and 13th ribs. Once thawed, under refrigerated conditions (4–7 °C), they were boned, weighed and placed in a pre-heated shell style electric grill for 10 min, until internal temperature reached 71 °C (AMSA, 1995). Cooked steaks were weighed and cooled to <10 °C. Eight 1.3 cm-diameter cores were extracted from each steak parallel to the muscle fiber orientation

Table 1
Means and standard errors for final weight (FW), average daily gain (ADG), final backfat thickness (BFT), average monthly backfat thickness gain (AMBFTG), final rib-eye area (REA), average monthly rib-eye area gain (AMREAG) and age at slaughter (AGE) for each slaughter group (SG).

Trait	Slaughter group (date of slaughter) (n)									
	SG1 (04/17) (37)		SG2 (07/03) (34)		SG3 (07/10)(34)		SG4 (09/11)(33)		SG5 (10/30)(24)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
FW (kg)	447	27.4	514	37.99	520	42.91	513	42.54	560	51.84
ADG (g/day)	697	51	717	52	727	59	648	51	645	62
BFT (mm)	6.74	0.87	6.58	0.69	6.92	0.89	5.78	1.08	5.95	1.50
AMBFTG (mm/30 days)	0.321	0.081	0.270	0.051	0.289	0.055	0.205	0.048	0.171	0.043
REA (cm^2)	56.66	6.93	56.46	6.39	57.73	6.73	58.65	7.17	58.84	6.64
AMREAG (cm^2/30 days)	1.78	0.48	1.70	0.34	1.86	0.32	1.53	0.34	1.47	0.32
AGE (day)	596	13.9	669	14.7	679	14.6	740	14.1	784	17.4

and sheared once across the middle using a Warner–Bratzler shear machine (model 3000; G-R Manufacturing CO., Manhattan, Kansas, USA).

2.5. Muscle and fat color

Color and pH were determined in steaks from the 8th and 11th ribs on 1 day and 7 day aged samples respectively. Color was measured with a portable reflectance spectrocolorimeter BYK Gardner 45° Gloss following the guidelines of AMSA (1991) with 10° observer and illuminant D65. Before measurement, a blooming time of 45 min at 5 ± 1 °C was allowed for an adequate color development. Results were expressed according to the CIELab system. Muscle pH and temperature were measured between the 10th and 11th rib using a Testo pH meter (model number 205, Testo, Ciudad Autónoma de Buenos Aires, BA, Argentina) equipped with a glass pH electrode and a temperature probe, with 4.0 and 7.0 buffers for calibration.

2.6. Sensory panel analysis

Laboratory facilities allowed the analysis of about 100 samples. So, analyses were performed on 7 day aged meat coming from steers in every slaughter groups, trying to include animals carrying the least frequent genotypes for both genetic markers and sired by bulls having progeny sizes as large as possible as to provide reliable results. Thus, 114 steers were chosen for these analyses. Number of steers in each genotype class within marker is shown in Table 3. Steaks were obtained from the 12th rib, and then they were thawed, deboned and cooked in the same way as described for WBSF. After cooking, each steak was deboned and trimmed of fat and the *longissimus* muscle was sliced into 1 cm × 1 cm cooked steak thickness cubes that were immediately served to an eight-member trained sensory panel. Each panel member (Cross, Moen, & Stanfield, 1978) evaluated two cubes taken at random from each steak in a cabin built according to ISO 8589:1998 under green light. Each member was provided with an evaluation form, a salt-free cracker and a glass of distilled water for rinsing. The samples were evaluated using a nine-point scale for juiciness, initial and sustained tenderness, beef flavor intensity and amount of connective tissue (1 = extremely dry, extremely tough, extremely bland and very much to 9 = extremely juicy, extremely tender, extremely intense and nothing respectively). Raw slices of *longissimuss dorsi* muscle were compared with USDA scales for Marbling by a panel of 5 members (1 = traces to 8 = abundant; Romans, Jones, Costello, & Carlson, 1985).

2.7. Statistical analyses

Monthly weight and ultrasound measurements were used to calculate individual average daily gain (ADG), average monthly backfat thickness gain (AMBFTG) and average monthly rib-eye area gain (AMREAG) by regression. Final weight (FW), backfat thickness (BFT) and rib-eye area (REA) correspond to the last recorded measurement of each trait before slaughter. Traits were analyzed by mixed models, using PROC MIXED of SAS (1998). Fixed effects and covariates included in the models differed according to the trait. Preliminary analyses showed that neither BFT, FW nor age at slaughter (AGE) had significant effects on the variables studied. An analysis of another data set (Papaleo Mazzucco et al., 2010) has suggested a possible interaction between genotype and length of aging period for WBSF. In the present paper, the inclusion of a Genotype × AT interaction term in the WBSF model of analysis would have produced an unbalanced design matrix, given the low numbers in some genotype groups. Hence, it was decided to analyze separately the WBSF 1 day and WBSF 7 day data sets, which was not the case for muscle and fat color. Included fixed effects for the analysis of WBSF in 1 day and in 7 day aged samples were slaughter group (SG), CAPN1 316 genotype and SG × CAPN1 316 interactions. The natural logarithm of 24-hour postmortem pH (lnpH) was included as a covariate. Meat and fat color were analyzed with a model that included CAPN1 316, E2FB, SG, aging treatment (AT) and SG × AT as fixed effects. For the analyses of the sensory panel traits the terms included in the models were chosen according to Caine et al. (2003). Tenderness and flavor were analyzed with a model including CAPN1 316, E2FB and SG as fixed effects and juiciness, lnpH as covariates. The model for juiciness included CAPN1 316, E2FB, SG as fixed effects and connective tissue and lnpH as covariates. The model for marbling included CAPN1 316, E2FB, SG as fixed effects and connective tissue and BFT as covariates. Differences in SG are expected to include differences in environmental conditions, including differences in availability and quality of pastures and handling of animals and carcasses.

The sire was included as random effect in all the analyses. Least squares means were compared with a Bonferroni-adjusted test to take into account multiple comparisons. A canonical correlation analysis was performed to test the association among traits measured during fattening with WBSF of 1 day and 7 day aged samples, meat color and fat color of 1 day aged samples. The proportion of variance explained by canonical variables was calculated according to Hair, Anderson, Tatham, and Black (1999). For all the analyses $P = 0.05$ was considered.

3. Results and discussion

3.1. Phenotypic information

Number of records, mean and standard deviation of each recorded trait are presented in Table 1. FW, REA and age increased with days on fattening. Lower values for ADG and AMBFTG in SG 4 and 5 are the consequence of decay in quality and quantity of forage along the year.

3.2. Genetic markers

Frequencies found among the 15 bulls identified as sires of steers were 0.13 (C) and 0.87 (G) for the CAPN1 316 alleles; and 0.37 (C) and 0.63 (T) for the E2FB marker alleles. Allele frequencies in the initial sample of steers ($n = 206$) for CAPN1 316 were 0.076 and 0.923 for C and G, respectively. Allelic frequencies vary among breeds (Van Eenennaam et al., 2007). A very low frequency of the C allele seems to be a common feature of the Hereford breed (Corva et al., 2007; Page et al., 2004). For E2FB allele frequencies were 0.271 and 0.729 for C and T respectively. Chi Square on both markers indicated that samples were in Hardy–Weinberg equilibrium ($P > 0.05$). These allele frequencies differ from the 50:50 reported elsewhere for the allele frequencies ratio in British and Continental European cattle (Schenkel et al., 2005; Kononoff, Deobald, Stewart, Laycock, & Marquess, 2005).

3.3. Association of WBSF with growth traits

A canonical correlation analysis between WBSF of 1 day aged and WBSF of 7 day aged samples (WBSF set) and a group of variables measured during the fattening period (G set: ADG, FW, BFT, REA, AMBFTG, AMREAG and AGE) was conducted. Only the first canonical correlation was significant ($P < 0.05$), with a value of 0.46. The canonical variable of the G set explained 20.86% (square canonical correlation) of the variance in the canonical variable of the WBSF set. The first canonical correlation accounted for 83% of the association between the canonical variables of the sets, and the canonical variables in the G set were able to predict 9.6% of the variance in the individual original WBSF variables. The contribution of each variable to the association was studied through the observation of the correlations between them and the canonical variable of the other set (Table 2). The correlation between WBSF of 1 day aged and WBSF of 7 day aged samples with the canonical variables of the G set were −0.44

Table 2
Correlations of growth traits and the first canonical variable of the WBSF set.

Correlations with	FW[a]	ADG	BFT	REA	AMBFTG	AMREAG	AGE
WBSF variables[b]	0.24	0.35	0.24	0.13	0.18	0.27	−0.05

[a] FW: final weight; ADG: average daily gain; BFT: final backfat thickness; REA: final rib-eye area; AMBFTG: average monthly backfat thickness gain; AMREAG: average monthly rib-eye area gain; AGE: age at slaughter.
[b] Correlations between the growth variables and the first canonical variable of the WBSF variables.

and −0.007 showing that the contribution of WBSF of 7 day aged samples to the association between the sets is negligible. The correlations of growth variables with their canonical variables show that ADG was the most important in the association, followed by AMREAG, FW and BFT. The magnitude of the correlations as well as the proportion of variance in WBSF explained by canonical variables of the G set, indicate a moderate degree of association between both sets. The low coefficient for AGE showed that its contribution to the association was weak.

Considered overall, these results suggest that traits recorded during the growing period are associated with WBSF of 1 day but not 7 day aged meat. In this association, growth rate was more relevant than live weight at slaughter. According to these results and since the correlation of WBSF of 1 day aged meat with the canonical variable of the G set was negative, higher ADG led to lower WBSF, probably because steers were younger at slaughter. Purchas, Burnham, and Morris (2002) found that fast growing animals produced more tender meat than slow growing ones. AMREAG followed this same trend. The contribution of FW and of BFT, each of which may be thought as representing the degree of maturity of steers, was lower than that of growth rate.

3.4. The effects of CAPN1 316 on WBSF

Since there were only two animals with the CC genotype for CAPN1 316, they were excluded from the analysis. CAPN1 316 and the CAPN1 316×SG interaction had no effect on WBSF of 1 day aged meat, whereas SG and lnpH were statistically significant ($P<0.05$). Least squares means for GC and GG were 5.19±0.27 kg and 5.44±0.12 kg respectively. Corva et al. (2007) for CAPN1 316 genotypes, in 1 day aged samples of a *Bos taurus* crossbred population found higher WBSF values than those observed in the present study: CC: 7.86±0.65 kg; CG: 8.73±0.51 kg; GG: 9.21±0.54 kg. In their study, CC and GG were statistically different ($P<0.05$). These authors reported that both the lack of an aging period and the cooking method could have contributed to obtaining such high values.

The effects of CAPN1 316 and the SG×CAPN1 316 interaction were statistically significant ($P<0.05$) for 7 days aged samples only, suggesting that the differential effect of CAPN1 alleles depends on the existence of an aging period and of other undetermined factors associated with the slaughter group. Despite the existence of a SG×CAPN1 316 interaction, there were no differences between genotypes within SG after the Bonferroni adjustment. Least-squares means for genotypes were: CG 3.87±0.28 kg and GG 4.50±0.14 kg which are consistent with previous results reporting C as the favorable allele for tenderness (Page et al., 2004; Van Eenennaam et al., 2007). The low frequency of the C allele prevented the comparison of the extreme homozygote genotypes. Also, the CG class was in a very low frequency and the standard error of its mean was high. Assuming no dominance effects ($d=0$), the CAPN1 316 marker explained only 3.08% of the variability (Falconer & Mackay, 1996).

Curi et al. (2010) in a population of males and females of Nellore and Nellore×*B. Taurus* bred in feedlot, Papaleo Mazzucco et al. (2010) in Brangus steers finished on pasture and White et al. (2005) in a population that included germplasm from *B. taurus* and *Bos indicus*, found differences between CG and GG slightly lower than those found in this study (−0.36 kg, −0.38 and −0.18 kg, respectively).

The effect of connective tissue on WBSF of samples aged for 7 day was analyzed on the samples that were submitted to the sensory panel. Two models were fitted: model 1 included sire as a random effect and CAPN1 316, SG, SG×CAPN1 as fixed effects and connective tissue and lnpH as covariates. Model 2 included the same sources of variation except connective tissue. With Model 1, connective tissue was statistically significant ($\beta_1=0.39\pm0.07$, $P<0.0001$) as well as CAPN1 316 ($P<0.03$) and SG×CAPN1 ($P<0.001$). As connective tissue increased in the samples, meat tenderness measured through WBSF, decreased. With Model 2, CAPN1 316 was close to significance ($P=0.056$) and SG, SG×CAPN1 316 were statistically significant. SG was not statistically significant when connective tissue was included in the analysis, suggesting that some of the differences in SG may be explained by differences in the amount of connective tissue. CAPN1 316 least squares means were: Model 1: CG: 3.85±0.34 kg and GG: 4.59±0.18 kg; Model 2: CG: 3.73±0.38 kg and GG: 4.48±0.19 kg, although the statistically significant interaction with both models indicated that CAPN1 316 effect depended on SG.

Slaughter group influenced WBSF in 1 day and 7 day aged samples. Growth rate influenced mainly tenderness meat that was aged for 1 day and CAPN1 316 was associated with 7 day aged meat. This result is consistent with the fact that μ-calpain is a protease that degrades muscle structure post mortem (Koohmaraie, 1996).

Meat tenderness, as measured by WBSF, was largely affected by environmental conditions during growth, handling of meat and CAPN1 316, although marker effects were somewhat lower than those reported in the literature. The proportion of variance explained by the factors considered in this study was low. Warner, Greenwood, Pethick, and Ferguson (2010) concluded that besides pre- and post-slaughter factors able to be controlled, there are unidentified animal or environmental factors that contribute to the variation in tenderness.

3.5. Sensory panel results

3.5.1. CAPN1 316 and E2FB SNP effects on trained sensory panel attributes and marbling

Results on initial and sustained tenderness, flavor, juiciness and marbling obtained by the panel for each CAPN1 316 genotype are shown in Table 3. The panel evaluated a limited number of 7 day aged samples as explained in Materials and methods. There were not significant differences between genotypes of CAPN1 316 for any of the attributes evaluated by the sensory panel, despite the already mentioned significant difference in WBSF between CAPN1 316 genotypes ($P<0.05$). Marbling showed significant differences between genotypes, with CG having 19% higher marbling values than GG.

According to Caine et al. (2003), reported correlations between WBSF and sensory assessment of beef tenderness are highly variable. Correlations of initial and sustained tenderness with WBSF in the present study were −0.46 and −0.42, respectively. These estimates are lower than the correlation of −0.77 found by Shackelford, Wheeler, and Koohmaraie (1999), but within the range (−0.32 and −0.94) cited by Szczesniak (1968), according to Caine et al. (2003). Destefanis, Brugiapaglia, Barge, and Dal Molin (2008) found that consumer evaluation of tenderness has a correlation of −0.72 with sensory panel evaluations, and that consumers can only discriminate tender, intermediate and tough beef. Miller et al. (1995) found that consumers can detect a difference in WBSF of about 1 kg if meat tasting occurs in a restaurant, whereas it is about 0.5 kg if tasting occurs at home. All these results show a variable correlation between sensory and instrumental assessment of beef tenderness. Sullivan and Calkins (2007) indicated that there is variability in tenderness of muscle, depending in the location of the meat where the samples are taken. Therefore, differences in the WBSF and panel tenderness evaluations might be explained, in part, by the differences in contribution of connective tissue and muscle fiber

Table 3
Least squares means and standard errors for the effect of CAPN1 316 and E2FB genotypes on beef quality traits evaluated by a sensory panel.

Trait	Genetic marker genotype				
	CAPN1 316 (n)		E2FB (n)		
	CG (19)	GG (95)	CC (9)	CT (45)	TT (60)
Initial tenderness	5.24 ± 0.18 a	5.17 ± 0.11 a	5.46 ± 0.25 a	5.02 ± 0.13 a	5.13 ± 0.12 a
Sustained tenderness	5.50 ± 0.16 a	5.60 ± 0.09 a	5.65 ± 0.24 a	5.46 ± 0.11 a	5.55 ± 0.10 a
Flavor	5.43 ± 0.12 a	5.66 ± 0.07 a	5.44 ± 0.18 a	5.67 ± 0.09 a	5.52 ± 0.07 a
Juiciness	5.44 ± 0.19 a	5.07 ± 0.10 a	5.48 ± 0.29 a	5.17 ± 0.14 a	5.12 ± 0.12 a
Marbling	2.26 ± 0.16 a	1.90 ± 0.09 b	1.85 ± 0.23 ab	2.06 ± 0.12 b	2.32 ± 0.10 a

a, b: Within genetic marker, means not sharing a common letter in the same row differ ($P<0.05$).

tenderness to WBSF and sensory tenderness evaluations due to different location of samples, caused by the method for extracting samples for evaluation by WBSF and by sensory panel.

According to the present results, 1 day aged meat would not show differences in tenderness among CAPN1 316 genotypes. Therefore, if meat is consumed soon after slaughter, selecting animals based on their CAPN1 316 genotype would not be advantageous. There are probably other genetic markers associated with differences in tenderness that do not depend on the aging of meat. Moreover, in this case the sensory panel did not discriminate between genotypes as WBFS did, but this could be a consequence of genotype distribution. After studying different cuts and evaluating tenderness through households who used different methods of cooking, Lorenzen et al. (2003) concluded that it is difficult to predict from objective data how consumers will rate meat at home.

E2FB had a statistically significant effect for marbling only (Table 3). The covariates, as well as SG, were statistically significant ($P<0.05$). Animals with the TT genotype had more marbling than those individuals carrying CT and CC genotypes. These results are in agreement with those obtained by Kononoff et al. (2005) in *B. taurus* crossbreeds.

Flavor was affected by juiciness but it was the only attribute for which SG was not statistically significant. Differences between SG could be a consequence of feeding conditions and/or age. In this sense, previous work has established a lack of association between juiciness and flavor and maturity of the animal on an age basis (Moon, Yang, Park, & Joo, 2006). SG and connective tissue were statistically significant in the analysis of juiciness.

3.6. Muscle color

3.6.1. Association of muscle color with growth traits

A canonical correlation was calculated between parameters L*, a* and b* of muscle color on 1 day aged samples and the G set of traits. The first ($P<0.0004$) and second ($P<0.02$) correlations were statistically significant. The first canonical correlation between the sets was 0.39 and the first canonical variable of the G set explained 15% of the variation in the first canonical variable of the muscle color parameters set. The first canonical variable of the G set was able to predict 9.54% of the variance in the original variables of the color of muscle set. Correlations of L*, a* and b* with the first canonical variable of the G set were 0.39, 0.22 and 0.30 respectively and −0.05, −0.01 and 0.11 with the second. Correlations of ADG and AMREAG with the 1st canonical variable of the muscle color set were the highest (Table 4): animals that grow faster produced lighter muscle. These findings could be associated with those obtained by Allingham, Harper, and Hunter (1998) in Brahman cross steers. They found that animals with a compensatory growth (high ADG) after a low ADG period produce lighter meat than animals with a constant body weight gain. Higher age at slaughter has been correlated with darker color of meat (Moon et al., 2006). In this sense, correlation between AGE and the 1st canonical variable was also important and negative. Treatment mediated differences in carcass fatness have been suggested as responsible for differences in meat color and lightness due to the increased fat content in muscle (Fiems et al., 2000), and to a slower cooling rate that corresponds to a faster pH decline (Young, Priolo, Simmons, & West, 1999). This was not totally reflected in the present study because the correlation between the 1st (and 2nd) canonical variables with BFT was small.

The second canonical correlation between the sets was 0.32, the second canonical variable of the G set explained 10% of the variation in the second canonical variable of the muscle color parameters set. The second canonical variable of the G set was able to predict only 0.5% of the variance in the original variables of the color of muscle set because correlations of muscle parameters with the second canonical variable were very low (Table 4).

3.6.2. CAPN1 316 and E2FB SNP effect on muscle color

Table 5 shows the least-squares means and standard errors for muscle and fat color according to CAPN1 316 and E2FB genotypes. The effect of CAPN1 316 genotypes was statistically significant for L*, a* and b* of muscle. Animals with CG genotype had higher L*, a* and b* values than animals with GG genotype ($P<0.05$). Since it is expected from CG genotypes to be more tender than GG, more tender genotypes would have higher values of L*, a* and b*, an observation that agrees with Wulf et al. (1997). They have found negative correlations between parameters of color and shear force. In Brangus, Papaleo Mazzucco et al. (2010) found differences in L* (CG was higher than GG) but not in a* or b*, although the means followed the same trend as in the present study. Furthermore, recently Reardon, Mullen, Sweeney, and Hamill (2010) found an association between calpastatin genotypes and the three CIELAB parameters. As calpastatin is an inhibitor of calpains, these two results together suggest an important effect of the calpain/calpastatin system on meat color. This effect could be explained by two mechanisms: i) the proteolytic activity of the calpains could affect the proteins responsible for the color (myoglobin and others), and/or ii) the effect of the calpain activity on the redox state of the myocytes, that can affect the color stability.

Table 4
Correlations of the original variables of the G set with the first and second canonical variable of the color of muscle set.

Correlations with	FW[a]	ADG	BFT	REA	AMBFTG	AMREAG	AGE
1st canonical variable of Color of muscle set[b]	−0.04	0.32	0.12	−0.003	0.17	0.27	−0.26
2nd canonical variable of Color of muscle set[c]	0.23	0.07	−0.05	0.20	−0.13	0.14	0.20

[a] FW: final weight; ADG: average daily gain; BFT: final backfat thickness; REA: final rib-eye area; AMBFTG: average monthly backfat thickness gain; AMREAG: average monthly rib-eye area gain; AGE: age at slaughter.
[b] Correlations between the growth variables and the first canonical variable of the color of muscle set.
[c] Correlations between the growth variables and the second canonical variable of the color of muscle set.

Table 5
Least-squares means and standard errors for the effect of CAPN1 316 and E2FB genotypes on muscle and fat color.

Color parameter[a]	Genetic marker genotype				
	CAPN1 316		E2FB		
	CG	GG	CC	CT	TT
Muscle					
L*	32.22 ± 0.69 a	30.51 ± 0.36 b	31.90 ± 0.87 a	31.14 ± 0.47 a	31.06 ± 0.42 a
a*	12.16 ± 0.44 a	10.79 ± 0.29 b	12.54 ± 0.52 a	10.84 ± 0.34 b	11.04 ± 0.32 b
b*	11.58 ± 0.36 a	10.51 ± 0.20 b	11.54 ± 0.44 a	10.76 ± 0.25 a	10.83 ± 0.23 a
Fat					
L*	73.65 ± 0.95 a	69.36 ± 0.60 b	72.60 ± 1.13 a	71.03 ± 0.71 a	70.89 ± 1.06 a
a*	3.01 ± 0.36 a	3.31 ± 0.19 a	3.81 ± 0.44 a	2.97 ± 0.25 ab	2.70 ± 0.22 b
b*	18.92 ± 0.50 a	19.46 ± 0.26 a	19.39 ± 0.63 a	19.34 ± 0.34 a	18.83 ± 0.31 a

a, b: Within genetic marker, means not sharing a common letter in the same row differ ($P<0.05$).
[a] L* luminance, a* redness, b* yellowness.

The E2FB genotype CC was different from CT and TT for a* (Table 5). Since this marker has been associated with differences in meat composition, it would also be expected that differences in the color of the meat be found. Moreover, in this study E2FB genotypes were also associated with marbling and the different fat content in muscle could be influencing a*. It was observed that SG and SG × AT were statistically significant for a* and b*, but only SG × AT was significant for L*. The differences in SG and interactions found indicate an important effect of environmental conditions on meat color.

3.7. Fat color

3.7.1. Association of fat color with growth traits

A canonical correlation was estimated between parameters of fat color on 1 day aged samples and the G set of variables. The first correlation was the only statistically significant ($P<0.05$). The first canonical correlation between the sets was 0.45; the first canonical variable of the G set explained 20% of the variability from the first canonical variable of the parameter set for fat color. The first canonical variable of the G set was able to predict 10% of the variance in the original variables of the color of fat set. The color parameter b* had the highest correlation with the first canonical variable of fat color traits (0.41), while the correlations of the other color parameters were lower (−0.18 and 0.31 for L* and a*, respectively). The correlation of 0.45 was mainly explained by the association of b* with age and, to a lesser extent, by the negative association with AMBFTG, ADG, AMREAG and BFT, all of them weighting very similarly in the association (Table 6).

3.7.2. CAPN1 316 and E2FB SNP effect on fat color

Table 5 shows that the effect of CAPN1 316 genotype was statistically significant ($P<0.05$) only for L*, the larger value for CG implying a brighter fat for this genotype. Meanwhile, E2FB was statistically significant only for a*: fat from CC steers had a higher score for redness than fat of TT steers. Results from analyses of variance indicated that SG and SG × AT were statistically significant for L*, a* and b* while AT was statistically significant only for a*, showing a large influence of environmental conditions on fat color as expected.

Table 6
Correlations of the original variables of the G set with the first canonical variable of the color of fat set.

Correlations with	FW[a]	ADG	BFT	REA	AMBFTG	AMREAG	AGE
1st canonical variable of color of fat set[b]	0.14	−0.29	−0.23	−0.01	−0.31	−0.25	0.39

[a] FW: final weight; ADG: average daily gain; BFT: final backfat thickness; REA: final rib-eye area; AMBFTG: average monthly backfat thickness gain; AMREAG: average monthly rib-eye area gain; AGE: age at slaughter.
[b] Correlations between the growth variables and the first canonical variable of the color of fat set.

4. Conclusions

Growth rate of grazing steers was associated with tenderness estimated as WBSF and also with fat and meat color of 1 day aged meat. In all cases, post weaning growth and carcass traits accounted for about 10% of the variance of the meat quality traits that were evaluated. CAPN1 316 was associated with WBSF of 7 days but not of 1 day aged beef. Connective tissue may have contributed to differences in meat tenderness as measured by WBSF. There was no difference between genotypes of CAPN1 316 in tenderness, juiciness and flavor as evaluated by a trained sensory panel. Differences among E2FB genotypes were found for traits associated with composition of meat such as marbling, and parameter a* of muscle and fat color. Although significant efforts were made in the design and management of the present experiment, the most important sources of variation in tenderness and color of meat remained unaccounted for. Those sources of variation must be identified and controlled in order to produce consistently high quality beef.

These results confirm that marker assisted selection for beef tenderness is advisable only in those situations in which aging is a common practice for meat produced in grazing systems. We expect that they highlight genetic and management aspects that must be considered by those responsible for finishing cattle on pastures and aiming to improve the quality of meat.

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References

Allingham, P. G., Harper, G. S., & Hunter, R. A. (1998). Effect of growth path on the tenderness of the semitendinosus muscle of Brahman-cross steers. *Meat Science*, *48*, 65–73.
Altmann, M., & Von Borell, E. (2007). Leptin as an indicator for carcass composition in farm animals. *Animal Science Journal*, *78*, 449–459.
AMSA (1991). *American Meat Science Association.* Guidelines for meat color evaluation. Illinois: Published by the American Meat Science Association. www.meatscience.org.
AMSA (1995). *American Meat Science Association.* Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. Illinois: Published by American Meat Science Association in cooperation with National Livestock and Meat Board 48 pp.
Belk, K. E., Scanga, J. A., Smith, G. C., & Grandin, T. (2002). *The relationship between good handling/stunning and meat quality in beef, pork, and lamb.* American Meat Institute Foundation, Animal Handling and Stunning Conference on February 21–22.
Buchanan, F. C., Fitzsimmons, C. J., Van Kessel, A. G., Thue, T. D., Winkelman-Sim, D. C., & Schmutz, S. M. (2002). Association of a missense mutation in the bovine

leptin gene with carcass fat content and leptin mRNA levels. *Genetics and Selection Evolution*, *34*, 105–116.
Caine, W. R., Aalhus, J. L., Best, D. R., Dugan, M. E. R., & Jeremiah, L. E. (2003). Relationship of texture profile analysis and Warner–Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science*, *64*, 333–339.
Corva, P. M., Soria, L. A., Villarreal, E. L., Schor, A., Perez Cenci, M., Motter, M., et al. (2007). Association of polymorphisms on the CAPN1 and CAST genes with meat tenderness in beef cattle of Argentina. *Genetics and Molecular Biology*, *30*(4), 1064–1069.
Cross, H. R., Moen, R., & Stanfield, M. S. (1978). Training and testing of judges for sensory analysis of meat quality. *Food Technology*, *37*, 48–54.
Curi, R. A., Chardulo, L. A. L., Giusti, J., Silveira, A. C., Martins, C. L., & de Oliveira, H. N. (2010). Assessment of GH1, CAPN1 and CAST polymorphisms as markers of carcass and meat traits in *Bos indicus* and *Bos taurus–Bos indicus* cross beef cattle. *Meat Science*, *86*, 915–920.
Destefanis, G., Brugiapaglia, A., Barge, M. T., & Dal Molin, E. (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. *Meat Science*, *78*, 153–156.
Eilers, J. D., Tatum, J. D., Morgan, J. B., & Smith, G. C. (1996). Modification of early-postmortem muscle pH and use of postmortem aging to improve beef tenderness. *Journal of Animal Science*, *74*, 790–798.
Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics.* Essex, England: Longman Group Limited 365 pp.
Fiems, L. O., De Campeneere, S., De Smet, S., Van de Voorde, G., Vanacker, J. M., & Boucqué, Ch. V. (2000). Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness. *Meat Science*, *56*, 41–47.
Hair, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (1999). *Análisis multivariante* (5a ed.). Madrid: Prentice Hall Iberia 799 pp.
Jiang, T., Busboom, J. R., Nelson, M. L., O'Fallon, J., Ringkob, T. P., Rogers-Klette, K. R., et al. (2010). The influence of forage diets and aging on beef palatability. *Meat Science*, *86*, 642–650.
Johnston, D. J., & Graser, H. -U. (2010). Estimated gene frequencies of GeneSTAR markers and their size of effects on meat tenderness, marbling and feed efficiency in temperate and tropical beef cattle breeds across a range of production systems. *Journal of Animal Science*, *88*, 1917–1935.
Kononoff, P. J., Deobald, H. M., Stewart, E. L., Laycock, A. D., & Marquess, F. L. S. (2005). The effect of a single leptin nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. *Journal of Animal Science*, *83*, 927–932.
Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science*, *43*, S193–S201.
Kropf, D. H. (1980). Effects of retail display conditions on meat color. *Proc. 33rd Recip. Meat Conf. Am. Meat Sci. Assoc., Champaign, IL* (pp. 15–32).
Lorenzen, C. L., Miller, R. K., Taylor, J. F., Neely, T. R., Tatum, J. D., Wise, J. W., et al. (2003). Beef customer satisfaction: Trained sensory panel ratings and Warner–Bratzler shear force values. *Journal of Animal Science*, *81*, 143–149.
Marshall, D. M. (1999). Genetics of meat quality. In R. Fries, & A. Ruvinsky (Eds.), *The genetics of cattle* (pp. 605–636). Oxon: CABI Publishing.
Miller, M. F., Hoover, L. C., Cook, K. D., Guerra, A. L., Huffman, K. L., Tinney, K. S., et al. (1995). Consumer acceptability of beef steak tenderness in the home and restaurant. *Journal of Food Science*, *60*, 963–965.
Miquel, M. C., Villarreal, E. L., Mezzadra, C., Melucci, L., Soria, L. A., Corva, P., et al. (2009). The association of CAPN1 316 marker genotypes with growth and meat quality traits of steers finished on pasture. *Genetics and Molecular Biology*, *32*(3), 491–496.
Monsón, F., Sañudo, C., & Sierra, I. (2005). Influence of breed and ageing time on the sensory meat quality and consumer acceptability in intensively reared beef. *Meat Science*, *71*, 471–479.
Moon, S. S., Yang, H. S., Park, G. B., & Joo, S. T. (2006). The relationship of physiological maturity and marbling judged according to Korean grading system to meat quality traits of Hanwoo beef females. *Meat Science*, *74*, 516–521.
Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., et al. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science*, *80*, 3077–3085.
Page, B. T., Casas, E., Quaas, R. L., Thallman, R. M., Wheeler, T. L., Shackelford, S. D., et al. (2004). Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. *Journal of Animal Science*, *82*, 3474–3481.
Papaleo Mazzucco, J., Melucci, L. M., Villarreal, E. L., Mezzadra, C. A., Soria, L., Corva, P., et al. (2010). Effect of ageing and μ-calpain markers on meat quality from Brangus steers finished on pasture. *Meat Science*, *86*, 878–882.
Purchas, R. W., Burnham, D. L., & Morris, S. T. (2002). Effects of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. *Journal of Animal Science*, *80*, 3211–3221.
Reardon, W., Mullen, A. M., Sweeney, T., & Hamill, R. M. (2010). Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. *Meat Science*, *86*, 270–275.
Romans, J. R., Jones, K. W., Costello, W. J., & Carlson, C. W. (1985). *The meat we eat. South Dakota State University.* Danville, Illinois: The Interstate Printers & Publishers, Inc.
SAS (1998). The SAS system for Windows. Version 8. Cary, NC. USA: SAS Institute Inc.
Schenkel, F. S., Miller, S. P., Ye, X., Moore, S. S., Nkrumah, J. D., Li, C., et al. (2005). Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, *83*, 2009–2020.
Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1999). Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. *Journal of Animal Science*, *73*, 3333–3340.
Sullivan, G. A., & Calkins, C. R. (2007). *Ranking beef muscles for Warner–Bratzler shear force and trained sensory panel ratings.* Nebraska Beef Cattle Reports. Lincoln, Nebraska: Animal Science Department, University of Nebraska-Lincoln. http://digitalcommons.unl.edu/animalscinber/90.
Szczesniak, A. S. (1968). Correlations between objective and sensory texture measurements. *Food Technology*, *22*, 981–986.
Van Eenennaam, A. L., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., Franke, D. E., et al. (2007). Validation of commercial DNA tests for quantitative beef quality traits. *Journal of Animal Science*, *85*, 891–900.
Van der Werf, J., & Kinghorn, B. (1999). *Beef breeding technologies 3. Marker assisted selection.* DPI&F Note. : Tropical Beef Centre Queensland Australia. http://www2.dpi.qld.gov.au/beef/2240.html Accession date: 11/Oct/2007.
Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. *Meat Science*, *86*, 171–183.
Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., & Smith, S. B. (1990). Effect of post-mortem treatments on the tenderness of meat from Hereford, Brahman and Brahman-cross beef cattle. *Journal of Animal Science*, *68*, 3677–3686.
White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., et al. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus* and crossbred descent. *Journal of Animal Science*, *83*, 2001–2008.
Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., et al. (2003). Effects of fatty acids on meat quality: A review. *Meat Science*, *66*, 21–32.
Wulf, D. M., O'Connor, S. F., Tatum, J. D., & Smith, G. C. (1997). Using objective measures of muscle color to predict beef Longissimus tenderness. *Journal of Animal Science*, *75*, 684–692.
Ye, S., Dhillon, S., Ke, X., Collins, A. R., & Day, I. N. M. (2001). An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Research*, *29*, E88.
Young, O. A., Priolo, A., Simmons, N. J., & West, J. (1999). Effects of rigor attainment temperature on meat blooming and colour on display. *Meat Science*, *52*, 47–56.