Australian Journal
of Experimental Agriculture

*. . . a journal publishing papers at the cutting edge
of applied agricultural research*

Meat quality and the calpain system of feedlot steers following a single generation of divergent selection for residual feed intake

M. B. McDonagh[A]*, R. M. Herd*[BF]*, E. C. Richardson*[C]*, V. H. Oddy*[D]*, J. A. Archer*[E] *and P. F. Arthur*[E]

Cooperative Research Centre for Cattle and Beef Quality
[A]School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351, Australia.
[B]NSW Agriculture, Beef Industry Centre, Armidale, NSW 2531, Australia.
[C]NSW Agriculture, Agricultural Research Institute, Wagga Wagga, NSW 2650, Australia.
[D]Meat and Livestock Australia, 165 Walker Street, North Sydney, NSW 2060, Australia.
[E]NSW Agriculture, Agricultural Research Centre, Trangie, NSW 2823, Australia.
[F]Author for correspondence; e-mail: robert.herd@agric.nsw.gov.au

Abstract. Residual feed intake (RFI) is calculated as the difference between an animal's actual feed intake and its expected feed intake based on its size and growth over a specified test period. Following a single generation of divergent selection for postweaning RFI, Angus steers and Angus × Hereford, Angus × Poll Hereford and Angus × Shorthorn crossbred steers born in 1996 and 1997 were fed in a feedlot. Cohorts of steers were slaughtered at the same age and had attained similar ($P>0.05$) final liveweights: 467 kg for steers selected for low RFI (high efficiency; HE, $n = 91$) and 459 kg for steers selected for high RFI (low efficiency; LE, $n = 98$). The HE and LE steers had similar ($P>0.05$) carcass weight (247 and 244 kg), dressing percentage (53.1 and 53.2%) and eye-muscle area (58.9 and 60.3 cm^2). The HE steers had slightly less subcutaneous fat over the rib than the LE steers (9.2 *v.* 10.1 mm, $P<0.05$), and there was a trend towards less fat over the rump of HE steers (11.5 *v.* 12.1 mm, $P = 0.10$).

For meat samples taken from the *M. longissimus dorsi* (LD) there were no differences ($P>0.05$) between the HE and LE steers in content of intramuscular fat (5.4 and 5.3% fresh weight), marbling scores, meat colour and fat colour. There were also no differences ($P>0.05$) between HE and LE steers in shear force and compression values for samples of LD aged for 1 day (4.6 and 4.6 kg shear force, 1.45 and 1.44 kg compression), or for 14 days (3.8 and 3.5 kg, 1.36 and 1.32 kg). Myofibril fragmentation index (MFI) measures the breakdown of these structural elements which occurs as an initial step in the process of protein degradation and meat tenderisation. MFI was lower (i.e. less fragmentation; $P<0.05$) in LD samples from HE steers than from LE steers, both in samples aged for 1 day (67.7 *v.* 72.5 units) and in samples aged for 14 days (87.8 *v.* 91.1 units). The rate of decline in MFI between 1 and 14 days post slaughter was similar in the LD samples from both lines of steers. There were no differences ($P>0.05$) between HE and LE steers in the activity of m-calpain and μ-calpain in LD immediately after slaughter (HE steers: 1.9 and 2.3 units, LE steers: 1.8 and 2.1 units). The level of calpastatin in LD from the HE steers was 13% higher than in the LD from the LE steers (5.2 and 4.6 units respectively, $P<0.05$). Rate of myofibril fragmentation was positively correlated ($P<0.01$) with the ratios of both m-calpain and μ-calpain to calpastatin, but not ($P>0.10$) with levels of either calpain or calpastatin. A single generation of divergent selection for RFI produced differences in calpastatin and myofibril fragmentation that may, with on-going selection for low RFI, negatively affect meat tenderness.

Additional keywords: cattle, efficiency, meat quality, tenderness, calpain, calpastatin.

Introduction

Genetic selection for improved feed efficiency aims to reduce the cost of feeding in cattle production and thereby improve profitability. In beef cattle there is considerable variation in feed intake that is independent of size and growth rate, and can be calculated as residual (or net) feed intake (RFI) (Arthur *et al.* 1997). Postweaning tests for RFI on British breed cattle fed a medium-quality ration have demonstrated that genetic variation in RFI exists, and that

the trait is moderately heritable (Archer *et al.* 1998). Selection to reduce RFI has resulted in progeny that eat less with no compromise in growth performance: in bulls and heifers tested between 8 and 12 months of age (Herd *et al.* 1997), and in yearling steers fed in a feedlot (Richardson *et al.* 1998).

The genetic associations between RFI and carcass traits and meat tenderness in Australian cattle are unknown. If antagonisms between improved RFI and the latter traits exist,

they need to be identified as they could reduce the economic benefit achieved from breeding to reduce the cost of feeding cattle. A study of feedlot steers resulting from divergent selection for RFI has shown that selection to improve RFI has been accompanied by a reduction in subcutaneous fat thickness (Richardson *et al.* 1998).

The increase in tenderness that accompanies post mortem aging of meat is thought to be due to several processes including the disassembly of muscle myofibres by the proteolytic activity of μ-calpain and the specific inhibition of calpain by calpastatin (McDonagh 1998). Rate of myofibre disassembly and improvement in objective measures of meat tenderness have been shown to be positively correlated with levels of proteolytic activity of the calpain system in muscle at slaughter. Higher rates of myofibre disassembly may also be indicative of higher rates of protein breakdown in muscle in the living animal (McDonagh 1998). Protein turnover in living animals is an energetically expensive process and variation in protein metabolism has been shown to accompany genetic selection for growth and other traits in domestic animals (reviewed by Oddy 1999). Selection for RFI may produce changes in the calpain system, due to its association with efficiency of energy use in muscle, and may therefore affect the tenderness of meat.

This study investigated the consequences of a single generation of divergent selection for postweaning RFI on carcass and meat quality traits, including tenderness, and the calpain system in steers finished in a feedlot.

Materials and methods
Animals and their management
Residual feed intake is calculated as the difference between an animal's actual feed intake and its expected feed intake based on its size and growth over a specified test period. More efficient cattle have low RFI and eat less then expected for their size and growth rate. Cattle breeding and postweaning tests for RFI were done at the NSW Agriculture Research Centre, Trangie, NSW. Postweaning growth and feed intake from 8 to 12 months of age were measured on Trangie-bred Angus bulls and heifers, and Angus, Shorthorn, Hereford and Poll Hereford heifers purchased from industry herds. Details of these tests are given by Arthur *et al.* (1997). At the end of each test, heifers and bulls were ranked for RFI. The top 50% of heifers were then mated to the top 5% of bulls, and the bottom 50% of heifers mated to the bottom 5% of bulls to produce progeny bred for either low RFI (i.e. high efficiency, 'HE') or high RFI (i.e. low efficiency, 'LE'). The entire drops of steer progeny born in March and April of 1996 and 1997 were available to this study. The 1996-born steers were the progeny of 4 HE and 4 LE bulls selected from young bulls tested for RFI in 1995, and the 1997-born calves were the progeny of 4 HE and 5 LE bulls selected from young bulls tested for RFI in 1996.

The calves born in 1996 were weaned in October 1996 and trucked from Trangie to the CRC research feedlot 'Tullimba', west of Armidale, NSW. They were grown on pasture until they reached feedlot entry liveweight.

The Angus steers entered the feedlot in November 1996. After a 2-week introductory period, they were fed a ration containing 70% rolled oats, 15% hay, 5% protein meal, plus molasses and mineral additives. This ration had a dry matter (DM) content of 89.0%, which was analysed *in vitro* to have a metabolisable energy (ME) content of

10.7 MJ/kg DM and to contain 14.3% crude protein. The steers, which had *ad libitum* access to this ration, were killed in 2 groups in April and June 1997. After being fed the feedlot ration for about 180 days, they had attained an average age of 14 months and liveweight of 426 kg.

The crossbred steers entered the feedlot in February 1997. After a 2-week introductory period, they were fed a ration containing 75% rolled barley, 10% hay, 5% protein meal, plus molasses and mineral additives. This ration had a DM content of 90.1%, which was analysed *in vitro* to have a ME content of 12.0 MJ/kg DM and to contain 15.8% crude protein. The steers were killed in May 1997 after being fed on this ration for 112 days, and attaining an average age of 14 months and 508 kg liveweight. Details of the management and performance of these steers are given by Richardson *et al.* (1998).

The calves born in 1997 were weaned in September 1997. In December 1997 they were trucked from Trangie to the CSIRO Pastoral Research Centre at Chiswick, south of Armidale, NSW, to be grown on pasture to feedlot entry liveweight.

In April 1998 the steers entered the CRC research feedlot. After a 2-week introductory period, they were fed a ration containing 75% rolled barley, 10% hay, 5% protein meal, plus molasses and mineral additives. This ration had a DM content of 84.4%, which was analysed *in vitro* to have a ME content of 12.3 MJ/kg DM and to contain 15.0% crude protein. The steers had *ad libitum* access to this ration. They were slaughtered in August 1998 after being fed on this ration for 104 days, and attaining an average age of 16 months and liveweight of 462 kg.

Slaughter and sampling
Two days before departure for slaughter the cattle were weighed (no fasting) and subcutaneous rib (12/13th) and rump (Australian P8 site) fat depths were measured using an Aloka 500 ultrasound scanner. The area of the eye-muscle (*M. longissimus dorsi* at the 12/13th rib) was measured subsequently by computer analysis of stored images.

The Angus steers born in 1996 were trucked 500 km to the CSIRO Food Science abattoir at Cannon Hill, Brisbane, for slaughter. After an overnight curfew without food but with water available, the steers were killed using a captive-bolt pistol, and then bled. Electrical stimulation was not applied. Muscle samples (about 20 g) from all carcasses were removed from the right side of the eye muscle (*M. longissimus dorsi*: 'LD') between the 11th and 12th ribs within 30 min of slaughter. The samples were taken from a small incision made by knife. For the first group, killed in April 1997 (8HE:9LE), the samples were immediately wrapped in aluminium foil and stored in liquid nitrogen for subsequent determination of calpain system enzymes. The carcasses were halved, hung by the Achilles tendon and chilled at 1°C overnight. Dressing percentage was calculated as carcass weight relative to final liveweight in the feedlot. At 24 h post mortem the left half of each carcass was weighed, quartered between the 12th and 13th ribs and the LD was excised caudal to the 12th rib. The LD sample was sealed in plastic and stored frozen for subsequent analysis. For the second group of steers, killed in June 1997 ('group 2'; 8HE:8LE), a small sample of LD was taken immediately after death and a subsample was wrapped in aluminium foil and immediately placed in liquid nitrogen for storage and subsequent determination of myofibril fragmentation. The remainder of the muscle sample was homogenised immediately in buffer for separation and functional assay of the calpain system proteins. To compare levels of the calpain enzyme system components in muscle samples taken soon after death, and presumed to be representative of *in vivo* levels, to those in muscle samples taken up to 24 h post mortem, additional muscle samples were taken from the right side of 4 carcasses of low-RFI line steers. The samples were taken at 8, 16 and 24 h after slaughter and immediately homogenised in buffer and placed on columns. For all the group 2 steers, the LD sample for objective measurements of meat tenderness was taken from the right side of the carcass, divided into 3 sections of equal size and the samples were vacuum-packaged in plastic. The 3 samples from each animal

were then assigned randomly to aging at 1°C for 1, 7 and 14 days, and after reaching their assigned days of aging were then frozen (–20°C).

In May 1997, the crossbred steers were trucked to a commercial abattoir near Inverell, NSW, held in lairage for 1 day (water only available), and killed the following morning. The steers were killed using a captive-bolt pistol, and then bled. Electrical stimulation was not applied. After overnight storage at 1°C the carcasses were weighed and quartered between the 12th and 13th ribs. The exposed surface was scored by a trained assessor for meat colour, fat colour and marbling, by comparison against industry-standard coloured strips and photographs. Meat colour was scored on a scale from: 1, light pinkish red; to 9, dark red. Fat colour was scored from: 1, white; to 9, yellow. Marbling was scored from: 0, nil visible; to 5, abundant. The carcasses were then moved to the boning room where the LD was removed, vacuum-packed in plastic and frozen following this single day of aging.

In August 1998, the 1997-born steers were trucked to a commercial abattoir near Grantham, Qld. At about 20 min after death a small sample of LD was removed by knife, wrapped in aluminium foil and immediately placed in liquid nitrogen for storage and subsequent analysis of calpain system enzymes. After overnight storage at 1°C, the carcasses were weighed and quartered between the 12th and 13th ribs. The exposed surface was scored by a trained assessor for meat colour, fat colour and marbling, using the same scoring system as used the previous year. The carcasses were then moved to the boning room where the LD was removed, halved and vacuum-packed in plastic. To ensure that all steers had a sample of LD that was aged for both 1 and 14 days, the identities of the steers had been sorted by breed (Angus or crossbred), selection line (HE or LE) and final liveweight. Based on this order, the cranial end of the LD from alternate steers was assigned to either 1 day of aging (i.e. frozen immediately) or 14 days of aging at 1°C, then frozen (–14°C), and the corresponding caudal end was stored for 14 days or 1 day.

Objective measurements of meat tenderness

Shear force and compression values were determined on LD samples following the methods of Bouton *et al.* (1971). The samples were thawed (4°C) for 48 h and trimmed into 250 g blocks to remove endomysial connective tissue. Cooking was at 70°C for 1 h in a waterbath, after which samples were cooled and stored at 4°C overnight before measurement. Objective measurements of meat tenderness were determined with a Lloyd LRX instrument (Lloyd Instruments Ltd, Hampshire, England). Change in shear force and compression with days of aging was calculated as the change from day 1 to day 14, divided by 13 days, with a negative value representing a decline in shear force and compression and indicative of meat becoming more tender.

Myofibril fragmentation index (MFI) for samples of LD was determined by the method of Olson *et al.* (1976), with the following modifications. A 0.5 g subsample was homogenised (Sorval Omni-mixer, Dupont, Australia) in an ice-cold vessel for 2 bursts of 30 s on high with 60 volumes (w/v) of buffer (100 mol/L KCl, 20 mol/L potassium phosphate, 1 mmol/L EDTA, 1 mmol/L NaNO$_3$; pH 7.0). Myofibril suspensions were filtered (1 by 1 mm polyethylene mesh) to remove connective tissue with a further 20 volumes (w/v) of buffer used to facilitate the passage of myofibres through the filter. Filtrates were centrifuged at 1000 *g* for 15 min at 2°C and the supernatant decanted. The pellets containing the myofibres were resuspended in 20 volumes (w/v) of buffer with a vortex at slow speed and sedimented again by centrifugation. Myofibres were washed 3 more times by repeating this process, and finally re-suspended in 20 volumes (w/v) of buffer. The protein concentration of the suspension was determined (Bio-Rad DC kit, Bio-Rad) and aliquots of the triplicate suspensions were diluted in buffer to a final concentration of 0.5 mg/mL. MFI was quantified as described in Olson *et al*. (1976). The rate of change in MFI (myofibril fragmentation rate: MFR) during aging was calculated as the change in

MFI from day 1 to day 14, divided by 13 days, with a positive value indicative of greater fragmentation.

Separation and functional assay of calpain system proteins

Five grams of trimmed and diced lean muscle was homogenised in 10 volumes of extraction buffer to ensure a low protein load on the columns. Sample preparation for separation and functional assay of the calpain system proteins was as described in McDonagh *et al.* (1999), with the following modifications. Fresh samples were homogenised in extraction buffer (40 mmol/L Tris-HCl, 10 mmol/L EDTA, 7.5 mmol/L MCE and enzyme inhibitors; pH 7.5). Frozen samples were homogenised in extraction buffer as above, but with pH at 8.3. After centrifugation (30 min), pH was checked to ensure that pH of homogenates was 7. In most cases they were equal or greater than 7.

Homogenates were loaded onto washed DEAE Sephacel columns. Columns were washed with 100 mL of washing buffer (40 mmol/L Tris-HCl, 0.05 mmol/L EDTA, 20 mmol/L NaCl, 7.5 mmol/L MCE; pH 8.0). Sephacel columns were eluted using increasing stepwise NaCl gradient and decreasing stepwise pH gradient in washing buffer. Flow rates were controlled at 0.5 mL/min using Technicon autoanalyser pumps. Optimal separation of the calpain system components was achieved using 40 mL of 100 mmol/L NaCl (pH 8.0) for calpastatin, 30 mL of 150 mmol/L NaCl (pH 7.0) for μ-calpain, and 30 mL of 350 mmol/L NaCl (pH 7.0) for m-calpain. The absorbance of a diluted aliquot of each fraction (0.1 mL in 0.9 mL washing buffer) was measured at 278 nm (Ultraspec, Hitachi, Japan) and plotted against fraction number to determine the protein elution profile for each column (Fig. 1).

Protein peaks were functionally assayed to identify fractions containing calpastatin, μ-calpain and m-calpain activity. Calpastatin is a heat stable protein, whereas μ- and m-calpain are not. To confirm separation of μ-calpain from calpastatin, fractions containing calpastatin inhibitory activity were identified by heating duplicate aliquots of 250 μL (tube A) and aliquots of 300 μL (tube B) from each fraction at 95°C for 3 min. The heated aliquots were allowed to cool at 4°C. Fifty microlitres of concentrated m-calpain was added to the tube A duplicates. Tubes were incubated with either 300 μL of CaCl$_2$ or EDTA assay buffers for 1 h at 25°C. Reactions were stopped and proteolytic activity determined using casein as a substrate. As shown in Figure 1, fractions containing calpastatin in tube A inhibit the activity of m-calpain. To confirm that μ-calpain was not contaminating the calpastatin peak, the caseinolytic activity of tube B for each fraction was compared with the caseinolytic activity profile of non-heated fraction aliquots determined previously.

Intramuscular fat percentage (IMF%)

A subsample of LD from all steers was minced and freeze-dried to determine DM content, and then ground again. Intramuscular fat content of the LD was determined in duplicate on a 5 g dried sample by weight lost following 20 h extraction with chloroform in a soxhlet apparatus.

Data analyses

Data analysed were for the 33 (16HE:17LE) 1996-born Angus steers described in Richardson *et al.* (1999), the 41 (19HE:22LE) 1996-born crossbred steers described in Richardson *et al.* (1998), and 38 (14HE:24LE) Angus and 77 (42HE:35LE) crossbred 1997-born steers. Data for final liveweight, carcass traits, IMF% and objective measurements for meat tenderness from the Angus and the crossbred steers were analysed together using the general linear models (GLM) procedure in SAS (1989). Year-of-birth (1996 or 1997), breed (Angus or crossbred), selection line (HE or LE) and their interactions were included in the models. Preliminary analyses showed there to be no significant (*P*>0.05) interactions between selection line and year-of-birth, or breed and year-of-birth, indicating that for these traits the relative performance of the 2 selection lines and 2 breed types was
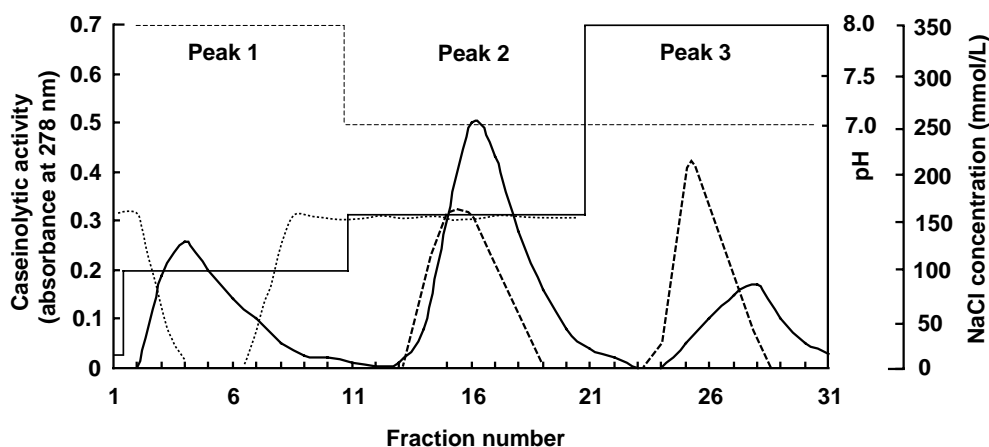
Figure 1. The bovine muscle protein elution profile (thick solid line), caseinolytic activity profiles (dashed line) of μ-calpain (peak 2) and m-calpain (peak 3), and the m-calpain inhibitory profile (dotted line) of calpastatin (peak 1) eluted as 3 mL fractions from DEAE Sephacel. Stepwise gradients for pH (dashed line) and NaCl (thin solid line) are also shown.

similar across birth years. The final GLM model used contained birth year, breed, selection line and the breed × selection line interaction. Because there were unequal numbers of animals in some groups within and between the 2 birth years the results are presented as least squares mean ± s.e. Data for marbling score and meat colour were analysed using the categorical data modelling procedure (CATMOD) in SAS (1989). There was no variation in fat colour and hence no analysis was undertaken for this trait.

Analysis of variation in calpain system activity in the Angus steers used GLM models that included whether the samples were analysed fresh (group 2 of 1996-born steers) or after storage in liquid nitrogen (mean ± s.e.) for 73 ± 1 days (first group of 1996-born steers) or for 11 ± 6 days (1997-born steers), and whether samples were taken soon after death (41 ± 1 min) or after a longer delay (121 ± 1 min) inadvertently caused by a mechanical failure in the abattoir which delayed sample collection for 16 (7HE:9LE) 1997-born steers. Preliminary analysis showed there were no significant (P>0.05) interactions for selection line with time to sample biopsy, or with storage time to chemical analysis, for the 3 components of the calpain system measured. The final GLM model contained time to biopsy (2 levels), storage time to analysis (3 levels) and selection line (2 levels). Least-squares means for time to biopsy calculated with storage time to analysis equal to fresh, and for storage time to analysis with time to biopsy equal to short, were calculated using the estimate statement option within the GLM procedure. Least-squares means for selection lines were calculated with time to biopsy equivalent to short (41 min) and storage time to assay equivalent to fresh samples (immediate preparation).

Post-slaughter calpain system activity and its relationships with objective measurements of meat tenderness were determined using data from the second group of 16 Angus steers killed in 1997. This group was chosen because it was the only one to have had the components of the calpain system measured in fresh LD taken immediately after slaughter, and to have objective measurements of tenderness for LD samples aged for 0, 1, 7 and 14 days. Correlations between post-slaughter calpain system activity and post mortem changes in meat tenderness attributes were determined as partial correlations from a multivariate analysis of variance, within the GLM procedure, with selection-line included as the fixed effect.

Results

Carcass traits

Following a single generation of divergent selection for RFI, there was no difference in final liveweight, carcass weight and dressing percentage between the HE and LE feedlot steers (Table 1). The HE steers had slightly less (P<0.05) subcutaneous fat at the rib, and there was a trend (P = 0.10) towards less fat over the rump site of HE steers, compared with the LE steers. There were no significant (P>0.05) interactions between selection line and year-of-birth, or breed, indicating that for these traits the relative performance of the HE against the LE selection line was similar across birth years and breed types. For eye muscle area, there was a significant (P = 0.02) interaction between selection line and breed, but not with birth year, such that crossbred HE steers had a smaller eye muscle area

Table 1. Carcass characteristics of feedlot steers following a single generation of selection for low RFI (high efficiency) or high RFI (low efficiency)

Values are least squares means ± s.e. for Angus and Angus-crossbred steers born in 1996 and 1997

	Low RFI line	High RFI line	Signif.
No. of animals	91	98	
Final weight (kg)	467 ± 4	459 ± 4	n.s.
Depth of rib fat (mm)	9.2 ± 0.3	10.1 ± 0.2	*
Depth of rump fat (mm)	11.5 ± 0.3	12.1 ± 0.3	P = 0.10
Eye muscle area (cm^2)	58.9 ± 0.6	60.3 ± 0.6	n.s.
Carcass weight (kg)	247 ± 2	244 ± 2	n.s.
Dressing percentage (%)	53.1 ± 0.2	53.2 ± 0.2	n.s.
Intramuscular fat (% FW)	5.4 ± 0.2	5.3 ± 0.1	n.s.

* P<0.05; n.s., not significant (P>0.10).

Table 2. Meat tenderness attributes of the *M. longissimus dorsi* of feedlot steers following a single generation of selection for low RFI (high efficiency) or high RFI (low efficiency)

Values are least squares means ± s.e. for Angus and Angus-crossbred steers born in 1996 and 1997

	Low RFI line	High RFI line	Signif.
Shear force (kg)			
Day 1	4.6 ± 0.2	4.6 ± 0.2	n.s.
Day 14	3.8 ± 0.2	3.5 ± 0.2	n.s.
Shear force change (g/day)	−60 ± 23	−94 ± 24	n.s.
Compression (kg)			
Day 1	1.45 ± 0.02	1.44 ± 0.02	n.s.
Day 14	1.36 ± 0.03	1.32 ± 0.03	n.s.
Compression change (g/day)	−8 ± 3	−10 ± 3	n.s.
Myofibril fragmentation index (MFI units)			
Day 1	67.7 ± 1.8	72.5 ± 1.9	*
Day 14	85.6 ± 1.2	89.5 ± 1.3	*
Myofibril fragmentation rate (MFI units/day)	1.38 ± 0.14	1.32 ± 0.14	n.s.

*$P<0.05$; n.s., not significant ($P>0.05$).

than crossbred LE steers (58.4 and 61.6 cm^2; $P<0.05$), whereas there was no difference between purebred HE and LE steers (59.6 and 59.0 cm^2; $P>0.05$). The LS means for eye muscle area in Table 1 include this interaction and indicate that the HE and LE comparison was not significant.

There was no difference between the HE and LE steers in IMF% in the samples of LD (Table 1), and no significant ($P>0.05$) interactions between selection line and year-of-birth or breed, indicating that IMF% was similar in the HE and LE selection lines in both birth years and in both breed types. Chiller assessment data, including marbling score, were not obtained for the 1996-born Angus steers. Marbling scores for all the other steers were 0, 1 or 2. Analysis of this categorical data revealed no difference ($P>0.05$) between selection lines in the proportion of steers scored 0 (HE: 0.27; LE: 0.33), 1 (HE: 0.56; LE: 0.54) and 2 (HE: 0.17; LE: 0.12). Meat colour data for 156 steers contained only 2 samples that were graded as '2', 1 sample graded as '3' and 1 other sample graded as '4'. Data for these samples were combined and analysed as if all were graded '2', that being slightly darker red than colour score '1'. Meat colour score 1 was subdivided into 1B (lighter) and 1C. There was no difference ($P>0.05$) between selection lines in the proportion of steers scored 1B (HE: 0.56; LE: 0.54), 1C (HE: 0.41; LE: 0.43) and 2 (HE: 0.03; LE: 0.02). All carcasses were graded as having a fat colour of 1 (white).

Meat tenderness

Results for objective measurements of meat tenderness of LD samples are presented in Table 2. There were no significant ($P>0.05$) interactions of selection-line with breed or birth year indicating that the relative difference between HE and LE steers was the same in both years and in both breeds. The interactions were dropped from the final GLM models. There was no difference between selection lines in shear force or in compression of LD samples aged for 1 day or for 14 days, or in rate of decline in shear force and compression with aging. Myofibril fragmentation (MFI) was higher in LD samples from LE steers than from HE steers, both in samples aged for 1 day and in samples aged for 14 days. However, the rate of decline in MFI with aging (MFR) was similar in the LD samples from both lines of steers. Thus, by this aspect of meat tenderness, the LE steers had a 5 unit, or 7% higher level of myofibre breakdown over the HE steers in LD soon after slaughter, an increment that was largely maintained for the 14-day aging period in this study.

Calpain system activity

Activities of components of the calpain system examined over the first 24 h post mortem in 4 carcasses declined within 8 h of slaughter (Fig. 2). Calpastatin activity declined by 44% within the first 8 h of post mortem aging, with 43% of initial activity remaining at 24 h post mortem. The activity of
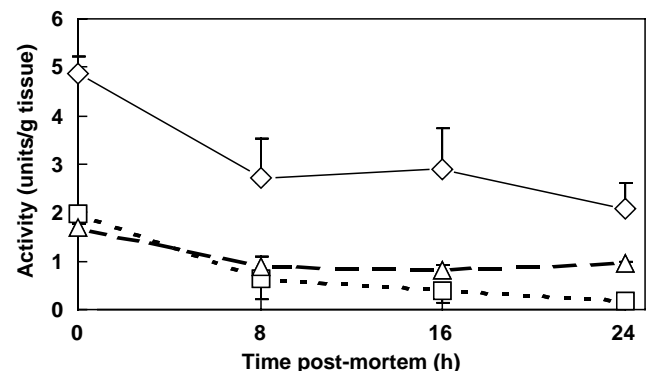

Figure 2. Extractable activity of calpastatin (◇), μ-calpain (□) and m-calpain (△) from bovine *M. longissimus et thorasics lumborum* during the first 24 h of post mortem aging at 1°C from 4 carcasses of low-RFI Angus steers. Values are mean ± s.e.

μ-calpain declined by 67% within the first 8 h of post mortem aging, with only 10% of the initial activity remaining at 24 h post mortem. The activity of m-calpain declined by 49% within the first 8 h of post mortem aging, changing little in activity with further aging to 24 h.

Substantial reduction in the activity of the calpain system in bovine *M. longissimus dorsi* is known to occur during post mortem aging and to be most rapid during the first 24 h of aging. Further, the decline in μ-calpain is more rapid than for calpastatin, and much less rapid for m-calpain (Koohmaraie *et al*. 1987; Wheeler and Koohmaraie 1991; Geesink *et al*. 1992; Boehm *et al*. 1998). The decline in the activity of the calpain system may be even more rapid than indicated by levels at 24 h post mortem. In the 4 carcasses sampled at 8-h intervals after death, the majority of changes in calpain system activity evident at 24 h after slaughter had occurred by 8 h post mortem (Fig. 2). One consequence of these differential rates of decline of components of the calpain system is that the ratio of μ-calpain to calpastatin, that is of protease to inhibitor, in muscle changes substantially between death and 24 h post mortem. In these 4 carcasses the ratio increased from about 1:2.5 at death to 1:6 after 8 h, to 1:10 after 16 h and to 1:12 after 24 h post mortem.

Analysis of calpain/calpastatin data for the Angus steers slaughtered in 1997 and 1998 revealed that storage in liquid nitrogen reduced the levels of components of the calpain systems compared with those in muscle analysed fresh. Calpastatin levels were 4.9 ± 0.2 units in muscle samples analysed fresh and similar (*P*>0.05) to levels after 11 days of storage (4.4 ± 0.2 units), but higher than levels after about 73 days of storage (3.0 ± 0.2 units; *P*<0.05). Levels of μ-calpain were 2.2 ± 0.1 units in fresh samples and higher (*P*<0.05) than levels after 11 and 73 days of frozen storage (0.7 ± 0.1 and 0.9 ± 0.1 units, respectively). Levels of m-calpain in fresh material and in muscle stored frozen for 11 days (1.8 ± 0.1 and 2.3 ± 0.1 units respectively) were higher (*P*<0.05) than in muscle stored for 73 days (1.1 ± 0.1 units). Koohmaraie (1990) also reported that frozen storage (in liquid nitrogen, with storage at –70°C) resulted in lower levels for components of the calpain system

Table 3. Calpain system activity after slaughter in the *M. longissimus dorsi* of feedlot steers following a single generation of selection for low RFI (high efficiency) or high RFI (low efficiency)

Values are least squares means ± s.e. for Angus steers born in 1996 and 1997

	Low RFI line	High RFI line	Signif.
No. of animals	30	41	
m-Calpain (units/g tissue)	1.9 ± 0.1	1.8 ± 0.1	n.s.
μ-Calpain (units/g tissue)	2.3 ± 0.1	2.1 ± 0.1	n.s.
Calpastatin (units/g tissue)	5.2 ± 0.3	4.6 ± 0.3	*
m-Calpain/calpastatin	0.36 ± 0.03	0.42 ± 0.03	*
μ-Calpain/calpastatin	0.47 ± 0.03	0.47 ± 0.03	n.s.

*P<0.05; n.s., not significant (P>0.05).

compared with levels measured in fresh muscle, with the effect biggest for levels of calpastatin. In recognition of these effects, the LS-means presented in Table 3 were calculated with time to biopsy equivalent to short and storage time to assay equivalent to being analysed as fresh samples.

A single generation of divergent selection for RFI resulted in no significant differences between selection lines in the activity of m-calpain and μ-calpain in LD immediately after slaughter (Table 3). However, level of calpastatin in LD from the HE steers was 13% higher than in LD from the LE steers. The ratio of m-calpain to calpastatin (i.e. of protease to inhibitor) differed between the selection lines as a result of the higher levels of calpastatin in the HE line. The ratio of μ-calpain to calpastatin did not differ between the selection lines.

The rate of myofibre fragmentation between 1 and 14 days post slaughter was positively correlated with the ratios of both m-calpain and μ-calpain to calpastatin (Table 4 and Fig. 3), although not significantly (*P*>0.10) correlated with levels of either calpain or calpastatin. That is, higher ratios of these proteases relative to their inhibitor were associated with more rapid myofibril fragmentation. There were no significant interactions between selection line and

Table 4. Means for post mortem changes in objective measures of meat tenderness and partial correlation coefficients with activity of components of the calpain system immediately after slaughter in samples of the *M. longissimus dorsi*

Samples are from group 2, 1996-born Angus steers following a single generation of divergent selection on the basis of RFI
(*n* = 8 low RFI line; 8 high RFI line)
μ-Cal is μ-calpain, m-Cal is m-calpain, Calp is calpastatin

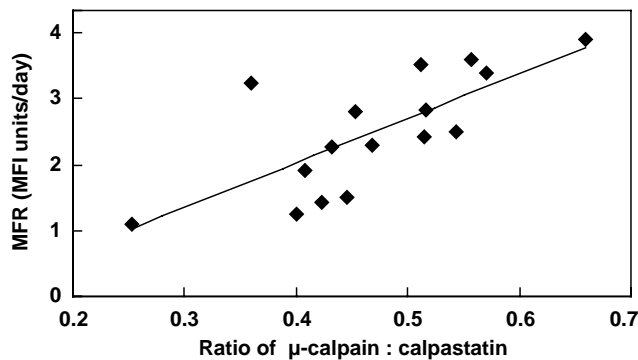| Trait	Change in meat tenderness Mean	Partial correlations μ-Cal	m-Cal	Calp	μ-Cal:Calp	m-Cal:Calp
MFR (MFI units/day)	2.52	0.29	0.25	−0.41	0.71**	0.75**
Shear force (g/day)	−108	0.03	−0.18	−0.29	0.35	0.33
Compression (g/day)	−12	0.37	0.31	0.30	0.09	−0.15

**P<0.01.

Figure 3. Rate of myofibril fragmentation (MFR) is positively correlated with the μ-calpain:calpastatin ratio in the *M. longissimus dorsi* of Angus (same steers as in Table 4). The equation of the line is:

$$y = 6.75x - 0.67 \ (R^2 = 0.53; \ P<0.01)$$

protease/inhibitor traits for MFR, indicating that relationships between MFR and protease/inhibitor traits were similar within both selection lines. There were no statistically significant relationships between changes in shear force and compression during aging and the activities of calpain system proteins (Table 4).

Variation in myofibril fragmentation was associated with variation in shear force and compression. Figure 4 shows that higher levels of MFI are associated with lower values for shear force and compression in samples of LD from the 16 'group 2', 1996-born Angus steers aged for 1, 7 and 14 days. Analysis of all samples of LD taken from all steers in this study that were aged for 1 or 14 days, showed that shear force and compression declined by 0.027 ± 0.006 kg and 0.003 ± 0.001 kg per unit increase in MFI. Variation in

MFI explained 17% (*P*<0.01) of the variation in shear force and 14% (*P*<0.01) of the variation in compression. This was calculated from GLM models with variation due to birth year, breed and selection line accounted for before fitting MFI.

Discussion

A single generation of divergent selection of cattle on the basis of RFI has produced 2 lines of steers that differed in feed conversion ratio in the feedlot (Richardson *et al*. 1998). There was no evidence that a single generation of selection had been accompanied by change in final liveweight, carcass weight or dressing percentage, or by changes in 2 objective measurements of tenderness: shear force and compression of LD muscle samples. There was evidence that after just this single generation of selection, steers selected for low RFI (high efficiency) had slightly less subcutaneous fat, a lower index of myofibril fragmentation in LD muscle samples and higher levels of calpastatin in LD muscle at slaughter. There was no evidence that selection on RFI had been accompanied by change in IMF percentage, marbling score, meat colour and fat colour.

A single generation of divergent selection for RFI has been reported to produce a small change in body composition in young steers as measured by subcutaneous fat thickness (Richardson *et al*. 1998) and whole-body chemical analysis (Richardson *et al*. 1999). The small reduction in subcutaneous fat thickness in the HE steers was not accompanied by a reduction in carcass weight or eye muscle area. This might be expected to result in an increase in retail meat yield (Wolcott *et al*. 1997) from the carcasses of the HE steers: potentially a desirable outcome that has yet to be confirmed for these cattle. Small changes in body
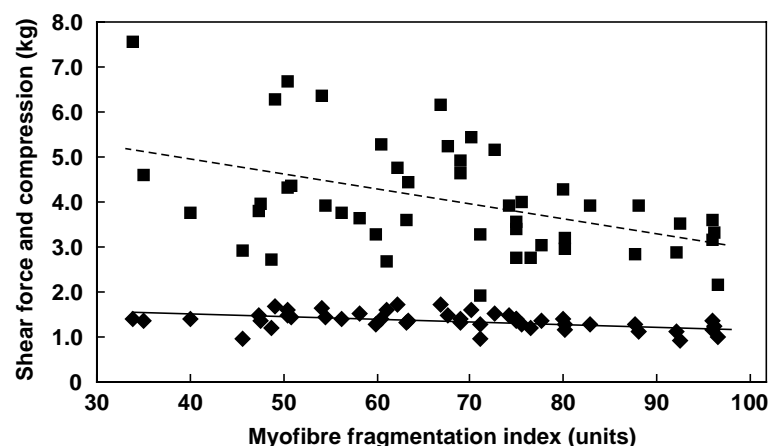

Figure 4. Shear force (■) and compression (◆) are negatively correlated with myofibril fragmentation index in samples of *M. longissimus dorsi* aged for 1, 7 and 14 days from Angus steers (same steers as in Table 4). The equations of the lines are:

Shear force (dashed line): $y = -0.033x + 6.26 \ (R^2 = 0.21; \ P<0.01)$
Compression (solid line): $y = -0.0049x + 1.69 \ (R^2 = 0.19; \ P<0.01)$

composition favouring lean gain are consistent with the moderate negative genetic correlation ($r_g = -0.43$) between postweaning RFI and carcass lean content in British Hereford bulls (Herd and Bishop 2000), and between feedlot steer RFI and subcutaneous rib fat ($r_g = 0.26$) and P8 rump fat ($r_g = 0.28$; Robinson *et al.* 1999). However, a difference in subcutaneous fat thickness was not observed between young HE and LE Angus bulls and heifers tested for RFI from 8 to 12 months of age (Herd *et al.* 1997). Further, Richardson *et al.* (1999) concluded that small changes in fatness accounted for only a small fraction of the observed differences between HE and LE steers in feed intake and feed efficiency. If the observed difference in fatness between the HE and LE steers does reflect an underlying genetic association with RFI measured postweaning, then on-going selection to reduce RFI might lead to reductions in subcutaneous fatness in steers such that animals fail to meet minimum market specifications for fatness.

With shear force values of less than 4.2 kg and compression values of less than 1.6 kg, the LD meat samples aged for 14 days from the steers of both selection lines would be considered acceptably tender by Australian consumers (Shorthose *et al.* 1988). However, there was evidence that after just this single generation of selection, steers selected for low RFI (high efficiency) had a lower index of myofibril fragmentation in LD muscle samples and higher levels of calpastatin in LD muscle at slaughter. Calpastatin is a powerful inhibitor of calpain activity: one unit of calpastatin being able to inhibit 4 units of calpain (Dransfield 1999). Associations between calpastatin activity at 1 day post mortem and toughness in bovine *M. longissimus dorsi* following 14 days of post mortem aging have been shown previously (Whipple *et al.* 1990; Morgan *et al.* 1993; Wulf *et al.* 1996). In this study, the ratio of μ-calpain to calpastatin from post-slaughter samples was positively correlated with rate of myofibril fragmentation (Fig. 3), and was similar to results obtained previously in lamb (McDonagh *et al.* 1999) and electrically stimulated beef (McDonagh 1998). That higher levels of protease-to-inhibitor were associated with more rapid myofibril fragmentation may reflect a linkage between activity of the calpain enzymes and inhibitor activity (Steen *et al.* 1997). Therefore, the higher level of calpastatin in LD muscles from the HE steers may have reduced myofibre fragmentation through inhibition of calpains and could provide a mechanism by which on-going selection for reduced RFI might be accompanied by less tender meat. If higher levels of calpastatin are accompanied by lower rates of protein breakdown in muscle in the living animal it may contribute to the efficiency of energy use in muscle in HE steers.

This study has shown that following just a single generation of divergent selection there were correlated responses in carcass fatness, and in calpastatin and myofibril fragmentation, that may, with on-going selection for low RFI,

affect market suitability and meat tenderness respectively. Given the present lack of understanding of the biological basis to variation in RFI and of its genetic association with these traits, selection for RFI should be accompanied by monitoring for any correlated response in meat tenderness. Should such correlated responses be confirmed, they could be managed though application of techniques that enhance post-slaughter tenderisation of meat.

References

Archer JA, Arthur PF, Herd RM, Richardson EC (1998) Genetic variation in feed efficiency and it's component traits. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production* **25**, 81–84.

Arthur PF, Archer JA, Herd RM, Richardson EC, Exton SC, Wright JH, Dibley KCP, Burton DA (1997) Genetic and phenotypic variation in feed intake, feed efficiency and growth in beef cattle. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12**, 234–237.

Boehm ML, Kendall TL, Thompson VF, Goll DE (1998) Changes in the calpains and calpastatin during postmortem storage of bovine muscle. *Journal of Animal Science* **76**, 2415–2434.

Bouton PE, Harris PV, Shorthose WR (1971) Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. *Journal of Food Science* **36**, 435–439.

Dransfield E (1999) Meat tenderness — the μ-calpain hypothesis. *Proceedings of the 45th International Congress of Meat Science and Technology* **1**, 220–228.

Geesink GH, Ouali A, Smulders FJM, Talmant A, Tassy C, Guignot F, van Laack HLJM (1992) The role of ultimate pH in proteolysis and calpain/calpastatin activity in bovine muscle. *Biochimie* **74**, 283–289.

Herd RM, Bishop SC (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. *Livestock Production Science* **63**, 111–119.

Herd RM, Archer JA, Arthur PF, Richardson EC, Wright JH, Dibley KCP, Burton DA (1997) Performance of progeny of high vs low net feed efficient Angus cows. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12**, 742–745.

Koohmaraie M (1990) Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. *Journal of Animal Science* **68**, 659–665.

Koohmaraie M, Seideman SC, Schollmeyer JE, Dutson TR, Crouse JD (1987) Effect of post-mortem storage on Ca^{++}-dependent proteases, their inhibitor and myofibril fragmentation. *Meat Science* **19**, 187–196.

McDonagh MD (1998) Calpain activity affects muscle protein turnover and meat tenderisation. PhD Thesis, The University of New England, Armidale, Australia.

McDonagh MB, Fernandez C, Oddy VH (1999) Hind-limb protein metabolism and calpain system activity influences post-mortem change in meat quality in lamb. *Meat Science* **52**, 9–18.

Morgan JB, Wheeler TL, Koohmaraie M, Savell JW, Crouse JD (1993) Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. *Journal of Animal Science* **71**, 1471–1476.

Oddy VH (1999) Genetic variation in protein metabolism and implications for variation in efficiency of growth. *Recent Advances in Animal Nutrition in Australia* **12**, 23–29.

Olson DG, Parrish FC, Stromeyer MH (1976) Myofibril fragmentation and shear force resistance of three bovine muscles during postmortem storage. *Journal of Food Science* **41**, 1036–1041.

Richardson EC, Herd RM, Archer JA, Woodgate RT, Arthur PF (1998) Steers bred for improved net feed efficiency eat less for the same feedlot performance. *Animal Production in Australia* **22**, 213–216.

Richardson EC, Herd RM, Oddy VH, Woodgate RT, Archer JA, Arthur PF (1999) Body composition explains only part of the intake difference between high and low efficiency Angus steers. *Recent Advances in Animal Nutrition in Australia* **12**, 4A.

Robinson DL, Oddy VH, Smith C (1999) Preliminary genetic parameters for feed intake and efficiency in feedlot cattle. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **13**, 492–495.

SAS Institute Inc. (1989) 'SAS/STAT Users Guide. Version 6 (4th edn).' (SAS Institute Inc: Cary, NC)

Shorthose WR, Harris PV, Hopkins AF, Kingston OL (1988) Relationships between some objective properties of beef and consumer perceptions of meat quality. *Proceedings of the 34th International Congress of Meat Science and Technology* **1**, 667–669.

Steen D, Claeys E, Uytterhaegen L, De Smet S, Demeyer D (1997) Early post-mortem conditions and the calpain/calpastatin system in relation to tenderness of double-muscled beef. *Meat Science* **45**, 307–319.

Wheeler TL, Koohmaraie M (1991) A modified procedure for simultaneous extraction and subsequent assay of calcium-dependent and lysosomal protease systems from a skeletal muscle biopsy. *Journal of Animal Science* **69**, 1559–1565.

Whipple G, Koohmaraie M, Dikeman ME, Crouse JD (1990) Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. *Journal of Animal Science* **68**, 4193–4199.

Wolcott ML, Thompson JM, Ferguson DM, Skerritt JW, Robinson DL (1997) Prediction of retail beef yield from real time ultrasound scans recorded at weaning, the commencement of finishing and pre-slaughter. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12**, 734–737.

Wulf DM, Tatum JD, Green RD, Morgan JB, Golden BL, Smith GC (1996) Genetic influences on beef longissimus palatability in Charolais and Limousin sired steers and heifers. *Journal of Animal Science* **74**, 2394–2405.