Genetic and environmental effects on the muscle structure response post-mortem

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Abstract

This paper reviewed the mechanisms by which glycolytic rate and pre-rigor stretching of muscle impact on meat quality. If muscle is free to shorten during the rigor process extremes in glycolytic rate can impact negatively on meat quality by inducing either cold or rigor shortening. Factors that contribute to variation in glycolytic rate include the glycogen concentration at slaughter and fibre type of the muscle. Glycolysis is highly sensitive to temperature, which is an important factor in heavy grain fed carcasses. An alternative solution to controlling glycolysis is to stretch the muscle pre-rigor so that it cannot shorten, thus providing an insurance against extremes in processing conditions. Results are presented which show a large reduction in variance (both additive and phenotypic) in tenderness caused by pre-rigor stretching. Whilst this did not impact on the heritability of shear force, it did reduce genotype differences. The implications of these results on the magnitude of genotype effects on tenderness is discussed.
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1. Introduction

Koohmaraie, Kent, Shackelford, Veiseth, and Wheeler (2002) proposed that the tenderness of muscle can be considered as a function of three main components; the connective tissue content/composition, sarcomere length and the extent of proteolysis of the myofibrillar proteins. The relative contribution of these three components to ultimate tenderness will vary with muscle, animal, pre- and post-slaughter factors and the length of time and temperature at which the product is stored post-mortem. The consensus of several reviews (e.g. Ouali, Demeyer, & Raichon, 1992; Sentandreu, Coulis, & Ouali, 2002) was that the amount and chemical composition of connective tissue was largely a function of the age of the animal at the time of slaughter and should be considered as 'background toughness'. Whilst Purslow (2005) has challenged this view in the post-genomic era, there are currently few treatments that allow the contribution of connective tissue to tenderness to be manipulated.

An optimal slaughter process could be considered as one that during the post-mortem period maximized the degree of proteolysis and/or minimized shortening, or even

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stretched the muscle, during the rigor process. Whilst there has been much documented about the mechanisms involved in proteolysis (Hopkins & Thompson, 2002) and the effect of minimizing shortening, or stretching muscle pre-rigor (Tornberg, 1996), there has been little discussion of the magnitude of either environmental and genetic manipulations on these processes. This paper will briefly review the mechanisms by which these two events impact on tenderness and examine the evidence of genetic or environmental effects on these processes. It is by understanding these interactions that progress will be made in the improvement of tenderness.

2. Early post-mortem metabolism

After death, the muscle continues to metabolise. Initially energy is supplied by ATP and creatine phosphate within the muscle. With the cessation of blood circulation at death the muscle quickly becomes anaerobic and the muscle glycogen is metabolized to replace ATP reserves. Circulatory failure means that waste products can no longer leave the tissue and the subsequent build up of lactate and the associated hydrogen ions gradually lower the pH of the muscle from neutrality to mildly acidic (Marsh, 1993). Glycolysis is eventually halted by either depletion of the substrate glycogen, or when the pH of the muscle becomes acidic enough to deactivate the enzymes associated with post-mortem glycolysis (Lawrie, 1992).

2.1. The onset of rigor

With the cessation of glycolysis, ATP reserves within the muscle are no longer replenished and as the muscle continues to metabolise these ATP reserves will be depleted. Rigor mortis in the muscle is defined as the stage when ATP supplies are depleted (Bendall, 1969). As discussed by Hwang, Devine, and Hopkins (2003) the onset of rigor does not occur simultaneously across all muscle fibres, but rather in individual fibres as they become depleted in ATP. This produces successive rigor contractions in individual fibres that gradually increase overall muscle stiffness (Honikel, Roncales, & Hamm, 1983; Jeacocke, 1984). As the number of fibres entering rigor increases the stiffness of the muscle as a whole increases and is significant when the muscle reaches a pH of *ca.* 6.

The temperature at which the muscle enters rigor will impact on the degree of muscle shortening. The classic study by Locker and Hagyard (1963) showed that minimal shortening occurred when muscle samples were held at approximately 15 °C. As discussed by Hwang et al. (2003), for temperatures above this optimum, fibre contracture occurs at rigor, whilst at temperatures below optimal the fibre contracture occurs before rigor. Thus, shortening above 15 °C is a consequence of rigor shortening and occurs when the muscle fibres are depleted of ATP, whereas at temperatures below 15 °C pre-rigor contracture takes place until rigor is completed.

2.2. Cold shortening

Cold shortening caused by pre-rigor contracture is driven by increased cellular calcium as falling temperatures cause increased inactivation of the ATP-driven calcium pumps of the sarcoplasmic reticulum (Bendall, 1978; Honikel & Hamm, 1978) and increased release of Ca^{2+} through leakage from the sarcoplasmic reticulum (Kanda, Pearson, & Merkel, 1977; Pearson, Carse, Davey, Locker, & Hagyard, 1973). The increased cellular calcium stimulates the binding between actin and myosin whilst activating the Ca^{2+}-dependant myosin ATPase, which hydrolyses ATP to energise the muscle contraction.

2.3. Rigor shortening

At the other extreme, muscles maintained at higher temperatures for long periods post-mortem tend to have greater glycolytic rates due to increased activity of glycolytic enzymes and, thus, a faster rate of pH decline. Accelerated pH decline combined with high muscle temperature can induce a form of shortening known as heat shortening or rigor shortening (Lee & Ashmore, 1985). At the higher muscle temperatures increased cellular calcium, induced by the onset of rigor, initiates muscle contraction in a similar fashion to that of cold shortening. However, mitochondrial release of calcium ions will not occur if ATP is still available (Mickelson, 1983) and therefore the contraction associated with heat shortening occurs at the onset of rigor rather than before (Hwang et al., 2003). Due to a lack of ATP to energise the contraction it is far less severe than that of cold shortening.

2.4. Post-mortem proteolytic activity

The activity of cysteine protease enzymes, in particular the calpains, has been shown to be sensitive to the prevailing pH and temperature of the meat (Dransfield, 1994b). Calpains are Ca^{2+} dependent proteases that specifically attack certain proteins of the Z-line and are considered primarily responsible for the degradative changes which occur during post-rigor conditioning at low temperatures (Gault, 1992). Calpains not only digest important structural proteins (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995) but also themselves and their inhibitor calpastatin and the extent of tenderisation is dependant upon net proteolysis resulting from the activity and inactivation of calpains (Tornberg, 1996).

It has been shown that the combination of low pH and high temperature conditions pre-rigor favour the autolysis and reduction of μ-calpains, which subsequently reduces ageing potential (Dransfield, 1994a; Ducastaing, Valin, Schollmeyer, & Cross, 1985; Geesink, van Laack, Barnier, & Smulders, 1994; Hwang & Thompson, 2001). When chilling was slow, the activity of μ-calpain and calpastatin decreased with a rapid pH decline, whereas when the chilling was rapid, the activity of the μ-calpain was largely

unaffected by rate of pH decline (Hwang & Thompson, 2001). Other work showed an increase in μ-calpain and calpastatin activities at 3 h post-mortem in rapidly glycolysing muscle (O'Halloran, Troy, Buckley, & Reville, 1997). A rapid glycolytic rate results in early activation of the calpain system, but there is a balance between the early activation of calpains and self destruction of these enzymes exacerbated by a rapid drop in pH at high temperatures (Hwang et al., 2003). Dransfield (1994a) modeled the temperature and pH dependence of inactivation of calpains and found an intermediate temperature range between 10 and 25 °C was preferential for calpain activity.

3. Glycolytic rate

3.1. The effect of environmental factors on glycolytic rate

3.1.1. Muscle glycogen concentration

Given the importance of rate of pH decline relative to temperature for meat quality, it is essential to understand factors that influence either variable. Rate of pH decline is largely reflective of glycolytic rate, which in the post-mortem scenario is traditionally thought to be driven by the rate of ATP hydrolysis via various muscle ATPase systems. However, one factor potentially limiting glycolytic rate is substrate (glucose 6-phosphate) availability associated with muscle glycogen concentration.

Work by Daly, Richards, Gibson, Gardner, and Thompson (2002) showed that higher initial muscle glycogen concentration resulted in a faster rate of pH decline, which was reflected in a higher temperature at rigor (i.e. temperature at pH 6) in beef *m. longissimus dorsi*. In their analyses carcass weight was included as a covariate to account for the impact of differing cooling rates on glycolysis.

3.1.2. Carcass weight

Carcass weight is another important determinant of glycolytic rate, largely due to the effect of temperature on glycolysis. This was demonstrated by Daly (2005) who measured pH/temperature declines in 761 commercial carcasses. Carcass weight had a linear effect on rate of temperature fall per hour, with every 20 kg increase in carcass weight reducing temperature fall per hour by approximately 0.05 °C. There was a linear relationship between carcass weight and temperature at pH 6, whereby increasing carcass weight resulting in an increase in temperature at pH 6 (a 20 kg increase in carcass weight increased temperature at pH 6 by 1 °C). This change in temperature at pH 6 was largely a function of the increased muscle temperature causing a faster rate of pH fall. When temperature fall per hour was included in the statistical model, the significance of the effect of carcass weight was weaker (the F ratio was reduced from 22 to 9) where a 20 kg increase in carcass weight produced only a 0.2 °C increase in temperature at pH 6.

This impact of carcass weight on cooling rate, and therefore glycolytic rate, can potentially be misconstrued as a metabolic effect. For example in the Australian long-fed cattle industry problems currently exist with rapid rates of pH decline and thus rigor shortening, despite the elimination of any electrical inputs during processing. This has led to one potential hypothesis – that long-fed cattle are metabolically pre-disposed to rapid rates of pH decline. An alternative hypothesis is proposed using results from a recent Beef CRC experiment (Gardner, G.E, unpublished data) which studied rates of pH decline post-mortem in three different muscles of genetically related animals. Slaughters were carried out at 9 and 36 months, with the latter animals slaughtered after approximately 200 days on feedlot rations. All carcasses were halved and one half exposed to high voltage electrical stimulation. Aligning with industry observations, the rates of pH decline in the older and larger animals were much faster for almost all muscles, whether stimulated or not. This translated into much higher temperatures at pH 6. For this data set it was difficult to incorporate a correction factor for cooling rate, given the massive differences between carcass weights. However, when mathematical adjustments were made to correct for the impact of temperature on glycolytic rate (Bendall, 1978), the data indicated no difference in the rates of pH decline in the older animals compared to the younger cohort. These results highlight the importance of temperature correction when assessing metabolic influences on rate of pH decline. They also indicate that attempts to rectify the rapid pH decline in the long fed cattle industry may not require a metabolic solution, and in fact may lie in something as simple as increasing temperature loss from the carcass during the development of rigor.

3.2. The effect of genotype on glycolytic rate

The effects of genotype on glycolytic rate are likely to be driven through muscle fibre type, given that the metabolic properties of muscle fibres strongly affect muscle metabolism in the living animal and post-mortem carcass. The effect of genotype on muscle fibre type has been demonstrated by the comparison of two lines of Charolais bulls obtained by divergent selection on a combination of growth rate and feed efficiency. The animals selected for high growth and feed efficiency demonstrated an increased lean to fat ratio that was also associated with lower intramuscular fat content (Renand et al., 1994), lower oxidative metabolism especially in oxidative muscles (Cassar-Malek et al., 2003), a greater number of fibres, a higher proportion of fast glycolytic fibres and a lower proportion of slow fibres (Duris, Renand, & Picard, 1999).

Results from a recent Beef CRC study (Gardner G.E., unpublished data) demonstrated the influence of fibre type on rates of pH decline in cattle. Using steers sired by Piedmontese, Angus or Waygu sires the results showed that the rate of pH decline was related to the proportion of type IIB fibres and the activity of lactate dehydrogenase within

the *mm. semimembranosus, semitendinosus* and *longissimus dorsi*. A similar response has been shown in the post-mortem muscle of lambs selected for increased muscle (Gardner, Pethick, Greenwood, & Hegarty, 2006). Proportionately more Type IIB fibres in a muscle was associated with faster rates of pH decline, although in the cattle data this was only in electrically stimulated (and therefore fast glycolysing) muscle tissue. These results suggest that the role of genotype must be considered in abattoirs looking to standardize rates of pH decline between genetically different lines of cattle.

4. Pre-rigor stretching of muscle

As already discussed, the degree of shortening in an unrestrained muscle is largely a function of temperature at rigor (Locker & Hagyard, 1963). *In situ* muscles are attached to the bones and the degree of shortening will depend upon the temperature and the tension that is placed on the muscle during rigor. Tenderstretching, or pelvic hanging, is a means by which the carcass is re-hung by the pelvis after slaughter and before rigor so most of the muscles of the hindquarter are either stretched, or prevented from shortening during the rigor process. This increased tension is aimed at either minimising shortening, or stretching the muscles with subsequent improvement in tenderness (Bouton, Carroll, Harris, & Shorthose, 1973; Hostetler, Link, Landmann, & Fitzhugh, 1972; O'Halloran, Ferguson, Perry, & Egan, 1998).

Whilst the process of pre-rigor stretching is a simple mechanical treatment, in that tension is placed on the muscle fibres to prevent shortening, the mechanisms by which stretching pre-rigor impacts on eating quality appear to be more complex. Early proposals centred on the degree of overlap of actin and myosin (Marsh & Carse, 1974), changes in gap filaments (Locker, 1982), in collagen orientation (Purslow, 1999; Rowe, 1974) or changed contributions from both myofibrils and connective tissue components (Bouton et al., 1973; Hostetler, Landmann, Link, & Fitzhugh, 1970). Hopkins and Thompson (2001) examined the energy required to dissociate the actomyosin complex in carcasses subjected to a range of stretching treatments pre-rigor and therefore different levels of actin/myosin overlap. They showed no relationship between the amount of energy to dissociate the actin/myosin complex and shear force, which suggested that mechanisms other than actin/myosin overlap were responsible for the improvement in palatability, with tenderstretching possibly associated with more rapid degradation of structural proteins at the junction of the Z disk and intermyofibre filaments.

A number of workers have shown that the magnitude of the effect of pre-rigor stretching treatments of muscle varied according to muscle and the temperature at which the samples were cooked. (Bouton et al., 1978, Bouton, Harris, & Shorthose, 1975; Eikelenboom, Barnier, Hoving-Bolink, Smulders, & Culioli, 1998; Moller, Kirkegaard, & Vestergaard, 1987). High connective tissue muscle, in both the raw state or when cooked to low internal temperatures, had a higher shear force for stretched samples than Achilles hung samples. In the experiment by Eikelenboom et al. (1998) it was not until cooking temperatures increased to 80 °C that tenderstretch samples had a lower shear force than Achilles hung samples. For low connective muscles such as the *m. longissimus dorsi* the magnitude of the interaction was not as clear, although the crossover temperature appeared to be lower.

Tornberg (1996) proposed a model to explain the interaction between stretched muscle and cooking temperature on tenderness, whereby in the raw state stretched muscle was tougher due to a smaller viscous component in the muscle structure. Upon heating to 60 °C or above, connective tissue began to contract which reversed this effect because of the smaller extracellular space in cooked stretched muscle which gave less room for connective tissue to contract without being restricted by the myofibrillar mass, hence, a more tender muscle. From this model, Tornberg (1996) proposed that the interaction between stretching and cooking temperature was likely to be greater in muscles with high connective tissue content.

4.1. Environmental effects on pre-rigor stretching

4.1.1. Rigor temperature

A number of studies have reported that the tenderstretch effect is variable and is largest under rapid chilling conditions (Hostetler, Carpenter, Smith, & Dutson, 1975; Sorheim et al., 2001), i.e. under the conditions where cold shortening is most likely to occur. Thompson (2002) reported taste panel results for cooked samples of the *m. longissimus dorsi* from 195 beef carcasses where one side had been tenderstretched and the other normally hung. In this data set tenderstretching reduced the variance in palatability scores by 25% compared to samples from the normally hung sides, which was in agreement with Sorheim et al. (2001). Of particular interest was the magnitude of the difference between stretched and unstretched sides, which varied from 0 to 40 taste panel units on a 100 point scale. In other words tenderstretch conveyed little or no advantage for some carcasses, whilst for others the gains were substantial. The greatest gains due to tenderstretching were in the carcasses with the poorest palatability scores in the normally hung sides.

The significance of this variable response to tenderstretching becomes clear when considering the results from Thompson et al. (2005). In this study palatability of several muscles was evaluated in tenderstretch and normally hung sheep and lamb carcasses. As shown in Fig. 1, in the normally hung carcasses there was a curvilinear relationship between tenderness score and rigor temperature (temperature at pH 6), whereby the highest tenderness score occurred at 21 °C. As discussed previously other workers have reported an optimal temperature at rigor of 15–18 °C (Devine et al., 1996; Locker & Hagyard, 1963). Clearly, in normally hung carcasses, extremes of both low
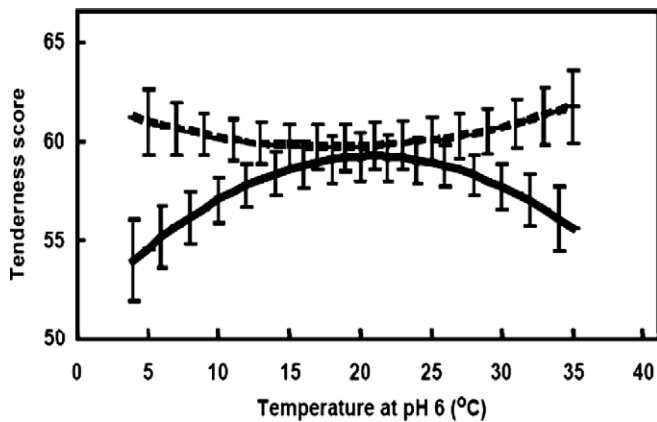
Fig. 1. The relationship between tenderness score as a function of temperature at pH 6 in sheep carcasses. The solid line represents the predicted response for achilles hung carcasses whilst the dashed line represents the predicted response for the tenderstretch carcasses. Data were adjusted for muscle, age category and post-mortem ageing time. pH and temperature were measured in the posterior portion of the *m. longissimus dorsi*. The vertical bars represent the standard errors of the predicted values. From Thompson et al. (2005).

and high temperatures at rigor are to be avoided for optimal eating quality. In contrast, in the tenderstretched carcasses there was no apparent relationship between tenderness score and rigor temperature. In other words tenderstretching provided insurance for palatability against processing carcasses at hot or cold rigor temperatures.

The other important message from Fig. 1 is that if carcasses were processed at an optimal rigor temperature then there was little difference between tenderstretch and normally hung carcasses. However, as discussed earlier in this paper, and by Thompson et al. (2005), the variability in rigor temperature due to carcass factors (whether the muscle is deep or superficial, its glycogen content, as well as carcass weight and fatness) and environmental effects (abattoir and day) means that under current processing conditions it is extremely difficult to achieve optimal processing conditions for all muscles and this underpins the potential of tenderstretch in the commercial environment.

4.2. Genetic effects on pre-rigor stretching

The literature on genetic parameters for meat quality traits shows considerable variation in the magnitude of the heritabilities across studies (see reviews by Marshall, 1999; Burrow, Moore, Johnston, Barendse, & Bindon, 2001). As discussed by Johnston, Reverter, Ferguson, Thompson, and Burrow (2003), confidence in many of these previously published estimates has been constrained by the low numbers of animals used and a concern that the post-slaughter conditions were not always controlled. Given that fatness and weight traits are moderately to highly heritable (Johnston et al., 2003), if carcasses are rapidly chilled without any control of glycolytic rate the leaner carcasses will chill more rapidly and could be exposed to cold shortening conditions. This may inflate the additive

genetic variance, not because the leaner sire groups were intrinsically tougher, but simply because their carcasses were processed under sub-optimal conditions.

Johnston et al. (2003) reported the genetic parameters for a large experiment in which post-mortem glycolysis was controlled using electrical stimulation. They showed that the additive variance and heritability estimates for meat quality of the *m. longissimus dorsi* were low for temperate breeds (h^2 for shear force and tenderness score of 0.09 and 0.18, respectively) whilst for tropically adapted breeds the estimates were moderate (h^2 of 0.30 and 0.31 for shear force and tenderness score, respectively).

As already discussed, tenderstretch has been shown to reduce the variance in shear force and sensory scores and is currently used as an industry tool to improve tenderness scores (Thompson, 2002). A large experiment was recently undertaken to investigate the effect of tenderstretch on the partitioning of variance for shear force and compression in *m. longissimus dorsi* samples from tropically adopted cattle (Burrow et al., 2003). Depending upon whether the reduction in variance was associated with the genetic or non-genetic components of tenderness the heritability of tenderness could be reduced or inflated for tenderstretch sides compared to normally hung sides.

Steers from Brahman and tropically adapted composite cattle genotypes were finished on grain and slaughtered at approximately 320 kg carcass weight. One side of the carcass was hung by the Achilles tendon whilst the other was tenderstretched and samples collected from the anterior end of the *m. longissimus dorsi* for meat quality evaluation. Preliminary results were reported by Burrow, Johnston, and Thompson (2006) and showed that for the normally hung sides the Brahman genotype had a higher shear force than the composites (Table 1). In the tenderstretched sides, shear force was less for both genotypes, and although genotype differences were still evident, they were less than for the normally hung sides. Tenderstret-

Table 1
Least squares means, additive genetic (σ_A^2) and phenotypic (σ_P^2) variance and heritabilities (h^2) for shear force and compression (both in Newtons) within Brahman and Tropical Composite steers

Breed/Trait	Shear force		Compression	
	AT	TS	AT	TS
Brahman	51.94	43.12	18.62	17.64
Composite	45.08	38.22	17.64	16.66
Sed	0.98	0.98	0.20	0.29
Brahman				
σ_A^2	3.63	0.78	0.98	0.98
σ_P^2	10.98	2.65	0.49	0.69
h^2	0.33	0.30	0.20	0.15
Composites				
σ_A^2	3.43	0.69	0.10	0.10
σ_P^2	10.68	2.25	0.49	0.39
h^2	0.32	0.30	0.20	0.27

AT, achilles hung; TS, tenderstretched (adapted from Burrow et al., 2006). Sed, standard error of the difference.

ching resulted in a 5-fold reduction in both the phenotypic and additive genetic variance for shear force in both the genotypes. The net result was little change in the heritabilities for shear force for both the normally hung and tenderstretched sides. The correlations between shear force in achilles hung and tenderstretch sides showed that the genetic correlations were higher than the phenotypic correlations, indicating that the relationship was strongest at the genetic level. Tenderstretching resulted in a small decrease in compression readings, although there was little impact on either the additive or phenotypic variance and consequently little change in heritabilities (Table 1). The relatively larger response in shear force compared with compression would suggest that most of the effect was via the myofibre axis rather than connective tissue.

From a practical point of view the heritability results indicated that genetic progress in shear force was possible if based on data from either tenderstretched or normally hung carcasses, although the smaller phenotypic and genetic variance in the tenderstretch sides would mean that progress would be very slow if selection of animals was based on meat quality measurements in the tenderstretched carcasses. Alternatively, the positive genetic correlations indicated that the progress resulting from selection for tenderness in normally hung sides will confer most of the advantage to carcasses that are tenderstretched. Given the effect of stretching on the magnitude of the genotype differences in shear force it would be interesting to investigate the effect of other post-mortem interventions, such as very high pressure or wrapping hot boned muscle pre-rigor, on the magnitude of genetic differences in tenderness. Techniques such as the Pivac (Troy, 2006) have the potential to further increase sarcomere length which may further reduce variance and potentially minimise genotype effects on tenderness.

5. Conclusion

This paper reviewed the mechanisms by which glycolytic rate and pre-rigor stretching of muscle impacted on tenderness. Glycolytic rate is a function of glycogen concentration with higher initial glycogen resulting in faster pH decline, independent of the temperature effect caused by heavier carcass weight. The very rapid decline in pH decline which is observed in long fed feedlot carcasses appeared to be explained by a simple temperature effect, rather than a metabolic deficiency as sometimes inferred. Genotypes with increased proportions of type IIB muscle fibres have a faster rate pH decline. Therefore, efforts to standardise glycolytic rate between carcasses may need to adjust for genotype in addition to other factors such as initial glycogen levels and the weight and fatness of the carcass. Alternatively, pre-rigor stretching of muscle can effectively provide an insurance against the effects of extremes in glycolytic rate. In addition, pre-rigor stretching of muscle cause a marked drop in both additive and phenotypic variance in shear force and, whilst heritabilities were not chan-

ged, genotype differences were reduced. The large reduction in variances (both additive and phenotypic) resulting from tenderstretching suggested that more extreme stretching of the muscle pre-rigor may potentially minimise genotype effects on tenderness.

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