Muscle metabolism in relation to genotypic and environmental influences on consumer defined quality of red meat.

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Summary

This paper discusses the management of consumer defined beef palatability using a carcass grading scheme which utilizes the concept of total quality management. The scheme called Meat Standards Australia (MSA) has identified the Critical Control Points (CCPs) from the production, pre-slaughter, processing and value adding sectors of the beef supply chain and quantified their relative importance using large-scale consumer testing. These CCPs have been used to manage beef palatability in two ways. Firstly, CCPs from the pre-slaughter and processing sectors have been used as mandatory criteria for carcasses to be graded. Secondly, other CCPs from the production and processing sectors have been incorporated into a model to predict palatability for individual muscles. The CCPs from the production (breed, ossification and HGP implants), pre-slaughter and processing (pH/temperature window, alternative carcass suspension, marbling and ageing) sectors are reviewed. The paper then discusses the interacting roles of nutrition and genotype as determinants of muscle energy pattern with respect to glycogen and fat metabolism. In particular the roles of fibre type and/or pattern of muscle energy metabolism is discussed in relation to the high ultimate pH syndrome (dark cutting beef), the rate of *post mortem* glycolysis and the response to electrical stimulation. Finally the development of intramuscular fat is discussed in terms of growth and development, biochemical regulation and nutritional modification.

Keywords: beef, tenderness, palatability, Meat Standards Australia, genotype, breed, glycogen, ultimate pH, pH decline, intramuscular fat, muscle fibre type, metabolism

Introduction

Meat palatability is a function of production, processing, value adding and cooking method used to prepare the meat for consumption by the consumer. Failure of one or more links in the beef supply chain increases the risk of a poor eating experience for the consumer. A guarantee for eating quality can only be given if the links that most affect eating quality are controlled along the meat production chain.

An example of a 'paddock to plate' quality assurance system which manages meat quality along the entire length of the meat production chain is the new grading scheme called Meat Standards Australia (MSA), which is presently being implemented for the Australian domestic beef market by Meat and Livestock Australia (MLA).

This paper will initially overview the main factors used in the MSA system to describe the development and implementation of a quality assurance system which manages and describes the palatability of meat for the consumer. A more detailed analysis of MSA can be found in Thompson (2002). Next the paper describes the impact of genetic and nutritional

manipulation on the metabolism of muscle in relation to glycogen and lipid metabolism, which are important factors which may affect beef quality traits (Hocquette et al., 1998).

A total quality management approach to meat quality

The MSA grading scheme uses a total quality management approach to identify critical control points (CCPs) and to predict the quality of the final product. Much of the research undertaken by MSA was not new. New components included the use of a large-scale consumer testing system that allowed the effects of the CCPs to be quantified using a standard evaluation procedure. A second new feature was the introduction of a cuts-based-grading system to improve the accuracy of predicting palatability in beef and the need to grade all muscles in the carcass. Analysis of the MSA database showed that the variation in palatability explained by muscles was approximately 60 times greater than that explained by the variation between animals for the same muscle.

The consumer testing system

At the commencement of MSA the decision was made to use sensory results derived from untrained consumers as the means to describe palatability of beef. Although objective measurements (such as shear force) have the advantage of being relatively cheap, they are rather simplistic one dimensional measures of a complex set of interactions which occur when cooked meat is chewed and masticated in the mouth. Furthermore, studies in France showed that shear force may explain only up to 48% of total variability in tenderness and this proportion depends on the breed and the production system (Brouard et al., 2001).

The consumer sensory testing protocol used by MSA (Polkinghorne et al., 1999) was based on existing protocols in use by the American Meat Science Association protocols (AMSA 1995). Briefly, untrained consumers were asked to score tenderness, juiciness, flavour and overall acceptability on a scale of 0 to 100. They also graded the sample on the following word associations; unsatisfactory, good everyday (3 star), better than everyday (4 star), or premium quality (5 star). To combine the 4 sensory dimensions into a single palatability or meat quality score (MQ4), weightings were formulated from a discriminant analysis (0.4, 0.1, 0.2 and 0.3 for tenderness, juiciness, flavour and overall acceptability, respectively). The palatability scores were then used to calculate the optimum boundaries for the grades assigned by the consumers with 45.5 separating ungraded (fail) and 3 star categories, 63.5 for 3 and 4 star, and 76.5 and above for 5 star. The current iteration of the model in June 2004 is based on a data base which contains responses from 60,100 consumers

Components of the MSA model

The specifications for producers and processors to supply carcasses which are eligible for grading by MSA include compliance with a set of conditions aimed at reducing pre-slaughter stress and optimizing processing conditions.

Producers need to be registered and must adhere to MSA Cattle Handling Guidelines to minimize stress. They must declare the *Bos Indicus* % content of their cattle, and whether the cattle can be classed as milk fed calves. The time of loading must be supplied, the cattle trucked direct to slaughter, not mixed in lairage, and killed the day after dispatch.

Abattoir procedures are audited within a QA system to ensure pH and temperature relationships are within the prescribed window to achieve optimal palatability. To minimise variation in cooling rates carcasses must have an even distribution of fat with at least 3 mm of fat at the rib site. All carcasses must have an ultimate pH below 5.7 and a USDA ossification score (Romans et al. 1994) below 300.

A summary of the input factors which drive the palatability prediction model are:

- *Bos indicus %*: This is specified on the producer declaration and/or estimated as the hump height which is measured on the carcass and related to carcass weight. The magnitude of the *Bos indicus* effect varies with muscle. There were no other breed effects that could not be explained by the other commercial production parameters measured (marbling score, rib fat, ossification, pHu).
- Sex: A sex adjustment (steers versus heifers) is made which varies with muscle and is relatively small, being of the order of 2 palatability units. As yet beef from entire males is not included within the model.
- USDA Ossification score: This is used as an estimate of animal physiological age and proved more revealing than using traditional dentition classes which have previously been used by the Australian beef industry. As ossification score increases from 100 to 200 the consumer score declines 5, 10 and 12 points for the *m. longissimun lumborum (LT)*, *m. gluteus medius* (GM) and *m. semitendinosus* (ST) respectively indicating a greater effect of animal age in the leg muscles.
- Milk Fed Veal (MFV): Muscles from calves weaned immediately prior to slaughter (at approximately 8-10 months of age) receive a higher score than from weaned cattle of equivalent ossification score. The magnitude of the MFV effect is typically 5 to 6 palatability units.
- Carcass Hanging Method: This effect is applied on an individual muscle basis, with different values for each muscle and hang combination. Hanging methods are AT (Achilles tendon) or TS (Tenderstretch from the ligament or related procedures). Differences in palatability between AT and TS carcasses are in the order of 5-6 points for muscles which are under tension due to the TS process (GM and LL muscles).
- Intramuscular fat or marbling: As marbling score and rib fat were positively correlated, both parameters are used to assess the impact of marbling on palatability of individual cuts. An increase in USDA marble score from 250 to 550 (equivalent to an increase from 0 to 3 marble score on the AUS-MEAT system) results in an increase of 8 palatability units for the LL muscle. The adjustment made for marbling depends on the muscle as different muscles express different levels of intramuscular fat.
- Ultimate pH: A small improvement in eating quality occurs as pH declines from the threshold of 5.7 (ca. 1 palatability unit).
- Ageing: The rate of ageing is estimated differently for each muscle within each hanging option. MSA product cannot be sold to consumers before 5 days post slaughter and aging to 21 days increases the consumer score by up to 4 units.
- Hormonal growth promotants: Hormonal growth promotants as combinations of oestrogenic or androgenic steroids are sometimes used in Australia to increase the rate of lean tissue deposition. Recent MSA research has shown that use of these hormones reduce the palatability of beef particularly in the LL muscle. The effects are lower in other muscles and reduced further by aging from 5 out to 21 days.
- Cooking method and muscle: Palatability for individual muscles is predicted for a specific cooking method. Larger muscles generally have several cooking options. Grilling (25mm thickness) low connective tissue cuts resulted in the highest palatability scores. Roasting low connective cuts gave similar scores to grilling, whereas for the high connective cuts roasting gave higher palatability scores than did grilling. Stir frying (10mm) and thin slicing (4mm) gave similar results to grilling for low connective muscles, but relatively higher scores in the high connective tissue muscles. The magnitude of the muscle effect is large and in the order of 30-40 palatability units regardless of cooking method.

Muscle metabolism and the critical control points

The critical control points underpinning palatability are directly dependant on key biochemical parameters including on one hand, muscle characteristics of alive animals and, on the other hand, *post-mortem* muscle biochemistry. The former include glycogen metabolism, intramuscular adipocyte number and size (marbling), connective tissue chemistry, and pigment content which contributes to colour. The latter include rates of proteolysis during ageing. Among the muscle characteristics linked to metabolism of alive animals, the pH of meat (which depends on glycogen metabolism before slaughtering) and intramuscular fat content are the major factors associated with beef quality traits. This is true in both Australia (as shown before) and Europe. Indeed, studies with young bulls in France have shown that pH at 3 hours *post-mortem* and intramuscular fat content explain 52% and 56% of the variation in tenderness and flavour respectively (Dransfield et al., 2003). Furthermore, any change in growth rate (Cassar-Malek et al., 2004) and feeding conditions (Listrat et al., 2001) were shown to affect primarily muscle metabolic activity. This paper will thus focus on glycogen and fat metabolism of muscle.

Glycogen metabolism and ultimate pH

The MSA model has a requirement that the ultimate pH of the LL muscle (measured at the 12/13th rib) must be 5.7 or less. As ultimate pH increases beef becomes less juicy, has altered cooking properties, lacks visual appeal and has reduced shelf life (Shorthose 1989). In the pH range of 5.8 - 6.2, beef is also tougher (Purchas and Aungsupakorn, 1993). To achieve an ultimate pH of 5.5, muscle needs to contain at least 50-60 μmoles/g of glycogen immediately pre-slaughter to form sufficient lactic acid to lower pH (Tarrant, 1989).

Glycogen reserves at slaughter are a function of the initial levels of glycogen (i.e. on farm level) and the losses due to stresses placed on the animal during the immediate pre-slaughter period. It is proposed that both genotype and nutrition play an interacting role in determining this balance between the initial level and loss of glycogen from muscle.

One mechanism is based on differential metabolism of glycogen in the contrasting muscle fibre types. Muscles consist of distinct fibre types that can be differentiated on the basis of their contractile and metabolic properties. They are: (i) slow-twitch oxidative fibres (type I); (ii) fast-twitch oxidative-glycolytic fibres (type IIa); and (iii) fast-twitch glycolytic fibres (type IIb) (Peter et al., 1972). The metabolism of glycogen is different in the various fibres such that type I fibres have low levels of glycogen. Type IIa fibres have higher levels of glycogen, a high rate of glycogen resynthesis and are least affected by stress. Type IIb fibres

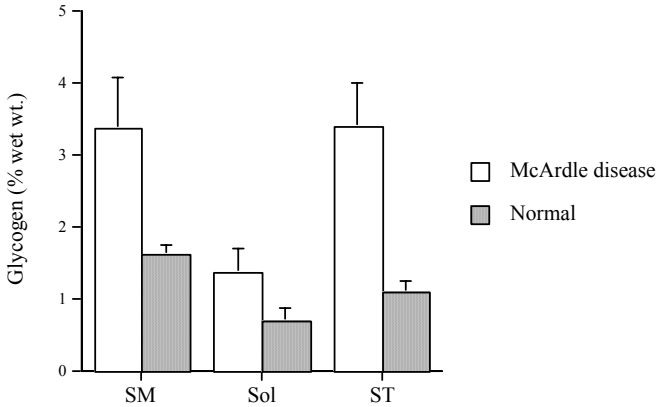

Figure 1. The level of glycogen in the m. semimembranosus (SM), m. soleus (Sol) m. semitendinosis (ST) of normal and McArdle disease affect sheep. Data is mean ± sem for 6 sheep per treatment.

have lower glycogen levels, slow rates of glycogen synthesis and are therefore the most susceptible to stress induced glycogen depletion (Monin 1981, Pethick et al. 1999). These differences can be largely explained by the different enzyme compliment of each fibre type of mammals (Saltin and Gollnick, 1983) including cattle (Talmant et al., 1986). Thus the very high activity of glycogen phosphorylase in combination with low activities of glycogen synthase and hexokinase mean that type IIb muscle fibres rapidly deplete and slowly replete glycogen levels when compared to type IIa. Clear evidence of this is shown in Figure 1 where the glycogen content of muscle was compared between normal and sheep suffering a congenital deficiency of glycogen phosphorylase within muscle known as McArdles disease (Kumar, 1998). The data shows that basal levels of glycogen increase dramatically in the absence of glycogen phosphorylase and are constant with glycogen turnover being a regulator of concentration. Further work is needed to access the rates of glycogen turnover in cattle. Some authors have speculated that the expression and/or activity of glucose transporters (which control the entry of blood circulating glucose in muscle cells) might regulate muscle glycogen content, and hence the final quality of beef (Hocquette & Abe, 2000).

The effects of fibre type and or pattern of energy metabolism on the response of muscle to nutrition are also dramatic such that the more aerobic *m. semimembranosus* (SM) shows a strong linear relationship between the extent of glycogen repletion during a 72h period after exercise depletion whereas the more anaerobic ST muscle showed no significant repletion during the same time period (Gardner et al., 2001). There are also important chronic effects of nutrition on the level of muscle glycogen (Pethick et al., 1999).

The occurrence of dark-cutting in beef carcasses in Australia has been reported to have a seasonal effect although the peak months of dark-cutting vary between years and region. Our studies have investigated the effect of season (Pethick et al. 1999). There was a strong seasonal influence on the concentration of glycogen in muscle with consistently low levels in winter and summer and high levels in spring. This drop in muscle glycogen concentration is partly explained by declining animal growth rate, which is driven by changes in pasture availability and quality. There was a positive correlation between live weight change and muscle glycogen concentration (r^2=0.69, *P*=0.04) and it was concluded that a growth rate of above 1kg/day is needed to assure a level of muscle glycogen that is sufficient to help reduce the incidence dark cutting for cattle grazing in southern Australia.

Recently we have completed an experiment employing the exercise depletion/repletion methodology of Gardner et al. (2001) to investigate rates of glycogen repletion in different breeds of cattle. Cross-bred cattle (9 months of age) from either Peidmontese, Angus or Wagyu sires were maintained on either roughage or concentrate rations. Marked differences were evident between the breeds in both levels of glycogen depletion through exercise (trotting at 9 km/h for 5 x 15min intervals with a 15 minute rest period between each interval resulting in a blood lactate of 2-3 mM at the end of exercise), with the Angus sired cattle depleting almost 60% more muscle glycogen than Piedmontese ($P<0.05$), and rates of glycogen repletion 72 hours following exercise, with the Wagyu sired cattle repleting about 50% more muscle glycogen ($P<0.05$, Figure 2). These responses are apparent after adjustment for live weight, fat depth (P8), metabolisable energy intake, and starting muscle glycogen concentration, and therefore cannot be attributed to these factors. Given that Wagyu are noted for a propensity to accrete fat at the intramuscular adipocyte, the repletion response appears to be consistent with a general trend for increased substrate deposition (either fat or glycogen) by this breed at the level of the muscle. We are currently profiling the muscle tissue for fibre type and aerobic/anaerobic enzyme activity to test the hypothesis that these differences are related to fibre type and/or pattern of energy metabolism within muscle.

In a recent study we have investigated the effect of sire on the level of glycogen both at pasture and subsequently at slaughter. Ten month old cross bred cattle sired by one of 3 Simmental bulls (22 progeny per bull) were used in the study. Muscle glycogen content was

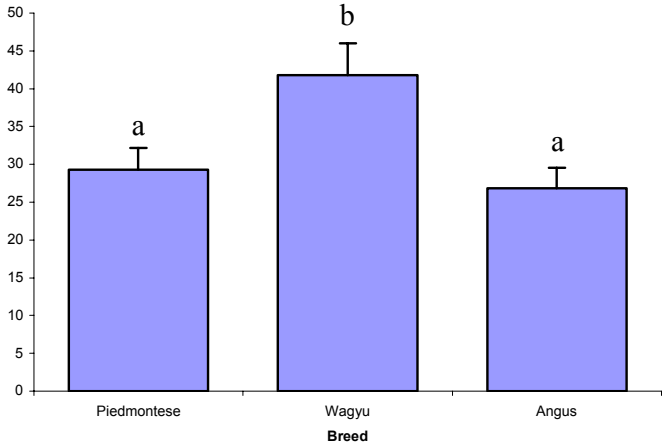

Figure 2. The effect of sire breed on muscle glycogen repletion during 72 h following exercise in cattle. Values are least square means ± sem (adjusted).

measured on biopsy samples taken at pasture and then on muscle collected immediately post mortem. Sire 3 had a significant effect on ST glycogen concentration at slaughter but not at pasture (Table 1, P<0.05). Progeny from sire 3 had about 15% more glycogen within the ST when compared to the other two sires. The fact that this response was found only for the ST suggests that the differences were associated with stress, that is sire 3 progeny were most probably less stress sensitive. The basis of this response could clearly be complex but points to the possibility of selecting sires on the basis that their progeny loose less glycogen from muscle when subjected to commercial stressors.

Table 1. Effect of sire on glycogen content (%) of the *m. semimembranosus* (SM) and *m. semitendinosus* (ST) at different times pre-slaughter. Values are Means ± sem.

	Sire 1	2	3	Significance of Effect
SM (Slaughter)	1.50 ± .037	1.54 ± .055	1.61 ± .034	ns
ST (Slaughter)	1.15 ± .042[a]	1.14 ± .062[a]	1.28 ± .039[b]	.044
SM (pre-slaughter)	1.61 ± .051	1.55 ± .081	1.70 ± .054	ns
ST (pre-slaughter)	1.33 ± .074	1.25 ± .118	1.33 ± .078	ns

Values within rows followed by different letters are different (P<0.05).

Glycogen metabolism and the pH/temperature window

The pH/temperature window was one of the initial specifications for the MSA 'carcass pathways' grading scheme. The concept of the window originated from the results of Locker and Hagyard (1963) who showed that myofibrillar shortening occurred when pre-rigor muscle was held at either low or high temperatures. At low muscle temperatures extensive shortening occurred and the subsequent increased toughness was termed 'cold shortening'. Pearson and Young (1989) considered that for cold shortening to occur the muscle pH had to be greater than 6.0 with ATP still available for muscle contraction and the muscle temperature to be less than 10°C. At high muscle temperatures some shortening also occurred, in some cases (but not all) leading to increased toughness (Uruh et al., 1986; Simmons et al., 1997). This effect

was termed rigor or heat shortening and was considered to be due to the combination of high temperature and low pH in the muscle causing early exhaustion of proteolytic activity and so reduced tenderisation during aging (Dransfield, 1993; Simmons et al., 1996; Hwang and Thompson, 2001) and increased drip loss (Denhertogmeischke et al., 1997). These studies lead to the development of the MSA pH/temperature window, whereby controlled use of chilling and/or electrical inputs during processing were managed to achieve a pH/temperature relationship of greater than pH 6 for muscle temperatures greater than 35°C, and a pH of less than 6 for muscle temperatures less than 12°C (Figure 3). The pH/temperature window is currently measured for the LL muscle at the level of the 12/13th rib. Currently further work is underway to optimize the pH/temperature window of the deeper muscles (such as the GM muscle) which are more susceptible to heat shortening.

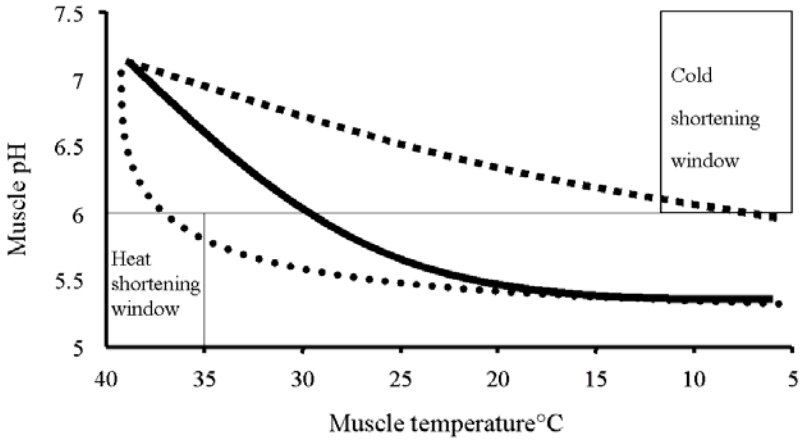

Figure 3. The pH/temperature window used by MSA to optimise the decline in pH relative to the temperature of the muscle. The solid line represents an optimal rate of decline, the dashed line a cold shortening, and the dotted line, a heat shortening scenario.

Electrical stimulation can elicit several benefits in tenderness because (i) it can be used to prevent of cold shortening, (ii) it causes increased fracturing and disruption of the myofibrillar structure of muscle, and (iii) it accelerates post mortem proteolysis (Fergusson et al. 2001). Whereas electrical stimulation is widely used in Australia, it is almost not used in some European countries, especially in the South of Europe. This contributes among other factors (breeds, fattening systems) to the differences in the final quality of beef between these two parts of the world. When the MSA pH/temperature window was implemented as part of the abattoir audit it was found that many Australian abattoirs had an over use of electrical stimulation, with carcasses entering the heat shortening region. This was due in part to other electrical inputs being installed in the slaughter chain (eg immobilisers and rigidity probes), which along with electrical stimulation, accelerate glycolytic rate (Petch and Gilbert 1997). It is clear that differences between abattoirs in the positioning of the stimulator, effectiveness of contact electrodes, and speed of the chain make it impossible to recommend a uniform protocol for stimulation and so MSA chose to regularly audit individual abattoirs.

The extent to which genotype and nutrition can influence the extent of pH drop due to electrical stimulation and then the subsequent rate of pH decline is not clear. Traditionally the rate of pH decline is thought to be driven by the rate of ATP hydrolysis via various muscle ATP'ase systems. In a recent study sheep were used to test the influence of muscle glycogen level pre slaughter on the pH decline post slaughter in 3 different muscles. Delta pH was calculated as the difference between the electrically stimulated and non-stimulated sides immediately following stimulation. Rate of pH decline was modeled using an exponential

function from which pH at 3 hours post slaughter (pH3) was calculated. Temperature and pH readings were corrected to account for temperature differences and the impact of temperature on rate of pH decline. Muscle glycogen concentration was found to affect both delta pH and pH3. The dependence of delta pH on muscle glycogen concentration differed between muscles, with no relationship in the SM and LT, but a strong positive relationship in the more anaerobic ST ($P<0.01$, Figure 4). Generally delta pH values were higher in the ST ($P<0.01$) indicating that the ST is capable of very fast rate of ATP hydrolysis and so glycolytic rates but only when adequate substrate (muscle glycogen) is available. Alternatively, the more aerobic SM and LT muscles do not have the same glycolytic potential, and therefore the availability of muscle glycogen is not the rate limiting factor, over the ranges measured in this study.

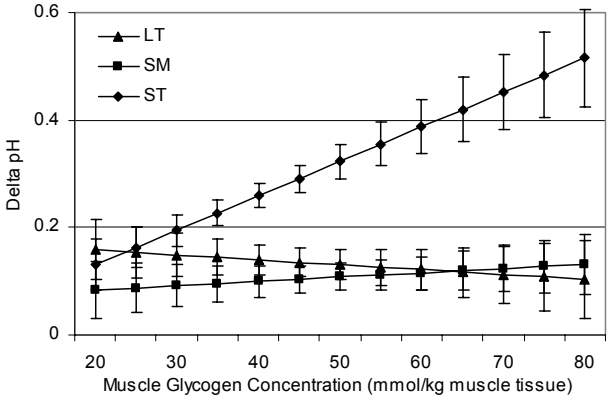

Figure 4. Effect of muscle on the response to electrical stimulation (delta pH) at different muscle glycogen concentrations.

pH3 was markedly reduced as muscle glycogen concentration increased (Figure 5), indicating a faster rate of pH decline. This response was curvilinear ($P<0.001$), plateauing at a muscle glycogen concentration of about 60 umoles/g (after adjusted for the starting pH immediately following stimulation in both stimulated and non-stimulated sides). pH3 also differed between muscles, with the ST having the fastest rates of pH decline ($P<0.001$), with pH3 values approx 0.1 pH units lower than the SM and LT at all glycogen concentrations (Figure 5). Electrical stimulation generally resulted in lower pH3 values and subsequently faster rates of pH fall ($P<0.01$, Figure 5), however it also made the relationship between pH3 and muscle glycogen concentration more pronounced ($P<0.05$). Generally these results support a role for muscle glycogen concentration to impact on pH change in the more anaerobic muscle types. The impact of muscle glycogen concentration is further supported by data collected in cattle over a 6 month period at a commercial abattoir by Daly et al. (2002). In both stimulated and non-stimulated carcasses increasing muscle glycogen concentration resulted in faster rates of pH decline and this response was curvilinear in stimulated groups, plateauing at about 90 umoles/g muscle glycogen.

There is some further evidence of genotypic effects on muscle glycogen metabolism. For example, double-muscled cattle which have more glycolytic muscle tissue than normal cattle (see below) and are also characterized by an accelerated post-mortem glycolysis resulting in a rapid fall of the post-mortem pH and a rapid rise in lactic acid (Clinquart et al., 1994; Fiems et al., 1995). Additionally, double-muscled cattle are more sensitive to stress, which may result prior to slaughter in reduced carbohydrate stores (Monin, 1980), especially in hypertrophic muscles (Fernandez et al., 1997).

Given this information we conclude that nutrition will effect pH decline, especially in response to electrical stimulation, via making a contribution to elevating the initial glycogen

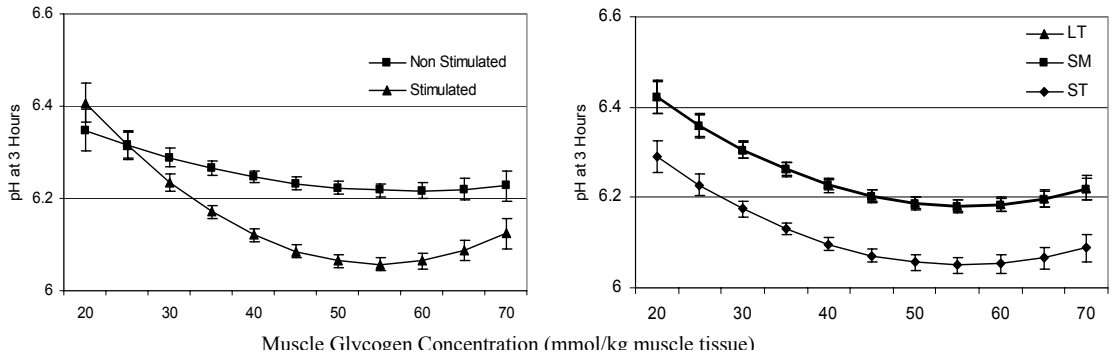

Figure 5. Effect of muscle glycogen concentration on pH at 3 hours post-mortem (rate of pH decline) in stimulated and non-stimulated carcasses (main effect across all muscles), and in the SM, ST, and LT (main effect across stimulated and non-stimulated carcasses).

content of muscle. Furthermore we also predict that genotype will influence pH decline, especially the response to electrical stimulation (delta pH). Any genetic effects to increase the type IIb fibre proportion would increase the response to electrical stimulation. Future studies need to quantify such effects so as the processing sector can better optimize pH decline via chilling and electrical inputs especially in countries where there is a broad base of animal genotypes and production systems such as in Australia. In the longer term, better control of electrical inputs, in conjunction with a prediction model to allow the stimulation requirements to be specified for different classes of cattle being processed at specific abattoirs, may be the answer.

Genotype as a determinant of muscle fibre type and pattern of energy metabolism

Given that the metabolic properties of muscle fibres will strongly effect the metabolism of muscle glycogen in the living and post mortem animal what evidence is there that genotypic differences can effect muscle fibre type proportions and the final pattern of energy metabolism ? Double-muscled cattle display strong muscle hypertrophy (about 20%) and lower fat deposition in the carcass. Their muscles are more glycolytic since they contain a higher proportion of fast glycolytic fibers than bovines with 'normal' muscle mass. Double muscle cattle are also characterized by an enhanced muscle sensitivity to insulin (Hocquette et al., 1998), and by lower levels of triiodothyronine, insulin and glucose plasma concentrations (Hocquette et al., 1999), underlining the importance of the metabolic and hormonal status in the control of carcass composition and muscle characteristics. As a general rule, whichever the species and by whatever mechanism, this type of selection induces an increase in glycolytic muscle energy metabolism and an enhanced sensitivity to insulin (Hocquette et al. 1998). Indeed, the comparison of two lines of young Charolais bulls obtained by divergent selection on growth rate and feed efficiency demonstrated that an increased lean to fat ratio was associated with lower intramuscular fat content (Renand et al. 1994), lower oxidative metabolism especially in oxidative muscles (Cassar-Malek et al., 2003), a greater number of fibers, a higher proportion of fast glycolytic fibers and a lower proportion of slow fibers (Duris et al. 1999). Furthermore, the Blonde d'Aquitaine breed, in which neither deletion nor mutation in the myostatin gene have been yet identified (Grobet et al. 1998), shows similar muscle characteristics to those of double muscle cattle (Listrat et al. 2001).

In conclusion, whatever the genetic basis (breeds with or without mutations in the myostatin gene, genetic selection on growth or muscle parameters within a breed), muscle hypertrophy is associated with a higher glycolytic muscle metabolism and a lower intramuscular fat content. The extent to which these genetic differences will impinge on glycogen metabolism is not known but would seem highly probable based on the above

discussion. Thus beef cattle with a more glycolytic muscle metabolism would need more careful management with respect to pre slaughter nutrition and stress management so as to prevent the elevated ultimate pH syndrome. In addition such cattle will show a different pH/temperature decline post slaughter with the response to electrical stimulation being greater and modulated by the pre slaughter initial glycogen concentration. There would almost certainly be effects on retail colour stability in the form of metmyoglobin formation which is effected by myoglobin content and post mortem oxygen consumption rate (Trout, 2002), both of which will be influenced by the nature of the energy metabolism within muscle.

Intramuscular fat or marbling

Role in palatability

Although marbling is generally an integral part of any beef grading scheme the literature suggests that it has only a minor association with palatability. Dikeman (1987) concluded that marbling accounted for only 10 to 15% of the variance in palatability. The MSA research would agree and showed that the contribution of marbling to palatability was significant but importantly just one of several factors. However, Thompson (2001) concluded that as variations in tenderness are controlled by schemes such as MSA, marbling will become a more important determinant of palatability due to its specific contribution to juiciness and flavour of grilled steaks for Australian consumers.

There is also a concern that at very low levels of intramuscular fat found in young highly muscled lean cattle (double-muscled genotypes, young bulls from Belgian Blue or Blonde d'Aquitaine for instance), that the meat is perceived as dry and less tasty. This is especially true for French and Belgian beef breeds which have been selected in favour of high muscle growth potential and low fat deposition. Savell and Cross (1986) concluded that the minimum requirement for ether extractable fat in order to achieve acceptable consumer satisfaction for grilling cuts was 3% on a fresh uncooked basis.

Development of intramuscular fat

A common conclusion from animal developmental studies is that intramuscular fat is late developing (Vernon 1981). Indeed the usually quoted developmental order is abdominal, then intermuscular, then subcutaneous, then finally intramuscular. However, because fat is deposited at a greater rate than lean tissues later in life, the concentration of fat in muscle will inevitably increase later in an animal's life. Therefore the commercial trait, marbling, visible intramuscular fat or actual percentage intramuscular fat is late maturing. This does not mean that the rate of fat accretion in intramuscular adipocytes is also late maturing. The study of Johnson et al. (1972), showed that the proportional distribution of fat between carcass pools is found to be constant over a wide range of carcass fat contents (in the range from 5 to over 150 kg total fat) indicating that the major fat depots grow in the same proportion as animals fatten. The results of Pugh et al. (2004) are also consistent with this observation.

The development of intramuscular fat is shown in Figure 6. The data suggests a period of minimal change of intramuscular fat content in young ages followed by a linear increase between a carcass weight of about 200 – 400 kg at least for American Angus x Hereford (Duckett et al., 1993), Australian Angus (Pugh et al., 2004) or Japanese Black x Holstein (Aoki et al., 2001) type cattle undergoing prolonged grain feeding. Based on this data we hypothesise two key genetic drivers of intramuscular fat development (i) the initial or 'starting' intramuscular fat content at ≤ 200kg carcass weight, and (ii) the potential for muscle growth.

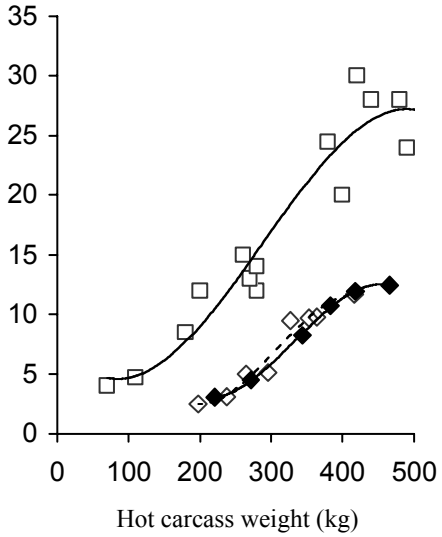

Figure 6. The relationship between carcass weight and intramuscular fat content of the m. longissimus lumborum of American Angus x Hereford (◊, Duckett et al. 1993), Australian Angus (◆, Pugh et al. 2004) and Japanese Black x Holstein cross cattle (□, Aoki et al. 2001).

The initial or 'starting' intramuscular fat content at ≤ 200kg carcass weight is likely driven by the genetic predisposition for development of adipocytes at the intramuscular site relative to other depots. Importantly there is a proportional developmental difference that is maintained when the American or Australian cattle are compared to the Japanese Black cross cattle such that the starting (2 vs 4%) and final (13 vs 27%) intramuscular fat contents are proportionally different at about 2 fold (Figure 6). Cellular studies in rabbits (Gondret et al., 1998) have shown that intramuscular fat develops due to both an increased number and size of clustered adipocytes with associated increases in lipogenic enzyme activity (Gondret et al., 1997). The cattle data would suggest that the potential for cellular development of adipocytes is fixed relatively early in life and there after changes in either size and or number of cells occurs in proportion to the initial cell number and/or lipogenic proteins and so we propose the theoretical response shown is Figure 7. This would clearly indicate that a variety of 'fat'

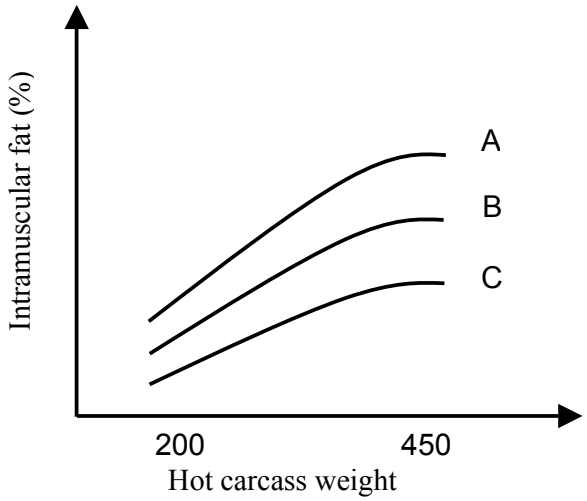

Figure 7. The effect of initial intramuscular fat (%) content (A >B >C) on final value in cattle of similar muscle growth potential.

measurements taken on muscle tissue in early life would hold great potential for predicting subsequent intramuscular fat development. Examples would include intramuscular fat content

(perhaps by non invasive methods such as ultrasound), marker of adipocytes such fatty acid carrier proteins and/or functional lipogenic enzymes involved in fatty acid biosynthesis. Certainly cattle breeds with a higher propensity to deposit fat have higher expression of key lipogenic enzyme either within adipose tissue (expressed per mg protein) or muscle (Bonnet et al. 2003).

Another feature associated with the development of intramuscular fat is the fibre type or metabolic pattern of energy metabolism expressed by the muscle tissue. Within the one animal genotype the more glycolytic muscle types (e.g. ST) have lower levels of intramuscular fat (Gondret et al., 1998; Hocquette et al., 2003). Across genotypes a similar response can be found. Thus in the study by Hocquette et al., (2003) where 2 muscle types were contrasted across 3 breeds of cattle with disparate propensity to accumulate intramuscular fat, there was a strong correlation between intramuscular fat and the aerobic markers cytochrome-*c*oxidase and isocitrate dehydrogenase as well as the adipose specific fatty acid binding protein. Of course studies across genotypes which correlate the extent of anaerobic muscle metabolism to the level of intramuscular fat accumulation are confounded by the observation that highly muscled cattle are more glycolytic and also have less carcass fat. However, these studies as well as others in rabbits (Gondret et al., 2004) suggested that intramuscular fat content results from a balance between catabolic and anabolic pathways rather than from the regulation of a specific biochemical pathway. It has thus been speculated that a high fat turnover (which is a characteristics of oxidative muscles) would favour fat deposition (Hocquette et al., 2003).

The propensity to lay down muscle tissue will also be an important component which will determine the expression of intramuscular fat. As animals develop or fatten the rate of muscle gain declines while the rate of fat gain is maintained (Owens et al. 1995). Logically therefore increased intramuscular fat (%), relies on continued fat synthesis within muscle combined with a decreasing rate of muscle growth as animals mature. Therefore we propose that the developmental curve for intramuscular fat will be shifted to the right in animals with a high propensity to grow muscle (and in this case with the same propensity to marble at maturity, Figure 8). This 'right shift' would also occur in response to metabolic modifiers such as hormonal growth promotants, ß agonists and organic chromium supplementation, all of which can increase muscle growth. In very highly muscled animals it might be that intramuscular fat does not reach the 'linear accumulation' phase discussed in Figure 6 within normal commercial slaughter weights (that is the right shift described in Figure 8 is profound). This would appear to be the case for the modern pig genotypes where intramuscular fat % does not increase over a wide range of commercial carcass weights (Dunshea and D'Souza, 2003).

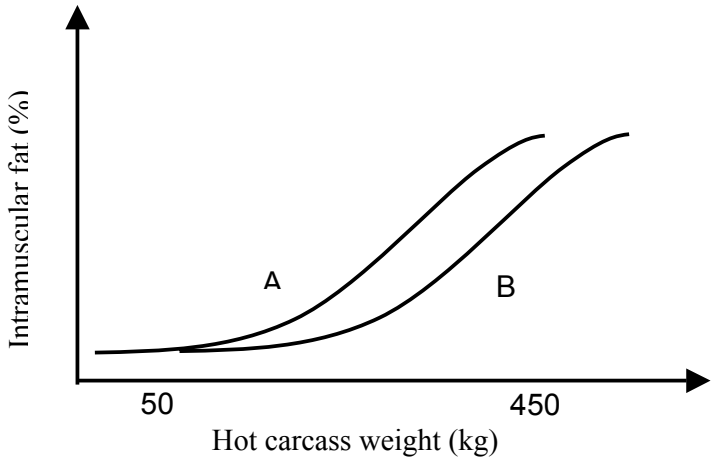

Figure 8. Hypothetical graph showing the development of intramuscular fat in cattle of different mature body weight (B >A).

The role of nutrition in the regulation of intramuscular fat deposition has been reviewed by Pethick et al. (2004). The key factors centre on the availability of net energy for fat deposition and possibility that glucose availability might accelerate lipogenesis within intramuscular adipocytes.

Conclusion

Nowadays, farming and agri-food sectors are faced with a general saturation of meat markets in developed countries including Europe and with an increasing demand from consumers for high-quality products. The major current questions are thus how to define and increase the quality of animal products to satisfy the requirements of modern consumers while maintaining efficient production systems.

This is the reason why research must now aim to construct decision support tools for use throughout the beef production chain to provide consistent quality beef to consumers. The Meat Standards Australia is a successful system which is the culmination of many years of research. It has been, however, constructed based on the characteristics of the beef production chain in Australia. The beef production systems will differ in other countries and factors such as the *Bos indicus* effect and the influence of hormonal growth promotants would not need to be addressed by European systems. However many of the production factors within the MSA system would be directly applicable to beef supplychains within Europe such as ossification, pH and temperature decline, ultimate pH, marbling, days of meat aging, muscle and cooking method. Basic research will still need to be conducted to better understand how to manipulate the major animal characteristics which may have an important impact on beef quality. The pattern of glycogen and fat metabolism within muscle are among them. It is clear that glycogen metabolism is differently regulated depending on breed, selection for muscle yield, nutrition and the use of electric stimulation. It is also obvious that beef contains less fat in many European countries than in Australia or America. However we believe that the basic biological mechanisms are the same for any animal type. Therefore, understanding the development of intramuscular fat in the Australian context is of general interest for the rest of the world.

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