Effect of growth path on the calpain system and shear force in Angus steers.

Thomson, B.C*., Dobbie, P.M*., Bass, J.J.*, Singh, K* and Muir, P.D.#
* AgResearch, Ruakura Research Station, Hamilton, New Zealand
# AgResearch, Poukawa Research Station, Havelock North, New Zealand

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Background

One of the important characteristics contributing to overall meat quality is tenderness. A number of factors affect meat tenderness including breed and nutritional status. The reported effects of nutritional status on meat tenderness in ruminants are variable and appear to be related to the effect of nutrition on protein turnover. Aberle *et al.* (1981) suggested that the growth rate prior to slaughter may be a major factor determining tenderness with faster growing animals being more tender. The mechanism by which this occurs is not completely understood. One of the systems reported to be involved in the post slaughter tenderisation of meat is the calcium-dependent protease system (calpains, E.C. 3.4.22.17) (Koohmaraie et al., 1995). This system consists of at least two enzymes (μ-calpain and m-calpain) and their endogenous inhibitor calpastatin.
This experiment compared steers fed ad libitum on a feedlot from 9 months of age and another group fed at pasture until 25 months of age and then allowed to compensate, and slaughtered at approximately 800 kg liveweight to determine the effects on the calpain system and to relate these differences to differences in shear force.

Methods

Twenty six, 9 month old Angus steers were randomly allocated to one of two treatment groups. One group of 13 steers was placed on the feedlot at nine months of age (F9) and fed ad libitum on 70 % maize grain and 30 % pasture silage, until slaughter at 30 months of age (mean liveweight 801 kg) after growth rate had ceased for 8 weeks. The second group of 13 steers was fed at pasture until 25 months of age (F25) at which point they were 164 kg lighter than the F9 group. The F25 group was then introduced to the feedlot and placed on the same ration as the F9 steers. While on the feedlot the F25 steers grew faster than the F9 steers. The compensating Angus steers (F25) were slaughtered 83 days after the F9 group when their mean liveweight was not significantly different from the F9 steers.

All steers were slaughtered in a commercial abbatoir. At slaughter longissimus muscle samples were collected over the 13 rib for the analysis of the components of the calpain system. After splitting, carcasses were chilled to 4 °C in a commercial blast chiller. Longissimus muscles were removed and placed on ice. Shear force was determined using a MIRINZ pneumatic tenderometer 48 hours post slaughter and after aging at -1 °C for 21 days (Frazerhurst and MacFarlane, 1983, Graafhuis *et al.*, 1991).
After removing dissectible fat, the activities of the components of the calpain system were determined in muscle samples homogenized 2 and 24 hours post slaughter. Approximately 5 g of homogenized muscle was loaded onto a DEAE-sephacel column and eluted with a step-wise NaCl gradient (Sainz *et al.* 1992, Thomson *et al.*, 1992; Dobbie *et al.*, 1995). Enzyme activity was defined as the increase in absorbance at 278 nm after 60 minutes at 25 °C in the presence of casein and calcium chloride (Wheeler and Koohmaraie, 1991). The extracted protein concentration was determined using the BCA protein assay kit (Pierce, Rockford, Illinois, USA) using bovine serum albumin V as the standard.

Data were analysed using ANOVA and regression procedures (Minitab for Windows; Version 10.1)

Results and Discussion

Shear forces determined after aging for 21 days at -1°C were higher in the muscles from F9 steers than those from F25 steers (6.9 vs 5.9, SE 0.23, $P < 0.05$). The activity of calpastatin increased over the first 24 hours post slaughter in the F9 steers ($P<0.01$) but not in the F25 steers. μ-calpain activity declined with time post slaughter ($P < 0.001$) while m-calpain activity tended to increase in both groups of steers ($P < 0.10$). The F25 steers had the highest calpastatin activities 2 hours post slaughter ($P < 0.05$) but the effects had disappeared by 24 hours post slaughter. The activity of μ-calpain was lower in the F9 steers ($P < 0.001$), however, these differences disappeared within 24 hours of slaughter. Time on the feedlot had no effect on m-calpain activity at either 2 or 24 hours post slaughter (Table). There were no associations between aged muscle shear force and calpastatin or m-calpain activity 2 or 24 hours post slaughter. There was a weak, negative association between shear force (kg) and μ-calpain activity 2 hours post slaughter (Unit / g muscle). Shear force = 8.66 - 4.84 μ-calpain activity 2 hours post slaughter ($R^2= 0.35$; $P < 0.001$).

The reported effects of nutrition on the activity of the components of the calpain system are variable. For example, Higgins *et al.* (1988) found that nutritional status did not affect the calpain system in lambs fed above maintenance, whereas, restricting intake in weaned lambs resulted in a lower ratio of calpastatin: μ-calpain activity (Thomson *et al.*, 1992). These studies did not determine tenderness. Studies on the effect of nutrition on the rate of protein degradation in skeletal muscle have also produced a range of responses in different types of animals. In small laboratory animals improving nutritional status increases the rate of protein degradation in muscle, in ruminants a similar effect occurs when nutritional status is raised to maintenance from lower levels. However, in ruminants fed above maintenance the effects on protein degradation rate appear to be more variable. For example improving the nutritional status above maintenance in Merino cross sheep increased the rate of protein degradation (Oddy, 1993), while in Suffolk cross and Romney cross wethers (Harris *et al.*, 1992, Thomson, 1994) the rates of protein degradation remained relatively constant. Thomson *et al.* (1992) found a lower ratio of calpastatin :μ-calpain activity and a higher rate of protein degradation in the hind-limb of lambs on a restricted level of feeding as compared to those fed *ad-libitum*. These inconsistencies in the response of protein turnover and protein degradation rate may explain why results investigating the effect of nutritional status on meat tenderness are also variable.

Conclusion

Growth path did affect the calpain system and tenderness as determined by shear force. The steers growing fastest prior to slaughter (F25) were more tender and had higher μ-calpain and calpastatin activities 2 hours post slaughter. A high level of μ-calpain activity is likely to be related to a high rate of protein degradation and this should result in an increase in the myofibrillar fragmentation and therefore tenderness. The μ-calpain activities had a weak negative association with shear force while there was no association with calpastatin suggesting that in this situation the level of μ-calpain activity close to slaughter was more important in determining tenderness than calpastatin activity.

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Table 1. The effect of breed and growth path on the activity of the components of the calpain system (U/g fresh muscle) 2 hours post slaughter

Time post slaughter (d)	F9	F25	SE[1]	P[2]
Calpastatin				
2	1.6	2.3	0.10	0.014
24	2.2	2.4	0.11	0.44
P	0.01	0.45		
μ-calpain				
2	0.38	0.69	0.026	0.001
24	0.17	0.19	0023	0.36
P	0.001	0.001		
m-calpain				
2	0.73	0.66	0.045	0.34
24	1.03	0.76	0.17	0.15
P	0.08	0.07		
Ratio[3]	5.3	3.4	0.67	0.16

SE[1] standard error of the mean, P[2] probability of making a type I error,
Ratio[3] = ratio of calpastatin to μ-calpain activity.