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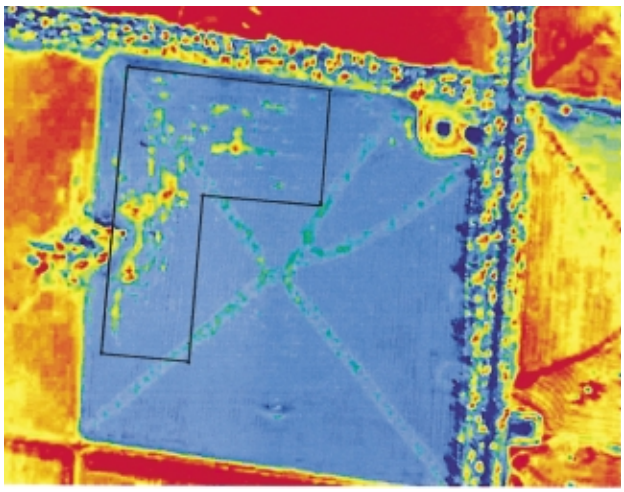

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Factors affecting beef palatability — farmgate to chilled carcass

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Abstract. The potential eating quality of beef is set by the intrinsic structural and compositional characteristics of muscle. However, the extrinsic factors that prevail during the production of the animal, slaughter and processing of its carcass and finally, cooking can produce changes in these structural and compositional characteristics that ultimately manifest as large variations in beef palatability. The conditions that apply in the 24–48 h immediately before and after slaughter are recognised as having the largest influence on beef palatability. This review specifically examines the critical pre- and post-slaughter factors and discusses their putative effects on biochemical and physical changes in muscle and the consequences to beef palatability. Areas for future research within this domain are also discussed.

Introduction

In the delivery of consistent quality to the consumer, it is important to recognise that beef palatability is not simply a function of the genetics or production history of the animal. Palatability, particularly tenderness, can be affected by a range of critical factors that begin with the animal's genotype and conclude with the final process of cooking.

Given this, a critical control point approach similar to that used in food safety programs (e.g. HACCP) lends itself to eating quality assurance schemes. This approach, underpins the Meat Standards Australia beef-grading scheme recently introduced in Australia (Ferguson *et al.* 1999; Polkinghorne *et al.* 1999; Thompson *et al.* 1999*a*, 1999*b*).

Of all the critical factors, the management of the animal immediately before slaughter and the carcass processing conditions that apply during the first 24 h after slaughter are by far the most influential in the context of palatability. To place this into perspective, when cattle were processed under conditions designed to minimise post-slaughter variation in tenderness/toughness, Robinson *et al.* (2001) estimated the genetic variance in shear force, an objective measure of tenderness/toughness, to range from 8% (temperate breeds) to 32% (tropically adapted breeds). The latter result is quite encouraging as it indicates that toughness may be reduced by genetic manipulation, particularly in tropically adapted breeds. However, the salient feature of their results was that the total variance in shear force was only 0.75 kg^2. This is quite small compared with the variation that can occur in commercial situations where the post-slaughter treatment of carcasses is suboptimal. For example, in the application of different, albeit commercially relevant, post-slaughter treatments, Butchers *et al.* (1998) showed that shear force could effectively double from 4.5 kg to 9 kg. Thus, the effect of the post-slaughter environment can not only result in irreversible losses in eating quality but can also negate any genetic advantage in tenderness an animal may have.

The purpose of this review is to examine the ante- and post mortem factors that influence beef quality. Specifically, it provides an overview of the normal course of biochemical events that occur during the conversion of muscle to meat and how these events can be controlled in order to maximise beef palatability.

Pre-slaughter management of cattle

The process of harvesting animals for slaughter results in inevitable losses in both product quality and quantity. The magnitude of these losses will depend on the intensity and duration of the various stressors that apply between the farmgate and abattoir and also the susceptibility of the animal to stress. During the pre-slaughter phase, cattle can be exposed to several stressors that include: (i) fasting; (ii) dehydration; (iii) novel/unfamiliar environments; (iv) transport; (v) increased human contact; (vi) changes in the social structure (i.e. through separation and mixing); and (vii) sudden climatic changes.

These stressors or stimuli result in a perturbation of the animal's homeostasis. An adaptive response is then initiated to restore balance, which might involve a simple alteration of behaviour (e.g. movement away from an aggressive contemporary) or more complex autonomic and/or neuroendocrinal changes may be invoked. This response is often non-specific and considerable variability exists between animals in their perception of the stressor and the co-ordination of the physiological response. Both are modulated by several intrinsic factors (e.g. genetics, sex, age, and physiological state) and by past experiences and

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acquired learning (Moberg 1985; Boissy 1995). In the context of selecting for better-adapted cattle, the genetic variance in stress perception and response is quite important.

Stress response

Localised regions within the hypothalamus and brain stem of the central nervous system (CNS) are responsible for activating and regulating the stress response. These in turn may interact with other CNS elements such as the amygdala and hippocampus (Chrousos 1998). The amygdala in particular coordinates the animal's response in relation to fear (Gregory 1999).

The stress response is elicited via 2 integrated peripheral systems namely, the sympatho-adrenalmedullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes.

The SAM system is designed for emergency or threatening situations and underpins the classic 'fight–flight' response theory developed by Cannon (1929). Activation of it results in increased release of the catecholamines, epinephrine, norepinephrine and dopamine.

The sympathoneural component is a branch of the autonomic nervous system that on activation releases norepinephrine at nerve endings that binds to specific receptors on tissues and organs including salivary and sweat glands, vasculature, the splanchnic region, kidneys, heart, skin and skeletal muscle (Goldstein 1990). By virtue of the targeted response, norepinephrine release via the sympathetic nerves provides an effective regional control measure (Clark *et al.* 1997).

The second component of the SAM system involves the adrenal medulla. Upon stimulation, chromaffin cells within it release epinephrine and norepinephrine into the blood stream thus affecting the majority of the body's systems. Obvious physiological changes include increased heart rate and force and contraction, increased respiration rate, heightened alertness, elevated body temperature and redistribution of visceral blood volume towards skeletal muscle and the brain. Catecholamines elicit other significant changes in muscle metabolism, such as increased glycogenolysis (epinephrine only) and lipolysis (Clark *et al.* 1997) and an anabolic effect on protein metabolism through decreased protein degradation (Sensky *et al.* 1996; Rooyackers and Nair 1997).

Activation of the second peripheral system, the HPA axis, promotes the increased release of glucocorticoids by the adrenal cortex. Regulation of the HPA axis comes under the control of corticotrophin releasing factor (CRF). CRF is viewed as the central element in the co-ordination of endocrine, autonomic and behavioural responses to stress (DeSouza *et al.* 1991; Clark *et al.* 1997). It is stimulated by a wide variety of neurotransmitters such as serotonin, norepinephrine and glutamate and is inhibited by γ-aminobutyric acid (GABA), vasopressin, glucocorticoids and other neuropeptides (e.g. opioids, neurotensin, substance P) (Goldstein 1990; Clark *et al.* 1997; Jessop 1999). Discharge of CRF into the hypophyseal-portal system initiates production of adrenocorticotrophic hormone (ACTH) by cells in the anterior pituitary. ACTH is then released into the bloodstream, giving rise to increased synthesis of glucocorticoids by the adrenal cortex.

Increased secretion of glucocorticoids amplifies the mobilisation of energy induced by the catecholamines (Dantzer 1994) although the response is slower. The primary metabolic function of glucocorticoids is to moderate the demand for glucose by some tissues while concomitantly stimulating hepatic gluconeogenesis to increase glucose delivery to skeletal muscle and the brain. Increased secretion of glucocorticoids also suppresses muscle protein synthesis (Sugden and Fuller 1991). Glucocorticoids serve another important role by attenuating the cellular reaction to stress and/or trauma (i.e. anti-inflammatory action).

The stress-induced release of catecholamines and glucocorticoids clearly invokes marked changes to protein and glucose metabolism in muscle. The consequences, particularly the accelerated depletion of muscle glycogen reserves and the suppression of protein degradation (catecholamines), are potentially very important to beef quality.

Effects of pre-slaughter stress on beef quality

Sustained efforts by the animal to restore balance with its environment incur inevitable costs. During the pre-slaughter period, one of the most obvious costs is weight loss. Cattle deprived of both feed and water will lose about 0.75%/day of initial liveweight (Shorthose and Wythes 1988). This will vary depending on the treatment of the animals (e.g. duration of fast, transport conditions etc.) and their condition. Furthermore, weight loss is not linear with time. The majority of it, predominately gutfill, is lost within the initial 24 h post farm. Further losses will accrue depending upon the duration of feed and water restriction and the level of physical activity. Losses in carcass weight are generally not observed until about 48 h after feed and water withdrawal with the latter taking on far greater importance. Dehydrated cattle given access to water will quickly rehydrate with noticeable increases in muscle weight and moisture content after 3 h of rehydration (Wythes *et al.* 1980).

In relation to meat quality, pre-slaughter losses in muscle glycogen reserves are unequivocally the most critical. In healthy well-fed cattle, muscle glycogen concentrations typically range from 60–120 μmol/g muscle (about 1–2% of wet muscle weight) (Howard 1963; Tarrant 1989, Lambert *et al.* 1998 and Pethick *et al.* 1999), although levels as high as 200 μmol/g have been reported for grainfed cattle (Pethick *et al.* 1999). Muscle glycogenolysis occurs during pre-slaughter handling of cattle through the combined effects of increased physical activity and adrenal activation. In his review, Tarrant (1989) reported glycogenolytic rates to vary

from 1.3 μmol/g.day (fasting heifers) to 11 μmol/g.h (mixed penning of bulls). The intensity of physical activity is quite critical, as physical activity *per se* may not always result in glycogen depletion. For example, Lambert *et al.* (1998) demonstrated that fast-walking cattle at a speed of 8 km/h over 5 km did not affect glycogen concentration in *M. longissimus dorsi* (LD). Similarly, electrical immobilisation of cattle resulting in isometric contraction of the musculature resulted in small, non-significant losses (2–7 μmol/g) in muscle glycogen after 15 min of immobilisation (Crouse and Smith 1986).

Glycogenolysis will also vary between muscle and fibre types (Tarrant 1989). Once again, this is governed by the intensity of the physical activity and adrenal activation. Muscles along the back and in the hind limbs appear most prone to glycogen depletion in cattle (Tarrant and Sherrington 1980). The association between exercise intensity, fibre type and glycogen mobilisation was shown in an elegant study by Richter *et al.* (1982). During high frequency stimulation of perfused rat muscle, the effect of epinephrine on glycogenolysis was most pronounced in slow twitch fibres, whilst there was virtually no effect in the fast twitch fibres. In contrast, the exact opposite was observed when the muscle was exposed to low-frequency stimulation. By extrapolation, the emotional state of the animal is therefore probably more critical in relation to glycogen loss during events that are not physically demanding (i.e. during transport).

Some loss in glycogen before slaughter can be accommodated without any deleterious effects on meat quality. However, if the pre-slaughter glycogen reserves fall below a threshold of about 40–57 μmol/g (Howard 1963; Tarrant 1989), then there is insufficient substrate to convert to lactic acid and consequently, the ultimate pH (pH_u) of the meat will progressively increase (refer section on postmortem muscle biochemistry). Meat with a high pH_u (>5.9) is typically referred to as dark cutting or dark, firm and dry (DFD). It is characterised by a darker colour, higher water-holding capacity and, depending on the pH_u, increased toughness (especially between 5.9 and 6.2, see Purchas and Aungsupakorn 1993). DFD beef therefore lacks both visual and organoleptic appeal and has a predisposition to increased microbial spoilage (Shorthose 1989).

Australian surveys of the incidence of DFD (pH_u >5.8) in beef carcasses range from 8 to 10% (Shorthose 1989; Warner *et al.* 1988). However, levels as high 40% (pH_u >6.0) have been reported in 1 Victorian survey on 3168 cattle (Stevenson *et al.* 1996). The reasons for the unusually high incidence are not clear although seasonal conditions during the survey period were implicated. In stark contrast, for directly consigned cattle accustomed to handling and finished on moderate quality pasture or grain, the incidence level may fall to 0.5–3.0% (D. Perry, J. M. Thompson and D. M. Ferguson unpublished data). It is generally accepted that feedlot cattle have a lower predisposition to dark cutting (Warner *et al.* 1988). This can be attributed to their higher muscle glycogen levels (Pethick *et al.* 1999) and the influence of increased exposure to handling and human contact in feedlots compared to cattle off pasture.

The financial penalties for dark cutting are quite substantial. For example, Walker *et al.* (1999) reported discounts of $0.45/kg carcass weight in some Victorian abattoirs. Moreover, quite stringent pH_u limits are common to beef specifications used by retailers and the food service sector. For example, carcasses with a pH_u >5.7 (LD) are excluded under the new Meat Standards Australia beef grading scheme.

The following section examines the impact of common pre-slaughter stressors on beef palatability and quality. A summary of the results from several studies in this area is presented in Table 1.

Transport

The distances cattle are transported in Australia cover a considerable range. Distances greater than 1000 km are not uncommon, particularly in northern Australia. Furthermore, with the current rationalisation of slaughter capacity resulting in the closure of some abattoirs, the average distance cattle are transported is unlikely to reduce and may, in fact, increase.

The influence of transport stress in cattle varies depending on the type of animal and the conditions prevailing during the journey (Tarrant 1990). From the studies examining either transport distance or time, the results suggest that transport *per se* at least over moderate distances (<400 km) is unlikely to affect pH_u (Table 1; Eldridge and Winfield 1988, Tarrant 1989). Small increases of 0.1–0.2 pH units might be expected over much greater distances as illustrated by Wythes *et al.* (1981) and Tarrant (1989). However, there are caveats to such statements. The magnitude of any effect will also be governed by the condition of the cattle, their nutritional history and also the holding time in lairage (see Table 1). Changes in stocking density over moderate distances did not significantly affect the pH_u (Eldridge and Winfield 1988), although in another study by Eldridge *et al.* (1988), reducing the space allowance per animal did result in increased heart rates and movement scores over a moderate journey. The other interesting feature of the latter study was the observation that once cattle were habituated to transport, their heart rates were only 15% higher than while grazing.

Social reorganisation or mixing

Pre-slaughter mixing of unfamiliar animals resulting in agonistic behaviour will cause rapid losses in muscle glycogen and therefore predispose to dark cutting. This is largely due to the strenuous efforts and emotional stress associated with the re-establishment of the social hierarchy. Mixing probably elicits the most detrimental effect on glycogen loss and should be avoided at all costs (Grandin

1993). Agonistic behaviour is particularly prevalent when young bulls are mixed and this would account for the extremely high prevalence of dark cutting (about 50%) in 2 studies highlighted in Table 1 (Price and Tennessen 1981; Warriss 1984). Unfortunately, there is very little available data involving mixed consignments of steers or heifers. In Australia, the practice of mixing small mobs of cattle before slaughter is common when cattle have been marketed through saleyards. In contrast, mixing rarely occurs when cattle are directly consigned to the abattoir.

Method of marketing

The practice of selling cattle via saleyards is still widely practiced in Australia, particularly in the southern states. It is estimated that 40–50% of all prime cattle are still marketed through saleyards (ABARE 1999). Marketing method appears to influence beef quality although the results are equivocal. In a Queensland survey by Shorthose (1989), higher levels of dark cutting were observed in saleyard cattle. This contrasts with 2 Victorian surveys (Warner *et al.* 1988; Stevenson *et al.* 1996) that indicated no difference in pH_u between saleyard and direct consignment cattle. The Victorian survey results are intriguing given the fact that saleyard cattle are typically exposed to increased handling and holding times between farm and slaughter. However, some care should be exercised when drawing conclusions from surveys such as these. In particular, within each marketing method, it is unlikely that all vendor groups were

Table 1. Results from selected studies examining the effects of various pre-slaughter factors on beef quality attributes

pH_u, ultimate pH of meat; DFD, dark cutting or dark, firm and dry meat

Reference	pH_u	DFD (%)	Muscle glycogen (mg/g)	Shear force (kg)	Cooking loss	Experimental conditions
			Transport distance			
Wythes *et al.* (1981)	5.58, 460 km 5.71, 2055 km					160 cows trucked 460 *v.* 2055 km; both had same duration between farm and slaughter (8 days)
Tarrant (1989)	pH_u increase 0.1–0.2					Steers trucked for 1 *v.* 24 h
Eldridge & Winfield (1988)	No effect					48 steers trucked 360 km with different stocking densities (0.9, 1.2 and 1.4 m^2/animal)
			Regrouping/mixing			
Price & Tennessen (1981)		73% mixed group				112 bulls; groups were either mixed or not before trucking (150 km)
Warriss (1984)		50% mixed group				30 bulls; groups were either mixed or not before slaughter (16 h)
			Marketing method			
Warner *et al.* (1988)		12% DC 8% SY				Survey of 2714 saleyard (SY) and 706 directly consigned (DC) cattle; pH_u >5.8
Shorthose (1989)		10% DC 22% SY				Survey of 1160 saleyard and 6400 directly consigned cattle; pH_u >5.7
Warner *et al.* (1998)	5.51 DC 5.58 SY		Signif. decline in SY cattle	3.65 DC 4.03 SY		60 steers; 2 nutrition × 2 marketing treatments (direct consigment *v.* saleyard selling)
			Pre-slaughter holding time			
Warner *et al.* (1986)	5.41, control 5.37, FI 5.51, FO	0% across treatments	5.82, control 6.48, FI 5.15, FO			50 steers; no holding period (control), fasted indoors 72 h (FI) and fasted outdoors for 72 h (FO). Fasted cattle had access to water.
Grosskopf *et al.* (1988)	5.72, nil 5.76, 3 h 5.75, 24 h					45 steers held and fasted for 0, 3 and 24 h before slaughter. Water provided in lairage.
Wythes *et al.* (1988)	5.48, 2.5 h 5.46, 26.5 h			9.2, 2.5 h 7.8, 26.5 h	30.9, 2.5 h 30.9, 26.5 h	22 steers held and fasted for either 2.5 h or 26.5 h before slaughter (water available) after a short trucking journey
Jones *et al.* (1990)	5.62, nil 5.70, 24 h 5.72, 48 h			6.3, nil 7.0, 24 h 7.7, 48 h		80 steers held for 0, 12, 24, 36, 48 h before slaughter (no access to feed or water)
Purchas (1992)	5.64, 4 h 5.98, 28 h			11.8, 4 h 11.3, 28 h	28.6, 4 h 24.8, 28 h	159 mixed sex cattle held 4 or 28 h before slaughter
Walker *et al.* (1999)		0% either treatment	10.8, 3 h 9.7, 24 h			8 consignments of 20–30 vealers held for 3 or 24 h before slaughter (fasted with access to water). Cattle held for 24 h were exposed to washing and extra handling.

treated the same. Confounding due to variations in holding times and the possible influence of mixing cannot be ignored.

Perhaps the outcomes of the recent study by Warner *et al.* (1998) (see Table 1) are more informative. They observed small increases in ultimate pH and shear force and darker meat colour from saleyard cattle compared with their directly consigned contemporaries. Moreover, their results showed that the level of nutrition before slaughter influenced the magnitude of the differences in meat quality between the 2 selling methods. They showed that cattle finished on poor quality pasture had an increased risk of dark cutting due to reduced glycogen reserves in muscle.

Lairage management

In general, the critical issue of lairage management of meat animals has not received the level of scientific scrutiny it deserved. On arrival at the abattoir, the lairage phase provides the first real opportunity for animals to recover from the physical and emotional exertions of transport and handling. However, the issues of what is an appropriate period of recovery for cattle and whether there are strategies to enhance their recovery are not fully understood. In relation to meat quality, it is difficult to draw general conclusions as the effect of lairage will vary depending on the pre-slaughter muscle glycogen concentrations, animal sex, production history and transport duration/distance.

As discussed, the pre-slaughter glycogen levels in muscle will dictate whether dark cutting occurs. Clearly, if glycogen levels are in excess of the critical threshold then there is no benefit in extending the lairage time. However, the deficiency in this logic is that there is no accurate, practical way of knowing the animal's muscle glycogen status on arrival at the abattoir. Second, and probably more importantly, the animal welfare implications of this scenario need to be considered. On these grounds, a small period of recovery is therefore desirable. Moreover, apart from the Australian veterinary public health regulations stipulating that cattle are rested on arrival at the abattoir, some lairage time is inevitable in view of the logistics associated with receiving and scheduling cattle for slaughter (Wythes 1990).

Potentially, rest not only enables cattle to rehydrate but also allows glyconeogenesis to occur. Glycogen repletion is important when the pre-slaughter levels are near or below the critical concentration to achieve a normal pH_u. However, the rates of glycogen repletion in ruminants are considerably slower than those observed in monogastrics. Repletion rates range from 0.1 to 1.0 μmol/g.h depending on the extent of glycogen depletion, period of inanition and conditions during recovery, notably access to feed (Tarrant 1989; Pethick *et al.* 1999). Therefore, for cattle with subcritical concentrations of muscle glycogen, a longer period of recovery (>48 h) would be necessary to avoid the dark cutting condition. This was demonstrated by Shorthose *et al.* (1972) and Wythes *et al.* (1980) involving cattle transported considerable distances (i.e. >1000 km).

In contrast, for cattle that have been transported less onerous distances or minimally stressed, there is a suggestion that reduced holding periods (<12 h) may be more desirable. Although the data across studies are not totally consistent (Table 1), the results of Purchas (1992) and Jones *et al.* (1990) indicate a reduced incidence of high pH meat in cattle slaughtered within 4 h post-farm compared with cattle held for 24 h or longer. Unfortunately, in the study by Jones *et al.* (1990), there were other confounding effects in the 24 h lairage treatment (mixing and water deprivation) therefore it cannot be determined whether the effect was simply due to holding time. Less uncertainty exists in the case of bulls where there is a clear reduction in the incidence of dark cutting through reduced lairage periods (Fabiansson *et al.* 1984; Jedlicka *et al.* 1984).

Apart from a reduced incidence of dark cutting, shorter pre-slaughter holding periods may also provide benefits in terms of eating quality as demonstrated in several Canadian investigations (Jones *et al.* 1986; Jeremiah *et al.* 1988*a*, 1988*b*). The results of Jeremiah *et al.* (1988*a*, 1988*b*) are particularly salient as they demonstrated significant improvements in both sensory texture and flavour intensity scores in feedlot cattle exposed to minimal stress (transported 4 km) and slaughtered within 4 h post-farm compared with cattle exposed to moderate stress (transported 160 km, mixed in lairage, no access to water) and a longer lairage period of 24 h. No differences were observed in shear force, although improvements in meat colour and ultimate pH were reported (Jones *et al.* 1986).

In support of these findings, Newsome *et al.* (1999) showed that there were positive improvements in beef flavour and juiciness when feedlot cattle were not fasted before slaughter. Once again, no change in sensory tenderness or shear force measurements was observed between treatments. In this study, the cattle were trucked to the abattoir 4 days before slaughter and fed/fasted in lairage.

The positive improvements in beef palatability achieved by minimising pre-slaughter stress (i.e. reduced periods of fasting and/or handling) as shown by Jeremiah *et al.* (1988*a*, 1988*b*) and Newsome *et al.* (1999) clearly require further research. However, in future studies, consideration must also be given to other factors, notably food safety implications. Any reduction in lairage may result in cattle with increased rumen volume at slaughter and this may increase the risk of rupture and contamination during processing.

Pre-slaughter supplements

The use of pre-slaughter supplements, largely consisting of mixtures of sugars and electrolytes, has also been investigated for their attenuating effects on transport stress (see review by Schaefer *et al.* 1997). Commercial feed supplements or soluble preparations are now available and

these can be can be provided either on farm immediately before transport or while in lairage. Schaefer *et al.* (1997) reported that the administration of these supplements to cattle resulted in reduced losses in live and carcass weights and reduced incidence of dark cutting. The results of Australian research, while encouraging, have been relatively inconclusive (Pethick *et al.* 1999). Suffice to say that electrolyte supplements did elicit small reductions in glycogen loss.

Pethick *et al.* (1999) also investigated the use of hyperglycaemic agents that were amenable for use in ruminants. A mixture of 3.5% glycerol and 1.5% propylene glycol was deemed the most effective and its administration resulted in positive increases in blood glucose concentration 4 h after oral administration in lambs. Another interesting and highly relevant effect was that it doubled the lambs' water intake.

Reduced glycogenolysis has also been observed in studies where animals were given magnesium supplements before a stressful event (D'Souza *et al.* 1998; Pethick *et al.* 1999). The role of magnesium as a means for attenuating glycogen loss is predicated on the fact that it has a profound effect on neuromuscular stimulation (Galland 1991). Specifically, magnesium acts as a calcium antagonist and has also been shown to lower the secretion of acetylcholine by motor-nerve endings (Hubbard 1973).

The role of supplements designed to either attenuate pre-slaughter stress or the effect of it on beef quality requires further investigation. The potential of supplements of this kind is quite considerable given the nature of cattle production in Australia, with its large transport distances and the diversity in the marketing and management of cattle before slaughter.

Slaughter and carcass processing

With the cessation of blood supply to the musculature, a complex series of biophysical events is initiated. The rate and extent of these changes is critical in terms of palatability, particularly the tenderness/toughness of the myofibrillar component.

An understanding of the biophysical changes in post mortem muscle is therefore necessary in order to develop technologies and management practices that are designed to maximise beef palatability.

Post mortem muscle biochemistry

There are several detailed reviews of the post mortem biochemical changes in muscle (e.g. Bendall 1973; Lawrie 1992). However, for the purposes of this paper, only a summary of significant events is given here.

The post mortem rate and extent of glycolysis and proteolysis are the primary biochemical changes that govern myofibrillar tenderness/toughness. Although proteolytic weakening of connective tissue has been reported (Stanton and Light 1990; Nishimura *et al*. 1998), it is generally accepted that, relative to the myofibrillar component, the post-slaughter changes in the connective tissue contribution to toughness are quite small.

Following the loss in blood supply (exsanguination), the muscles continue to produce ATP anaerobically in an attempt to maintain functionality. ATP in this instance is provided by glycolysis in which glycogen is converted to lactate. Loss of muscle extensibility is the first obvious physical sign post mortem and this coincides with insufficient levels of ATP. ATP is required to separate the 2 major contractile proteins, actin and myosin. In its absence, an irreversible bond forms between them (actomyosin) thereby leading to muscle shortening and stiffening and this is typically referred to as rigor mortis. Failure to generate ATP occurs either through a lack of available substrate (i.e. glycogen) to produce it or, more commonly, because the fall in pH (7.1–5.5) due to the build up of lactate results in inactivation of 1 or more of the glycolytic enzymes (Lawrie 1992).

In the context of meat tenderness and other quality issues (e.g. colour, water-holding capacity), glycolytic rate and the temperature at rigor are paramount. The seminal work of Locker and Hagyard (1963) clearly demonstrated that the degree of muscle shortening (relative to pre-rigor length) was highly dependent on the temperature at rigor. In their study, minimal shortening was observed between 15 and 20°C. More recent studies from Sweden by Hertzman *et al.* (1993), Olsson *et al.* (1994) and Devine *et al.* (1999) indicate that the optimal rigor temperature range might be lower at 10–15°C. However, there is general agreement that rigor temperatures below 10°C and above 20°C resulted in increased muscle shortening and meat toughness. The increased meat toughness observed at low (<10°C) and high (especially >30°C) rigor temperatures gave rise to the conditions known as cold and heat shortening, respectively. Both conditions are governed by the rate at which glycolysis proceeds and the rate at which muscles cool.

The extent of post mortem proteolysis is the other major factor governing myofibrillar tenderness/toughness. In his review of the proteolytic mechanisms involved in post mortem tenderisation, Ouali (1992) identified 3 main endogenous proteinase systems: (i) calcium-dependent calpain system (calpains I and II and their inbitor calpastatin); (ii) lysosomal cathepsins (cathepsin B, D, H and L); and (iii) proteosome (also known as multicatalytic proteinase).

Considerable debate has ensued regarding the relative contributions of these proteinases, particularly the calpains and cathepsins, however, on the basis of the *in vitro* data, calpain I appears to be responsible for the majority of the post mortem tenderisation (Koohmaraie 1996; Dransfield 1999). Although it must be emphasised that there are still significant gaps in our knowledge regarding the exact role and specific activities of these proteinases *in situ*. The primary arguments against the involvement of cathepsins in

post mortem tenderisation are centred on the fact that they must first be released from the lysosomes. Second, once released into the cytosol, they can still be inhibited by cystatins, a high affinity inhibitor of cathepsins. Finally, and probably the most compelling argument, is the lack of apparent degradation of their known substrates (e.g. myosin and actin) during aging (Roncales *et al.* 1995).

Calpains require calcium for activation. The reported requirements range from 5 to 50 μmol/L for calpain I and 400 to 1000 μmol/L for calpain II (Goll *et al.* 1995). Paradoxically, the levels of free calcium in living muscle (1.5 μmol/g, see Lawrie 1992 and Dransfield 1999) are well below that required for activation. Moreover, the increase in free calcium during rigor (100 μmol/L, see Jeacocke 1993*)* at best would be sufficient only to activate calpain I (Dransfield 1999).

Once activated, calpains target several cytoskeletal proteins (e.g. desmin, vinculin, titin, nebulin) as well as costameric proteins (e.g. vinculin and dystrophin) (Koohmaraie 1996). These proteins are responsible for structural integrity and with their degradation there is a weakening of the myofibrillar matrix thus leading to tenderisation. Calpains are inhibited by a specific inhibitor, calpastatin and they also undergo autolysis. The prevailing pH and temperature conditions govern the rate of calpain activation and inactivation (Dransfield 1994; Simmons *et al.* 1996). For example, Dransfield (1994) predicted that calpain activities would be 6 times greater following rapid glycolysis (i.e. pH 5.5 at 2 h post mortem) compared with more standard rates of glycolysis (i.e. pH 5.5 at 20 h) under standard cooling conditions. However, the corollary of rapid glycolysis, especially at high temperature, is rapid autolysis resulting in reduced tenderisation by the calpain system.

Non-enzymatic mechanisms have also been implicated during post mortem tenderisation. The effective doubling in ionic strength during rigor has been shown to cause conformational changes in proteins and, therefore, increased susceptibility to proteolysis (Ouali 1990). The 'calcium theory' of tenderisation, which is based on the translocation and fragmentation of cytoskeletal proteins in the presence of 0.1 mmol/L Ca^{2+}, has also been proposed by Takahashi (1999).

Our knowledge of the regulation of proteolysis in post mortem muscle is far from complete and both enzymatic as well as non-enzymatic mechanisms may be involved. Furthermore, the post mortem activities of these enzyme systems are also probably governed by the *in vivo* status of protein turnover in muscle (McDonagh *et al.* 2001).

Best practice carcass processing

The concept of best practice carcass processing is predicated on our knowledge of the biophysical changes in the conversion of muscle to meat and their implications to meat quality. Essentially, the aims of best practice carcass processing are to minimise the degree of myofibrillar shortening (i.e. optimise rate of glycolysis and temperature decline) and maximise the extent of proteolysis whilst ensuring compliance with necessary microbiological standards.

To that end, there are several current commercial options including: (i) controlled chilling; (ii) electrical stimulation; (iii) alternative carcass suspension (Tendercut and Tenderstretch); and (vi) calcium chloride infusion.

Controlled chilling

With respect to rigor temperature, the simplest available method for control is via the chilling regime. Effective chiller design and control can ensure that the rate of temperature decline is optimal in the majority of the carcass. Ideally, rigor should be completed between 10 and 20°C in order to minimise the degree of myofibrillar shortening (e.g. Locker and Hagyard 1963). In practice, this is not always achievable simply because there are differences in cooling rates between different regions of the carcass as well as regions within muscles. Moreover, these differentials will vary depending on the carcass weight, amount of fat cover and air flow patterns within a chiller.

In Europe recently, an alternative chilling practice known as 'ultra rapid chilling' has been investigated for beef (Bowling *et al.* 1987; Demeyer *et al.* 1996) and lamb (Sheridan 1990; McGeehin and Sheridan 1999) carcass processing. Ultra rapid chilling involves cooling the carcass or excised muscles at extremely low temperatures (e.g. lamb carcasses chilled at –20°C for 3.5 h followed by conventional cooling at 4°C, McGeehin and Sheridan 1999). In view of the risks of cold shortening, the practice might seem somewhat dubious. However, results from some studies indicate that there are minimal differences in tenderness between ultra fast and conventionally chilled product (Bowling *et al.* 1987; Sheridan 1990; McGeehin and Sheridan 1999). Two plausible reasons have been put forward to explain this somewhat incongruous result. Sheridan (1990) put the view that cold shortening was avoided by skeletal restraint caused by crust hardening of the outer surface of the carcass. Enhanced proteolytic activity has also been postulated (Jaime *et al.* 1992). This was based on the fact that at low temperatures the sarcoplasmic reticulum fails in its function to retain calcium thus giving rise to increased calcium levels in the cytosol (Davey and Gilbert 1974) and therefore enhanced proteolysis. Unfortunately, failure to control the calcium flux is also the underlying reason for cold shortening. Jaime *et al.* (1992) suggested that the intense tenderisation that occurs might mitigate against the loss in tenderness due to cold shortening.

While both postulates are attractive, other workers (e.g. Demeyer *et al.* 1996) demonstrated that cold shortening was a real issue with ultra fast chilling and therefore its commercial application would seem somewhat limited.

Electrical stimulation

Electrical stimulation is used to hasten the onset of rigor by accelerating post mortem glycolysis. Its application therefore allows carcasses and hot boned meat (i.e. pre-rigor) to be rapidly chilled with minimal risk of cold shortening occurring. Bendall (1980) and Chrystall and Devine (1983) provide excellent reviews on the development, scientific basis and methods of electrical stimulation.

The typical pattern of pH decline in electrical stimulated and non-stimulated muscles is illustrated in Figure 1.

There are 2 apparent changes in the pH profiles of electrically stimulated muscles. First, during stimulation there is a sharp decrease in pH (ΔpH about 0.4–0.5 pH units). Second, the rate of pH decline subsequent to stimulation is generally faster (1.5–2.0 fold) than that observed in non-stimulated muscle (Chrystall and Devine 1978). However, there has been some debate over whether the latter effect is real or merely due to differences in muscle temperature (Bendall 1980). Based on the results from Daly (1997), differences in temperature are unlikely to be the sole contributor as he reported a 50–75% increase in post-stimulation pH decline compared with non-stimulated muscle when both were held at constant temperature (35°C). The mechanisms underpinning the accelerated fall in pH post-stimulation are not fully understood although several novel postulates have been put forward including increased phosphorylase *a* activity (Horgan and Kuypers 1985) and ADP induced Ca^{2+} efflux from the sarcoplasmic reticulum (Daly 1997).

The magnitude of the ΔpH and the post-stimulation rate of pH decline is contingent on the voltage (Carse 1973; Chrystall and Devine 1978; Bendall 1980), frequency (Chrystall and Devine 1978; Bouton *et al.* 1980), current and wave form (Chrystall and Devine 1978) and duration of stimulation (Chrystall and Devine 1978; Butchers *et al.* 1998; Hwang *et al.* 1999). Moreover, intrinsic muscle properties such as the pre-stimulation pH (Chrystall and Devine 1978) and the fibre morphology (Devine *et al.* 1984) also influence the glycolytic response to electrical stimulation. In these studies, larger changes (ΔpH and pH decline) were elicited in those muscles with a higher pre-stimulation pH and/or high proportion of glycolytic Type IIb fibres.

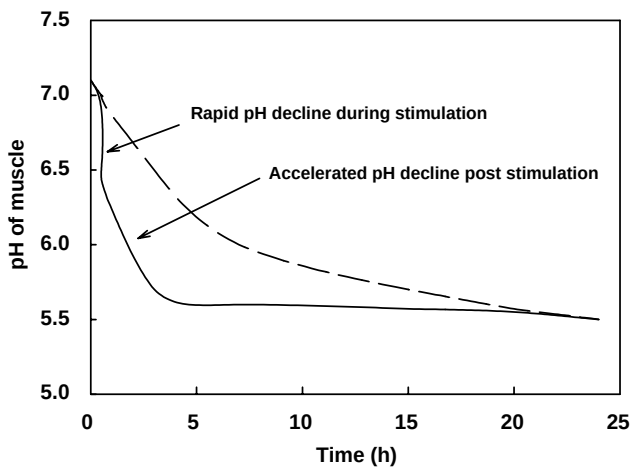

Figure 1. Indicative pH profiles for electrically stimulated (solid line) and non-stimulated (dashed line) muscle.

Perhaps one of the most critical issues in relation to electrical stimulation is the time it is applied post mortem. In general, the glycolytic response is reduced with delayed application of electrical stimulation post mortem. This can be attributed to the reductions in the pre-stimulation pH, muscle temperature and nerve reactivity (voltage dependent) (Chrystall and Devine 1983). In practice, carcasses are electrically stimulated within 1 h after slaughter. In Australian abattoirs, extra low voltage stimulation (<45 V) is generally used within 5 min after slaughter, while high voltage (800–1200 V) stimulation is typically applied within 20–50 min after slaughter. Low voltage stimulation is applied early after slaughter as its efficacy was considered to be dependent on nervous recruitment. However, some workers (Hwang *et al.* 1998) have shown that low voltage stimulation as late as 40 min post mortem is still effective. From a commercial perspective, low voltage systems offer advantages over high voltage electrical stimulation through reduced capital costs and improved occupational safety to abattoir workers. Comparative studies of the effects of high and low voltage stimulation on beef quality suggest that the differences are small (Eikelenboom *et al.* 1985; Hwang *et al.* 1998) when applied at the same time post mortem. When compared under commercial conditions (i.e. low voltage <10 min and high voltage 30–40 min post mortem), high voltage stimulation tended to elicit a more favourable improvement in tenderness (Aalhus *et al.* 1994) largely through a more moderate rate of pH decline.

Electrical stimulation can elicit other benefits in tenderness as well as the prevention of cold shortening. Takahashi *et al.* (1987) and Ho *et al.* (1996) have shown that stimulation caused increased fracturing and disruption of the myofibrillar structure in the LD. On the other hand, several workers (e.g. Ducastaing *et al.* 1985; Dransfield *et al.* 1992; Uytterhaegen *et al.* 1992; Ferguson *et al.* 2000) have demonstrated that electrical stimulation accelerates post mortem proteolysis. However, the rapid activation of calpains by virtue of the increased rate of glycolysis may reduce the total aging response due to the concomitant increase in their autolysis (Geesink *et al.* 1994*a*; Hwang *et al.* 1999). The enhanced proteolysis may also be linked to increased catheptic activity as electrical stimulation has also been shown to cause increased lysosomal rupture (Dutson *et al.* 1980; O'Halloran *et al.* 1999).

Rapid rigor development at high temperatures (i.e. heat shortening) has been a recent cause for concern regarding current electrical stimulation protocols in Australian

abattoirs. For example, in one abattoir, pH_u had been achieved in LD within 1–3 h post mortem (Butchers *et al.* 1998). The problem is certainly more apparent when the stimulation is applied within 5 min after slaughter (Hwang *et al.* 1998) or when the slow chilling regimes are utilised. Heavy, fat carcasses are also problematic as they cool slower, therefore, electrical stimulation is generally not recommended for carcasses >300 kg. Furthermore, the additive effects of other electrical inputs on the slaughter floor (e.g. immobilisers) can also contribute to rapid rigor development (Petch and Gilbert 1997). To address this issue in Australia, each abattoir is now assessed on a case-by-case basis. Rather than recommending uniform protocols for electrical stimulation, the protocols are adjusted to suit the conditions within the abattoir. Factors for consideration include the type of cattle being processed, the presence of other electrical inputs and the chilling practices.

Alternative carcass suspension

Another method for minimising the degree of shortening is to physically prevent the muscles from contracting during rigor. This can be achieved by suspending the carcass or side by the pelvic bone or sacro-sciatic ligament. Tenderstretching, as it is commercially known, is an alternative method of carcass suspension that prevents shortening in most commercially important hindquarter muscles (Hostetler *et al.* 1972; Bouton *et al.* 1973; Ferguson *et al.* 1999).

Bouton *et al.* (1973) and Ferguson *et al.* (1999) demonstrated that most hindquarter muscles are improved following tenderstretching with the exceptions of the *M. psoas major* (tenderloin) and *M. semitendinosus* (eye round). Both of these muscles are stretched when carcasses are normally suspended. An interesting feature of the study by Ferguson *et al.* (1999) (see Table 2) was that the carcasses were also electrically stimulated. This indicates that the combination of electrical stimulation and tenderstretch were additive in terms of their improvements to eating quality. Although the design of this study precluded any firm conclusions to be drawn about an additive effect, earlier research on ovine carcasses by Bouton *et al.* (1984) would reinforce the view that electrical stimulation can elicit further improvements in tenderness in stretched muscle.

Another characteristic of tenderstretch is that there is a lower aging response (Bouton *et al.* 1973; O'Halloran *et al.* 1998). This is of major commercial benefit as it means reduced storage times to achieve a desired level of tenderness. The reasons for this effect are not clear because proteolysis does not appear to be retarded in stretched muscle (O'Halloran *et al*. 1998). Rather it is postulated that the proteolytic weakening of the myofibrillar matrix is less significant when the density of the matrix is dramatically reduced (i.e. stretched).

Wang *et al.* (1994) have patented another alternative means of skeletal restraint known as Tendercut. Here bone, ligaments and tendons are severed at specific points on the carcass in order to increase the tension on muscles and thus prevent them from shortening. The main advantage over tenderstretch is that the sides are conventionally hung. Results to date suggest that Tendercut is an effective means for improving tenderness (Wang *et al.* 1994, 1996; Beatty *et al.* 1999) in commercially valuable hindquarter muscles.

Calcium chloride infusion

The infusion of $CaCl_2$ into the carcass (ovine) immediately following slaughter has been shown to dramatically accelerate post mortem tenderisation (Koohmaraie *et al.* 1988, 1990). The technique is also directly amenable to the tenderisation of post-rigor meat (Wheeler *et al.* 1993). The increased levels of calcium ions are believed to enhance the activation of calpains and perhaps directly contribute to destabilisation of muscle proteins (Takahashi 1999). Super-contraction in muscle has also been observed following pre-rigor $CaCl_2$ infusion (Geesink *et al.* 1994*b*) and this could also contribute to a weakening of the myofibrillar matrix. Geesink *et al.* (1994*b*) demonstrated that accelerated tenderisation could also be achieved with pre-rigor infusion with NaCl. This effect was not associated with any increase in calpain activity rather it was postulated that the increase in ionic strength increased the susceptibility of the myofibrillar proteins to proteolysis (Ouali 1990).

While improvements in tenderness were achieved, Geesink *et al.* (1994*b*) also showed that pre-rigor infusion of

Table 2. Least squares means for sensory scores (CMQ4) for different beef muscles from electrically stimulated tenderstretched and normally hung (achilles tendon) sides (Ferguson *et al.* 1999)

CMQ4 score (1–100) is a consumer panel score based on weighted assessments of tenderness, juiciness, flavour and overall liking

Primal cut	Muscle	Tender stretched	Normally hung	Signif.
Forequarter				
Brisket	*Pectoralis profundus*	31.9	34.7	n.s.
Blade	*Triceps brachii*	55.3	55.8	n.s.
Oyster blade	*Infraspinatous*	61.3	62.4	n.s.
Cube roll	*Longissimus thoracis*	65.2	62.9	$P<0.05$
	Spinalis dorsi	74.6	75.6	n.s.
Hindquarter				
Striploin	*Longissimus lumborum*	61.2	55.3	$P<0.001$
Tenderloin	*Psoas major*	70.9	73.5	$P<0.01$
Rump	*Gluteus medius*	63.9	56.9	$P<0.001$
Topside	*Semimembranosus*	44.9	37.8	$P<0.001$
Outside flat	*Biceps femoris*	50.4	46.7	$P<0.001$
Eye round	*Semitendinosus*	48.3	47.3	n.s.
Knuckle	*Rectus femoris*	50.3	48.0	$P<0.05$

either $CaCl_2$ or NaCl gave rise to increased drip losses and reduced colour stability. These negative effects were less evident when the muscle was infused post-rigor (Wheeler *et al.* 1993). There are also some practical constraints associated with the commercial application of pre-rigor infusion, notably the ability to do this aseptically.

Conclusions

Research reviewed in this paper suggests that a holistic approach must be adopted to achieve consistent beef eating quality. That said, the pre- and post-slaughter conditions that apply to the animal and its carcass remain the most critical as irreversible losses in beef quality will arise, if conditions such as dark cutting or cold shortening occur.

The review has focused largely on tenderness/toughness by virtue of its considerable impact on consumer satisfaction with beef. Improvements in the pre-slaughter management of cattle and the adoption of best practice carcass processing have given rise to quantum improvements in beef tenderness. The consumer endorsed success of the recently introduced MSA beef grading scheme is testament to this fact (MLA unpublished data). With continued improvement in beef tenderness, other palatability factors such as juiciness and flavour will take on greater significance in the future.

In relation to the pre- and post-slaughter environments, further improvements in beef palatability, particularly its consistency is achievable through improved understanding of a number of key areas. In particular, the effect of the immediate pre-slaughter conditions and the post mortem glycolytic and proteolytic rates requires closer investigation. New data is emerging suggesting that the management and handling of animals immediately before slaughter can affect both the glycolytic rate (Simmons *et al.* 1997; Butchers *et al.* 1998) and rate of aging (Daly *et al.* 1995). Inherent variability in either will ultimately influence the effectiveness of post-slaughter technologies such as electrical stimulation. Second, in relation to the pre-slaughter management of cattle, the issue of lairage time warrants attention. Directly consigned cattle are typically rested in lairage between 12 and 36 h. For cattle that have only travelled moderate distances (<400 km), shorter lairage times may be more desirable to optimise meat quality. Third, achieving the optimal rigor temperature throughout the majority of the high value cuts in the carcass is still a major challenge in some abattoirs. Far greater attention must be given to the additive impact of the electrical inputs on the slaughter floor, the type of stock being processed and the chilling regimes. The fourth issue extends beyond the pre-slaughter period but relates to the production background of the animal, specifically its growth history. It is still not clear how changes in the rates of either protein synthesis or degradation leading up to slaughter translate in terms of altered states of post mortem proteolysis.

Gains in our understanding of these issues will facilitate improvements in pre- and post-slaughter practices and therefore continue the improvement in beef palatability.

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