1990s
- Changes to agriculture support
- Steady growth of chilled lamb
- Further EU enlargement
- EU beef stocks and export subsidies
- GATT UR provides stability in access

1995 and beyond
- Food safety, HGP, residues, BSE, animal welfare, environmental issues, HACCP, ISO
- Smaller families/households
- 'Faster' food
- Shelf life, presentation
- Increased travel
- Internationalisation/concentration
- Closer customer relationships

Beef

1950 to 1980
- 1950s sales to Britain
- 1960s boneless beef to North America
- 1970s US market collapse
  Australia moves to develop Japan
  NZ access to USA matched by production increases

1985 to 1994
- 1988 Korean beef market reopened
- 1989 Japan beef market liberalized
  Changing impact of MIL
- NAFTA Agreement – Mexico

1995 . . .
- GATT Agreement
- South American access to USA
- EU stockpile eliminated
- Mexican market collapse
- EU and Eastern Europe opportunities

Chicken Competition in the US

" As we move into the next century, I predict chicken will make significant in-roads into the ground red meat and breakfast meat markets – the last bastion of dominance by the beef and pork industries. Further, I see beef hamburger products becoming a combination product market. That is, I envision that instead of 100 percent beef hamburgers, the most common meat patty after the year 2000 will be a 50/50 beef-poultry blend burger."

Joe Frank Sanderson, Jr Chairman, National Broiler Council, July 1994

Changes is meat consumption

Countries/ Regions	Beef & Veal	Pigment	Poultry	Sheepmeat
Canada	-17.9	-8.6	39.6	15.7
United States	-10.4	-5.0	55.5	-4.3
EU	-10.8	13.2	32.1	17.6
Australia	-24.5	18.8	42.9	3.2
Japan	78.6	17.7	31.7	-46.7
Argentina	-21.3	28.2	28.2	-31.3

Source: WTO - International Markets for Meat 1994-5

Proteases and meat quality

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Consumers judge the quality of meat at the point of sale on colour, visible fat content and odour. However, on eating meat, characteristics such as juiciness, flavour and texture also assume importance. Supermarket surveys of customer preferences have indicated that tenderness is the single most important meat quality characteristic that influences whether consumers are repeat buyers. In New Zealand, the industry standard for tenderness is that shear force must be less than 11 kg F for exported meat. The meat industry recognises, however, that 11 kg F is the upper limit for shear force and that for discerning markets, tenderness values of <5 kg F are required if the markets are to be retained or improved. This presentation will emphasise the biochemical factors contributing to meat tenderisation and future research that will contribute to the production of meat with tenderness values < 5kg F.

1 Post-mortem enzyme changes and meat tenderisation

Biochemical components of meat tenderisation include the activity of enzymes involved in: 1) regulating the conversion of glycogen to lactic acid which lowers meat pH; 2) generating ATP which affects muscle contraction and the final sarcomere length of muscle fibres - a contributor to meat tenderness and 3) the breakdown of muscle myofibrillar proteins - another contributor to meat tenderness (Greaser, 1986; Etherington, 1991). This breakdown of myofibrillar proteins is carried out by proteolytic enzymes which are broadly divided into the lysosomal cathepsins and the cytoplasmic ubiquitin and calcium dependent proteases. All three biochemical processes are inter-connected.

A research challenge is to unravel the sequence of post-mortem metabolic events that dictate the final quality of meat

and to establish whether this sequence is dependent on farm factors such as the nutritional, environmental and genetic background of the stock and/or processing factors such as pre-slaughter stress, type of electrical stimulation and chilling regime. Current evidence suggests that on-farm factors contribute up to 40% of the variability in meat tenderness and meat processing 60-70% (Koohmaraie *et al.*, 1995). Thus, a good quality animal processed correctly yields excellent meat, processed incorrectly poor quality meat. However, a poor quality animal can never be converted by normal processing into excellent meat. Clearly, vertical integration involving selecting quality stock, correct method of processing the stock and selecting the ideal chilled storage conditions, is essential for the production of high quality meat. This vertical integration concept alone should contribute to maintaining the long term survival of the meat industry.

It is now recognised that biochemical indicators are required to identify animals which are pre-disposed to yielding meat with the necessary colour, pH, fat content and tenderness. They are also required during post-mortem meat processing to monitor any changes in meat quality characteristics. Recently researchers have focussed on the role of muscle proteolytic enzymes, particularly the calcium dependent calpain-calpastatin system, in determining some important meat quality characteristics. Proteolytic enzymes have been identified because of their perceived dual role in regulating muscle protein degradation ante-mortem and rate of meat tenderisation post-mortem. This role, however, is conflicting since low calpain, high calpastatin activities promote muscle growth (Goll *et al.*,1992) whilst high calpain, low calpastatin activities, are associated with post-mortem tenderisation (Koohmaraie *et al.*, 1995). This change in the relative importance of individual enzyme activities in the calpain-calpastatin system in ante- and post-mortem muscle and their action on muscle myofibrillar proteins is an area of active investigation, (Dransfield, 1993; Ouali, 1990; Koohmaraie *et al*, 1995; Taylor *et al*, 1995). Clearly, any factor which influences the activity of any one of the individual proteolytic enzymes will impact on muscle growth ante-mortem or meat tenderisation post-mortem. Possible fruitful areas of research into proteolysis in the post-mortem conversion of muscle to meat are outlined below.

Dransfield (1993) used a Computer Programme to predict the rate of meat tenderisation based on the relative activities of the calpains, temperature and pH changes over the tenderisation period. The key assumptions in this Model are:

1. μ-calpain and not calpastatin activity is driving the rate of meat tenderisation,
2. *in vivo* activity of μ-calpain and not calpastatin is an important regulatory parameter,
3. m-calpain activity has a minor role, and
4. enzyme changes that occur within the 0 to 24h post-mortem period (rather than enzyme changes after 24h) dictate the majority of any subsequent meat tenderisation.

Figure 1 shows that enzymatic changes in μ-calpain and calpastatin activities over the initial 24h post-mortem tenderisation period mirror the observed rate of meat

FIGURE 1: Post-mortem changes in (-calpain (■), m-calpain (▲) and calpastatin (♦) in Longissimus dorsi muscle from electrically stimulated lambs (from Morton *et al.*, 1996).

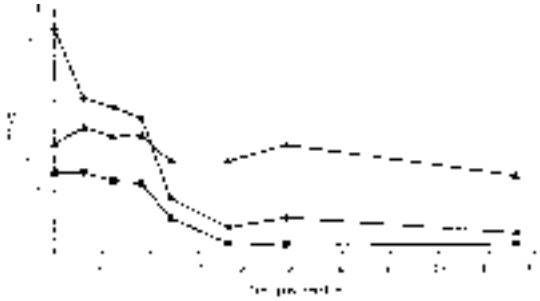
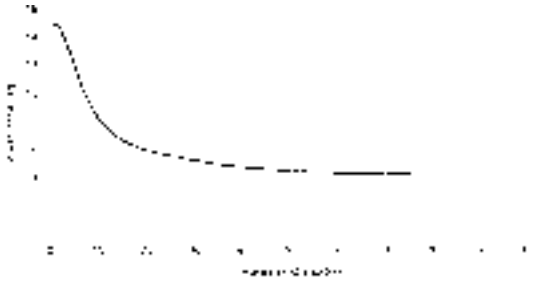


FIGURE 2: Comparison of measured shear force values (+) of Longissumus dorsi muscles from electrically stimulated lambs and shear forces predicted by the Dransfield tenderisation model (-) (Morton *et al.*, 1996).



tenderisation in Figure 2 (Morton *et al.*, 1996). The enzymatic changes in Figure 1, together with temperature and pH profiles, were submitted to the Dransfield Predictive Meat Tenderisation Programme at Lincoln University to predict the rate of meat tenderisation. Figure 2 shows there was a close fit between the computer predicted and observed rate of meat tenderisation indicating that the assumptions used in the Predictive Model may be valid and that μ-calpain is the determinant of meat tenderisation. This conclusion has also been reached by Dransfield (1993) and Taylor *et al.*, (1995). Nevertheless, the question still remains - "is the level and specific activity of μ-calpain the primary regulator of tenderisation or is the activity of calpastatin that regulates μ-calpain more important"?

Technically, it is not the calpain activity measured *in vitro* which is important but the *in situ* activity. As yet noone has measured the *in situ* activity of either μ-calpain or calpastatin. In the Predictive Meat Tenderisation Programme, Dransfield (1993) calculates the *in situ* activity of calpain from the initial calpain activity and rate of calpain inactivation according to the temperature and pH of meat and the *in vitro* enzymatic kinetic changes over the tenderisation period. To measure the enzymatic kinetic changes requires a considerable amount of laboratory work and a strict control of processing conditions. Our work at Lincoln University (Morton *et al.*, 1996) has shown a strong correlation between the rate of meat tenderisation and the kinetic changes in μ-calpain and calpastatin activities over the first 24h post-mortem. Figure 1 shows that after 24h the *in vitro* activity of μ-calpain has disappeared. Nevertheless, tenderisation continues

for at least another 2 weeks. This result raises the question "what enzyme is responsible for the additional tenderisation after 24h"? Is it residual μ-calpain activity that failed to be measured by our existing analytical techniques through lack of sensitivity or is it due to the post-mortem conditions activating m-calpain or the cathepsins? These issues need to be resolved by improving the sensitivity of all the analytical techniques to measure the activities of individual proteolytic enzymes.

2 Breed effects on enzymes and tenderness

Remarkably, the majority of meat from various breeds of sheep, if processed correctly by the recommended MIRINZ accelerated conditioning (AC) and ageing (A) process have shear force values < 11 kg F. Similarly, most *Bos taurus* breeds of cattle, after electrical stimulation and ageing, yield acceptable Warner-Bratzler shear force values of < 5.65 kg. Such results imply that management and processing have a greater effect on meat tenderness than genetics of the animal. Thus control of stock management and method of processing will produce sheep and cattle meat with 'Acceptable Tenderness' but if the management and method of processing is adapted and linked to the genetics of the animal then 'Guaranteed Tender' meat will result (sheep < 5 kg F, beef < 4.1 kg). The scientific issue is whether different breeds have different initial proteolytic enzyme levels and is there an interaction between these levels with the enzymatic kinetic changes that occur during processing (Koohmaraie, *et al.,* 1995; Shackelford *et al.*, 1994).

3 Inter-muscular variations in enzymes and tenderness

Intermuscular variation in tenderness within an animal is well established (Cridge *et al.*, 1994; Ouali and Talmant, 1990; Koohmaraie, *et al.*, 1995; Shackelford *et al*, 1995). The reasons for this variation are not known. Furthermore, within the same animal, there are no correlations between tenderness in the various muscles i.e. an animal that produces a tender fillet does not automatically produce a tender strip loin. Variation in tenderness between muscles could be due to several factors that distinguish individual muscles from each other such as their location in the body, the dynamic functional properties of the muscle, fibre sacromere length characteristics, glycogen content and metabolic type as judged by the level and activity of specific oxidative and glycolytic enzymes. Our results at Lincoln University have linked tenderness to the metabolic type of muscle and the kinetic changes in μ-calpain and calpastatin over the 24h post-mortem period using individual muscles. The results are in agreement with the observations of Ouali and Talmant (1990), but whether these differences are linked to the genetic expression of calpastatin (Ilian and Forsberg 1992; Speck *et al*, 1993) within a specific muscle or to different isoforms of calpastatin (Lee *et al*, 1992) still needs to be established.

4 Effect of pre-slaughter stress

Pre-slaughter stress can increase ultimate meat pH from < 5.8 to >5.8. Associated with the variations in pH are differences in meat tenderness (Devine *et al*, 1993; Purchas & Aungsupakorn, 1993). Recent work has indicated that the stress associated with isolating and restraining sheep consistently generated differences in meat pH and tenderness (Apple *et al*, 1995). This result raises the question as to whether the stress factors influence the proteolytic enzymes directly or whether there is a pH effect which directly or indirectly affects the proteolytic enzymes. At Lincoln we have evidence that stress on particular breeds of sheep affects their meat pH, the activity of the calpain-calpastatin system and the process of meat tenderisation. Preliminary results suggest pH-dependent and pH-independent stress factor(s) influence muscle pre- and post-mortem μ-calpain activities and by these mechanisms the rate of meat tenderisation. The interesting question is, what is the sequence of events and is this sequence breed dependent? Possible scenarios are as follows:

Scenario 1: Exposure of Breed A to stress rapidly mobilises muscle glycogen; consequently meat pH > 5.8, post-mortem calpain-calpastatin activities respond to the pH changes, net muscle proteolytic activity is reduced and meat toughens.

Scenario 2: Exposure of Breed B to stress partially mobilises muscle glycogen and residual muscle glycogen is sufficient to reduce meat pH < 5.8, a stress factor independently of pH is released and affects calpain-calpastatin levels and/or their kinetic changes. The net result is that muscle proteolytic activity is reduced and, meat toughens. There is evidence for both of these scenarios.

5 Effect of genotype

The toughness of the longissimus muscle can increase by up to 90% in cattle as the percentage of *Bos indicus* increases in the crossbreed (Crouse *et al*, 1989). In sheep, *longissimus* shear force values can increase by 248% if sheep possess the callipyge genes, an autosomal non-recessive gene on chromosome 18 linked to increases in muscle mass (Koohmaraie *et al*, 1995). Associated with these differences in meat toughness in *Bos indicus* cattle and callipyge sheep, are increases in pre- and post-mortem activities of calpastatin.

These results prompted Koohmaraie *et al.,* (1995; 1995a) to suggest that genetic selection of animals with low post-rigor muscle calpastatin activity may be advantageous in producing animals that yield tender meat. Unfortunately, fast growing high muscular yielding animals require high pre-rigor calpastatin activities which infers a molecular switch is required to switch off the calpastatin gene (Killefer and Koohmaraie, 1994) prior to slaughter or tough meat will eventuate. At Lincoln we are examining two aspects of this problem by: characterising the calpastatin promoter region to identify a molecular switch and; determining whether natural polymorphic variants of calpastatin exist. In sheep three polymorphic alleles of ovine calpastatin have been found (Roberts *et al.*, 1996). Preliminary evidence indicates that the alleles are associated with differences in the muscle calpain-calpastatin system and in meat tenderness. If confirmed,

this observation will be the first step towards identifying a gene to select animals pre-disposed to yield tender meat or, alternatively, to identify flocks with a high frequency of the desirable allele(s) and a propensity to yield tender meat. Future work needs to establish whether the alleles are actually responsible for differences in muscle calpastatin activity or whether the alleles are linked to another gene which is contributing to calpastatin activity and/or meat tenderisation.

6 Enzyme substrates

The proteolytic enzymes have to act on substrates that form the structural framework of skeletal muscles. Several studies have indicated that degradation of the Z-disks is a major contributor to post-mortem tenderisation. However, recent work (Taylor *et al*, 1995) has investigated the breakdown of nebulin and titin (constituents of the N_2 lines which extend to the I bands) together with vinculin, desmin and dystrophin (constitutents of the costameres); all these myofibrillar linking proteins are substrates of calpains. During tenderisation, breakdown of the costameres and N_2 lines occurred prior to the degradation of the Z-disks. No doubt, future work will identify the specific substrates in more detail and which part of the muscle structural framework is most susceptible to calpain action. Consequently blocking or enhancing the availability of the substrates to calpains may offer an alternative approach to manipulating the process of tenderisation rather than manipulating the activity of the proteolytic enzymes themselves. In other words, using the analogy that the process of tenderisation is a black tenderisation box with a lock and key (Fig. 3); research to date, has concentrated on identifying the codes of the keys that fit the locks of the tenderisation box on every muscle in an attempt to identify the code of the master tenderisation key. Some of the necessary codes for this key have been identified and are listed in Fig. 3. An alternative research approach is to change the tenderisation lock (substrates) of the individual muscles. No doubt, future research will indicate which approach is the most successful i.e. breaking the key code or identifying the lock mechanism, in producing a uniform and consistent quality meat product for the consumer.

FIGURE 3: Schematic representation of the parameters that act on muscle myofibrillar proteins during meat tenderisation.