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## Calpain, calpastatin and tenderness comparisons in *M. longissimus dorsi* samples from weight-selected and control Angus cattle

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### ABSTRACT

Meat samples (*M. longissimus dorsi*) from a control Angus herd (ACO) and associated selection herd (AS1) were compared for calpain/calpastatin concentrations and shear-force tenderness, after the herds had been subjected to 22 to 24 years of random replacement or selection for increased adjusted yearling weight. The objective was to monitor changes in meat tenderness at fixed slaughter date resulting from genetic selection, and associated changes in muscle calpain or calpastatin concentrations. Bull calves (n=94), from 3 successive calf crops raised on pasture, were slaughtered immediately after arrival at the abattoir at about 20 months of age on one day each year (May 1995, 1996 and 1997). Carcasses were electrically stimulated, weighed and hot boned. Meat samples for calpain/calpastatin analysis were taken within 30 minutes of slaughter from the *M. longissimus dorsi*, with further samples after 24 hr of ageing at 10°C. Shear-force tenderness was assessed on the same muscle at intervals from 24 hr until one month of ageing at -1°C. Calpastatin, milli- and micro-calpain activities were assayed using casein as a substrate. Yearling live weights, hot carcass weights and muscle weights were respectively 19.0%, 16.5% and 17.3% greater in the AS1 than the ACO herd (all P<0.001). The AS1 samples were on average 8 to 16% more tender than ACO samples (P<0.05) on days 1 and 3 *post mortem*, but there were no significant herd differences in tenderness at later ageing times. The between-animal repeatability of shear-force tenderness from days 1 to 28 was 0.44±0.07. Millicalpain concentrations were significantly lower in the AS1 than ACO samples by an average of 13% (P<0.001), and there were smaller differences in microcalpain and calpastatin between herds in the same direction. These results show that early *post mortem* tenderness, millicalpain and possibly microcalpain and calpastatin concentrations were associated with genetic differences in growth.

**Keywords:** cattle; liveweight; selection; tenderness; calpain

### INTRODUCTION

Tenderness is an important attribute of meat, with a significant impact on consumer acceptability of the product. Considerable variation in meat tenderness exists even under modern production and handling systems (Morgan *et al.*, 1991). Final tenderness of meat is determined by the extent of *post mortem* proteolysis of key myofibrillar proteins. The calpain proteolytic system plays a major role in the tenderisation of meat *post mortem* (Koochmaraie, 1992). Given the possible role of the calpain system in normal growth and metabolism (Goll *et al.*, 1989), it is likely that differences exist between animals selected for improved growth performance, and this may impact on meat tenderness *post mortem*.

Few studies have examined the impact of genetic improvement for growth on beef meat quality and in particular meat tenderness. Burrow *et al.* (1991) have demonstrated that shear values for tenderness from chilled meat samples did not differ in lines of cattle selected for increased weight at a given age. The purpose of the current study was to assess the impact of persistent selection for yearling weight on components of the calpain system and the possible relationship with meat tenderness.

### MATERIALS AND METHODS

#### Animals

The selection and control herds which provided meat samples for this comparison were Angus cattle extensively sampled from industry sources to establish a foundation herd in 1969-72 (Baker *et al.*, 1986). Selection for yearling weight in one herd (the AS1 herd), alongside an unselected control (ACO) herd, began in 1971 at Waikite Station near Rotorua, as described by Baker *et al.* (1991). The cow herd was transferred to Rotomahana Station near Rotorua after the 1988 calvings and then to AgResearch's Whatawhata Research Station near Hamilton in 1991. The cow numbers calving in September/early October at Whatawhata have averaged about 50 per herd each year. The AS1 herd achieved a genetic increase in live weight of 37.7 kg over the first 15 years (2.51 kg/year), representing a total of 16.0% of the mean weight of control bulls (Baker *et al.*, 1991). Only small increases have been achieved since then, mainly as a result of the reduced herd numbers.

Bull calves from the calf crops born in 1993, 1994 and 1995 at Whatawhata were the subject of the present study, and they were grown out after weaning at AgResearch's Tokanui Station near Te Awamutu. Calves grazed on ryegrass-white clover pasture, with no concen-

trate feeding, but they received minimal supplementation with hay or silage in winter. Apart from two or three bulls per line per year used for yearling mating, all bulls grazed together at Tokanui until slaughter on one day at about 20 months of age in May 1995, 1996 or 1997. The fastest periods of pasture growth and animal growth at Tokanui were in spring/early summer, when bulls were between 13 and 15 months of age. The AS1 herd (1993-, 1994-, and 1995-born bull calves) was heavier than the ACO herd as yearlings by an average of  $19.0 \pm 1.5\%$ , and in carcass weight at 20 months by an average of  $16.5 \pm 2.0\%$ .

**Slaughter and measurement procedures**

*Slaughter.* The animals were first weighed off pasture at Tokanui, three days (1995) or one day (1996 and 1997) before slaughter, then transported to a commercial abattoir in Hamilton and slaughtered immediately after arrival. Animals were handled together at all times, and pre-slaughter handling (which could have affected ultimate pH) was the same for all animals. Carcasses were stimulated at 70 volts for up to 90 seconds at a frequency of 15 pulses per second. Immediately after slaughter and dressing, each carcass was split into left and right sides, and the hot carcass weight was obtained from the two side weights.

The *M. longissimus dorsi* were obtained from the left side of each carcass. The muscles were removed during the hot boning procedure within 30 minutes of slaughter and transported to a temperature controlled room (10°C) for pH and temperature monitoring. Twenty four hours after slaughter, muscles were portioned and vacuum packed before being placed at -1°C for sampling at intervals through to about one month post mortem (35 days in the first year; 28 days in the other two years).

*Tenderometer studies.* The *M. longissimus* portions (approximately 6 cm in width) were placed inside a plastic cooking bag and heated in a waterbath at 85°C, until they reached an internal temperature of 75°C. They were then removed, cooled rapidly in ice to an internal temperature of 2°C (Graafhuis *et al.*, 1991), and shear-force measurements were taken using a MIRINZ tenderometer (Frazerhurst and MacFarlane, 1983).

*Calpain and calpastatin determination.* Samples for calpain and calpastatin determination were removed from the distal end of the *M. longissimus*. Samples were processed within 30 minutes of slaughter, and in 1996 at 24 hours *post mortem* also. Fresh samples (approximately 5g) were accurately weighed and then processed for calpastatin, milli-calpain and micro-calpain assays by stepwise gradient in DEAE-Sephacel columns, as previously described in detail by Thomson *et al.* (1996). All fractions were assayed for proteolytic activity and the fractions containing enzyme or inhibitory activity were pooled and reassayed. Enzyme activity was defined as the increase in absorbance at 278 nm after 60 minutes at 25°C in the presence of casein and Ca Cl<sub>2</sub> (Wheeler and Koohmaraie, 1991).

**Statistical methods**

Results were analysed by analysis of variance (Genstat, 1994). Effects were fitted for herd, sampling day, year of

birth (or year of kill) and their two-way interactions. Preliminary statistical models were fitted to test for the significance of two possible fixed factors in addition: age of dam and date of birth covariate. Where any of these latter two effects or the two-way interactions were not significant, they were removed and the model was re-run.

**TABLE 1:** Effect of herd on the selection criterion (yearling weight), pre-slaughter live weight, hot carcass weight and *M. longissimus* weight (left side), for bull calves born in 1993-95 and slaughtered in May 1995, 1996 and 1997.

Trait	Selection herd, AS1	Control herd, ACO	AS1-ACO ± s.e.d.	Signif.	σ <sub>p</sub> <sup>a</sup>
Yearling weight <sup>b</sup> , kg	261.9	220.1	41.8±3.4	***	25.0
Pre-slaughter live weight, kg	478.6	408.0	70.6±7.6	***	31.8
Hot carcass weight, kg	257.8	221.3	36.5±4.5	***	18.3
Muscle weight, kg	2.17	1.85	0.32±0.05	***	0.19
Number slaughtered					
1995	23	13			
1996	11	12			
1997	17	18			

<sup>a</sup> Phenotypic standard deviation.

<sup>b</sup> Both sexes at selection weight

**RESULTS**

Table 1 shows the numbers of animals slaughtered and the least squares means for live and carcass weights by herd. The mean yearling (i.e. selection) weight was 19.0% higher (P<0.001) in the AS1 herd (both sexes) than in the ACO herd. On average the AS1 herd bulls for slaughter then had a 17.3% greater live weight at 20 months and 16.5% greater hot carcass weight than ACO herd bulls (both P<0.001). There was a significant herd effect on *M. longissimus* muscle weight (P<0.001), with AS1 muscles being heavier by 17.3%, on average.

Shear-force measurements showed that the AS1 meat was more tender than ACO meat, as it required 8.2% less shear force than ACO meat (12.62 vs 13.75 kg, respectively) on day 1 after slaughter (P<0.05), 15.9% less force (8.65 vs 10.28 kg, respectively) on day 3 (P<0.05) and 9.5% less force (7.31 vs 8.08 kg, respectively) on day 7 (P<0.10). On days 14, 21 and one month after slaughter, the meat from both herds was tender (5.98 kg F), and not significantly different between herds. The shear force required on these latter days was less than half of that required on day 1 (13.19 kg F). It was notable that the shear-force variability also declined by a half from days 1 to 14, as indicated by the root mean square errors (phenotypic standard deviation overall = 1.66 kg F). Ageing rate for 1996 muscles, as determined by the equations of Dransfield (1992), highlighted the fact that meat from the AS1 line aged at twice the rate of meat from the ACO line.

The repeatability estimate (among days 1-28) for tenderness measurements was  $0.44 \pm 0.07$ , indicating that tenderness/toughness rankings on day 1 from animal to

**TABLE 2:** Effect of herd (selected, AS1, or control, ACO) on calpains and calpastatin (activity units per g muscle) in *M. longissimus dorsi* samples at two times after slaughter.

Effect	m-calpain	μ-calpain	calpastatin
<b>30 minutes after slaughter (3 years of data)</b>			
Herd: AS1	0.97	0.65	2.81
ACO	1.12	0.72	3.02
AS1-ACO	-0.15±0.04	-0.07±0.04	-0.21±0.17
Significance	***	n.s.	n.s.
Root mean square error	0.19	0.19	0.80
Interaction (herd x year)	***	n.s.	n.s.
Interaction (herd x time; 1996 data only)	n.s.	*	n.s.
<b>1996 data only</b>			
Time after slaughter: 30 min (x)	1.35	0.77	3.21
24 hr (y)	1.22	0.43	2.44
(x-y)	0.13±0.07	0.34±0.06	0.77±0.20
Significance	n.s.	***	***
<b>Interaction of herd and time effects (1996 data only)</b>			
Herd effect at 30 min AS1-ACO		-0.12±0.08	
Herd effect at 24 hr AS1-ACO		+0.13±0.07	

<sup>a</sup>This interaction represented a change in magnitude of the herd differences from year to year, without a change in rank.

animal tended to persist through to day 28. The regression of shear force on within-herd carcass weight (fitted simultaneously with a date of birth covariate) was not significant when considered across years. Younger animals were significantly more tender (by 2.6% per week;  $P < 0.05$ ).

Table 2 shows the results of millicalpain, microcalpain and calpastatin assays from the AS1 and ACO herds, and the effect of time of muscle sampling (for 1996 only). There was a significant herd difference in millicalpain activity ( $P < 0.001$ ). Averaging over years and times *post mortem*, the AS1 herd activity was  $13 \pm 4\%$  less than the ACO activity. There was a significant herd by year interaction (although it did not lead to any reranking of the herds), but there was no significant effect of time since slaughter (1996 data). For microcalpain and calpastatin, the herd effects were not significant, although the AS1 means tended to be smaller than the ACO means. For both assays, mean activities found at 24 hr *post mortem* were significantly lower than at 30 minutes *post mortem*.

From the 1996 and 1997 samples, there were residual correlations between 30-minute calpastatin activity and shear force at day 1 (0.34), day 3 (0.39), day 7 (0.32) and day 28 (0.29) *post mortem* (all  $P < 0.05$ ), and from one year (1996) there were data to estimate residual correlations between 24-hour calpastatin activity and shear force and these averaged 0.18 (not significant).

## DISCUSSION

The present study has demonstrated a significant effect of enhanced growth performance on the ageing rate and tenderness of *M. longissimus dorsi* at 1 and 3 days and possibly at 7 days, which in itself has important implica-

tions with respect to processing meat from lines of animals even of the same breed. The results highlight the fact that meat derived from animals almost completely devoid of any external carcass fat has the potential to provide a product which is highly desirable in terms of tenderness. The current study was a more sensitive test than the serial slaughter study by Morris *et al.* (1995), where no significant herd differences in tenderness were found in the *M. longissimus* of bulls aged for five days *post mortem* at 1°C.

Our results confirm the findings of Koohmaraie (1992) that increased calpastatin inhibitory activity is associated with increased toughness of meat. In the current study we found a positive (within-herd) relationship between 30-minute but not 24-hour calpastatin and shear force at various intervals *post mortem*. For comparison, the studies of Shackelford *et al.* (1994) obtained a genetic correlation of  $0.50 \pm 0.22$  and a phenotypic correlation of  $0.27 \pm 0.04$  between 24-hour calpastatin activity and 7 to 9 day shear force in steers. Our study used bulls, primarily as a consequence of the genetic selection and future breeding aspects of the overall experiment.

There was also an effect of genotype on millicalpain (i.e. consistently lower activities in the AS1 selected cattle) but not on microcalpain activities. Reduced millicalpain activity is associated with increased proportions of glycolytic (fast-twitch) muscle fibres. Muscles with higher glycolytic content age more quickly and are more tender (Monin and Ouali, 1991). From the millicalpain and tenderness results observed in the two herds in this experiment, it is suggested that selecting Angus cattle for increased liveweight leads to altered muscle fibre composition. This possibility is now being investigated further.

## ACKNOWLEDGEMENTS

This study was funded by the New Zealand Foundation for Research, Science and Technology. We are grateful for assistance from Mr A.E. Graafhuis (Meat Industry Research Institute of New Zealand) for assistance with controlled cooling of samples in 1996.

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