CSIRO PUBLISHING

Animal Production Science, 2014, **54**, 482–493
http://dx.doi.org/10.1071/AN12338

Review

Techniques to reduce the temperature of beef muscle early in the post mortem period – a review

Robin H. Jacob[A,C] *and David L. Hopkins*[B]

[A]Department of Agriculture and Food WA, 3 Baron Hay Court, South Perth, WA 6151, Australia.
[B]NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, PO Box 129, Cowra, NSW 2794, Australia.
[C]Corresponding author. Email: robin.jacob@agric.wa.gov.au

Abstract. A review of the literature was conducted on the effects of high temperature and low pH (HTLP) on meat quality, with a focus on interventions that increase the rate of cooling post slaughter. HTLP can potentially change meat tenderness, water-holding capacity and colour due primarily to protein denaturation during the first 5 h post mortem. Deep muscles in large carcasses are susceptible to HTLP when cooled conventionally. Ante mortem and post mortem solutions that increase the rate of carcass cooling are discussed. Ante mortem solutions include access to feed and water, showering with water and provision of shade. Post mortem solutions included vascular flushing, hot fat trimming, opening seams, hot boning, spray chilling, blast chilling, immersion cooling, and very fast chilling. Accelerating rigor with electrical stimulation before HTLP remains controversial. Combinations of different techniques, that suit the specific requirements of a particular processing plant, is the likely best solution to HTLP, but further development of commercial solutions is suggested.

Received 27 September 2012, accepted 19 May 2013, published online 21 February 2014

Introduction

This review focuses on strategies to control the rate of cooling for beef carcasses with the aim of preventing conditions of high temperature and low pH (HTLP) occurring at the same time, in muscle early in the post mortem period. The effect of these conditions on meat quality, the importance of the rate of carcass cooling in creating these conditions and possible ways to manipulate carcass cooling are discussed.

There is good evidence that HTLP conditions, defined for the purpose of this review as pH less than 6 and temperature greater than 35°C, occur in many beef carcasses in Australia (Hopkins *et al*. 2007; Warner *et al*. 2014). The cause of HTLP could be; a slow reduction in temperature, a rapid reduction in pH, or a combination of these two effects that in part are co-dependent.

The temperature and pH interaction for meat

Muscle pH depends to some extent on temperature during the post mortem period because of the fundamental effect that temperature has on the rate of biochemical reactions. Heat provides molecules with the energy required to overcome the activation energy of a reaction between substrates. The rate constant (k) hence rate of reaction doubles for every change of 10°C as described by the Arrhenius equation (http://www.chemguide.co.uk/physical/basicrates/arrhenius.html, verified 22 May 2013).

Incubation of muscle at a high temperature favours a fast rate of pH decline (Hwang *et al*. 2004) due to the effect of temperature on enzyme activity and subsequently the rate of glycolysis (Newbold and Scopes 1967). A slow rate of cooling therefore favours the likelihood of low pH and high temperature conditions occurring together post mortem. This may be modified to some extent by the energy demands of cold shortening such that the minimum rate of glycolysis may actually occur at 17°C as suggested by Dransfield (1994*b*). Similarly Jeacocke (1977) suggested that the minimum rate of pH decline occurs at ~13°C and attributed this to ATPase activity, as the rate of lactate production is proportional to the rate of hydrolysis of ATP. Another complication is the freeze concentration effect that can increase reaction rate by increasing substrate concentration when meat begins to freeze at about −2°C (Dransfield 1998). While this is an important consideration with very fast chilling (VFC) systems (Jacob *et al*. 2012), temperature is unlikely to go below 0°C before rigor for conventional chilling systems, in which case the Arrhenius relationship can be expected to hold (Ferguson and Gerrard 2014).

Meat quality effects of high temperature and low pH

In addition to this direct effect, high temperature interacts with low pH to cause denaturation and subsequently a loss in functionality of the proteins that influence meat quality. Reduced water-holding capacity, reduced tenderisation, pale colour, premature browning during retail display and sarcomere shortening have all been attributed to such effects. Denaturation of myosin causes shortening of the myosin head and a reduction in filament spacing; inducing water to be expelled from the muscle cells into the extracellular space

(Offer *et al.* 1989; Offer 1991). The role of myosin rather than sarcoplasmic protein denaturation was confirmed by den Hertog-Meishke *et al*. (1997) in relation to the effect of HTLP on water-holding capacity. High ATP concentration in addition to low pH and high temperature may exacerbate denaturation of myofibrils (van Laack and Lane 2000). Denaturation of μ-calpain reduces proteolysis and tenderisation associated with aging (Dransfield 1994*a*; Kim *et al*. 2010) that may not be evident until 30 days aging (Warner *et al*. 2009). In contrast HTLP conditions can reduce shear force values in the first 10 days post slaughter compared with optimal conditions (Warner *et al*. 2009). Simmons *et al*. (1996) showed a clear interaction between temperature and pH at the time of rigor for calpain and calpastatin activities.

Denaturation of sarcoplasmic proteins and sarcomere shortening may increase the amount of light reflected from the surface making it paler in appearance (Swatland 2004). A reduction in mitochondrial activity will reduce oxygen consumption rate making the meat lighter and redder in colour (Li *et al*. 2011). This effect occurs early in the post mortem period but may persist during retail display. However, metmyoglobin reductase activity may decrease (Sammel *et al*. 2002) and metmyoglobin production increase (Ledward 1985) causing meat to be less stable in colour during retail display due to HTLP.

Of the effects attributed to HTLP, sarcomere shortening has probably received the greatest attention due to the effect on shear force and tenderness in the case of cold shortening (Locker and Hagyard 1963). Heat or rigor shortening is said to occur above 20°C and cold shortening below 15°C (Honikel *et al*. 1983) and both occur due to the release of calcium into the myofibrillar space when ATP concentration is high under anoxic conditions (Hertzman *et al*. 1993; Olsson *et al*. 1994). Honikel and Hamm (1978) state that rigor or heat shortening occurs because of the pH dependence of the sarcoplasmic reticulum membrane Ca^{2+} uptake system, which is optimal at pH 6.3 and decreases rapidly when pH drops below 6, although other factors may be involved. Cold shortening is attributed to the release of calcium from mitochondria and the sarcoplasmic reticulum, when ATP concentration is not limiting (>3.5 μM), due to anoxic and cold conditions (below 15°C) causing inactivation of the ATP-driven calcium pump (Honikel and Hamm 1978). Greaser *et al*. (1969) showed that the calcium accumulating ability of porcine sarcoplasmic reticulum was impaired when incubated at pH 5.6 and 37°C for 1 h and the dependency between pH and 'free' calcium levels has been clearly shown (Hopkins and Thompson 2002). Henderson *et al*. (1970) found that the values for temperature and pH at which shortening occurs depends on the species of animal and muscle type. For beef, shortening was maximal at 2°C, minimal in the range 16−25°C and high again at 37°C in beef (Henderson *et al*. 1970). Similarly Honikel *et al*. (1986) found that sarcomeres in excised unrestrained bovine *M. sternomandibularis* incubated in a water bath shortened by 70% below 6°C, by less than 10% between 6 and 18°C and by 40% between 20 and 38°C. Hwang *et al*. (2004) found however that sarcomere length was no different ($P > 0.05$) for excised *M. longissimus* (LL) incubated at 36°C (1.45 μm) than when incubated at 5°C (1.52 μm), with the least at 15°C (1.76 μm). Olsson *et al*. (1994) showed that the extent of shortening depended on the muscle; being less for *M. semimembranosus* (SM) than *M. longissimus dorsi* at all temperatures and attributed this to the *M. longissimus dorsi* containing relatively less white muscle fibres.

Summary of the meat quality effects of HTLP

With all this complexity, some confusion is evident in the literature with different names being used to describe the same HTLP scenario including; 'heat shortening', 'heat toughening', and the term 'pale, soft, exudative' sometimes being used for beef as well as pork (Offer 1991). Some variation exists about the boundaries for pH and temperature used to define HTLP, for example Kim *et al.* (2010) suggests that 38°C might be the critical temperature for protein denaturation and calpain autolysis whereas other authors suggest that 35°C (Warner and Kerr 2009) and even 30°C (den Hertog-Meishke *et al*. 1997; Sammel *et al*. 2002) may be the threshold value. However, the issue is more than just deciding which levels constitute a threshold. The terms shortening and toughening could only be accurate if sarcomere length and shear force had been measured for example, and toughening is likely to require time to be specified as well as shear force because reduced tenderisation is a function of aging. Some authors suggest that shortening may be less important than delayed tenderisation with HTLP (Kim *et al*. 2012). However, use of the word toughening in this context is inconsistent with the description of Koohmaraie *et al.* (1996) of a transient increase in shear force associated with sarcomere shortening in the first 24 h post mortem. Furthermore, both toughening and shortening place an emphasis on tenderness, even though associated water-holding capacity and colour affects may be equally important in some market scenarios, such as ground beef for example. Agreement on the most suitable term is therefore still needed, unless the condition can be subclassified further in some way.

Obviously the meat quality effects associated with HTLP could vary. The degree of shortening depends on the amount of ATP available at the time that shortening conditions prevail (Hertzman *et al*. 1993) and this might vary between animals and processing conditions. Another possible reason for variation might be the effect of time and this is rarely described with HTLP conditions. For example, the system adopted by Meat Standards Australia for benchmarking commercial scenarios relies on a plot of pH (*y*-axis) versus temperature (*x*-axis) both of which are dependent on time (Thompson *et al*. 2005). While time is implicit in this relationship, presumably the nature of this could vary. The length of time that HTLP conditions prevail is likely to be important because proteins are more likely to denature when this period of time is extended (Offer 1991). Thomson *et al*. (2008) demonstrated an increase in shear force of 30% after 21 days aging for meat with a pH of 5.6 incubated at 35°C for 3 h compared with 1 h. Conceivably if a period of inertia was to occur for temperature, the HTLP conditions could prevail for an extended period of time and the effect on meat quality would be greater compared with a muscle for which temperature declined quickly. The effect of temperature on rate of pH decline (Jeacocke 1977) might also be more pronounced in this scenario, making an extended period of HTLP conditions more likely. However, an effect of time

could depend on the type of protein in question. Offer (1991) states that the fraction of myosin denatured increases with the rate of pH fall, then reaches a maximum at a rate of pH fall that depends on the chilling conditions, and finally decreases. Within a muscle, individual muscle fibres will enter rigor at different rates depending on ATP concentration (Jeacocke 1984). This could add further variation to the effects of HTLP on meat quality if early onset of rigor protected myosin from denaturation (Offer 1991). As stressed by Hwang *et al*. (2003), caution is needed when extrapolating from studies based on excised muscle to those with *in situ* muscle as the former creates a very different pre-rigor temperature. Notwithstanding the inherent variation in the meat quality response, HTLP conditions are likely to be time critical, for the reasons outlined above and because they occur early in the post mortem period. So interventions to prevent HTLP will also be time critical, in a process management context.

Ante mortem factors related to carcass cooling

Body temperature at the time of death represents the starting point from which the temperature declines after slaughter. Ante mortem body temperature could therefore influence post mortem temperature and be influenced by prior management; on-farm, during transport and in lairage at abattoirs (Gregory 2010).

Hyperthermia in cattle ante mortem

The normal body temperature range for cattle is 38.0–39.3°C (Ensminger 1987) and hyperthermia is said to occur when rectal temperature exceeds 39.5°C (Radostits *et al*. 1994). Beatty *et al*. (2006) reported that the diurnal range of core body temperature for cattle is 1°C and heat stress signs occur when core body temperature increased by 2°C.

Hyperthermia in cattle during the ante mortem period seems to be largely unreported, but presumably occurs under Australian conditions. Factors such as dehydration, excessive muscular activity, high ambient wet bulb air temperature and toxin ingestion (Radostits *et al*. 1994; Bryden 2012) could potentially cause hyperthermia in groups of cattle during the ante mortem period. Other metabolic reasons for elevated body temperature are outlined by DiGiacomo *et al*. (2014).

Dehydration and exercise

Muscle activity associated with exercise during transport, unloading, mixing in lairage and movement from lairage to the knocking box can increase body temperature. Jacobson *et al*. (1997) found that activity 3 h before slaughter increased body temperature at slaughter. The authors developed a prediction system for high ultimate pH based on body temperature profile before slaughter and have patented this method (United States Patent 6862550).

High ambient temperature

Ambient conditions can be sufficiently high to cause excessive heat load for cattle during summer in Australia, but animal factors are likely to be important as well. *Bos taurus* breeds are more susceptible than *Bos indicus* to heat stress (Beatty *et al*. 2006) and dark coated cattle are more susceptible than light coloured cattle (Sakaguchi and Gaughan 2004). Obesity causes animals to be more susceptible to heat stress. Brown-Brandl *et al*. (2006) found that respiratory rate was 6.8% higher in finished animals compared with lean animals for heifers kept in a feedlot when ambient temperature was greater than 25.5°C dry bulb. A 9-point classification system was used for condition score, with lean animals having a score less than 8 and finished animals greater than or equal to 8. Furthermore the effect of breed and condition score were additive such that finished Angus (dark coat) heifers had a 31.6% higher respiration rate than a lean Charolais (light coat).

Toxins

Toxins that cause hyperthermia in livestock are principally the ergot alkaloids, ergotamine and ergovaline, a subset of indole alkaloids (Radostits *et al*. 1994). These toxins are produced by the fungal species, *Claviceps purpurea* and *Acremonium coenophialum* (Ross *et al*. 1989), that parasitise the seed heads of various grass species. Once ingested, ergot alkaloids cause contraction of all smooth muscle resulting in vasoconstriction as well as oxytocic effects (van Rensburg and Altenkirk 1974). Vasoconstriction interferes with heat loss mediated by circulation of blood in the vascular system. The mechanism for this effect is thought to be a direct action on smooth muscle rather than being nerve mediated. Depression in plasma prolactin concentrations, reductions in feed intake, liveweight gain and milk production are seen with ergotism (Radostits *et al*. 1994).

Ergot of rye and sorghum

Outbreaks of hyperthermia in feedlot cattle have been attributed to *Claviceps purpurea* ingestion in New South Wales (Ross *et al*. 1989) and Western Australia (Peet *et al*. 1991). Ross *et al*. (1989) found that Hereford cattle fed a ration containing 3.75 g/kg of ergots displayed symptoms of hyperthermia 3 days after feeding the infested ration began. In one animal the rectal temperature reached 41.75°C. Jessep *et al*. (1987) reported symptoms in cattle when feed contained just 0.02% ergots w/w, while Peet *et al*. (1991) reported death due to hyperthermia in feedlot cattle which had consumed rations contaminated with *Claviceps purpurea.*Blaney *et al*. (2011) demonstrated the effects of hyperthermia in Hereford steers fed a ration containing sorghum infected with *Claviceps africana.*

Fescue toxicoses

The fungus *Neotyphodium coenophialum* (formerly *Acremonium coenophialum*) grows on tall fescue grass (*Festuca arundinacea*) and produces the toxin ergovaline. Ergovaline can cause vascular constriction, impairment of heat loss and hyperthermia although it is less potent than ergotamine. Paterson *et al*. (1995) found that feed intake was reduced in cows grazing endophyte-infected tall fescue, but only when the ambient air temperature exceeded 32°C. Under periods of heat stress, animals eating the infected grass had reduced ability to dissipate body heat, and blood flow to peripheral (rib skin), core-body (duodenum colon), and brain (cerebellum) tissues was decreased. Tall fescue grass is grown as a perennial pasture in Australia particularly in rainfall regions of 600 mm and above.

Ergotism at the time of slaughter

Clearly hyperthermia due to ergotism occurs in Australia, but there have been no reports of outbreaks occurring at abattoirs, so the extent to which signs might persist to the point of slaughter is difficult to ascertain. In the outbreak reported by Ross *et al*. (1989) symptoms returned to normal at night when ambient air temperatures declined to 16°C from the day time high of 37°C. Moreover prolactin and body temperature values returned to normal 24 h after the feeding of the infested ration was ceased. Other biochemical effects of ergotamine may have implications for meat quality warranting further investigation. Lakritz *et al*. (2002) found that consumption of ergovaline exacerbated the oxidation of whole blood glutathione caused by heat stress in Simmental cows.

Ante mortem interventions to increase muscle cooling rate

Access to food and water

In Australia cattle are given access to water without food during abattoir lairage. The fasting period is longer than the lairage period and includes on-farm curfew and transport times. McLennan (2005) found no evidence of dehydration at one large abattoir in Western Australia so preventing dehydration during lairage appears to be easier with cattle than lambs (Jacob *et al*. 2006). Jacobson *et al*. (1997) found that body temperature of heifers declined during overnight lairage for some animals and attributed this to a reduction in heat produced by rumen fermentation due to fasting. Variations in fasting time before slaughter might therefore account for some variation in ante mortem body temperature. The National Model Codes of Practice for Animal Welfare stipulate maximum allowable times for cattle to be deprived of food and water being 48 and 36 h for food and water, respectively (Anonymous 2002). Prolonging the length of fasting to reduce body temperature seems an unrealistic option because this is likely to reduce meat yield (Thompson *et al*. 1987; Warriss 1990), although it may have little effect on muscle glycogen concentration (Toohey and Hopkins 2006), provided the animals are not stressed (Jacob *et al*. 2005).

Showering with water

Direct application of water to cattle with a sprinkler system can be an effective method of relieving heat stress in cattle under feedlot conditions (Mader and Davis 2004), and could be a way of reducing body temperature at the time of slaughter. Long and Tarrant (1990) found that showering Landrace and Large White pigs before slaughter could reduce the loin temperature by 3.5°C, when measured 40 min post slaughter at depths of 3 and 6 cm. The magnitude of this effect was decreased to 2°C when the duration of the shower period was decreased from two periods of 30 min separated by a 30-min break to two periods of 15 min with a 15-min break. The effect was also less in summer than winter. Showering had no effect on loin pH, but improved colour and drip loss.

In the ante mortem context, showering has been advocated to reduce post slaughter temperature of pigs (Hambrecht 2004) and to improve carcass hygiene for all livestock (Maltin *et al*. 2006), although the value of the latter has attracted some debate. In the study with pigs reported by Long and Tarrant (1990), showering failed to overcome homeothermic regulatory mechanisms shown by rectal and deep leg muscle temperatures. If showering reduced the temperature of superficial, but not deep muscles its value would be limited given that deep muscles are more susceptible to HTLP. Nevertheless showering could be of some value in preventing hyperthermia under heat stress conditions. Potential negative effects of showering are glycogen depletion in response to cold stress as demonstrated by Jacob *et al*. (2003) in lambs, potentially leading to an elevated ultimate pH, and the potential to increase the spread of pathogenic bacteria.

Provision of shade

Provision of shade in lairage may be a simple, but important technique for reducing body temperature at the time of slaughter. Bourke (2003) found that solar radiation made an important contribution to hyperthermia in cattle that ingested ergot when air temperatures were relatively low and this is likely also the case for cattle with dark coats. Blackshaw and Blackshaw (1994) state that shade can reduce an animal's radiant heat load by 30%. They concluded that providing cattle with shade can prevent increases in rectal temperature and respiratory rate due to hot weather.

Post mortem factors related to carcass cooling

Under Australian conditions, the post mortem period has two distinct components in relation to temperature control. The slaughter period when ambient temperature conditions may not be controlled; lasts from stunning until when 'dressing' is complete up to ~60 min later. This is followed by the refrigeration period when the dressed carcass is subjected to controlled and low ambient temperature conditions.

Carcass cooling

Carcass temperature changes with time after slaughter and subsequently during refrigeration before being maintained at a constant temperature ~0°C for storage. Various computer-based models have been developed to quantify this complex process, principally for optimising refrigeration systems (Pham *et al*. 2009). The specialised and complex nature of these mathematical models makes their description well beyond the scope of this review, but there are some salient aspects of carcass temperature change relevant to HTLP.

Homeostatic mechanisms such as blood circulation maintain temperature at a constant level in the live animal. Cessation of these mechanisms at the time of death allows body temperature to subsequently change. The rate of temperature change post mortem is governed mainly by heat loss although some heat production occurs in the immediate post mortem period. Various biochemical reactions, including glycolysis, continue to produce heat in the early post mortem period. Morley (1974) measured heat production in *M. semitendinosus* and *M. extensor carpi radialis* excised and enclosed in an insulated container. The rate of heat production reached a maximum level of 1.5 kJ/kg h that was maintained until 4–5 h post mortem before decreasing to zero. Total heat production in *M. semitendinosus* was 6.4 kJ/kg being equivalent to a temperature rise of 1.8°C. The heat

production measured per unit decrease of pH averaged 5.4 kJ/g for *M. semitendinosus* and 6.8 kJ/kg for *M. extensor carpi radialis*.

The rate of heat loss from a carcass is influenced by heat transfer between the surface and ambient environment and the rate of heat movement from within the muscle to the surface. The heat transfer coefficient (χ) between the surface and the environment consists of three components; forced convection, evaporation and radiation although heat loss due to radiation from carcasses is practically negligible (Levy 1986). The type of cooling media and the refrigeration settings used can alter the heat transfer coefficient hence the rate of cooling of a carcass. Liquid cooling systems have a higher heat transfer coefficient compared with air cooling systems (van Moeseke *et al*. 2001) and are used to cool poultry, but not beef carcasses. For air cooled systems the temperature, humidity and speed of the air surrounding the carcass are critical to the heat transfer coefficient. Lovatt (2004) provides a detailed description of the importance of these factors for cooling including the roles of air temperature and the use of fans to increase air speed.

The passive rate of heat transfer within a medium depends on the thermal conductivity of the material, the heat gradient and the inverse of the distance between the points measured. The thermal conductivity of beef is 0.45 *W*/(m.°C) (Fontana *et al*. 1999), similar to wood and low by comparison to copper [401 *W*/(m.°C), http://www.engineeringtoolbox.com/thermal-conductivity-d_429.html, verified 22 May 2013]. The effect of distance from the surface on heat transfer is partly evident in the difference between muscles for cooling rate post mortem. Stolowski *et al*. (2006) measured rate of temperature decline in seven major muscles in beef carcasses chilled conventionally for 48 h at 2°C and found they fell broadly into three groups. For rate of temperature decline post mortem, the LL was the most rapid as this muscle is relatively flat in shape and located superficially. Larger and deeper muscles such as the SM, *M. semitendinosus*, *M. vastus lateralis* (VL) and *M. triceps brachhi* were intermediate and the *M. biceps femoris* (BF) and *M. gluteus medius* were the slowest to lose heat of all the muscles examined. Refrigeration settings can change the rate of cooling at the surface, but the rate of heat transfer within a muscle will still be limited by the thermal conductivity of the meat. Cooling the surface will increase the temperature gradient between the surface and the centre of the carcass, but the thermal conductivity of the meat remains unchanged. Tarrant (1977) demonstrated the effect of depth on temperature gradient in an experiment that compared hot boning with cold boning. For the cold-boned SM there was a 15°C difference in temperature between the surface and a depth of 8 cm compared with 5°C for the cold-boned SM 3 h post slaughter. For this reason the thickness of the meat is important and VFC protocols for example require portions to be no greater than 80 mm in thickness (van Moeseke *et al*. 2001).

The mass of a carcass will determine the amount of energy to be lost during cooling so is important in addition to the shape of the mass to be cooled. Meade *et al*. (1992) estimated the energy removed from sides during chilling and related the amount of energy to body mass with the formula. Q = mass * Cp * ($T_0 - T_f$), where Q = Heat removed (KW), Mass = weight (kg), T_0 = initial temperature, T_F = final temperature, CP = specific heat (0.74 Watt hours/kg°C). Interestingly the rate of weight loss during cooling is related to heat loss, due to the role of evaporation, with rate of weight loss being greater when rate of heat loss is high (Levy 1986). This can be modified with spray chilling that is discussed later in the paper.

In summary, the rate of temperature change varies between and within a carcass being slowest for large carcasses and deep muscles. Typically the rate of change in temperature varies with time from death and can be described as a monotonic exponential decay for superficial muscles and a sigmoid shape decay for deep muscles in large carcasses (Kaliszan *et al*. 2005). For the latter, the period of temperature inertia at the beginning of the post mortem period is likely due to; (i) metabolic heat production that continues for several hours post mortem and (ii) meat having a relatively low thermal conductivity coefficient that limits the rate of heat movement from within a muscle to the surface.

Slaughter interventions to increase muscle cooling rate

Several techniques are available in the slaughter period to influence the rate of temperature and or pH change immediately or later during refrigeration including; electrical simulation, vascular flushing, opening seams, hot fat trimming and hot boning.

Electrical stimulation

As well as stimulation to accelerate rate of pH decline, electrical stimulation can be used to immobilise the animal and to assist with hide removal. While glycolysis and muscle twitching produce heat (Morley 1974), the findings about the effect of electrical stimulation on carcass temperature have been equivocal, despite the predictions of (Davey and Pham 1997) that a temperature increase as large as 4°C may occur due to electrical stimulation.

In the study of Jeremiah *et al*. (1985) there was no effect of electrical stimulation on temperature change over time in a range of cattle genotypes. The electrical stimulation unit was a Best and Donovan hog stunner applying 550 V, AC 50–60 cycles/s, 20 pulses of 3-s duration with a maximum output of 5A. This was confirmed by Li *et al*. (2006) who found no effect of low voltage electrical stimulation on the temperature of Chinese Yellow crossbred bulls. Stolowski *et al*. (2006) found that electrical stimulation increased the temperature of the LL, but not the SM, ST, BF, and VL. The difference seen in the LL was still evident after a 21-h cooling period. Fjelkner-Modig and Ruderus (1983) found that electrical stimulation raised meat temperature of bull carcasses by 2°C when the bulls had not been stressed. For bulls that were stressed, shown by high ultimate pH, the temperature was high anyway. A continuous stimulation with a frequency of 14 Hz, a peak voltage of 85 V and an individual peak width of 5 ms was used for a stimulation time of 32 s.

Notwithstanding a variable temperature effect, effective electrical stimulation will likely reduce muscle pH in cattle, although the magnitude of this drop may depend on a range of factors including the nature of the electrical signal (Chrystall and Devine 1978), other electrical inputs such as immobilisers (Simmons *et al*. 2008), and variation in response between

individual carcasses (Pearce *et al*. 2006; Hopkins *et al*. 2014). In so doing, electrical stimulation will increase the likelihood of HTLP when the rate of temperature decline is slow. However, the understanding of the use of electrical stimulation when temperature is high appears complicated and incomplete. Rosenvold *et al*. (2008) suggested that electrical stimulation may in fact offer some protection against HTLP conditions by accelerating rigor, consistent with the reasoning of Offer (1991) that attachment of myosin heads to actin protects myosin from denaturation. Kim *et al*. (2012) found that electrical stimulation may protect myosin, but not μ-calpain against denaturation by advancing the onset of rigor. Electrical stimulation at high temperatures will not shorten sarcomere length (Kim *et al*. 2012), presumably because it reduces ATP concentration (Hertzman *et al*. 1993), so is unlikely to toughen meat in this way. However, denaturation of μ-calpain will likely reduce tenderisation due to aging (Hwang and Thompson 2001). Using electrical stimulation to protect myosin and improve water-holding capacity may be appropriate depending on the expected aging period and market scenario. Electrical stimulation might also cause meat to be lighter and redder in colour during retail display by reducing oxygen consumption rate (Sammel *et al*. 2002; Hopkins *et al*. 2014). However, HTLP conditions will favour denaturation of metmyoglobin reductase enzymes (Sammel *et al*. 2002), so the net result of these two effects on colour may depend on the muscle.

Vascular flushing

Farouk *et al*. (1992) showed that a solution could be administered to lamb carcasses by vascular infusion at 10% by weight and this changed the rate of glycolysis and the extent of muscle shortening. Further work (Farouk and Price 1994) used the same infusion process and revealed that infusion of a solution containing maltose, glycerin, dextrose and sodium and potassium tripolyphosphates lowered the temperature by ~2°C compared with non-infused carcasses during the early post mortem period (3 h), but also increased the rate of pH decline. Glycolysis was completed in 6 h compared with 12–24 h for the control.

Rinse and Chill (MSPC, Inc., Eden Prairie, MN, USA) is a commercially available technique that involves flushing solutions into the body via the left carotid artery, immediately after exsanguination and before evisceration. This process effectively removes residual blood from the carcass; replacing it with a water-based solution to enhance bleed, lower pH, and reduce temperature earlier and more rapidly (http://mpscinc.com/about_us.html, verified 22 May 2013) and this system is used in several Australian abattoirs.

Studies have been conducted to examine the effects of various solutions on several aspects including tenderness, colour stability, flavour and yield (Yancey *et al*. 2002; Hunt *et al*. 2003). Depending on the solution used, this technique can accelerate the rate of pH decline (Dikeman *et al*. 2003), but the effect on carcass cooling is not clear from existing research. Dikeman *et al*. (2003) found that vascular flushing had no effect on the rate of temperature decline when solutions kept at temperatures of 25 and 28°C were used. Further research is required to determine if flushing with very low temperature fluids could influence the rate of temperature decline post mortem and avoid HTLP conditions.

Another way to introduce solutions is direct injection into the muscle pre rigor. Stephens *et al*. (2006) found that injecting pig loins 50 min after death with a solution of either sodium citrate or sodium acetate kept at 17°C, lowered loin temperature in the first few minutes after injection, but did not affect chill rate after 1 h. The citrate solution had a greater cooling effect than the acetate treatment.

Hot fat trimming

Removal of subcutaneous, kidney, pelvic, and heart fat before chilling has been advocated to improve the yield of subprimal cuts (Ahmed *et al*. 1992; Miller *et al*. 1995) and to reduce refrigeration costs. Miller *et al*. (1995) found that hot fat trimming reduced carcass weight on entry to the chiller by ~5% in both Brown Swiss and English crossbred steers. They also found that breed can influence the location of fat on a carcass while not affecting the total amount of fat removed.

Hot fat trimming has been investigated as a method of preventing pale soft exudative meat in pork. Milligan *et al*. (1998) found that hot fat trimming reduced the internal ham temperature by ~1°C from 3.5 h after chilling with a conventional chilling system. This difference was increased to 5°C when accelerated chilling was used. A disadvantage of hot fat trimming could be a reduction in price paid to the producer if the trim occurred before the carcass being weighed for valuation purposes. If so, then a change in the price structure may be required to fairly represent the value of the carcass at the point of sale.

Opening seams

'Opening seams' is an industry term that refers to dividing muscles along fascia planes, done usually to prevent the forelimbs of large bodies from contacting the floor. In this case a knife cut is made medial to the shoulder blade to loosen the attachment of the shoulder to the thoracic wall. A light steel frame is then used to lift and hold the forelimbs in a higher position than is normally possible. Theoretically at least this technique could increase the surface area of the carcass in contact with air and subsequently the rate of heat loss from the surface. No reports were found in the literature to provide any quantitative information on the effect of this on muscle temperature. Some investigation may be warranted to determine if this can reduce the temperature of cuts prone to HTLP, particularly if it could be applied to the hind limb.

Hot boning

Boning operations that occur before completion of rigor are known as either hot or warm boning. For hot boning, muscle is removed from skeletal restraint pre rigor without delay while warm boning occurs after a short period of cooling (Murray 2001). The temperature of meat for cold, warm and hot boning is less than 8, 15–24, and 38°C, respectively (Bell 1999). Hot cutting is another variant that refers to cutting meat while hot, but with the bone left in (Joseph 1996). Partial hot boning methods can potentially be combined with other technologies such as rapid chilling methods (Meade *et al*. 1992) or stretching technology

such as SmartStretch (Taylor and Hopkins 2011; Taylor *et al*. 2012). Sammel *et al.* (2002) described a procedure where the 'inside semimembranosus' muscle was partially removed from the carcass to effectively increase the rate of temperature decline and mitigate the effects of HTLP on this muscle. Furthermore segregation of product may occur after hot boning, according to the quality of the cut. Low quality cuts may be frozen immediately for manufacturing purposes (Toohey *et al*. 2012) and high quality cuts chilled and aged to optimise eating quality (Taylor *et al*. 2011).

Temperature effects of hot boning. Given that thermal conductivity is a physical property difficult to change, hot boning is a logical way to reduce both the length of the temperature inertia period as well as the subsequent rate of temperature decline, by changing mass and shape. The effect of hot boning on the rate of temperature decline was clearly demonstrated in a study by Jeremiah *et al*. (1985). In this study the removal of muscles 40 min after exsanguination reduced temperature by ~5°C for SM, 3°C for LL and 10°C for *M. triceps brachii* 2 h after excision. The different magnitudes of the effect could suggest that hot boning is more efficacious for large deep muscles than for flat superficial muscles for reducing the risk of HTLP. The LL had the highest rate of temperature decline when conventionally chilled and had the smallest relative increase in rate of temperature decline of the three muscles when hot boned. As well as accelerating heat loss by reducing the mass of muscle to be cooled, hot boning may allow different cooling regimes to be applied to different cuts using techniques such as immersion chilling as summarised in the review by Simmons *et al*. (2006).

pH effects of hot boning. By decreasing temperature it might be expected that hot boning would decrease the rate of pH decline post mortem as the rate of glycolysis is temperature dependent. Accordingly Tarrant (1977) found that hot boning had a significant negative effect on the rate of glycolysis post mortem indicated by ATP concentration and pH. The rates from 1 to 6 h post slaughter were 0.07 pH units/h in the hot-boned SM compared with 0.25 pH units/h in the cold-boned product, 8 cm below the surface. This and the associated higher temperatures emphasise the importance of location in the carcass for HTLP, being more likely in deep muscles. Furthermore the rate of pH and temperature decline was more uniform throughout the muscle for hot-boned compared with cold-boned SM. However, Jeremiah *et al*. (1985) found that hot boning decreased muscle pH by 0.5 units 2 h after muscles were excised in the SM and *M. triceps brachii*, but not in the LL. Tarrant (1977) found a negative effect of hot boning on phosphocreatine concentration and speculated that the excision process may have had a muscle stimulation effect on glycolysis. Such an effect might explain the conflicting result for pH decline recorded by Jeremiah *et al*. (1985) for which there is no other explanation.

Hot boning applications. Several authors have cited multiple benefits of hot boning compared with cold boning. These advantages include reductions of up to 50% in energy and space requirements for chilling, reduced labour requirements, reduced shrinkage, reduced holding times, and reduced transport costs (Jeremiah *et al*. 1985; Pisula and Tyburcy 1996). Hot boning may also allow muscles or cuts to be shaped in preparation for portion cutting using technologies such as Smartshape (Taylor *et al*. 2011). Reducing the weight of material to be cooled will reduce the amount of energy that needs to be removed from the carcass. In relation to meat quality, Sammel *et al*. (2002) found that hot boning prevented the two tone colour seen with cold-boned beef topside as well as improving colour stability. This together with the findings of Tarrant (1977) that hot boning will improve water-holding capacity of meat suggests that hot boning is an effective way to reduce HTLP.

On the other hand several negative effects of hot boning have also been cited including dark colour due to increased mitochondrial respiratory rates (Brown *et al*. 1988), propensity to cold shorten, shape distortion with 'hot cut' meat and muscle separation (Pisula and Tyburcy 1996). Rapid cooling may be necessary to avoid the risk of increased microbial proliferation after packaging when meat has been boned hot (Reichel *et al*. 1991; Meade *et al*. 1992). The Australian Quarantine and Inspection Service require Hazard Analysis Critical Control Point-based temperature control systems for meat hot boned in Australia (Murray 2001). Hot boning also requires changes to meat inspection procedures during slaughter, although hot boning may actually facilitate the use of techniques designed to reduce the risk of contamination of meat with central nervous system tissues during the slaughter process (Ramantanis 2006). Such techniques involve removing the vertebral column without splitting the carcass.

Concerns about meat quality, the cost of change, production difficulties, product acceptance by consumers and the inability to grade carcasses before boning are issues that are likely to hinder adoption of hot boning. Simmons *et al*. (2006) reports that hot boning is used for high value meat in New Zealand and estimates that ~20% of New Zealand beef production is currently processed hot, but in Australia hot boning is limited to a few plants processing low quality meat (Taylor *et al*. 2012).

Refrigeration interventions to increase muscle cooling rate

With conventional refrigeration, carcasses are cooled in air the temperature of which is usually in the range of 1−2°C. Air speed is varied with fans (1–5 m/sec) to change rate of evaporation hence rate of heat loss. Methods to further accelerate cooling compared with conventional refrigeration include cold water showering, blast chilling, freezer chilling and liquid immersion (Springer *et al*. 2003). There are many variations and systems possible making comparison of different accelerated chilling studies complex (Evans 2012). Accelerated chilling can be applied to either a whole carcass or hot-boned primal cuts and delivered in a single phase or a double phase where cooling is accelerated after rigor.

European Union regulations require carcass temperature to be less than 7°C before the carcass can be removed from the chiller or processed for food safety reasons. This will take ~24 h for beef carcasses with conventional single-stage chilling regimes (James 1996). Australian Meat Standard AS4696:2007 stipulates that all carcasses must be placed under refrigeration within 2 h of stunning and the surface temperatures of carcasses, sides, quarters or bone-in major separated cuts must be reduced to 7°C within 24 h of stunning (Spooncer *et al*. 2007).

In the UK relatively slow rates of cooling have been favoured to avoid cold shortening (Joseph 1996), such that meat

temperature reaches 10°C ~10 h post mortem. This goal is based on the studies that suggest tenderness is optimal when rigor is reached at 12−15°C (Devine *et al*. 1999; Geesink *et al*. 2000) independently of the cooling method used. VFC (Joseph 1996) is the only system that aims to move away from this paradigm.

Spray chilling

Spray chilling is defined as the intermittent spraying of cold water on carcasses during the first 3–8 h post mortem (Hippe *et al*. 1991). Spray chilling is widely used in the USA and has recently been investigated for commercial application in Australia (Brereton *et al*. 2011). The prime motivation is to reduce carcass weight loss during cooling rather than to change cooling rate. There are regulations about the amount of water retained at the end of the process that processors using this system need to be aware of (Dorian 2002). Hippe *et al*. (1991) sprayed beef carcasses with 9.1 L of water at 2°C over a 5-min period every 15 min for 5 h in a chiller with an air temperature of 2−4°C. They found no effect of spray chilling on the internal temperatures of ST, LL or *M. serratus ventralis* measured at 0, 2, 4, 6, and 24 h post mortem.

Blast chilling and chiller freezing

Blast chillers use high speed air flows to remove heat from products before going into conventional chillers and have had particular application to cooked food. Chiller freezing on the other hand utilises very low air temperatures such as −32°C (Springer *et al*. 2003). These methods have been shown to successfully reduce the post mortem temperature of pig (Springer *et al*. 2003; Hambrecht *et al*. 2004) and beef (Li *et al*. 2006) carcasses. Li *et al*. (2006) showed that rapid chilling of intact beef carcasses reduced LL temperature at the 10th/11th rib interface relative to conventional chilling. Air temperature, relative humidity and wind velocity were 1 ± 1°C and 0.5 ms^{-1} in the conventional chill compared with −12°C and 2.5 ms^{-1} in the rapid chill treatments, respectively. Rapid chill was applied for 2 h then followed by normal chill for another 21 h. However, the difference in muscle temperature was limited to the middle part of the chilling process, between 3 and 7 h post mortem. Furthermore, the cattle used for this experiment were relatively small Chinese Yellow crossbred bulls with a liveweight of 527 kg. Interestingly this rapid chill system reduced the rate of pH decline only when carcasses were electrically stimulated.

Immersion cooling

Immersing an object in a liquid media increases the surface heat transfer coefficient compared with air (Fricke 2012), reducing cooling time and evaporative weight losses. The majority of birds processed in the UK and USA are chilled this way (Brown *et al*. 1988). Liquids used for chilling include propylene glycol (Meade *et al*. 1992) and brine solutions (Brown *et al*. 1988). Proplyene glycol (50% solution) is non-toxic, non-corrosive, colourless, and odourless. However, meat has to wrapped before immersing in propylene glycol to be classified as fresh (Redmond *et al*. 2001), so implementing immersion chilling is likely to require significant change to the processing system. Redmond *et al*. (2001) found that lamb carcasses lost less water by evaporation and had the same tenderness when chilled by immersion chilling compared with air chilling at the same temperature. However, the need to wrap carcasses reduced the effectiveness of immersion cooling.

Very fast chilling

VFC is a specific cooling regime, defined in European studies as reducing muscle temperature to −1°C by 5 h post mortem (Joseph 1996). The motivation for VFC appears primarily to be to increase cold carcass weight (Hippe *et al*. 1991), brought about by water condensing on the carcass when the surface is very cold. However, there has also been an interest in accelerated tenderisation via VFC, achieved when subzero temperatures are reached before the onset of rigor (Jaime *et al*. 1992; Jacob *et al*. 2012).

A range of different refrigeration methods can been used to achieve the cooling rate required for VFC. Irrespective of the method used, meat thickness should be less than 80 mm because of the low heat conductivity characteristics of meat (van Moeseke *et al*. 2001). For this reason, VFC would likely need to be combined with some form of hot boning in the beef scenario, to overcome the differences between cuts due to size and shape that are critical to cooling rate. There is a risk of inducing severe cold shortening and poor retail colour stability if the required temperature profile is not achieved (Jacob and Thomson 2012), and VFC has yet to be commercialised with pre-rigor meat.

Summary of solutions

A range of methods are available during the ante mortem as well as post mortem periods to control temperature during the post mortem period, and a combination of these could well be the best strategy. For example, accelerated chilling regimes can only commence 1 h post mortem at which time protein denaturation may have already started (Long and Tarrant 1990; Milligan *et al*. 1998). Li *et al*. (2006) confirmed that a chilling regime of −12°C did not change the loin temperature 1 h after slaughter for bulls that had a mean liveweight of 527 kg. Nonetheless they found an improvement in water-holding capacity due to rapid chilling in these animals indicating some decrease in the extent of protein denaturation. Long and Tarrant (1990) showed the effects of ante mortem showering and post mortem accelerated chilling were greater when combined than the sum of their individual effects applied alone. Milligan *et al*. (1998) found that hot fat trimming was much more effective at reducing ham temperature when combined with an accelerated chilling regime compared with a conventional chilling regime.

Some potential solutions such as hot boning might have a larger effect on carcass temperature than others such as better control of ante mortem body temperature for example. However, integration of a range of techniques in an overall strategy is likely to still be important. Furthermore, some measures such as hot boning may reduce the quality of cuts not susceptible to HTLP, unless combined with other techniques such as pre-rigor stretching technology in the form of SmartStretch (Taylor and Hopkins 2011), or done on part of the carcass only (Sammel *et al*. 2002).

Changing cooling systems in established meat processing plants may involve considerable capital costs that impede adoption of new cooling systems even when running costs

of the new system are lower than the existing system (Troy and Joseph 2001). Adapting existing cooling systems using combinations of different solutions therefore needs consideration to reduce the capital cost required to achieve the necessary outcome. However, some solutions such as vascular infusion and management of animals during lairage may require relatively little capital outlay. Importantly the market destination may also influence the reason for controlling HTLP conditions.

Conclusions

HTLP is an ephemeral event that occurs in the deep muscles of large carcasses, early in the post slaughter period. Temperature has a preeminent role in HTLP because it affects the rate of pH change with time, as well as interacting with pH to change protein functionality. Manipulating the rate of temperature change is a way of avoiding HTLP, but the success of intervention strategies is time critical and they must be effective during the first 5 h post mortem. A range of options are available to manipulate body temperature post mortem, but further development for the commercial application of many of these is needed in relation to the prevention of HTLP.

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