Effects Of Polymorphisms In The *Calpastatin* And *µ-Calpain* Genes On Meat Tenderness In Three French Beef Breeds

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Introduction

Meat tenderness is an important issue in beef cattle because it has a major impact on consumer satisfaction. However, there is no routine measure of beef meat quality so a classical selection based on records is not feasible. In these conditions, the study of genetic basis for variation in meat tenderness could be a solution to improve tenderness by developing a marker assisted selection. The *µ-calpain* gene (*CAPN1*), mapped on chromosome 29 (Smith *et al.* 2000), encodes the protease µ-calpain which degrades myofibrillar proteins postmortem and the *calpastatin* gene (*CAST*), mapped on chromosome 7 (Bishop *et al.* 1993), encodes its inhibitor (Koohmaraie 1996). The objectives of this study were to estimate the frequencies of genotypes for markers in the *CAST* and *CAPN* genes and the effects of these polymorphisms on meat tenderness, in the Charolais, Limousin and Blond d'Aquitaine breeds.

Material and methods

Animals. Purebred young bulls, progeny of 48 Charolais, 36 Limousin and 30 Blond d'Aquitaine sires, were randomly procreated in a large number of herds from mostly unrelated dams. After weaning in the farm of origin, bull calves entered the feed-lots at 40, 37 or 24 weeks of age on average (± 3 weeks) for the Charolais, Limousin and Blond d'Aquitaine breeds respectively. They were humanely slaughtered in commercial slaughterhouses when they reached 730 kg live weight on average for the Charolais progeny and 479 days or 417 days on average for the Limousin or Blond d'Aquitaine progeny respectively. A total of 1,114 Charolais, 1,254 Limousin and 981 Blond d'Aquitaine purebred young bulls were used in this study.

Traits. Traits analyzed were meat tenderness attributes: Warner-Bratzler shear force and tenderness score (Table 1). Two steaks of the *longissimus thoracis* muscle were vacuum-

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packaged and kept at 4°C for 14 d for ageing, before to be frozen. Before each measure session, the steaks were placed at 4°C for 24 hours to be thawed and they were cooked over an electric grill to an internal temperature of 55°C (rare cooking). The Warner-Bratzler shear force (N/cm²) was averaged on 10 core measures of the first steak. Tenderness was evaluated on the second steak by three different test panels, one for each breed, composed of 12 trained panellists. They scored tenderness on non-structured 100-point scales: from 1 (extremely tough) to 100 (extremely tender). The scores were averaged over the panellist for each animal.

Table 1: Overall mean, number of observations and phenotypic standard deviation (RSD) for shear force and tenderness score in the three breeds

Traits	overall mean	Charolais		Limousin		Blond d'Aquitaine	
		No.	RSD	No.	RSD	No.	RSD
shear force	39.9 N/cm²	1114	7.32	1252	7.47	977	10.41
tenderness score	60.9 /100	1113	7.87	1241	7.25	970	10.72

Genotypes. Three SNP in the bovine *CAST* gene were genotyped. Marker *CAST-1* is an adenosine/guanosine (A/G) substitution in intron 8 of the *CAST* gene. Markers *CAST-2* and *CAST-3* are A/G substitutions in the 3'untranslated region. They are in positions 97531815, 97574679 and 97576054, respectively on Btau4.0. Marker *CAST-2* was reported by Barendse (2002). Four SNP in the bovine *CAPN1* gene were genotyped. Marker *CAPN1-1* is an A/G substitution in exon 6. Marker *CAPN1-2* is a cytidine/guanosine (C/G) substitution in exon 9. Marker *CAPN1-3* is an A/G substitution in exon 14. Marker *CAPN1-4* is an A/G substitution in intron 19. They are in positions 45219395, 45221250, 45237834, and 45241089 respectively on Btau4.0. All the *CAPN1* markers were reported by Page *et al.* (2002). Markers *CAST-2* and *CAPN1-2* were included in GeneSTAR® Tenderness commercial test and marker *CAPN1-2* was included in Igenity Tenderness® commercial test.

Statistical analyses. Models were evaluated using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). To study the individual association of each marker with shear force and tenderness score, a mixed model of regression was performed: $Y_{ijkl} = \mu + C_i + \beta_j G_j + S_k + e_{ijkl}$ where Y_{ijkl} = phenotypic observations, μ = overall mean, C_i = fixed effect of contemporary group for shear force or the date of taste panel for tenderness score, G_j = number of copies of one of the two alleles present in the genotype, S_k = random effect of sire and e_{ijkl} = random error. To study the effect of haplotypes, a mixed model of multiple regression was used: $Y_{ijkl} = \mu + C_i + \sum_{j=i}^{n} \beta_j H_j + S_k + e_{ijkl}$ where μ, C_j, S_k et e_{ijkl} were the same effects than in the marker model and H_j was the number of copies of each haplotype j, with n the number of haplotypes segregating in the population. Analyses were conducted by breed.

Results and discussion

Allelic frequencies were relatively similar in the three breeds for each of the three SNP of the *CAST* gene, *CAPN1-3* and *CAPN1-4* (Table 2). For marker *CAPN1-1*, the frequency of the G allele (47%) in the Limousin breed was lower than in the Charolais (64%) and Blond d'Aquitaine (80%) breeds. For *CAPN1-2*, the frequency of the G allele was very high in the Charolais (91%) and Blond d'Aquitaine breed (96%) and lower in the Limousin breed (73%).

Table 2: Frequencies of the G allele for the seven markers studied among young bulls of the three breeds

Breeds	*CAST-1*	*CAST-2*	*CAST-3*	*CAPN1-1*	*CAPN1-2*	*CAPN1-3*	*CAPN1-4*
Charolais	0.75	0.18	0.35	0.64	0.91	0.76	0.45
Limousin	0.86	0.17	0.30	0.47	0.73	0.64	0.56
Blond d'Aquitaine	0.86	0.23	0.23	0.80	0.96	0.64	0.63

There was a significant effect of the marker *CAST-3* on shear force in the Charolais breed (+0.12 RSD) but not on tenderness score (Table 3). There were significant effects of the markers *CAPN1-2* and *CAPN1-4* on the two traits in the Charolais breed. The G allele was associated with a tougher meat for the two markers: the effect of the G allele of *CAPN1-2* was +0.22 RSD on shear force and -0.25 RSD on tenderness score and the effect of the G allele of *CAPN1-4* was +0.11 RSD on shear force and -0.11 RSD on tenderness score. The effects of the G allele of *CAPN1-2* were consistent with those found in the ASA, GPE cycle VII and VIII populations by Page *et al.* (2004) and White *et al.* (2005). In the Limousin breed, we showed a significant effect of the G allele of the marker *CAPN1-2* on shear force (+0.12 RSD) but not on tenderness score and a significant effect of the G allele of the marker *CAST-1* on tenderness score (-0.17 RSD) but not on shear force. In the Blond d'Aquitaine breed, the G allele of the marker *CAST-2* was associated with a tougher meat when studying the two traits (+0.18 RSD of shear force and -0.22 RSD of meat tenderness). These effects are in the same order than those found in the GPE cycle VII population by Casas *et al.* (2006). No effect was found for *CAPN1-3* in the three breeds as in the cycle VIII population (White *et al.* 2005). On the contrary, Page *et al.* (2004) found a positive effect of the G allele on meat tenderness in the ASA and cycle VII populations.

Table 3: Additive effects of the G allele divided by the phenotypic standard deviation (RSD) for shear force and tenderness score (Tend. Sc.) in the three breeds

Markers	Charolais		Limousin		Blond d'Aquitaine	
	Shear force	Tend. Sc.	Shear force	Tend. Sc	Shear force	Tend. Sc.
CAST-1	-0.08 ns	0.02 ns	0.08 ns	-0.17 **	0.06 ns	-0.10 ns
CAST-2	0.11 ns	0.06 ns	-0.03 ns	-0.04 ns	0.18 **	-0.22 ***
CAST-3	0.12 *	-0.07 ns	-0.004 ns	0.03 ns	-0.06 ns	0.05 ns
CAPN1-1	-0.03 ns	0.04 ns	-0.005 ns	0.05 ns	-0.08 ns	0.12 ns
CAPN1-2	0.22 **	-0.25 **	0.12 *	0.01 ns	-0.05 ns	0.06 ns
CAPN1-3	-0.07 ns	0.02 ns	-0.05 ns	0.05 ns	0.08 ns	-0.003 ns
CAPN1-4	0.11 *	-0.11 *	0.01 ns	0.02 ns	0.03 ns	-0.04 ns

ns = non significant, * < 0.05, ** < 0.01, *** < 0.001

We found a significant association between *CAST-2+CAST-3* haplotypes and phenotypes in the Blond d'Aquitaine breed (Figure 1). There was a high effect of the GA haplotype on shear force (+0.27 RSD) and tenderness score (-0.22 RSD). An association was shown between *CAPN1-1+CAPN1-2+CAPN1-4* haplotypes and meat quality traits in the Charolais breed (Figure 2). The ACA haplotype was associated with a low shear force (-0.24 RSD) and a high tenderness score (+0.30 RSD) and the AGG haplotype was associated with a high shear force (+0.22 RSD) and a low tenderness score (-0.22 RSD).

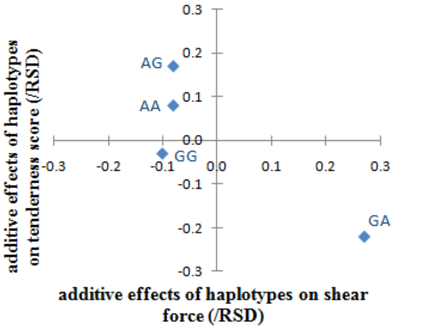

Figure 1: Additive effects of *CAST* haplotypes on tenderness traits in the Blond d'Aquitaine breed

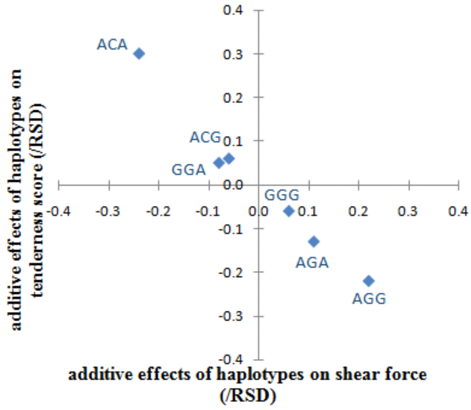

Figure 2: Additive effects of *CAPN1* haplotypes on tenderness traits in the Charolais breed

Conclusion

These results clearly show that effects of SNP and haplotypes, on meat tenderness traits, were breed-specific. Consequently, the current work demonstrates that effects of these markers cannot be extended to all *Bos taurus* breeds reared in France. Additional work is also required to identify other more appropriate markers for these French beef breeds.

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