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## Differences in the post mortem kinetics of the calpain system in meat from bulls and steers

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### ABSTRACT

This experiment investigated differences in meat tenderness as defined by shear force between bulls and steers, and how these related to differences in the post mortem kinetics of the components of the calpain system. Strip loins (*m. longissimus dorsi*) from bulls with acceptable (pH < 5.7) ultimate pHs were compared with strip loins from prime steers. Samples were aged for 1, 3, 7, 13, 24, 48 and 72 hours at 15 °C. The activities of calpastatin and  $\mu$ -calpain were higher in bulls than in steer samples, however, the rates of decline in the activities of calpastatin and  $\mu$ -calpain were more rapid over the first 24 hours post slaughter in the bull samples than in the steer samples.

**Keywords:** calpain; calpastatin; tenderness; steer; bull.

### INTRODUCTION

There is an increased consumer demand for lean beef products of a consistently high quality. One potential way of producing leaner beef is to use bulls for beef production rather than steers. Bulls appear to produce a poorer quality meat product in terms of tenderness, colour and storage life (Bailey *et al.*, 1966; Joseph and Connolly, 1974). Tenderness makes an important contribution to the consumers overall enjoyment of the meat (Jeremiah *et al.*, 1992).

The activity of calcium dependent protease (calpain, E.C. 3.4.22.17) system has been implicated in the regulation of protein degradation during meat tenderisation (Koochmaraie, 1992). This system consists of at least two enzymes ( $\mu$ -calpain and m-calpain) and their endogenous inhibitor calpastatin. At slaughter the activity of calpastatin appears to be higher in bull *longissimus dorsi* muscles than the activity in *longissimus dorsi* muscles from steers (Morgan *et al.*, 1993). Therefore, changes in the post slaughter activities of the components of the calpain system may be involved in any observed differences in shear force post slaughter between bulls and steers.

The objective of this study was to determine whether differences in the activities of the components of the calpain system could be contributing to differences in shear force values between bulls and steers.

### MATERIALS AND METHODS

Entire strip loins (*m. longissimus dorsi*) from 8 young bulls and 5 prime steers were purchased from a local abattoir immediately after hot boning using the abattoirs standard procedures. All strip loins had acceptable ultimate pHs (pH < 5.7; 24 hours post slaughter. Samples of each strip loin (approx 200 g) were aged at 15°C in a waterbath for 1, 3, 7, 13, 24, 48 and 72 hours post slaughter. After each aging period, the activities of the components of the calpain system, and shear force were determined.

Shear force was determined by cooking approximately 100 g of each muscle sample in a waterbath until their

internal temperature reached 75°C, as determined by a temperature probe. The cooked samples were cooled on ice until their internal temperature reached 4°C (Graafhuis *et al.*, 1991). The force (kg) required to shear across the grain of a 10 mm by 10 mm strip was determined using a MIRINZ pneumatic tenderometer. A mean of 10 determinations per sample was used (Frazerhurst and MacFarlane, 1983).

Calpastatin,  $\mu$ -calpain and m-calpain activities were separated on a DEAE Sephacel column using a NaCl stepwise gradient using the method of Sainz *et al.* (1992) as modified by Dobbie *et al.* (1995). After separation the activities of the different components were determined in the presence of casein and calcium chloride (Wheeler and Koochmaraie, 1991). The calpastatin inhibitory activity was determined using the same assay but in the presence of a known amount of m-calpain extracted from bovine lung.

Data were subjected to analyses of variance using the GLM program (Minitab for Windows, Version 10.1, Minitab Inc. State College, PA).

### RESULTS

Bull and steer strip loins had similar patterns of aging throughout the study period ( $P > 0.10$ , Table 1). Shear force declined by 40-46% at 72 hour post mortem as compared with shear force at 24 h ( $P < 0.05$ ).

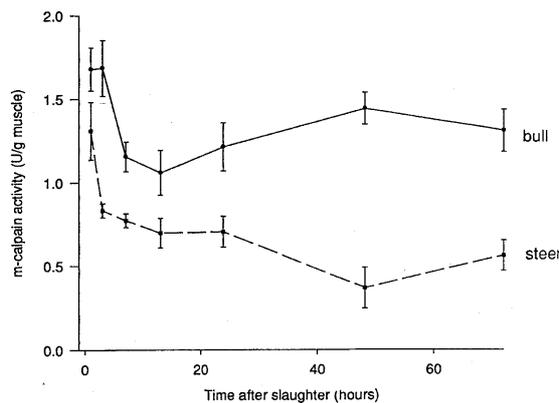
m-calpain activity did not significantly differ between bull and steer samples 1 hour post slaughter but by 3 hours post slaughter the m-calpain activity was higher in the bull samples (Figure 1). Steer samples tended to have lower calpastatin activities than bull samples one hour post slaughter ( $P < 0.07$ , Figure 2). There was no loss of calpastatin activity in the steer samples post slaughter while the bull samples lost approximately 27% of their calpastatin activity over the 72 hour aging period. This meant that by 24 hours post slaughter there were no differences in the calpastatin activities between the bull and steer strip loins. The activity of  $\mu$ -calpain was lower in the steer samples than in the bull samples one hour post slaughter (Figure 3;

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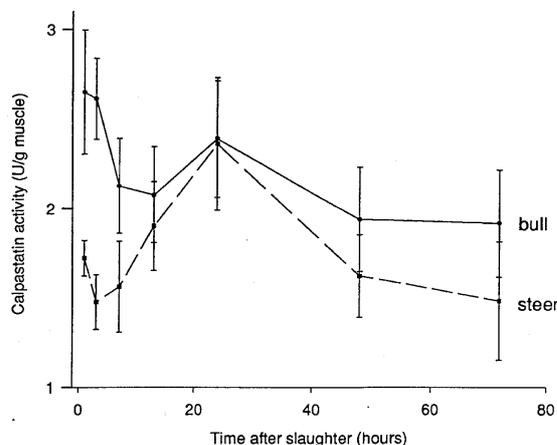
**TABLE 1:** Changes in shear force (kg) post slaughter and the ratio of calpastatin : $\mu$ -calpain activity one hour post slaughter in strip loins from 8 bulls and 5 steers

Time (hours)	Bull	Steer	SE	P
	Shear force			
24	11.1	12.5	0.70	NS
48	6.7	7.3	0.82	NS
72	6.7	6.7	0.76	NS
Calpastatin : $\mu$ -calpain	2.7	2.9	0.32	NS

**FIGURE 1.** Changes in the activity of m-calpain (U/g fresh muscle) in strip loins from steers and bull carcasses over time post slaughter when aged at 15°C (mean, SE).



**FIGURE 2** Changes in the inhibitory activity of calpastatin (U/g fresh muscle) in strip loins from steers and bull carcasses post slaughter when aged at 15°C (mean, SE).

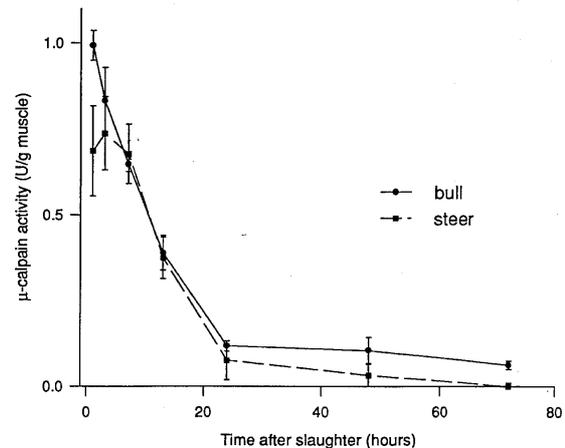


$P < 0.05$ ). Bull samples lost 26% of their  $\mu$ -calpain activity over the first 7 hours post slaughter while in the steer samples only 17% was lost over the same period. After 7 hours no differences in  $\mu$ -calpain activity were detected between the bull and steer samples (Figure 3).

## DISCUSSION

There were no differences in shear force between strip loins from bulls and steers 24 hours post slaughter or after aging for 120 hours. Results from tenderness comparisons between bulls and steers have been variable, with

**FIGURE 3** Changes in the activity of ( $\mu$ -calpain (U/g fresh muscle) in strip loins from steers and bull carcasses over time post slaughter when aged at 15°C (mean, SE).



many studies finding that the size of the difference between bulls and steers was less than the differences within each sex (Bailey *et al.*, 1966; Dransfield *et al.*, 1984).

In our study, m-calpain activity declined over the first 12 hours post slaughter in both steers and bull samples. Previous work by Koochmarraie *et al.* (1987) found no change in m-calpain over time in steers when the samples were aged at 4°C. Although Koochmarraie *et al.* (1995) presented a figure showing a 20% drop in m-calpain activity over the first 24 hours post slaughter, there was no further decline over the remainder of the aging period. These workers suggested that this decline was small relative to the changes in the activities of  $\mu$ -calpain and calpastatin. Goll *et al.* (1995) suggested that as m-calpain requires a higher calcium concentration for activation that its main role occurs towards the end of the aging process rather than in the initial stages. Therefore the importance of this decline in m-calpain and its role in tenderisation is unclear.

The trend for higher calpastatin activities in bull strip loins compared to steer strip loins one hour post slaughter is consistent with that of Morgan *et al.* (1993). This may contribute to the reported tendency for bull beef to be tougher than prime steer (Bailey *et al.*, 1966; Joseph and Connolly, 1974). In our study, although the activity of calpastatin tended to be higher in the bull samples, approximately 15% of the activity of calpastatin was lost within 24 hours of slaughter. This meant that by 24 hours post slaughter, the differences in calpastatin activity between the bull and steer samples had disappeared. Therefore, the calpastatin activity results support the data showing no differences in shear force from 24 to 120 hours post slaughter. The constant level of activity of calpastatin post slaughter in the steer samples is in contrast to previous reports where losses of around 50% have been recorded (Ducastaing *et al.*, 1985).

After the initial differences one hour post slaughter there were no further differences detected in  $\mu$ -calpain activity between samples from bulls and steers. In this experiment about 90% of the  $\mu$ -calpain activity disap-

peared within 24 hours of slaughter. Generally the reported rates of loss of  $\mu$ -calpain activity post slaughter have been slower than the losses in this experiment with losses of around 50 % in 24 hours in other studies (Ducastaing et al., 1985, Koohmaraie et al., 1987). These previous studies have generally reduced the temperature of the meat to 4°C over the first 24 hours and then aged at lower temperatures (-1 or 4°C). In this study aging occurred at 15°C. The higher aging temperature would result in a more rapid aging rate than in experiments carried out at -1 or 4°C (Dransfield et al., 1981) and a more rapid loss in  $\mu$ -calpain activity. As there were no differences in shear force in this experiment, the higher calpastatin activity one hour post slaughter in the bull samples appears to have been counteracted by their higher  $\mu$ -calpain activity at this time.

There were no differences in shear force between the bull and steer samples but there were differences in the activities of calpastatin and  $\mu$ -calpain one hour post slaughter. This suggests that the differences between bulls and steers in the activities of the components of the calpain system one hour post slaughter were not related to the degree of tenderisation. There were, however, no differences in the activities of calpastatin and  $\mu$ -calpain 24 hours post slaughter, nor were there any differences in shear force at this point. These samples all had acceptable ultimate pHs (below 5.7, 24 hours post slaughter).

#### ACKNOWLEDGEMENTS

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