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Overview

Muscle metabolism in sheep and cattle in relation to high rigor temperature – overview and perspective

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Abstract. An increasing number of Australian slaughter plants were found not to meet the Meat Standards Australia (MSA) pH–temperature window, due to high rigor temperatures, particularly at plants where grain-fed animals were slaughtered. Hence, the red meat processing industry in Australia supported a research program focused on resolving this issue, as carcasses that do not meet the MSA pH–temperature window are excluded from MSA grading. This special issue of *Animal Production Science* describes the outcomes of a major program identifying ante- and post-mortem factors related to heat-induced toughening in both beef and sheep meat through literature reviews and targeted research to find interventions to prevent the impact of high rigor temperature on meat quality, particularly tenderness. This paper provides an overview of the outcomes of the research program, some of which require further research before implementation. It is suggested that an entire supply-chain approach be applied to establish the most efficient and cost-effective way of reducing the incidence of high rigor temperature.

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Introduction

Factors leading to the variation in metabolism during the conversion of muscle to meat have consequences for initial and final tenderness, colour stability and water-holding capacity (WHC) (Savell *et al.* 2005). These attributes have a major impact on consumer satisfaction either at the time of purchase or during consumption. Factors such as WHC pose problems at both processing and retail level, due to water lost at boning or as purge in both vacuum-bags and retail packaging.

This special issue focuses on muscle metabolism in cattle and sheep and, more specifically, on the fact that an increasing number of beef carcasses were found to have high rigor temperature (defined as carcasses temperature >35°C at the point at which the loin pH reached 6.0) and thereby missed the Meat Standards Australia (MSA) prescribed pH–temperature window (Thompson 2002). In 2006, the proportion of beef carcasses with high rigor temperature was established in 2006 to be as high as 75% across seven abattoirs, ranging from 56% to 94% (Warner *et al*. 2014*a*). The current awareness of the importance of rigor conditions in the Australian beef and sheep meat industry is a likely outcome of the scientific results that led to the criteria used in the MSA cuts-based grading system for beef, in which an ideal pH–temperature window was deemed important. The problem with high rigor temperature has also been experienced in other parts of the world as witnessed by earlier and recent studies (Hunt and Hedrick 1977; Sammel *et al*. 2002; Seyfert *et al*. 2004). However, most of this work has focused on problems with colour, shelf life and consistency. Changes in the industry over the last decade have probably increased the incidence of high pre-rigor temperature in Australian beef. These changes include an increasing number of cattle being finished in feedlots, increased slaughter weights and increased electrical inputs in Australian abattoirs.

Australian feedlots first emerged in the 1960s as a way of utilising surplus grain supplies from summer and winter crops, and feedlotting was also used to mitigate the effect of drought and aid export to Japan until the Japanese market closed in 1974 (Chappell 1993). The liberalisation of the Japanese beef market in 1988 following the New Beef Access Agreement between Australia and Japan resulted in the rapid expansion of Australia's feedlot sector with ~375 000 cattle held in feedlots in 1996 at any one time; this had increased to almost 800 000 cattle in 2012 (Australian Lot Feeders' Association 2013). Simultaneously, average slaughter weights have increased from 180 kg/head in 1983–84, 219 kg/head in 1993–94 and 232 kg/head in 2003–04 (Department of Agriculture, Fisheries and Forestry 2005) to reach 287 kg/head in 2011 (MLA 2013). Finally, the amount of electrical inputs has also increased over the years. When electrical stimulation was introduced, it would have been the only electrical input on the slaughter floor. However, today, electrical inputs have increased and can occur at the immobiliser, bleed rail and the hide puller (Warner *et al*. 2014*c*). Days in a feedlot, heavier carcass weights and increased electrical inputs were indeed found to increase the incidence of high rigor temperature (Warner *et al*. 2014*a*).

The research discussed in this special issue of *Animal Production Science* focuses on the higher end of the MSA pH–temperature window, namely high temperatures coupled with a fast pH decline leading to an early onset of rigor. As most of the focus of the studies is on meat tenderness, this overview paper also focuses on tenderness.

Definition and general objectives of the studies

High rigor temperature carcasses in the context of the MSA pH–temperature window are those with a temperature >35°C and a pH <6 (Thompson 2002). In some, but not all, cases, muscle under these conditions may toughen due to shortening (called 'heat rigor' or 'rigor shortening'), but more likely, the muscle will fail to tenderise due to changes in protein functionality even if the meat is initially tender. This condition has generally been referred to as heat toughening, although a more precise description might be 'reduced tenderisation at elevated temperature' or potentially 'heat-induced toughening' as explained by Kim *et al*. (2014*b*). Typically, contractile proteins (myosin) will denature under these conditions, but only until the onset of rigor mortis, and this will have negative effects on WHC and colour stability as well as muscle texture. Of more importance to ageing potential and final tenderness is the inactivation of calpains due to the greater autolysis that occurs even post-rigor, as these proteins are not protected after entering into rigor, unlike myosin. The denaturation of myosin was shown by Offer (1991) to induce shortening of the myosin head and reduction in filament space, which forces water from the muscle cells into the extracellular space and causes a reduction in WHC. Poor WHC is of economic importance as it increases drip loss and purge, leading to excessive carcass weight loss and reduced yield and quality of fresh and processed meat (Savage *et al*. 1990; Wright *et al*. 2005). Poor WHC in beef may negatively affect consumer perception of juiciness, as was reported for high rigor temperature carcasses by Warner *et al*. (2014*c*). Pearce *et al*. (2011) also suggested that poor WHC could negatively affect the perception of tenderness, although it was emphasised that the interaction between the changes during cooking and the exact location of water within the muscle structure and the combined effect on quality may require further investigation before final conclusions on WHC and its impact on eating quality can be made.

The objectives of the studies included in this special issue were therefore to identify ante- and post-mortem factors related to heat-induced toughening through literature reviews and targeted research to find interventions to prevent or manage the impact of these factors on meat quality, in this case, tenderness, colour stability and WHC. In the review by Ferguson and Gerrard (2014) the authors emphasise that post-mortem energy metabolism plays an important role when muscle changes to meat and that this crucially impacts on the quality of the final product. They also rightfully acknowledge that although models of anaerobic metabolism in living muscle are used to understand transitions in this type of metabolism post mortem, there are gaps in our knowledge, more so for ruminants than for pigs. Furthermore, the effects of pre- and post-slaughter factors on the relevant enzyme actions are poorly understood.

Pre-slaughter interventions

From the review of Jacob and Hopkins (2014) on industry solutions to high rigor temperature, it seems that pre-slaughter management of both initial temperature and pH decline is difficult and is mostly a function of specific production systems and procedures. This was confirmed by Warner *et al*. (2014*a*), who conclude that carcass weights, fatness and electrical inputs are the most important culprits in the incidence of high rigor temperatures in beef slaughtered across Australia. Efficient chilling of carcasses in general and in particular of certain bulky muscle groups that are probably more prone to high rigor temperatures (buttock primals) under conventional chilling and cold deboning conditions will be a function of size and fatness of the carcass, which in turn is fixed/a given for certain production systems or markets. A worldwide push for more efficient production to feed the increasing world population has placed upward pressure on carcass size, which has been achieved by combined efforts of improved genetics, efficient feeding and utilisation of growth promoters (Capper 2011). In line with this, average slaughter weights in Australia increased from 180 kg/carcass in 1983–84 to 287 kg/carcass in 2011 (Department of Agriculture, Fisheries and Forestry 2005; MLA 2013), and Japanese feeder steers often weigh up to 450 kg, mostly from late-maturing breed types. Along with these changes in production systems, factors such as weather extremes, heat stress and illness may place an additional burden on initial carcass temperature and subsequent chilling rate.

In their review, DiGiacomo *et al*. (2014) add an interesting perspective to the fact that in production systems based on high-energy feeds resulting in heavy carcass weights and increased fat levels, carcass temperature is further challenged by insulin resistance and associated factors, and they hypothesise that this could lead to decreased heat tolerance and subsequent increased carcass temperatures at slaughter. This hypothesis is supported by findings that feedlot-finished cattle (150 and 300 days) had significantly higher body temperatures at slaughter than grass-finished cattle (Jacob *et al*. 2014*b*). It should be noted that the carcass weight of the feedlot cattle (401 kg for 150 days and 438 kg for 300 days feedlot finishing) was significantly higher than the grass-finished cattle (301 kg). The carcasses of the feedlot-finished cattle also fell outside the MSA pH–temperature window with loin temperatures >40°C when pH 6 was reached, increasing the risk of heat-induced toughening. Unfortunately, tenderness was not measured in this preliminary study. By contrast, Warner *et al*. (2014*a*) showed that for carcasses from grain-fed cattle the temperature at pH 6 showed no, or a poor, relationship with carcass fatness (P8 fat thickness), while a linear relationship for these parameters was reported for grass-fed cattle. Although most work on insulin resistance and associated abnormalities has been performed on mice and humans, similar abnormalities in body functions are reported for cattle under similar challenges to those that affect diabetics, as discussed by DiGiacomo *et al*. (2014). Warner *et al*. (2014*a*) confirm that insulin resistance is a function of duration of high-energy feeding. Insulin levels increased from normal levels (associated with grass-fed cattle) to a maximum at 250 days on grain. Increased age, increased weight and fatness conditions occurring under modern grain-fed systems are likely to raise blood glucose, insulin and leptin levels, causing insulin resistance over time. This condition, in turn, could lead to a thinner skin and reduced blood flow to the skin, which would impair heat dissipation through evaporation under challenging environmental conditions. In addition, since leptin levels are positively related to adipose tissue levels, an additional heat stress burden is placed on fat cattle due to the thermogenic properties of leptin (DiGiacomo *et al*. 2014). It is postulated that this effect could be exacerbated with heat production due to increased activity, stress and the associated catecholamines during transport and slaughter.

Options for pre-slaughter interventions to control initial temperature and subsequent chilling seem limited in the sense that carcass size and fatness are a function of the production system and current demand, while weather conditions, and seasons and places of extreme heat, cannot be regulated. Provision of shade, showering of animals, especially under heat-stress conditions selection of heat-tolerant breeds or types, and the provision of less feed before slaughter may all contribute to normal body temperatures at slaughter. In addition, DiGiacomo *et al*. (2014) refer to dietary interventions to reduce heat-induced toughening, with particular emphasis on the potential of betaine (trimethylglycine). Although most studies on betaine as a nutritional supplement have been carried out on pigs and poultry for the purpose of reducing heat stress and improving growth performance, reports for beef have shown that betaine was effective in lowering the incidence of high rigor temperature carcasses (Loxton *et al*. 2007), reducing rectal temperatures under both thermo-neutral and heat-challenging conditions in sheep (DiGiacomo 2011), and reducing heart and respiration rates in sheep (DiGiacomo *et al.* 2012). In addition, Wang *et al*. (2010*a*) showed that betaine was effective in reducing insulin resistance by decreasing non-esterified fatty acids (NEFA) in dairy cattle; further support for this effect in mice and humans was reported by Wang *et al*. (2010*b*) and Borgschulte *et al*. (2008). Various mechanisms of the involvement of betaine in thermoprotection and insulin resistance are discussed by DiGiacomo *et al*. (2014). Betaine has a sparing effect on methionine and acts as an osmoprotectant, thereby increasing feed efficiency, lowering maintenance energy (and probably basal metabolism) and protecting cells during thermal extremes. Furthermore, improvement of gut mucosal integrity is mentioned as a mechanism against the manifestations of heat stress. Amelioration of insulin resistance by betaine supplementation may indirectly decrease heat stress and therefore potentially the incidence of high rigor temperature. Mechanisms in this regard include lowering of NEFA, normalisation of pathways involved in gluconeogenesis and improved adipose tissue function. In view of this evidence, investigation of the use of betaine supplementation for grain-fed cattle to manage heat stress and insulin resistance may be worthwhile.

Likewise, the utilisation of dietary thiazolidinedione (TZD), chromium, zinc and vanadium as anti-diabetic compounds for grain-fed cattle is also worth looking into, according to the review of DiGiacomo *et al*. (2014). Chromium is mentioned for its improvement in insulin sensitivity in ruminants (Hua *et al*. 2012). High levels of dietary zinc may be used to manipulate adipogenesis through its insulin-mimetic action, although its action in successfully addressing insulin resistance needs to be investigated further. Likewise, vanadium is suggested as an active anti-diabetic agent in livestock based on successes with humans, although toxicity may be a concern. Smith *et al*. (2007) reported successful attempts with TZD to lower NEFA in dairy cattle. However, at the killing box, even without pre-slaughter extremes, a certain carcass weight and fatness remain a challenge that can only be addressed after killing due to the combination of low thermal conductivity of meat, the specific shape of the carcass and heat generation even after slaughter (Jacob *et al*. 2014*a*).

DiGiacomo *et al*. (2014) also refers to the role of specific heat-shock proteins involved in protection of cell function and integrity under heat-stress conditions. A negative relationship between small heat-shock proteins (sHSP, specifically sHSP70) and insulin resistance was reported for humans (Chung *et al*. 2008), while Moran *et al*. (2006) also reported malfunctioning of sHSPs in heat-intolerant subjects, which led to a lack of protection against protein denaturation. There is therefore potential that sHSPs could be used in the selection of heat-tolerant animals or as a parameter to ascertain whether animals have adapted to heat extremes. In addition, Bernard *et al*. (2007) indicated that sHSP27 was positively related to improved tenderness, flavour and juiciness of meat, which may or may not relate to heat tolerance or other stressors involved in meat quality expression. This study is supported by findings confirming the relationship between two gene families (heat-shock proteins and energy metabolism) and beef quality, although the markers found for beef quality could not be universally extrapolated (Hocquette *et al*. 2012). The interested reader is directed to a recent review on the role of sHSP in meat tenderness (Lomiwes et al. 2014), which gives an overview of the basic structure and function of sHSP and their possible role in post-mortem muscle and hence effects on meat quality.

While the challenges discussed relate to increased carcass temperature and the problems it poses to effective chilling, many of these factors also contribute to accelerated rate of glycolysis, further exacerbating the condition of high rigor temperature. DiGiacomo *et al*. (2014) refers to the work of Young (1990) explaining that the Q10 effect of elevated muscle temperatures on key enzymes involved in muscle metabolism. A Q10 value represents the amount of increase in enzyme reaction rate with each 1°C in muscle temperature, which relates to a 15–20% increase in activity per 1°C. According to Marsh (1954), there is an exponential increase in Q10 at the upper end of the temperature scale (in muscle) since the Q10 values for the temperature range 37−43°C are double that of the range 34−37°C. Pighin *et al*. (2014) suggest that although pre-slaughter stress or exercise may increase body temperature, it normally shows a negative relationship with muscle glycogen. Therefore, DFD (dark, firm, dry) meat caused by low pre-slaughter glycogen levels may more likely be a problem in stressed animals than high rigor temperatures, although the study did not report on the latter. However, Ferguson and Gerrard (2014) pointed out that type and duration of stressors will determine whether glycogen levels and, more important to this discussion, glycolytic rate will be affected. Fasting will deplete glycogen, but mixing of bulls before slaughter will increase glycogenolytic rate and hence be more likely to cause high rigor temperatures (Tarrant 1989). Emotional stress pre-slaughter resulting in an adrenal response, even naturally experienced, together with moderate activity, causes elevation of cAMP, one of the main drivers of glycolysis (Ferguson and Gerrard 2014). Obviously high fat levels and muscle mass (large carcasses) will then further exacerbate the effect on rate of glycogenolyses. Although level of pre-slaughter glycogen relates to ultimate pH (completion of glycolysis), there seems to be no consensus that high glycogen level pre-slaughter will increase the rate of glycolysis. High levels of glycogen elevate the active form of glycogen phosphorylase, the initiator of glycolysis, yet the work of Daly *et al*. (2006) suggested that differences even at low glycogen levels would lead to differences in pH decline.

Post-slaughter interventions

Heat dissipation

Efficient chilling of deep muscle in order to keep within the limits of the MSA pH–temperature window remains a challenge. While the flatter shaped, superficial muscles such as the loin are less difficult to chill to acceptable temperatures in the shortest time, larger and deeper muscles pose a problem for any technology implemented to dissipate heat (Stolowski *et al*. 2006). In addition, Tarrant and Mothersill (1977) emphasised that huge differences in glycolysis rate may occur within large muscles, depending on temperature differences at various depths. In the paper of Jacob and Hopkins (2014) various alternative chilling methods for intact carcasses are discussed besides conventional chilling, where wind speed, humidity and ambient temperature are optimised but with limited success. Such methods include spray chilling, blast chilling, immersion cooling and very fast chilling. However, although the temperature gradient between the muscle core and surface is increased by these methods, carcass size, fatness and specific muscle depth still limit their efficacy. Even so, considering the fact that the time muscle spends at temperatures >35°C and pH <6 has a negative effect on tenderness development (Thomson *et al.* 2008), it seems that faster chilling will be advantageous for any muscle within certain limits of pH decline, hence the search for more effective methods. Jacob and Hopkins (2014) confirmed that the first 5 h during which muscle conditions are above the MSA pH–temperature window are the most damaging to muscle physiology related to tenderness.

Heat pipes as a method to increase conductivity or increase the heat gradient were investigated by Jacob *et al*. (2014*a*). This method is based on the utilisation of latent heat (from the deep muscle) to vaporise a fluid in an evacuated pipe that has been driven into the core of the deeper muscles. Heat dissipation takes place when the liquid phase is turned into the gas phase, and since the pipe is placed in a certain manner the gas phase moves to the end exposed to cold air in the chiller, turns into liquid again and runs back into warmer part inside the muscle. Because the latent heat of vaporisation is greater than the heat capacity of a fluid or solid, this method works better than a solid conductor or water (Jacob *et al*. 2014*a*). While this method effectively reduced the time taken for the deeper part of a large muscle group to reach the critical temperature of 35°C, low heat conductivity of muscle necessitates placement of pipes fairly close to each other. More pipes may cause physical damage to muscles and/or become expensive and labour-intensive. As meat and eating quality measurements were not included in the study, the potential advantage of accelerated chilling with heat pipes has not been established and further investigation to establish this is required to determine whether the technique might represent a commercial solution to mitigate heat-induced toughening. The study of Jacob *et al*. (2014*a*) also showed that loin muscle would benefit more from methods that dissipate heat from the muscle surface, such as fat trimming or spray chilling, rather than by heat pipes, due to the flat shape, thickness and location of the muscle.

Application of electrical stimulation, and contradictory results

In most discussions on effects of high rigor temperature conditions, application of electrical stimulation (ES), or for that matter any electrical input, comes under scrutiny. The dangers of uncontrolled electrical inputs in modern slaughterhouses and suggestions for alternatives or moderation of electrical inputs have previously been discussed (Simmons *et al*. 2006, 2008). Further, the importance of keeping within the ideal pH–temperature window (Thompson 2002) as prescribed by MSA had the application of ES questioned by some under certain circumstances. However, several studies (Hopkins *et al*. 2007, 2014; Warner *et al.* 2014*a*, 2014*c*) have shown that the majority of heavier feedlot carcasses may overstep the ideal window due to slow temperature decline and/or too fast a decline in pH even without stimulation. It seems, therefore, that these three factors (ES, weight and fatness) contribute most to conditions favouring high rigor temperatures and thus potential tenderness and other quality problems, but singly they are unlikely to be the sole reasons for the occurrence of such conditions.

The seemingly simple solution to solving the problem of larger (or smaller) feedlot cattle that are slaughtered and deboned conventionally is to limit or control electrical inputs. However, Simmons *et al*. (2006) suggested that this might be a complicated procedure, because not all carcasses have the same specifications with regard to their reaction to electrical impulses. It should also be noted that ES advances pH decline (glycolysis) not only during the application but also after the application has ceased, as illustrated in Ferguson and Gerrard (2014), and that the effect of stimulation depends on the type and duration of the application. Intrinsic factors such as muscle type and glycogen level pre-slaughter may also affect pH decline rate. Although Ferguson and Gerrard (2014) agree that altered metabolism post mortem is profoundly a function of physiological changes that occur in tissue pre-slaughter, they are convinced that further investigation of the association between pre-slaughter glycogen concentration and post-mortem glycolytic rate is warranted given contrasting results in various studies on this topic. They are further convinced that data from other studies show that factors other than pre-slaughter glycogen levels are more likely to determine the rate and extent of post-mortem pH decline.

Muscle fibre type can affect glycolytic rate. The fibre type composition is a function of species, genotype and specific muscle but can also be modified by certain growth promoters, such as beta agonists. Subsequent ageing because of proteolytic enzymes is also implicated with fibre type and will be discussed later. Surprisingly, white fibres that are expected to show higher rates of pH decline responded with greater declines during stimulation but slower declines after stimulation than type I red fibres according to Devine *et al*. (1984), probably due to higher buffering capacity (Aalhus and Price 1991; Talmant *et al*.

1986). Of more importance to this discussion is the lack of consistent results with regard to tenderness when the MSA pH–temperature window is missed due to rapid pH decline and/or slow chilling rates. While Hopkins *et al*. (2014) showed consistently similar sensory tenderness results for beef loin falling within or without the ideal MSA pH–temperature window, Warner *et al*. (2014*c*), using the extensive consumer data of Watson *et al*. (2008), reported initial preferences for high rigor temperature cuts, but these were soon outperformed in MQ4 scores by ones within the ideal pH–temperature window when ageing continued beyond 14 days. At the latter point, high rigor temperature cuts reached their maximum tenderness level. In the study of Hopkins *et al*. (2014), tenderness values of high rigor temperature cuts created by ES ran parallel to those of cuts that were within the pH–temperature window (non-stimulated carcasses, NES) from slaughter to 14 days, and differentiation after that was probably not likely to happen with ageing taking place at 0−1°C, although this was not confirmed. Furthermore, ES muscles recorded no initial advantage (1 day) as would have been expected (Thomson *et al*. 2008; Kim *et al*. 2012; Warner *et al*. 2014*a*).

Warner *et al*. (2014*c*) indicated a muscle effect in that rump (*m. gluteus*) of normal pH muscle was always preferred by consumers irrespective of ageing. The study did not indicate the reasons leading to a proportion of carcasses with high rigor temperatures, but it can be assumed that a combination of carcass weight, fatness, ES and chilling conditions would have been involved (Watson *et al*. 2008). While some NES sides in the study of Hopkins *et al*. (2014) were also exposed to high rigor temperature conditions, all stimulated sides missed the ideal window of pH 6 at a loin temperature <35°C. After the work of Rosenvold *et al*. (2008) and Rosenvold and Wiklund 2011), a protecting effect of ES against denaturation of proteolytic enzymes and other proteins was proposed as a possible reason for the lack of negative effects of high rigor temperature conditions in the stimulated group. The reasoning behind this mechanism is that individual fibres go into rigor through their own time-course depending on initial glycogen level. Those that have reached rigor are no longer affected by low pH and high temperatures and, consequently, neither drip nor ageing potential should be affected since denaturation is then limited for both calpains and structural proteins (Rosenvold *et al*. 2008). The authors emphasised the fact that inefficient stimulation may lead to tougher meat and higher drip loss, probably through higher levels of protein denaturation as muscle spends more time in higher temperature zones during the course of rigor. Likewise, muscle of heavy carcasses that is not stimulated and chilled slowly pre-rigor will also be exposed to unfavourable conditions for a longer time, as also mentioned by Hopkins *et al*. (2014). Despite this convincing argument, it is difficult to understand why Warner *et al*. (2014*c*), supported by other studies (Devine *et al*. 1999; Hwang and Thompson 2001*a*, 2001*b*), reported a limited ageing potential for cuts above the MSA pH–temperature window.

Many of the controversies in this debate stem from methods used in the respective studies. Hwang *et al*. (2003) in their review on ES specifically refer to the problem of studying effects of rigor paths and conditions *in situ* as opposed to excised muscles that were restrained (or not) and held under specific controlled and/or constant conditions with regard to temperature (Rosenvold *et al*. 2008; Thomson *et al*. 2008; Kim *et al*. 2012), a point stressed by Hopkins *et al*. (2014). These conditions would differ from the steady temperature decline under conventional chilling conditions of intact carcasses. In the study of Rosenvold *et al*. (2008), for example, excised NES muscle spent longer at 35°C before the end of rigor mortis, which would have increased the likeliness of protein denaturation (Offer 1991). These conditions would differ from the steady temperature decline under conventional chilling conditions of intact carcasses such as in the studies of Warner *et al*. (2014*c*) and Hopkins *et al*. (2014). In the study by Warner *et al*. (2014*c*) the loin cuts showing heat-induced toughening would have entered rigor and completed rigor mortis at fairly high temperatures, but the duration of exposure to unfavourable pre-rigor conditions was not recorded, in contrast to the study of Hopkins *et al*. (2014). When considering figure 1 in the study of Hopkins *et al*. (2014) and the reasoning of Rosenvold *et al*. (2008), the ES muscles spent a fairly short period under less ideal conditions before reaching rigor mortis, which would have caused limited denaturation. However the same conditions may have applied to muscles in the study of Warner *et al*. (2014*c*) where toughening did occur at high rigor temperatures The question may be raised as to why toughening does or does not occur under similar conditions, and the only explanation is that different dynamics apply for different methods of investigation.

Stretching and heat-induced toughening

Based on consumer responses, Warner *et al*. (2014*c*) confirmed that tender stretching protects specific muscles from the negative effects of high rigor temperatures. Unfortunately, the number of carcasses tender-stretched was limited compared with samples in the normal pH–temperature range, but similar results were presented by Warner *et al*. (2014*b*) and Kim *et al*. (2014*a*) for sheep meat, while positive effects on WHC were also demonstrated in certain cases. The *m. rectus femoris* (RF) showed increased cooking losses (Warner *et al*. 2014*b*), whereas no effect was recorded for *m. longissimus lumborum* (LL), and a clear positive effect was apparent for *m. semimembranosus* (SM) (Kim *et al*. 2014*a*). The questions to be considered about stretching under high rigor temperatures are whether the stretching prevents toughening caused by heat rigor (shortening) or whether it improves tenderness by stretching itself, i.e. increasing the sarcomere length beyond its normal length. Dransfield *et al*. (1991) demonstrated that stretching increased tenderness in pork muscle but no further improvement was recorded because of ageing. Hopkins and Thompson (2001) reported higher MFI (myofibrillar fragmentation index) values in stretched beef, indicating potentially more myofibrillar fragmentation due to proteolysis, and they recorded an increase in tenderness of stretched muscle that was not attributed to stretching. However, the improvement in tenderness induced by ageing in non-stretched muscle was higher than in stretched muscle, whereas the overall effect on tenderness was higher altogether due to stretching. The first question extends to the debate whether the toughening mechanism of typical heat-induced toughening always includes sarcomere shortening. From the study of Warner *et al*. (2014*b*), it is not possible to tell whether sarcomere shortening caused toughening in high rigor temperature muscles, while the work of Kim *et al*. (2014*a*) showed shortening of the SM muscle under these conditions, but no consistent toughening (measured at day 1) as a result of shortening for the different rigor temperature treatments. The LL showed no effect, probably due to unintended stretching of the LL on the other side of the carcass.

Working on different muscles on sheep carcasses, Warner *et al*. (2014*b*) found no shortening effect due to high rigor temperatures for *m. gluteus medius* (GM), *m. semitendinosus* (ST) and SM cuts. The RF cuts shortened, probably due to the anatomical position and/or muscle-fibre type of this muscle but surprisingly also showed increased tenderness (lower Warner–Bratzler shear force) for non-stretched muscle. The conclusion was made that neither shortening nor increased water loss (for certain muscles) gave rise to toughness of the SM and GM. Therefore, protein denaturation most likely impaired the effect of calpains and caused lower initial and final tenderness in SM and GM cuts. Since these muscles are of a more glycolytic nature, they will be more prone to protein denaturation under unfavourable rigor conditions. Warner *et al*. (2014*c*) also concluded that the LL was more affected by high rigor temperatures than the GM (Rhee *et al*. 2004), since the former muscle had higher proteolytic activity, meaning enzymes activity will be exhausted earlier by unfavourable conditions. However, it should be noted that the chilling rate of the GM would be slower than that of the LL, especially in beef carcasses. In general, the explanation of decreased ageing ability associated with increased protein denaturation was also offered by other studies (Kim *et al*. 2014*a*; Warner *et al*. 2014*b*), rather than muscle shortening, at least for certain muscles. In all three studies (Kim *et al*. 2014*a*; Warner *et al*. 2014*b*, 2014*c*), measurements were conducted on muscle that underwent rigor *in situ*, therefore restrained. It is therefore expected that unrestrained muscle will be more prone to heat shortening under high rigor conditions and thereby contribute to toughness. However, studies by Kim *et al*. (2012) and Thomson *et al*. (2008) support the results of the present series of studies that toughening as a result of high rigor temperatures is rather attributed to impairment of ageing potential due to denaturation proteolytic enzymes than to muscle shortening. Warner *et al*. (2014*a*) mention a very likely explanation, i.e. rigor shortening as opposed to cold shortening takes place at the onset of rigor mortis so that the start of shortening and loss of extensibility are close together (Honikel *et al*. 1983); thus, the formation of irreversible actomyosin bonds at rigor onset most likely prevents sarcomere shortening. It is therefore safe to conclude that stretching prevents an adverse effect of heat-toughening conditions on tenderness by limiting the importance of post-mortem proteolysis for tenderness development after Kim *et al*. (2014*a*).

Hot boning

The option of hot boning together with various rapid-chilling methods as well as combining hot boning with different stretching methods as a viable method to overcome heat-induced toughening was outlined by Jacob and Hopkins (2014). This combination of methods obviously favours larger muscles of the hind-quarter such as the SM and GM, rather than the flat LL, based on the effects of mass and shape on thermal conductivity of the muscle. In addition to faster chilling, the rate of pH decline is also decreased, which will also contribute to alleviating or preventing the effects of heat-induced toughening on quality. Given these apparent advantages, the relatively poor uptake of hot boning should therefore be questioned. While Tarrant (1977) and Sammel *et al.* (2002) reported on the positive effects of hot boning on WHC and prevention of colour two-toning, negative factors are discussed by Jacob and Hopkins (2014). These include the development of brown colour in meat due to increased mitochondrial respiratory rates (Brown *et al*. 1988). In addition, the possibility of cold shortening should be recognised if shortening is not prevented by stretching or restraining of muscle, which in itself may be technical and costly. Finally, shape distortion and muscle separation that can occur because of hot boning may negatively influence consumer acceptability (Reichel *et al*. 1991), and stringent hygiene regulations enforced by authorities and difficulties with carcass grading before deboning (Murray 2001) could place a further impediment on the utilisation of hot boning. However, it is unknown how the above-listed quality implications of hot boning compare with those arising from high-temperature rigor. Further, it should be recognised that many New Zealand beef slaughter plants are using hot boning of all cattle classes to take advantage of the reduced energy cost associated with hot-boning and reduced space requirement, and achieve excellent quality on table cuts. It should be recognised that the New Zealand cattle being hot-boned are all grass-fed.

Conclusions and perspective

With the MSA system in place, which requires regular audits for abattoirs to maintain accreditation, an increasing number of abattoirs were found not to meet the MSA pH–temperature window, especially for plants that were processing grain-fed cattle. Indeed, when the incidence of high rigor temperature carcasses was established, it was found to be as high as 75%, and high rigor temperature was found to be associated with heat-induced toughening in most, but not all cases. The increased incidence of high rigor temperature is associated with changes in the system over recent years, such as substantially larger and fatter cattle that take longer to chill post-mortem, increased electrical inputs that increase the rate of the pH decline, and the change towards grain-finishing, which was hypothesised to induce insulin resistance and heat stress and was subsequently shown to increase the body temperature at the time of slaughter. Grain finishing in feedlots has primarily been adopted by the Australian beef industry to take advantage of access to high-value markets such as the attractive Japanese market, which can be achieved through this production system due to increased marbling and perceived improved meat quality. However, for grain-finished meat products to maintain their market value, it is of utmost importance that their eating quality is not questioned.

As shown in the body of research presented in this special issue of *Animal Production Science*, it may be possible to target several points along the supply chain to reduce the incidence of high rigor temperature carcasses. Pre-slaughter, there is evidence that feeding with supplements such as betaine may reduce the incidence of insulin resistance, which may in turn reduce the incidence of heat stress. This would reduce the occurrence of high temperature rigor, although it is currently unknown how effective a betaine supplement feed is in overcoming the problem of heat-induced toughening. Other pre-slaughter interventions are mostly management-related and often restricted by environmental challenges but could include minimum activity and handling pre-slaughter and provision of facilities that could alleviate environmental stressors such as high ambient temperatures and or/humidity. Post-slaughter, it was found that the electrical inputs contribute to the incidence of high rigor temperature, and it is considered that the duration and type of electrical inputs should also be considered so that their potential negative effects are limited. This is of particular importance where larger, fatter carcasses have become the norm and where grain feeding and associated insulin resistance potentially increase the incidence of high initial carcass temperatures. Electrical stimulation induces an immediate pH drop as well as a subsequent increased rate of glycolysis, and it could be argued that muscle is exposed to unfavourable conditions for shorter durations than non-stimulated carcasses chilling at a slower rate. However, there is no convincing evidence that such conditions favour quality outcomes, whereas controlling electrical inputs to maintain the preferred pH–temperature ratio still provides the best results.

Technologies to reduce carcass temperature faster were investigated using heat pipes. While the heat pipes were able to achieve faster chilling rates, the studies were preliminary and the practical implications such as related labour costs and potential damage to the meat cuts should be considered when this method is compared with other potential cooling interventions such as fat trimming, seaming of muscle groups before rigor or hot boning. For further information, the reader is referred to the review by Jacob and Hopkins (2014). For many of these interventions, the effects on meat quality have not been investigated yet. While hot boning was more thoroughly studied in relation to meat quality in earlier research, it is criticised for potential negative effects on meat quality and strict process control is required. However, it is considered important to weigh up the disadvantages of hot boning, and possible methods to overcome these, with negative effects of high rigor temperature conditions that are probably more difficult to manage. Stretching of muscle seems to have beneficial effects under high rigor temperature conditions, more probably by improving tenderisation and/or stretching of the muscle rather than preventing heat-induced shortening. Its effectiveness is, however, dependent on muscle type and anatomical position, and its limitations will be a function of practicality in the abattoir set-up, labour cost and economy due to additional chiller-space requirements unless combined with hot boning. An entire supply-chain approach should be applied to establish the most efficient and cost-effective ways of reducing the incidence of high rigor temperature, whether this be a combination of any of the above approaches, or whether the most cost-effective way is to simply reduce the time that grain-finished cattle spend in feedlot to reduce their carcass weights and/or fat levels.

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