PRODUCTION SYSTEMS AND QUALITY

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INTRODUCTION

Over the last few decades there has been an increasing amount of attention given to the influence of animal production systems on the quality the meat produced. Meat quality is not only assessed by its organoleptic properties but also its nutritional quality. Whilst the demand for goo quality meat produced at the lowest possible price remains, there is increasing scepticism by consumers (at least with the EC) over the means us to achieve this, particularly with what they regard as unnatural production practices. This, combined with concerns over the nutritional value meat, particularly its fat content, and recent health scares regarding possible zoonoses from eating meat has resulted in meat, especially fro ruminant animals, obtaining a bad image. There is thus increasing pressure on producers to produce an improved product with at least equal, if n better eating quality than untreated meat, in a safe, effective and humane manner. In this review some of the factors which relate the nutrition a treatment of the animal, pre and post slaughter to quality of meat produced are discussed.

EFFECTS OF GROWTH RATE ON MUSCLE FIBRE STRUCTURE AND EATING QUALITY OF MEAT

The relationship between muscle fibre structure and eating quality is complex because a number of factors are involved. Attempts to manipula growth by selective breeding, experimental diets or exogenous growth promoters may change muscle fibre type, mean fibre diameter and th physiological status of the muscle at the time of slaughter. Separating the effects of these factors on tenderness can be difficult, since, for examp glycolytic (white) fibres tend to have larger diameters than oxidative (red) ones. However, some studies suggest that the toughness of uncook meat, as judged from its shear value, may increase with overall fibre diameter. In a trial involving 120 cross-bred steers, shear force was positive correlated with fibre diameter, and negatively correlated with the percentage of oxidative fibres (Seideman *et al.*, 1987). It would therefore see that high fibre number which correlates with smaller fibres may be a relevant parameter which correlates with muscle (meat) quality. Fast growin strains of pigs and other animals have more muscle fibres than slower growing strains (Ezekwe and Martin, 1975; Miller *et al.*, 1975). Even withi the same strain of animal, pigs between 50 and 80 kg body weight showed positive correlations between fibre number and average daily gain an the gain:feed conversion ratio (Dwyer *et al.*, 1993). In younger pigs however (up to 25 kg body weight), average daily gain was correlated wi birth weight but not with fibre number. As fibre number is completed around the time of birth in mammals and cannot be increased postnatally, th effect of maternal feed intake on the fibre number of the progeny has been studied. Undernutrition has been shown to result in smaller progeny wit fewer fibres per muscle (Powell & Aberle, 1981). Doubling maternal feed intake of sows at different periods during pregnancy has been show to increase fibre number in progeny when maternal intake was doubled between days 25 and 50 of gestation (Dwyer *et al.*, 1994). Mea secondary:primary fibre ratio (which has been associated with muscle hypertrophy) was increased in progeny from sows whose intake was double at all time periods when compared to control animals. Administration of GH to pregnant sows has also been shown to result in increased musc fibre number at birth, but only when administered at early (10 - 24 days) gestation, at a time when muscle cell proliferation is maximal (Rehfeld *et al.*, 1993). Administration of GH in mid-gestation resulted in delayed muscle maturation, whilst administration in late-gestation resulted in a increased muscle fibre diameter, with no effect on muscle fibre number. The obvious mechanism for the effects of GH on muscle fibre number ar via increases in circulating IGF-I in the foetus (endocrine action) or via increases in local intramuscular IGF-I (paracrine or autocrine action). I has recently been demonstrated in humans that the placenta produces a variant form of GH (GH-v) which takes over from the pituitary durin pregnancy when the pituitary stops producing GH. This variant form of GH has been shown to produce the same insulin-like and lipolytic response in rat adipose tissue as normal GH (Goodman *et al.*, 1991). Thus it may be possible to manipulate the placental production of GH-v, whic presumably is able to cross the placenta and influence foetal IGF-I production. Similarly, in the double muscled (DM) syndrome, a hereditar condition in which cattle possess almost 40% more fibres than do normal cattle at birth, increased growth factor activity during early foet development has been detected in the serum of DM foetuses (Gerrard and Judge, 1993) which has been suggested to play a role in the muscle fibr hyperplasia exhibited by these animals. Myoblasts from DM foetuses have been shown to replicate more than those from normal foetuses (Quin *et al.*, 1990) and serum from such foetuses increased proliferation of cultured L6 myoblasts (Gerrard and Judge, 1993). Maximal local IGF-I mRNA expression has been shown to be delayed in DM foetuses (Gerrard and Grant, 1994) compared to normal foetuses, which may relate to IGF- II's positive effects on muscle differentiation i.e., differentiation is also delayed in DM foetuses which may enable greater proliferation to occur. Identification of other growth factor(s) and determination of whether they are DM-specific factor(s) or simply altered concentration(s) of typica growth factor(s) at a critical stage of growth may facilitate muscle fibre number to be increased in other species. Growth factors can either b positive or negative regulators of muscle cell growth and development. A negative regulator of skeletal muscle growth has recently been identifie in mice (McPherron *et al.*, 1997). Gene targeting to disrupt the GDF-8 gene, a member of the transforming growth factor-ß family, has been show to increase muscle mass by 2-3 times by a combination of muscle cell hyperplasia and hypertrophy.

Pigs with more muscle fibres also tend to have less fat (Stickland and Goldspink, 1975). Other indices of quality, eg toughness, may have also improved. (see above). Studies into the effects of dietary protein on tenderness have yielded variable results (see Soloman *et al.*, 1994). Essen Gustavsson *et al.*, (1994) found an increase in the toughness of the M longissimus dorsi muscle (LD) in barrows and gilts fed a high protein diet

These changes were associated with a decrease in intramuscular lipid and an increase in muscle fibre diameter. The correlation between large fibre diameter and the pale, soft, exudative (PSE) condition in pork is well-established. A study at the time of slaughter showed that the large fibres have poor vascularisation, high lactate and ammonia concentrations and low ATP and glycogen (Essen-Gustavsson *et al.*, 1992). Hence this condition too may be attributable to the metabolic state of the fibres, and only indirectly to their structure.

The underlying mechanisms behind these changes are beginning to be determined following both *in vivo* and *in vitro* studies. Differentiation of muscle cells in culture can be induced in most cell types by reduction of serum concentration in the culture medium, with the mechanism thought to be via changes in locally produced insulin-like growth factors (IGFs). Differentiation has been shown to be dependent upon other components of the cell culture medium, including the type of serum used (Doumit & Merkel, 1992), the type of medium (Dodson *et al.*, 1990; Pinset & Whalen, 1984) and the substratum on which the cells are grown (Dodson *et al.*, 1990). One report suggested (Dodson *et al.*, 1990) that differentiation was increased in low glucose Dulbecco's modified Eagle's medium (DMEM), compared with high glucose DMEM. However, we have found no effect of glucose concentration on differentiation of primary foetal sheep myoblasts, as measured by changes in intracellular creatine kinase activity (Brameld *et al.*, unpublished data). There was also no effect of glucose on expression of IGF-I or IGF-1 receptor genes. It has also been shown that the unsaturated fatty acid, linoleic acid, stimulates differentiation of rat satellite cells (Allen *et al.*, 1985). The mechanism for how these nutrients could possibly affect myoblast differentiation may be via effects on the GH-IGF axis, and in particular any locally produced IGFs, IGF-binding proteins and/or IGF-1 receptor, all of which will alter IGF bioactivity. However, it is still uncertain whether primary myoblasts and/or myotubes possess GH-receptors; whether they produce IGF-I themselves and if so, whether the IGF-I is GH-dependent.

Studies involving the manipulation of specific nutrients in the media are lacking and the published studies of direct nutritional effects on muscle cells in culture have involved mainly micro-nutrients. A lack of available zinc has been shown to inhibit C2C12 myoblast differentiation and to decrease expression of both MyoD and myogenin mRNA (Petrie *et al.*, 1996). Similarly, a lack of calcium can also inhibit muscle cell fusion (Merlie & Gros, 1976; Morris *et al.*, 1976), although effects on differentiated protein production appear to be concentration dependent (Morris *et al.*, 1976). The effects of calcium may be related to the increase in the level of m-calpain, the calcium dependent proteinase requiring millimolar concentrations of calcium, seen during differentiation of foetal chicken myoblasts (Kwak *et al.*, 1993). This increase in m-calpain correlated with the elevated cleavage of filamin which occurs during the fusion process, and may therefore play an important role in the cytoskeletal reorganisation required for myoblast fusion. This would also agree with the report that IGF-I, which stimulates differentiation, also increases m-calpain mRNA concentrations (Hong & Forsberg, 1994), although this observation was made in already differentiated rat L8 myotubes. Retinoic acid, the vitamin A derivative, has also been shown to induce myogenic differentiation and myogenin synthesis in the rat Rhabdomyosarcoma cell line BA-Han-1C (Arnold *et al.*, 1992). The effects of retinoids are mediated through two receptor subtypes, namely the retinoic acid receptors (RAR) and the retinoid X receptors (RXR), and expression of RAR mRNA has been shown to be repressed during C2C12 myoblast differentiation, while RXR mRNA was induced (Downes *et al.*, 1994).

The growth of domesticated animals is largely controlled by their nutrition. Evidence for effects of nutrition on muscle cell proliferation and differentiation comes from work in whole animals, with rates of foetal growth in the pig found to be very much dependent upon maternal nutrition and the supply of maternal nutrients to the foetus (see above). For example, a reduction in the uterine blood supply can lead to runting and runted pigs are born with fewer muscle fibres (Powell & Aberle, 1981). The effects of nutritional and hormonal manipulations on post-natal growth, and in particular the GH-IGF axis, has recently been reviewed (Brameld, 1997; Straus, 1994), and therefore will not be duplicated here. However, the evidence from studies in whole animals and cultured hepatocytes would seem to suggest that both the protein and energy constituents of a diet have direct effects on expression of growth-regulatory genes. In pigs, energy in the form of glucose, appears to directly increase expression of the GH-receptor (GHR) gene in liver and therefore enhance the effects of GH on IGF-I expression, with protein, in the form of amino acids, having a similar effect, although the mechanism may be slightly different. Interestingly, neither of these nutrients appear to alter IGF-I expression in muscle, but they have the opposite effects on muscle GHR expression to those seen in liver (see Brameld, 1997).

FAT CONTENT OF THE CARCASS

From nutritional, economic and meat quality points of view it would be better to reduce fat deposition in all depots except the intramuscular depot. The UK pig industry, for example, has already considerably reduced the total carcass fat. However, this reduction has been associated with a reduction in meat quality. The relative growth rates of different fat depots varies with the stage of maturity of the animal, with the intramuscular depot usually being one of the latest to be deposited. Thus the age of the animal will affect the distribution of fat and it is unclear how feasible it may be to manipulate selectively deposition in certain late-developing depots whilst minimising deposition in other earlier-maturing depots. Evidence of differences in metabolic responsiveness between fat depots has been found in a number of species but this may well be related to the age of the animal and the stage of development. Thus for example, in rats subcutaneous adipose tissue was the least responsive to the lipogenic effects of insulin and showed no inhibition of this response with glucocorticoids (Hauner and Pfeiffer, 1989). *In vitro* pig fat explant studies have also demonstrated differences between internal and external fat depots with respect to lipid metabolism (Budd *et al.*, 1994), with internal depots being more lipogenically and lipolytically active both at basal levels of activity and in response to hormone treatments. No differences in cell size were observed and results suggest there may be intrinsic differences in the hormonal responsiveness of subcutaneous pre-adipocytes themselves (Ramsay *et al*, 1989). Similar pig fat explant studies comparing different breeds of pig demonstrated that, at the conventional slaughter weight, the Large White was generally more lipogenically (both basal and hormone-stimulated) and lipolytically (basal only) active than the Meishan x Landrace, with the same differences between internal and external depots as described previously (Budd *et al.*, unpublished data). Differences in the metabolism of different fat depots have been recorded in other species, eg cattle (Dawson *et al.* 1993), sheep (Vernon, 1992). Previously these observations have largely come from *in vitro* studies. Recently, however, Greathead *et al.* (unpublished observations) was able to show that the rate of incorporation of acetate into total lipid and into triacylglycerol varied between fat depots (see Table 1). Thus a basic understanding of the mechanisms controlling fat metabolism in different depots may lead to the ability to manipulate specific fat depots via the animal's nutrition.

The effect of dietary regime on the fat to protein ratio in the carcass of the ruminant is much more complicated. This is clearly illustrated by effe of forage diets, especially grass silage upon the growth and carcass composition of cattle. Grass silage diets often give lower levels of performan in cattle and result in a greater proportion of fat in the gain compared to animals fed either dried grass (McCarrick, 1966; Lonsdale, 1976) concentrate diets fed at similar levels of energy and protein (Steen, 1991; 1994). This high carcass fat:protein ratio could either be due to impair protein metabolism or to enhanced fat deposition.

The relationship between whole body protein synthesis and total nitrogen intake of steers fed either dried grass or silage is shown in Fig. 1 a illustrates the impaired efficiency associated with silage. Young cattle fed grass silage alone have been shown to respond to dietary prote supplementation with increased growth rates which could be accounted for almost entirely by an increase in protein accretion (Gill *et al.*, 198 Fishmeal supplementation (150g/kg silage dry matter) was shown to increase the duodenal supply of amino acids. In hourly fed animals it w shown that 70% of this increase was due to an elevation of undegraded dietary protein flow with an increase in the flow of microbial prote accounting for the remainder. The efficiency of microbial protein production was also increased (Dawson *et al.*, 1988). Similar experiments wi twice-daily fed animals indicated that the increase in duodenal protein flow was solely due to an increase in the flow of protein escaping degradati in the rumen (Beever *et al.*, 1990). An asynchronous supply of nitrogen and energy release in the rumen is believed to contribute to the lo efficiency of capture of the rumen degradable nitrogen and low rates of microbial protein synthesis are often observed on silage diets. Balanci the supply of nitrogen and energy-yielding nutrients to rumen micro-organisms has been attempted in order to maximise the capture of rum degradable nitrogen and to optimise microbial growth rate and efficiency. The synchrony of nutrient supply in the rumen is affected by a numb of factors in addition to the fermentation characteristics of the individual feed ingredients, including the level of feeding, the number of meals a the timing of meals throughout the day. For example, supplementation of grass silage with fishmeal was shown to increase the efficiency of microbi protein synthesis when the diets were fed hourly, but not when fed twice daily (Dawson *et al.*, 1988; Beever *et al.*, 1990). This was attributed a more continuous supply of substrates for microbial growth when the diets were supplied hourly. Other workers investigating the effect of feedi dietary ingredients with different fermentation characteristics have observed mixed responses, some demonstrating significant improvements in t efficiency of microbial growth efficiency with more synchronised diets (Herrera-Saldama *et al.*, 1990, Sinclair *et al.*, 1993), others reporting litt effect (eg Henning *et al.*, 1993). Some evidence, albeit circumstantial, was obtained from our studies to indicate that this was associated with enhancement of the incorporation of peptides originating from the fishmeal (Dawson *et al.*, 1988).

Further studies in our laboratory have suggested to us that, at least in the young animal, the high carcass fat:protein ratio is probably also associat with the metabolism of the animal itself, in addition to the activity of the rumen micro-organisms. Fishmeal supplementation of grass silage to you cattle was shown to increase the flow of amino acids to and absorption from the small intestine, but the efficiency of utilisation of the absorb amino acids was found to be similar on both diets and considerably lower than expected (53%; Beever *et al.*, 1990). Several possible mechanis for the inefficient utilisation of amino acids exist including the possibility that the timing of nutrient arrival at the peripheral tissues may also asynchronous which may reduce protein deposition. Protein accretion in humans has been shown to exhibit continual diurnal changes which refle the meal-eating pattern with periods of anabolism and catabolism of muscle protein, the sum of which will control protein accretion (Millward 1985). These switches from protein gain to protein loss and vice-versa are the result of fluctuations in nutrient intake which are mediated via chang in protein synthesis and protein degradation with the amplitude of the diurnal cycles varying with protein intake. A similar phenomenon is like to occur in the non-steady state in the ruminant although the extent to which the rumen and liver are able to level out fluctuations in nutrient (amin acid and energy) supply to the peripheral tissues is still unknown. Thus high fat to lean ratio in the silage fed animal is thought to be largely d to impaired protein deposition. An additional question is, are there differences in the rate of fat metabolism in silage fed animals compared wi those fed dried grass?

Differences in fat metabolism have been noted (see Table 1) in individual fat depots between animals fed dried grass and silage following assessme of the *in vivo* incorporation of ^{14}C acetate into lipid and triacylglycerol. It should be noted that these animals were fed at varying levels of ME intak and that an increased rate of fat synthesis in the silage fed animals was observed when compared to animals fed dried grass at equivalent intake It is interesting to note that no differences in whole body fat metabolism, assessed by measuring entry rates through the plasma pool of palmitat and glycerol, were detected between young cattle fed *ad libitum* grass silage alone or supplemented with fishmeal, or a forage-concentrate diet (70% dried grass:30% barley) (Greathead *et al.*, 1994). The data presented in Table 2 confirm the observation of Lonsdale (1976) that at equivalent M intakes the carcass fat to protein ratio of silage fed animals is greater but also clearly illustrate the need to compare fat deposition at equivalent intak when assessing whether feeding silage produces fatter carcasses than dried grass.

Just as maternal nutrition influences muscle fibre number and thus muscle mass in the progeny, it is possible that it may also influence the propensi of progeny to deposit fat in later life. Both maternal over- and undernutrition during gestation of rats was found to increase adiposity of your adult progeny (Fioretto *et al.*, 1995). This was attributed to changes in nutrient supply which influenced foetal insulin status. Both foet hyperinsulinaemia and hypoinsulinaemia have been shown to increase post-natal fat deposition in rats (See Fioretto *et al.*, 1995 for references)

CONTROL OF FAT COMPOSITION

The nutritional quality of fat is normally judged by the proportion of saturated fatty acids it contains. Animal fats, particularly those fro ruminant animals, are usually perceived as being rich in saturated fatty acids. There have been many attempts to change the fatty acid compositio of animal tissues. These have been most successful in the non-ruminant animal where the tissue fatty acid profile tends to reflect that of the di (Enser and Wood, 1996; Noble,1996; Redshaw *et al.* 1996). This is not, however, true in the ruminant due to: (i) the inherently low fat diet the animal which means that most adipose tissue fatty acids are synthesized *de novo* and (ii) most dietary unsaturated fatty acids are hydrogenate by rumen microbes. There is, however, some evidence to suggest that gross changes in the diet of the ruminant can influence tissue fatty aci composition. Cattle fed forage have a higher percentage of saturated fatty acids than feedlot (grain) fed animals (Marmer *et al.*, 1984, Sumida *et al.* 1972, Westarling and Hendrick, 1979). However, as grain-fed animals were generally fatter the total amount of saturated fatty acids in th

tissues actually increased (Marmer *et al.*, 1984). Cattle fed an all concentrate diet showed a 20% increase in adipose tissue unsaturated fatty acids, primarily oleic acid, compared to those fed forage (Rumsey *et al.*, 1972). If sheep are fed a purified diet containing casein, glucose, starch and hydrogenated vegetable fat the adipose tissue content of the two major saturated fatty acids, palmitic and stearic acids, is reduced (Tove & Matrone, 1962). However, these extreme diets are unlikely to be commercially viable, feeding an entirely concentrate or purified diet is impractical and expensive.

An alternative approach has been to try and 'protect' the dietary unsaturated fatty acids from biohydrogenation. This approach has been used both as a way of manipulating the fatty acid profile of meat and milk and to enable more fat to be used in the ruminant diet to increase the energy density, particularly for high producing dairy cows where energy can be limiting (Scott & Ashes, 1993).

There are various processes which have been developed to protect fats from rumen biohydrogenation. These have been well reviewed by Scott & Ashes (1993). The earliest approach was to protect vegetable oil by emulsification with sodium caseinate and then treatment with formaldehyde. The principle being that the formaldehyde forms a cross-linked matrix which protects the protein and entrapped the oil, which is protected from attack by the rumen microbes. The acidic conditions of the abomasum weaken the aldehyde-protein link and the lipid is released allowing the adsorption in the small intestine. Another approach is to form calcium soaps of fatty acids this reduces the solubility in the rumen. Oilseed either whole or are partially crushed are also protect some of the lipid from the rumen. This is also taken a stage further by coating the seeds with calcium to further the protection in the rumen.

Scott & Ashes (1993) showed data that feeding formaldehyde treated and protected canola seed caused significant changes in the adipose tissue percentage fatty acid profile (see Table 3). However, there is some evidence to suggest that the benefits of changes in fatty acid profile brought about by feeding protected fats may be offset by an increase in total fat deposition (Scott & Ashes, 1993).

MEAT TOUGHNESS

The final eating quality of meat depends on a number of organoleptic properties including appearance, comprising colour and fat content, taste, texture and tenderness. Whilst colour and fat content are important in influencing meat purchase, consumer studies indicate that it is the degree to which muscle tenderises after slaughter that is the most important factor contributing to overall meat quality in cattle, sheep and pigs (Koohmaraie, 1994). After slaughter the loss of oxygen supply to tissues initiates anaerobic metabolism, resulting in the utilisation of primary energy stores. In the case of skeletal muscle, this means that muscle glycogen is depleted, producing an increase in lactic acid in the muscle, thereby reducing muscle pH. The rate of postmortem glycogenolysis can be altered in ruminants by circumstances which produce or mimic stress. For example, adrenaline infusion in cattle reduces the rate of pH decrease postmortem by depleting glycogen stores prior to slaughter, diving rise to dark, firm, dry (DFD) beef (Geesink *et al.*, 1992), with highly fragmented fibres and poor taste qualities. Similar observations have been made on pork carcasses following long-term stress (Warris *et al.*, 1989), whilst short-term stress increases the rate of pH fall, giving rise to pale, soft, exudative (PSE) meat.

Among the most important reactions influencing toughness are those catalysed by certain endogenous proteolytic enzymes which act in a highly selective manner on a small number of key intracellular muscle proteins to initiate tenderisation. Several candidate enzyme systems have been proposed over the last 10 years, including cathepsins and the multicatalytic protease (proteasome). However, in recent years a consensus has emerged which suggests that the most important proteolytic enzymes affecting tenderisation belong to the Ca^{2+}-dependent cysteine proteinase or calpain family and the most important substrates include myofibrillar, Z line and costamere proteins in muscle fibres (Koohmaraie, 1994; Taylor *et al.*, 1995).

The calpain system comprises at least three components, two of which have proteolytic activity *in vitro* at micromolar and millimolar concentrations of calcium, namely μ-calpain and m-calpain respectively, with the third important factor being a specific endogenous inhibitor known as calpastatin. The system is highly sensitive to fluctuating levels of calcium ion, pH and temperature, all of which change rapidly in the immediate postmortem period (Suzuki *et al.*, 1995). Recently, other calpains have been identified, including p94 which is almost exclusively expressed in skeletal muscle (Sorimachi *et al.*, 1995). Recent data suggest that p94 is tightly bound to the giant elastic protein titin close to a calpastatin-like region (Labeit and Kolmerer, 1995) and its impaired expression has been implicated as being of importance in pre-disposition to limb girdle muscular dystrophy in humans (Richard *et al.*, 1995). Unlike other proteolytic systems which may be active postmortem, the calpains are the only proteolytic enzymes in skeletal muscle which are known not to degrade the major myofibrillar proteins actin and myosin which both remain intact during the tenderisation process (Goll *et al.*, 1991, 1992). Further evidence has shown that $CaCl_2$ infusion into the carcass increases the rate of tenderisation in beef whilst infusion of $ZnCl_2$, a calpain inhibitor, reduces the rate of tenderisation in both beef and lamb (Geesink *et al.*, 1994; Koohmaraie, 1990). In addition, studies at Nottingham and in the USA have shown that, in sheep and cattle, β-adrenergic agonist administration over several weeks not only has a significant hypertrophic effect on muscle growth, but also has a remarkable effect on the level of activity of calpastatin in LD muscle (Higgins *et al.*, 1988; Wang and Beermann, 1988; Bardsley *et al.*, 1992; Parr *et al.*, 1992; Speck *et al.*, 1993). Long term β-agonist treatment is known to produce tough meat in cattle and sheep, presumably due to suppression of postmortem proteolysis by the elevated activity of calpastatin (Kretchmar *et al.*,1990; Wheeler and Koohmaraie, 1992). The rate of tenderisation also varies between the principle meat species, with beef taking longer to tenderise than lamb and pork tenderising more rapidly. Significantly, calpastatin levels at slaughter are greatest in beef and lowest in pork adding further evidence to the involvement of the calpain system in the tenderisation process (Koohmaraie *et al.*, 1991). Recent work in the U.S. has also shown that the callipyge lambs, characterised by their enhanced muscle growth and excessively tough meat, have extremely high levels of calpastatin (Koohmaraie *et al.*, 1995). Whilst this is not enhanced by β-agonist treatment, it can be resolved by infusion of $CaCl_2$ (Koohmaraie *et al.*, 1996; Clare *et al.*, 1997). Thus a genetic condition has been shown to alter the calpain system in a manner consistent with effects on tenderisation.

Recent work in Nottingham has shown that the same system is involved in the conversion of pig muscle to pork and that pre-slaughter stress can

alter the responsiveness of the calpain system postmortem (Sensky *et al.*, 1996a, 1996b, 1997a). Intravenous infusion of adrenaline or a place in two sibling-matched groups of pigs for a seven day period immediately prior to slaughter increased the calpastatin activity extracted immediate after slaughter by 80% and stabilised m-calpain activity during the conditioning phase (Table 4), possibly as a result of posttranslational modificati of the proteins concerned (Sensky *et al.*, 1996a). Screening the active extracts for calpastatin polypeptides by Western blotting revealed that t active fractions partially purified by hydrophobic chromatography were in fact heavily fragmented, though still inhibitory. Direct probing of t extracts without chromatographic separation from calpain resulted in them electrophoresing as single bands of apparent molecular weight 135 kD Adrenaline appeared to increase 135 kDa calpastatin in the same way as it increased m-calpain (Table 4). Analysis of meat quality traits, includi shear force and degree of myofibrillar fragmentation, on corresponding samples which had been vacuum packed 48 h after slaughter and aged 2°C for a further 6 days, revealed that there were generally no significant differences in tenderness between the adrenaline-treated group a controls, although classification of the samples as tough (shear force > 5 kg), normal (3.5-5 kg) and tender (< 3.5 kg) revealed a significant positi correlation between toughness and extractable calpastatin activity in samples taken immediately post slaughter (Sensky *et al.*, 1996b). From t study it was concluded that the calpain system in pigs was broadly affected by adrenergic stimulation in the same way as in other specie particularly with respect to calpastatin. However, the observations on postmortem m-calpain stabilisation represented a novel finding, clea demonstrating that the activity of the calpain system after slaughter can be altered by adrenergic stimulation prior to slaughter.

In a further trial, the effect of short term stress on the calpain system was investigated by varying the degree of skeletal muscle β-recept stimulation prior to slaughter by supplementing the standard finisher diet of 4 groups of pigs with either a high or low dose of either a β-antagon or a β-agonist 16 h prior to slaughter. A broad positive correlation was observed between the degree of β-receptor stimulation and extracta calpastatin at slaughter (Sensky *et al.*, 1997a). At this stage it could be concluded that if adrenergic effects on calpains are important, the stre related fluctuations around slaughter are likely to far outweigh pharmacological dosage preslaughter. Assessment of the role of adrener stimulation is always likely to be compounded by pH effects which in turn are related to muscle glycogen level.

As with the callipyge lambs, the presence of a mutation of the ryanodine receptor in muscle sarcoplasmic reticulum in a percentage of certain breeds, the so-called "Halothane" mutation, presents an opportunity to look at genetic differences in the calpain system in porcine muscle. It currently known that this mutation renders pigs highly susceptible to stress, produces an increased incidence of PSE meat and tends to produ tougher meat (Desmet *et al.*, 1996; McPhee and Trout, 1995). Whilst the mechanism of increased toughness in callipyge lambs seems to be direct through calpastatin, indications are that halothane positive animals have lower levels of m-calpain (Sensky *et al.*, 1997b).

It is difficult to determine the relative importance of genotype, husbandry, preslaughter or post slaughter treatment in controlling proteolytic activ however to be able to do this will go a long way in enabling the meat industry to consistently produce tender meat.

CONCLUSIONS

The future of meat science therefore lies in the improvement of meat quality. It is necessary to combine investigations at the molecular, cellular an whole animal levels in order to increase understanding of basic mechanisms affecting fat and lean deposition in farm livestock and thus fulfil th quality and health concerns of the consumer.

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Table 1. Rates of acute incorporation into total lipid (Rlipid) (μg acetate/min/g lipid) and into TAG (RTAG) (μg acetate/min/g TAG) in subcutaneous, omental and perirenal adipose tissues in steers fed silage (S) or dried grass (DG) at a range of ME intakes.

		Metolisable energy intake (x maintenance)							SED[a]	Intake effect[b]		Diet	I x D[b,c]	
		1.1	1.2	1.3	1.4	1.55	1.8	2.0	(12df)	Linear	Quadratic	effect[b]	Linear	Quadratic
Rlipid - subcut.	S	0.48	0.8	1.57	1.36	3.35			0.59	P<0.001	P<0.05	P<0.1	NS	NS
	DG	0.06	0.07	1.25	1.34	2.99	4.41	5.39						
Rlipid - omental	S	0.11	0.42	1.15	0.79	2.16			0.65	P<0.001	P<0.05	P<0.1	NS	NS
	DG	0.08	0.25	0.26	0.31	1.98	2.88	3.46						
Rlipid - perirenal	S	0.75	2.12	3.05	1.07	2.16			0.60	P<0.05	NS	P<0.001	NS	P<0.05
	DG	0.12	0.23	0.40	0.70	1.77	1.97	2.53						
RTAG - subcut.	S	0.39	0.56	1.29	1.13	2.99			0.50	P<0.001	P<0.05	NS	NS	NS
	DG	0.05	0.07	1.15	1.15	2.77	3.76	5.12						
RTAG - omental	S	0.10	0.32	0.87	0.67	1.89			0.60	P<0.001	P<0.01	NS	NS	NS
	DG	0.07	0.16	0.24	0.26	1.88	2.79	3.26						
RTAG - perirenal	S	0.64	1.68	2.34	0.93	1.84			0.54	P<0.01	NS	P<0.01	NS	P<0.05
	DG	0.09	0.21	0.35	0.61	1.63	1.90	2.35						

[a] Pooled standard error of the difference between means.
[b] Effects determined for data over the intake range 1.1x to 1.55x ME requirement for maintenance.
[c] Intake x diet interaction.

Table 2. Carcass composition (g/kg carcaass) of steers fed silage (S) or dried grass (DG) at a range of ME intakes.

		Metolisable energy intake (x maintenance)							SED[a]	Intake effect[b]		Diet	I x D[b,c]	
		1.1	1.2	1.3	1.4	1.55	1.8	2.0	(12df)	Linear	Quadratic	effect[b]	Linear	Quadratic
Crude protein	S	199.4	191.8	190.8	191.8	191.7			5.6	NS	NS	P<0.05	NS	NS
(N x 6.25)	DG	203.5	203.1	195.1	203.4	195.7	195.1	200.7						
Fat	S	76.0	78.7	87.0	93.0	93.4			8.5	P<0.05	P<0.1	P<0.01	NS	P<0.05
	DG	77.2	69.6	55.5	74.0	82.9	84.4	100.3						
Ash	S	56.6	50.9	49.9	56.0	53.8			2.1	P<0.1	P<0.1	P<0.001	P<0.1	NS
	DG	51.5	46.0	46.20	47.70	43.5	44.5	43.5						
Water	S	661.5	669.9	665.2	651.5	657.7			10.1	NS	P<0.05	P<0.05	NS	NS
	DG	660.6	675.9	695.8	666.6	669.0	666.77	645.6						

[a] Pooled standard error of the difference between means.
[b] Effects determined for data over the intake range 1.1x to 1.55x ME requirement for maintenance.
[c] Intake x diet interaction.

Table 3. Effect of feeding protected canola seed on the fatty acid profile (% by weight) of subcutaneous adipose tissue of steers.

Fatty acid	control	protected canola seed
14:0	2.9	2.6
16:0	26.1	20.3
16:1	4.7	3.6
18:0	13.8	14.2
18:1	45.6	48.7
18:2	1.5	5.3
18:3	0.3	1.9

Adapted from Scott & Ashes (1993)

Table 4. Changes in m-calpain activity and m-calpain and 135 kDa calpastatin immunoreactivity in adrenaline (A) and placebo (P) infused animals (n=6) over the first 8 hours postmortem

	m-Calpain activity ($x10^7$ fluorescence units/kg)		m-Calpain immunoreactivity (% change in absorbance intensity of band relative to placebo 0 h[a])		Calpastatin immunoreactivity (% change in absorbance intensity of band relative to placebo 0 h[a])	
Time after slaughter	P	A	P	A	P	A
0 h	2.48	2.62	100	142	100	184
2 h	1.14	1.89	44	125	103	135
4 h	0.36	1.39	64	87	92	76
8 h	0.47	1.48	43	60	40	57

[a] The decay in m-calpain/calpastatin immunoreactivity on western blots was examined for adrenaline and placebo paired animals over the 8 h time course, a pair of animals being examined per blot. The intensity of the placebo m-calpain/calpastatin immunoreactive band at 0 h postmortem was taken as 100% , with the other time points on the same blot being relative to this. An average % change was calculated across the immunoblots.

Figure 1. The effect of total nitrogen intake on rates of whole-body protein synthesis ($g/kg^{0.75}/d$) in steers fed grass silage and dried-grass.