THE CALPAIN SYSTEM IN 15 EUROPEAN CATTLE BREEDS AND RELATIONSHIP TO SHEAR FORCE

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Keywords: beef, μ-calpain, m-calpain, calpastatin, shear force

Introduction

This study, which is part of the European Project GEMQUAL (Genetics of Meat Quality), examined young bulls from 15 different breeds (both dairy and beef) representing the genetic diversity of European cattle. Post-mortem (p.m.) storage of beef at refrigerated temperatures is known to increase meat tenderness. The calpain system is believed to be involved in tenderisation of beef. Moderate correlations between calpastatin activity and shear force have been reported in *Bos indicus* crosses (r = 0.43, Whipple *et al.*, 1990), whereas lower correlations were reported for *Bos taurus* breeds (r = 0.27, Shackelford *et al.*, 1994). Here, we report some characteristics of the calpain system in European cattle breeds and correlations to Warner Bratzler shear force in *longissimus thoracis* muscle.

Materials and Methods

The experiment included 437 young bulls from 15 European cattle breeds. Muscle samples (ca. 20 grams) were taken within 45 minutes post-mortem (p.m.) from the right side of *M. longissimus thoracis*, chopped into very small pieces, immediately frozen in liquid nitrogen and stored at -80°C until use. *M. longissimus thoracis* (between 8th and 13th rib) from the left side of the carcass was stored at 3°C ± 1°C until 10 days p.m., a sample was cut, vacuum packaged and frozen for Warner-Bratzler (WB) shear force. Meat slices for determination of WB shear force were heated in a water bath at 80°C until a 75°C internal temperature was reached. Peak force was determined on 10 cores (1x1x2 cm) per slice cut perpendicularly to the fibre axis. Calpain and calpastatin activities were determined in one gram muscle representing a mixture of the very finely chopped muscle of 20 grams. Briefly, an extract was prepared by homogenisation in 10 volumes of extraction buffer. After centrifugation at 10,000 x *g* for 30 min, one aliquot for calpastatin determination was heated to 95 °C for 5 min, centrifuged, and the supernatant was assayed for calpastatin activity as described in Ertbjerg *et al.* (1999). Then, one aliquot of the supernatant was taken for calpain activity determination by casein zymography. Briefly, casein minigels with 26 wells were run at 80 V for three hours at 4 °C, removed, and incubated with calcium for 1 hour at room temperature. After staining and destaining, the density of each band was measured and quantified relative to reference standards within each gel and relative to activity of the standards in a casein assay (Ertbjerg *et al.*, 1999).

Results and Discussion

From Table 1, it appears that the two dairy breeds Danish Red Cattle and Holstein and the specialised beef breed Limousin contained significantly higher μ-calpain activity than the other breeds, whereas the beef breeds Piemontese and Marchigiana contained the lowest μ-calpain activity. The m-calpain activity was the highest in Danish Red Cattle and the lowest in the beef breed Pirenaica. The calpastatin activity was the highest in Danish Red Cattle and the lowest in the double-muscled Piemontese breed. Although the Asturiana de los Valles breed is also a double-muscled breed,, the calpastatin activity in this breed appeared to be intermediate as compared to the other breeds.

Partial correlations (i.e. corrected pearson correlations for the breed differences) between μ-calpain or calpastatin activities and WB shear force were significant ($P < 0.01$), but explained only 4 and 2% of its variation, respectively (data not shown). The poor relationship between the calpain system and WB shear force were breed-dependent. There was indeed a high negative correlation between μ-calpain and WB shear force in Casina ($r = -0.70$, $P < 0.001$) (Figure 1) and Jersey ($r = -0.39$, $P < 0.05$) but no significant correlation for other breeds. Piemontese showed a positive correlation between m-calpain and WB shear force ($r = 0.41$, $P < 0.05$). A significant correlation between calpastatin activity and WB shear force was found in Highland ($r = 0.52$, $P < 0.05$) and Pirenaica ($r = 0.60$, $P < 0.001$) (Figure 1), but not in any of the other breeds studied.

Table 1: Differences in μ-calpain, m-calpain and calpastatin activity between 15 European cattle breeds. Values are expressed as means ± standard deviations. Jersey (n=30), South Devon (n=27), Aberdeen Angus (n=30), Highland (n=29), Holstein (n=29), Danish Red Cattle (n=29), Simmental (n=20), Asturiana de los Valles (n=30), Casina (n=31), Avileña (n=30), Pirenaica (n=31), Piemontese (n=30), Marchigiana (n=29), Limousin (n=31) and Charolais (n=31).

	μ-Calpain (U/g meat)	m-Calpain (U/g meat)	Calpastatin (U/g meat)
Danish Red Cattle	9.62 ± 1.04^{a}	5.74 ± 0.96^{a}	34.74 ± 4.88^{a}
Holstein	9.30 ± 0.84^{a}	5.60 ± 0.84^{ab}	32.87 ± 4.08^{ab}
Limousin	9.07 ± 0.59^{ab}	5.23 ± 0.93^{bcde}	31.47 ± 4.66^{bcd}
Jersey	8.50 ± 1.57^{b}	4.67 ± 1.00^{fg}	32.16 ± 3.74^{bcd}
Charolais	8.43 ± 0.77^{b}	5.38 ± 0.56^{abcde}	33.08 ± 5.38^{ab}
Simmental	8.43 ± 0.62^{b}	5.52 ± 0.94^{abc}	32.98 ± 3.17^{ab}
Asturiana de los Valles	7.47 ± 1.35^{c}	5.43 ± 0.63^{abcd}	30.24 ± 4.13^{def}
Casina	7.28 ± 1.31^{cd}	5.48 ± 0.74^{abc}	31.26 ± 3.52^{bcd}
Highland	7.23 ± 1.23^{cd}	5.51 ± 0.66^{abc}	32.53 ± 2.76^{bc}
Avileña	7.04 ± 2.03^{cd}	4.99 ± 0.59^{efg}	32.92 ± 3.57^{ab}
Pirenaica	7.03 ± 1.66^{cd}	4.59 ± 0.97^{g}	30.67 ± 5.12^{cde}
South Devon	7.03 ± 1.44^{cd}	5.21 ± 0.74^{bcde}	28.90 ± 4.08^{efg}
Aberdeen Angus	6.93 ± 0.91^{cd}	5.04 ± 0.49^{def}	30.51 ± 3.32^{cdef}
Piemontese	6.68 ± 1.76^{d}	5.43 ± 0.88^{abcd}	27.19 ± 3.37^{g}
Marchigiana	6.68 ± 1.25^{d}	5.16 ± 1.13^{cde}	31.15 ± 3.31^{bcd}

[a-g] Within columns, values with the same letter are not significantly different

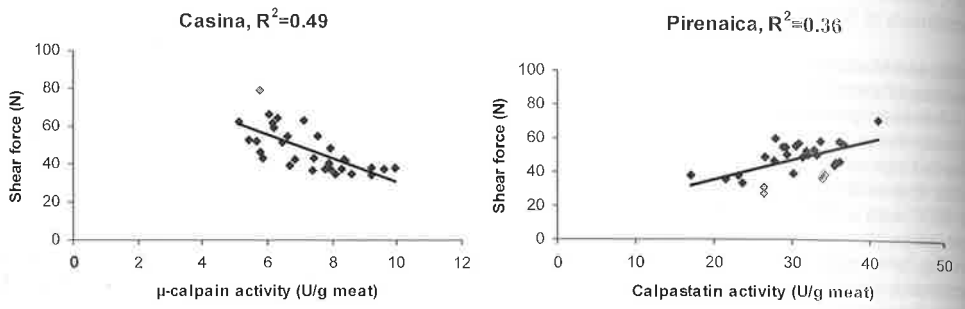

Figure 1: Correlation between μ-calpain activity and shear force in the Casina breed (left) and between calpastatin activity and shear force in the Pirenaica breed (right).

Conclusions

In conclusion, the dairy breeds showed relatively higher values for all components of the calpain system than beef breeds, whereas the double-muscled Piemontese breed had the lowest calpastatin activity. Overall, the 45 min post-mortem μ-calpain and calpastatin activity showed poor correlation to day 10 shear force. However, this relationship was strongly breed-dependent. Thus, 49% of the variation in shear force could be explained by μ-calpain in the Casina breed. Also, 36% of the variation in shear force could be explained by the calpastatin activity in the Pirenaica breed and 27% in the Highland breed. However, no strong significant relationship was revealed in other breeds.

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