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Effect of three single-nucleotide polymorphisms in *CAPN1* gene on beef tenderness (Brief Report)

Einfluss von drei Einzelnucleotidpolymorphismen im *CAPN1* Gen auf die Zartheit von Rindfleisch (Brief Report)

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Background

Meat tenderness is an important trait in beef cattle production, as consumers consider tenderness the most important attribute of beef palatability. There is ample evidence that *post mortem* proteolysis of myofibrillar proteins is responsible for the decline in shear force during storage. The bovine *micromolar calcium-activated neutral protease* (*CAPN1*) gene encodes the large subunit of μ-calpain, which is thought to be one of the most important enzymes involved in *post mortem* tenderization (KOOHMARAIE 1996). Three single-nucleotide polymorphisms (SNPs) on the *CAPN1* gene (316, 530 and 4 751 markers) have been associated with tenderness in different cattle breeds (PAGE *et al*. 2002, PAGE *et al*. 2004, WHITE *et al*. 2005). A more recent study confirmed that markers 316 and 4 751 had an effect on beef tenderness (VAN EENENNAAM *et al*. 2007). The objective of this research was to determine the existence of polymorphisms and to assess the effect of the reported SNP in the bovine *CAPN1* gene on tenderness from a sample of Angus and Brangus steers fattened on pasture.

Procedure

Sixty Brangus and 21 Angus steers were used in the study. The Brangus steers were chosen at random from three different commercial herds (n=20 each), which are representative of the breed located throughout Argentina. In all herds animals were purebreds, i.e. there were the progeny of either Brangus bull and cows, or Angus bulls and cows. Individual sire identifications were not available due to the multiple-sire mating system practiced in these herds. All Angus animals were produced at INTA Balcarce Experiment Station from cows belonging to a closed experimental herd. Animals were sorted into slaughter groups, classified by breed and herd origin. All steers from each group were slaughtered when at least 50% of them reached 6 mm of back fat thickness. Beef tenderness was estimated indirectly as Warner-Bratzler shear force (WBSF)

measured on samples taken from the *longissimus lumborum* muscle at 1, 7 and 14 days *post mortem* (CORVA *et al.* 2007). After slaughter, and following a 24-hour cooling period at 1-5 °C, the block of steaks corresponding to the 11, 12 and 13th ribs was removed from each left half carcass, deboned and divided into three pieces that were vacuum-packed and randomly assigned to three maturation treatments at 1-5 °C (1, 7 and 14 days). After ageing, meat samples were frozen and kept at −20 °C until they were thawed. DNA was isolated from blood by phenol/chloroform standard protocol. PCR-RFLP methods for markers 316 and 530 were carried out according CORVA *et al.* (2007). A 205 bp DNA fragment of marker 4 751 was amplified with the following primers:

forward – GAA GGG CTT GGG TTG GGA TGT CGG CAG AG and

reverse – AGG CTG GGA GGG GTG TTC TCT GAG TGC CA,

and digested with the *BsaJ*I restriction enzyme. The effects of the three markers on WBSF were tested simultaneously by fitting a mixed model using PROC MIXED of SAS (1998). Fixed effects were treatment (ageing at 1, 7 and 14 days), slaughter group , treatment × slaughter group interaction, main effects of markers 316, 530, 4 751, marker 316 × treatment, marker 530 × treatment, and marker 4 751 × treatment, and animal was considered random. Low frequencies in some of the marker genotypes, led to a lack of observations for different combinations of genetic markers, which prevented including the interactions among markers in the model. Meat samples from the same animal taken at different ageing times were considered repeated measures. The choice of covariance structure was tested with the Bayesian Information Criterion (BIC), and the variance components option in PROC MIXED was used for the analysis. Haplotyping was performed using the software PHASE (STEPHENS *et al*. 2001). Most likely haplotypes were included in the analysis if assigned with probabilities of 0.98 or more. Haplotypes were analyzed with a model that included fixed effects of treatment, slaughter group and treatment × slaughter group interaction and random effects of animal. Haplotype effects were fitted as a fixed regression in the number of haplotypes (i.e. 0, 1, 2). Tests of hypothesis were performed in a stepwise fashion (see for example CORDELL and CLAYTON 2002).

Results

A large proportion of animals were GG homozygous at markers 316 and 530. Only one homozygous AA at marker 530 was found in the entire sample, and was excluded from all statistical analyses. Genotype CT at marker 4 751 was the most frequent one. Only 4 haplotypes (CGC, GGC, GGT and GAT) were found among the eight possible. Tests of fixed effects showed that only treatment, treatment × slaughter group and marker 316 significantly ($P<0.05$) affected WSBF. Marker 316 showed a significant effect on WBSF at 7 days ($P<0.05$). At this ageing period, the difference between homozygotes CC and GG was 1.49 ± 0.69 kg. The heterozygous genotype showed an intermediate value. No significant differences were found between genotypes CC and CG, probably due to the low number of CC animals found in this study. At 7 days post-slaughter the shear force value decreased more in CC (31%) than in GG (10%). These results suggest that maturing periods longer than 7 days will not improve meat tenderness of steers carrying CC and CG genotypes at marker 316. For those carrying the GG genotype instead, 14 days were needed to reach

tenderness values similar to those obtained at 7 days from animals with genotype CG. The absence of the AA genotype at marker 530 in these data may have resulted in the lack of difference among genotypes. If differences among marker 4 751 genotypes are small, the number of animals sampled in the current study may not have been enough to detect them. All in all, the least squares means for marker 4 751 do not indicate a clear trend for the effect of the SNP on tenderness. Table 1 shows the results of the analyses performed on each haplotype. The individual tests indicate that the effect of CGC was statistically significant, and GGC almost reached significance. The haplotype CGC gathered three favorable alleles for tenderness. When the model included the effects of both markers, only CGC was significant. No significant effects on tenderness were found for all other haplotypes. This result agrees with the analysis of genotypes in that a statistically significant effect ($P<0.05$) was found for marker 316 on tenderness. Thus, the only marker having an effect on tenderness was 316, since the haplotype carrying CGC was the only one that displayed a significant effect. In the latter analysis, the increase in the number of C alleles from 316 was significant, whereas increasing the number of »favorable« alleles of either 530 or 4 751 showed no significant effects. The effects estimated by the analyses of the haplotypes GGC and GGT do not show a clear trend for the effect of 4 751, and this result agrees with the one obtained from the genotypic analysis. Of the three markers analyzed in this study, 316 was the only informative marker for tenderness at 7 days *post mortem*. There was little variation at 530 in the current experiment, whereas 4 751 displayed no clear effect on tenderness.

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Table 1
Tests of hypotheses for the haplotype effects
Tests von Hypothesen für die Haplotyp-Effekte

Marker 316/530/4751 haplotype	*N*			*P*-value	Estimated effect, kg
	0	1	2		
CGC	132	96	12	0.0044	−0.672 ± 0.23
GGC	90	114	36	0.0562	0.407 ± 0.21
GGT	114	108	18	0.7938	0.070 ± 0.27
GAT	210	30	0	0.4331	0.375 ± 0.47
Tests for haplotype effects in a model including both CGC and GGC					
CGC	132	96	12	0.0350	−0.618 ± 0.29
GGC	90	114	36	0.7589	0.078 ± 0.26

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