Calpain and calpastatin activity in muscle from genetic lines of lambs selected for divergent levels of fatness

J.D. MORTON, R. BICKERSTAFFE AND R.W. PURCHAS[1]

Animal and Food Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand.

ABSTRACT

There is widespread interest in the extent to which genetic factors influence meat quality. In this study we examined meat quality characteristics and the activity of the calpain protease system in *M. longissimus lumborum* (LL) from backfat selection lines of Southdown lambs.

The high fat line produced heavier carcasses with greater soft tissue depth GR. LL from the high fat line animals had a lower ultimate pH and was significantly more tender at 24h post-mortem using the MIRINZ tenderometer but not the Warner-Bratzler peak force.

There was a decline in μ-calpain and calpastatin activity but not m-calpain in the 9h post-mortem. There were no significant differences in the levels of the calpains or their inhibitor between high and low backfat lines. However, when the results were adjusted for pH, the 20 min calpastatin levels were highest for the sire group that had the highest peak force value.

Keywords: sheep ; selection lines ; backfat ; tenderness ; calpains.

INTRODUCTION

There has been a world-wide trend for meat-producing countries to improve the quality and consistency of their product. In New Zealand this is evidenced by the recent introduction of the Beef and Lamb Quality Mark and in Australia the new Eating Quality Standards grading system for beef. Surveys have shown both that tenderness is one of the most important quality attributes to the consumer (Koohmaraiae, 1996) and that a significant proportion of the meat available in New Zealand supermarkets is unacceptably tough (Bickerstaffe *et al*., 1997).

The final tenderness of meat is the result of a number of factors. These include the characteristics of the animal, on-farm practices, processing conditions and treatment by retailers and consumers. Efforts to improve livestock by breeding have focused on growth efficiencies and, more recently, leaner animals. It is important to determine whether by selecting for these characteristics breeders have also selected for animals which yield tougher meat. An extreme example of this is in the Callipyge sheep (Koohmaraie *et al*., 1995).

The biochemical processes involved in producing tender meat are still not fully understood although there is considerable evidence that the calpains, a family of calcium-dependent cytosolic proteases, have a key role in tenderisation (Koohmaraiae, 1996). Indeed, the toughness of meat from Callipyge sheep is associated with and ascribed to the abnormally high levels of calpastatin, the endogenous inhibitor of calpains (Koohmaraie *et al*., 1995). In this case it appears inhibition of the calpains promotes muscle growth and eye muscle area in the live animal but also inhibits the tenderisation process. Hence the increase in meat toughness.

The aim of the experiment described in this paper was to determine whether the *M. longissimus lumborum* (LL) from sheep selected for low backfat differed from those selected for high backfat with respect to meat tenderness or levels of the calpain proteases in muscle.

MATERIALS AND METHODS

Animals, carcasses and muscle sampling

Thirty two ram lambs (13-month old) from the Massey University Southdown backfat selection lines were processed to AC and A standards at Lamb Packers Fielding, Ltd. There were 16 lambs from each of the high and low backfat lines. Animals within each line originated from 2 sires. Samples (5g) of LL in the region of the first lumbar vertebra were taken at 20 min (prior to high-voltage electrical stimulation) and 9h post mortem. Measurements of pH and temperature of the right LL at the 12th rib of each carcass were made before electrical stimulation and at 8 and 33 h after slaughter using an Orion 8163 glass electrode and a temperature probe attached to a Hanna H19025 portable pH meter.

Carcasses were held in conditioning rooms at 12°C for 4h and then 4h at 8°C. Then about 150mm of the LL was removed from the left hand side of carcasses. These boneless loins were subsequently held at 3°C. A sample was removed from the anterior end of the loins at 9h for determination of calpains. At 24 h post-mortem each boneless loin was divided into two sections and frozen at -20°C until required. The portion immediately caudal to the calpain sample was used for determination of Warner-Bratzler shear. The remainder of the loin was used for analysis on the MIRINZ tenderometer.

Tenderness determination

MIRINZ Tenderometer

The samples were thawed at 2°C and cooked in plastic bags immersed in water at 80°C until they reached an

[1] Institute for Food, Nutrition and Human Health, Massey University, Priv. Bag 11-222, Palmerston North, New Zealand

internal temperature of 75°C and then cooled. Internal meat temperatures were measured by Fluke Type K Temperatures Probes attached to Fluke 52 meters. At least 6 test pieces (10x10mm cross-section x25mm along the length of the muscle fibres) were placed separately in the MIRINZ tenderometer (Chrystall and Devine, 1991) and the shear force (kgF) to cut across the fibres determined.

Warner-Bratzler

The samples were cooked for 90 minutes in a 70°C and then cooled. Twelve test-pieces (13x13mm) were analysed (Kadim *et al.* 1993).

Protease extraction and separation

Samples (5g) were homogenised immediately in 30ml of 100mM Tris-HCl, 10mM mercaptoethanol, 10mM EDTA, pH 8.3 using an Ultra-Turrax homogeniser (3x30s, full speed). The homogenate was centrifuged at 27,000g for 30 min at 4°C. The supernatant was filtered through a glass wool - cheese cloth 'sandwich', dialysed against 40mM Tris, 3mM EDTA, 10mM mercaptoethanol, pH 7.5 for at least 12 hours and centrifuged.

The supernatant was loaded onto a 20x1.5cm DEAE-Sepharose Fastflow (Pharmacia) column for separation of µ-calpain, m-calpain and calpastatin. After washing with 150ml of 40mM Tris, 0.5mM EDTA, 10mM mercaptoethanol, pH 7.5 (buffer A), a two stage elution of 0 to 175mM NaCl followed by a steeper linear gradient to 500mM NaCl both in buffer A.

Assay of calpains and calpastatin

Aliquots (1ml) of column fractions were incubated at 25°C with 1ml of 0.7% casein in 100mM Tris-HCl, 10mM mercaptoethanol, 1 mM NaN_3, pH 7.5 containing 100µl of either 0.1M $CaCl_2$ or 0.2M EDTA. After 30 minutes 2ml of 5% TCA was added and centrifuged at 3,000g for 15min. The absorbance of the supernatant was read at 278nm (1cm light path). A unit of calpain was defined as the amount which gave a Ca-dependent increase of 1.0 unit of absorption in one hour. Calpastatin was determined and expressed as inhibitory equivalents of m-calpain.

Analysis of Data.

Statistical analysis was carried out using the Minitab version 10 computer package.

RESULTS

Carcass Characteristics

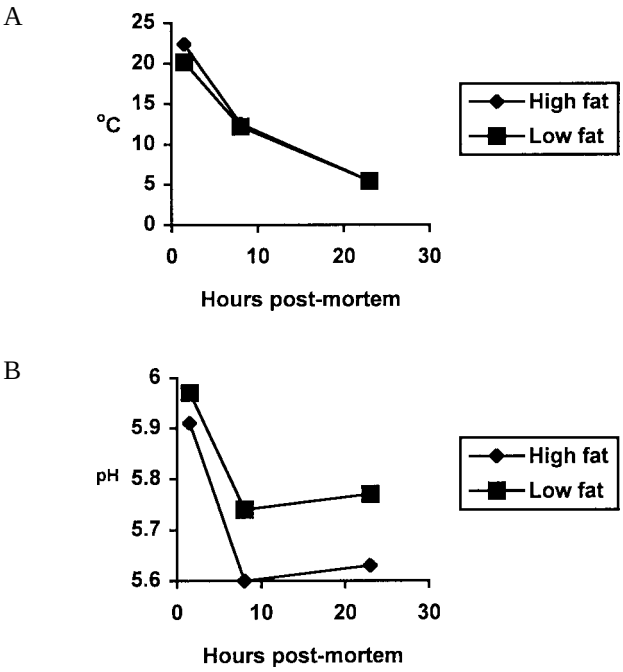
Table 1 shows that although the mean liveweight of the two lines of Southdown lambs was similar the high fat line produced shorter, heavier carcasses. As expected the high fat line had 70% more soft tissue (GR) than the low fat line. Associated with the high fat cover was a slightly slower carcass cooling rate (Figure 1A). All the carcasses reached their ultimate pH by 8h post-mortem with the carcasses from the low fat line having a significantly higher value (Figure 1B).

TABLE 1: Mean carcass characteristics of the high and low fat lines and their standard errors (in brackets). The GR and carcass length values have been adjusted to a constant weight by fitting carcass weight as a covariate.

	High fat	Low fat	Significance[a]
N	16	16	
Live weight (kg)	48 (1.2)	47 (0.9)	NS
Carcass weight (kg)	22.5 (0.67)	20.6 (0.53)	*
Carcass length (mm)	963 (6)	1013 (6)	*
GR depth (mm)	19.1 (0.8)	12.6 (0.8)	***
Ultimate pH	5.63 (0.011)	5.71 (0.039)	**

[a]NS, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001

FIGURE 1: Post-mortem decline in temperature (A) and pH (B) of the high and low fat lines.



Meat Quality

Meat from both lines were within tenderness values expected from AC and A processing (Table 2). The LL of the high fat line was significantly more tender at 24h post-mortem than meat from the low fat line using the MIRINZ tenderometer. The Warner Bratzler values showed a similar trend in peak force values but the difference between lines was not significant.

TABLE 2: Tenderness of the LL at 24h post-mortem of the high and low fat lines and their standard errors (in brackets).

	High fat	Low fat	Significance[a]
WB peak force	8.53 (0.44)	9.54 (0.56)	NS
MIRINZ shear force(kg)	8.56 (0.35)	9.84 (0.33)	*

[a]NS, p>0.05; *, p<0.05; **

Calpains

There was a decline in µ-calpain and calpastatin over the first 9 hours post-mortem (Table 3). There was no change in the level of m-calpain. Furthermore, there were no significant differences in the levels of the calpains or their inhibitor between the high and low backfat lines.

Sire Groups

When the results were analysed by sire group some interesting trends emerged (Table 4). The two low fat groups had a higher ultimate pH with 4 being the highest.

Comparison of the shear force values showed that sire group 3 produced the toughest meat. This was associated with higher levels of calpastatin, the calpain inhibitor. These trends were not significant. However adjustment of the data by covariance to a common ultimate pH showed there was a significant difference between the two low-backfat sire groups in WB peak force values. The group with the highest peak force values also had significantly higher levels of calpastatin after adjustment for pH.

TABLE 3: Levels of the calpains and calpastatin in the LL of the high and low fat lines and their standard errors (in brackets).

		High fat	Low fat	Significance[a]
μ-calpain	20 min	0.75 (0.04)	0.79 (0.04)	NS
	9 h	0.36 (0.04)	0.46 (0.05)	NS
m-calpain	20 min	0.94 (0.07)	0.84 (0.04)	NS
	9 h	0.88 (0.08)	0.87 (0.06)	NS
calpastatin	20 min	2.33 (0.19)	2.22 (0.12)	NS
	9 h	1.44 (0.18)	1.58 (0.16)	NS

[a]NS, $p>0.05$;

TABLE 4: Differences meat characteristics of the LL among sire groups.

	High fat		Low fat	
	1	2	3	4
N	10	6	8	8
Ultimate pH	5.63[a] (0.02)	5.63[a] (0.02)	5.73[ab] (0.05)	5.82[b] (0.05)
WB Peak force	8.31 (0.60)	8.91 (0.66)	10.29 (0.80)	8.78 (0.73)
MIRINZ shear	8.64 (0.39)	8.44 (0.71)	10.20 (0.56)	9.48 (0.34)
calpastatin (20 min)	2.38 (0.22)	2.26 (0.36)	2.62 (0.08)	1.59 (0.28)
calpastatin (9 h)	1.21 (0.20)	1.83 (0.32)	1.81 (0.19)	1.35 (0.21)

Values within the same row with different superscripts are significantly different at the ($p< 0.05$).

DISCUSSION

The carcasses in this trial showed a rapid decline in pH and had entered rigor by 8 hours post-mortem. This was consistent with results from other experiments with electrically stimulated lamb carcasses (Morton *et al*., 1997). The mean ultimate pH values were also comparable but the mean shear force at 24h was higher than expected. Although the carcasses were within AC and A specifications they would require further aging to ensure compliance with the Lamb Quality Mark.

The LL from the low-fat line was slightly tougher by all measurements with the MIRINZ tenderometer showing a significant difference ($p<0.05$) between the means. Kadim *et al*. (1993) previously found no significant differences between the lines in WB shear values for this muscle. The higher shear values for the low-backfat line was largely due to the influence of sire 3 (Table 4), but GR values for that group were similar to those for sire 4.

The mean levels of calpains were 40% lower than those measured previously (Bickerstaffe *et al*., 1996; Morton *et al*., 1997). The calpastatin levels were 30% lower. The resulting higher ratio of inhibitor to enzyme may explain the slower tenderisation in these carcasses. Although no difference was found between the calpain systems of high and low fat lines, an association between calpastatin levels and meat tenderness is suggested by the fact that, when adjusted for differences in ultimate pH by covariance, both the WB shear values and the 20-minute calpastatin were significantly higher ($p<0.05$) for sire-group 3 than sire-group 4.

CONCLUSION

These results suggest that selection for divergence in backfat may lead to small changes in meat quality and that sire groups within lines may also differ. There is also some limited support for the role of proteases in the tenderisation of meat.

ACKNOWLEDGEMENTS

The authors would like to acknowledge financial support from the Foundation for Research, Science and Technology.

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