Use of electrical stimulation and chilling to enhance meat tenderness of heavy lambs

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Pouliot, E., Gariépy, C., Thériault, M. and Castonguay, F. W. 2014. **Use of electrical stimulation and chilling to enhance meat tenderness of heavy lambs**. Can. J. Anim. Sci. **94**: 627–637. The aim of this study was to determine if electrical stimulation and chilling can modulate pre-rigor pH–temperature dynamics in order to enhance meat tenderness of heavy lambs produced in Quebec and determine if there is an optimum carcass temperature window to reach pH 6.0. A total of 128 heavy lambs (fasted body weight between 38 and 52 kg) were selected at the abattoir over 8 slaughter days (16 lambs d^{-1}) and assigned to four processing treatments in a 2×2 factorial design: electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling. Slow-chilled carcasses stayed warmer during the first 12 h postmortem ($P < 0.001$), but reached the same temperature as NC ones thereafter. They also had a lower pH between 2 and 12 h ($P < 0.05$). Stimulated carcasses had a lower pH than NES throughout the first 24 h postmortem ($P < 0.001$), while ultimate pH was similar ($P = 0.738$). Shear force values were improved ($P < 0.001$) by both ES and aging as expected, although chilling had no effect ($P = 0.400$). Stimulation × aging interactions for shear force values ($P = 0.019$) and myofibrillar fragmentation index ($P = 0.097$) indicate that aging began earlier following ES. Sarcomeres were longer for ES compared with NES carcasses ($P < 0.001$) indicating that the latter were subject to cold shortening. Meat from NES carcasses was more prone to be tough, although an important part of NES carcasses provided tender meat. This illustrates the importance of individual variations on tenderness and the multiplicity of factors involved in its development. No optimal window was observed for temperature at pH 6.0.

Key words: Chilling, electrical stimulation, lamb, meat, tenderness

Pouliot, E., Gariépy, C., Thériault, M. et Castonguay, F. W. 2014. **Utilisation de la stimulation électrique et du refroidissement pour améliorer la tendreté de la viande d'agneaux lourds**. Can. J. Anim. Sci. **94**: 627–637. L'objectif de cette étude était de déterminer si la stimulation électrique et le refroidissement peuvent permettre de moduler la dynamique de chute de pH et de température des carcasses afin d'améliorer la tendreté de la viande des agneaux lourds produits au Québec et déterminer s'il y a une fenêtre de température optimum dans laquelle les carcasses doivent atteindre le pH 6,0. Un total de 128 agneaux lourds (poids à jeun entre 38 et 52 kg) ont été sélectionnés à l'abattoir au cours de huit journées d'abattage (16 agneaux/jour) puis assignés à l'un des quatre traitements dans un plan factoriel 2×2 : stimulation électrique (ES – « electrical stimulation ») ou non (NES – « no electrical stimulation ») et refroidissement normal (NC – « normal chilling ») ou lent (SC – « slow chilling »). Les carcasses SC sont demeurées plus chaudes pendant les 12 premières heures postmortem ($P < 0{,}001$) puis ont atteint la même température que les carcasses NC. Elles ont également présenté un pH inférieur entre 2 et 12 h ($P < 0{,}05$). La stimulation électrique a engendré une chute plus rapide du pH durant les 24 premières heures ($P < 0{,}001$) postmortem, sans affecter le pH ultime ($P = 0{,}738$). Les forces de cisaillement ont été améliorées par la ES et la maturation ($P < 0{,}001$), tandis que la vitesse de refroidissement n'a eu aucun effet ($P = 0{,}400$). Les interactions stimulation × maturation pour la force de cisaillement ($P = 0{,}019$) et l'indice de fragmentation myofibrillaire ($P = 0{,}097$) indiquent que la maturation a débuté plus rapidement à la suite de la ES. Les sarcomères étaient plus longs pour les carcasses ES que les carcasses NES ($P < 0{,}001$), ce qui indique que les carcasses NES étaient sujettes au phénomène de contraction due au froid. La viande provenant des carcasses NES était plus susceptible d'être dure, bien qu'une proportion importante des carcasses NES ont produit une viande tendre, ce qui illustre l'importance de la variation individuelle sur la tendreté et la multitude de facteurs impliqués dans son développement. Aucune fenêtre optimale pour la température à pH 6,0 n'a été observée.

Mots clés: Refroidissement, stimulation électrique, agneau, viande, tendreté

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Abbreviations: **ES**, electrical stimulation; **LL**, longissimus lumborum; **LT**, longissimus thoracis; **MFI**, myofibrillar fragmentation index; **NC**, normal chilling; **NES**, no electrical stimulation; **SC**, slow chilling; **WBSF**, Warner–Bratzler shear force

Among all production factors, post-slaughter processes have the most important impact on lamb tenderness (Sañudo et al. 1998), with chilling and aging period being critical factors. In Quebec, most heavy lambs are slaughtered in multi-species abattoirs designed to chill beef, veal or pork carcasses (MAPAQ 2008). Due to their smaller size and their fibre type composition, ovine carcasses are susceptible to cold shortening and hence toughening of the meat in these conditions (Pearson and Young 1989). In fact, a recent study showed that meat tenderness from heavy lambs produced and processed in Quebec could be enhanced by electrical stimulation (ES), mostly through reduction in cold shortening (Pouliot et al. 2012a). Since lamb is perceived as a premium meat in Quebec and tenderness is one of the most important factors for consumer appreciation (Safari et al. 2002), established packing plant practices are needed to achieve optimal quality to meet consumer expectations and stay competitive.

Managing pH and temperature decline appear to be crucial control points, as these parameters affect cold and heat shortening (Honikel 2004), enzymatic activity (Dransfield et al. 1992; Uytterhaegen et al. 1992; Hwang and Thompson 2001a), protein denaturation (Penny 1969; Offer 1991) and hence meat quality. Temperature of lamb carcass at pH 6.0 appears to affect tenderness and eating quality and Thompson et al. (2005) suggested there was an optimal carcass temperature window to achieve pH 6.0. The Meat Standards Australia lamb and sheep meat program first targeted the 18–25°C window (Thompson et al. 2005; Toohey et al. 2008), which was subsequently increased to 18–35°C (Pearce et al. 2010).

Electrical stimulation of lamb carcasses is considered a postmortem processing procedure to attain the desired pH–temperature window. In addition to cold shortening reduction (Chrystall and Devine 1985; Devine et al. 2004), the benefits of ES on meat tenderness may be due to aging acceleration caused by faster ATP depletion, hastening rigor onset and allowing for tenderization to begin sooner and at higher temperature (Hwang et al. 2003; Simmons et al. 2008). It is well recognized that myofibrillar and cytoskeletal proteolysis occurs during aging and enhances meat tenderness (Koohmaraie 1994; Taylor et al. 1995; Hopkins and Taylor 2004), even if mechanisms are still under investigation. The calpain/calpastatin system plays a major role in the process (Dransfield 1993; Koohmaraie 1994; Koohmaraie and Geesink 2006), but other proteases (Ouali et al. 2006; Kemp and Parr 2012) or mechanisms (Wu and Smith 1987; Takahashi 1996) could be implicated. Faster proteolysis of cytoskeletal and myofibrillar proteins following ES have been reported (Uytterhaegen et al. 1992; Ho et al. 1996), as ES impacts on calpain/calpastatin system activities (Uytterhaegen et al. 1992; Lee et al. 2000; Hwang and Thompson 2001a).

However, there is extensive variation in ES response among studies, in part due to variability in ES protocols, pre-slaughter animal status and chilling conditions. Mechanisms by which ES enhances meat quality are not completely understood (Hwang et al. 2003). In this context, the aim of this study was to determine if electrical stimulation and chilling can modulate the prerigor pH–temperature dynamics in order to enhance meat tenderness of heavy lambs produced in Quebec and determine if there is an optimum carcass temperature window to reach pH 6.0.

MATERIALS AND METHODS

Treatments and Samplings

Between June and August 2010, 128 male market lambs were selected in a federally inspected abattoir over 8 slaughter days (16 lambs d^{-1}) based on weight (fasted body weight between 38 and 52 kg) and randomly assigned to one of four treatments (four lambs/treatment on each slaughter day) in a 2×2 factorial design:
NES_NC = no electrical stimulation (NES) and normal chilling (NC);
ES_NC = electrical stimulation (ES) and normal chilling (NC);
ES_SC = electrical stimulation (ES) and slow chilling (SC);
NES_SC = no electrical stimulation (NES) and slow chilling (SC).

Each ES carcass was hung by the hind legs and electrical stimulation was applied 5–10 min after slaughter for 30 s via a neck clip and a rectal probe using a commercial low-voltage system (21 V RMS; 0.25 A; 5-ms pulse width; Jarvis, Model ES-4, Middletown, CT). Both ES and NES carcasses were pelted, dressed, weighed and submitted to either a conventional (''normal'') or slow chilling process within 25 min post-mortem. The average room temperature for the NC treatment was 1.1 ± 1.4°C. For the SC treatment, carcasses were chilled for 3 h at 9.6 ± 1.0°C and they were then transferred in NC conditions, in an attempt to reach pH 6.0 between 18–25°C, as determined in preliminary tests. Temperature and pH decline were monitored along the left longissimus lumborum (LL) muscle at 0.75, 2, 3, 6, 12 and 24 h postslaughter. The pH meter (200 series pH meter, Beckman Instruments, Inc, Brea, CA) equipped with a puncture electrode (Mettler-Toledo LoT406-M6-DXK-S7/25, Mettler Toledo Ingold Inc., Bedford, MA) was calibrated at a near carcass temperature at every single time and a temperature compensation probe was used during measurements. Carcasses were graded according to standard commercial procedure (Agriculture and Agri-Food Canada 1992), based on visual assessment of shoulder, loin and leg conformation using a scale from 1 (deficient muscling) to 5 (excellent muscling) and ruler measurement of total tissue depth at the GR site (12th rib, 11 cm from the vertebral column). At 24 h postmortem, carcasses were cut into primal cuts (shoulder, loin, leg and flank). Left and right racks were separated from the front by a straight cut passing between the 6th and 7th ribs; from the short loin by a straight cut passing

behind (posterior to) the 13th rib and from the flank by a straight cut parallel to the backbone passing through the 13th rib, at the beginning of the costal cartilage. One-centimetre-thick slices were also collected at the caudal end of these racks for determination of myofibrillar fragmentation (MFI). Racks and slices were vacuum-packaged and assigned in balanced design to 3 or 8 d of aging at 4°C before being frozen at −30°C. Slices were assigned to the same aging time as the corresponding racks. Ultimate pH (pHu) was measured at 48 h on the left short loin (LL) maintained at 4°C. At the same time, a slice was taken and frozen at −20°C for sarcomere length analysis.

Laboratory Analyses

Color, Cooking Loss and Shear Force

Racks aged for 3 and 8 d were thawed at 4°C for 48 h. Color measurements (L^*, a^* and b^*) were taken in triplicate on longissimus thoracis (LT) muscle from a slice cut from thawed racks and exposed to air at 4°C for a 30-min oxygenation period with a colorimeter (Chroma Meter CR-300, Minolta Co., Ltd., Osaka, Japan) calibrated on a white tile. Racks were deboned and LT muscles were trimmed of fat and epimysium. The trimmed muscles were weighed and were then cooked in a convection oven (Market Forge 2600PHE, Market Forge Industries, Burlington, VT) to an internal temperature of 68°C as individually determined with thermocouples. Cooked LT sections were weighed to determine cooking loss. Warner–Bratzler shear force (WBSF) measurements were made on 1-cm^2 meat sticks following the procedure described by Pouliot et al. (2009) using a texturometer (TA-XT2i Texture Analyser, Stable Micro Systems, Godalming, UK).

Sarcomere Length

Sarcomere length was measured from meat slices aged for 48 h. Following homogenization of 2.5-g samples of muscle in 50 mL sucrose buffer (0.2 M) using a 40-s burst at 14 000 rpm (IKA T-18 Basic Ultra Turrax Homogenizer, IKA Works inc., Wilmington, NC), 25 images of myofibrils having at least 10 consecutive sarcomeres were taken for each sample with a phase contrast microscope (Nikon Eclipse TE2000-E, Nikon Canada Instuments inc., Missisauga, ON) equipped with a camera (ORCA-ER, Hamamatsu Photonics, Hamamatsu city, Japan). The average sarcomere length was determined using the open source image analysis software ImageJ (http://rsbweb.nih.gov/ij/).

Myofibrillar Fragmentation Index

Myofibrillar fragmentation index was determined on 1-cm-thick slices aged for 3 or 8 d using the approach described by Hopkins et al. (2000) based on Culler et al. (1978). A sub-sample of 160 slices was used (20 slices × 4 post-slaughter ES/chilling treatments × 2 aging times). Duplicate 2-g samples of muscle were homogenized on ice in 25 mL of buffer solution (KH_2PO_4 7 mM, K_2HPO_4 18 mM, KCl 0.1 M, EDTA 1 mM et NaN_3 1 mM) using two bursts of 30 s at 14 000 rpm (IKA T-18 Basic Ultra Turrax Homogenizer, IKA Works inc., Wilminaton, NC). Homogenates were filtered using a polyethylene strainer (1 mm^2). Myofibril suspensions were centrifuged (10 min, 2100 rpm, 2°C) and washed two times before being finally re-suspended in 40 mL cold buffer. Protein concentration was determined using the Biuret protein assay (Gornall et al. 1949) and samples were diluted to 0.5 mg mL^{-1} in triplicate. Absorption was measured at 540 nm using a spectrophotometer (Genesys 10 UV, Thermo Spectronic, Rochester, NY).

Statistical Analysis

Carcasses were randomly assigned to post-slaughter treatments (ES and chilling) in each of the eight blocks (days of slaughter) resulting in a generalized randomized complete block design. Out of the 128 carcasses, 21 were identified as DFD based on pHu higher than 5.8 (mean: 6.07; range: 5.86–6.65) and were removed from all analyses. The distribution of these high pHu carcasses was not different according to treatments or days of slaughter. The 5.8 threshold was chosen based on studies on lamb and beef quality (Purchas 1990; Watanabe et al. 1996; Geesink et al. 2001). Two NES carcasses were also excluded from the study due to abnormal pH decline. For the remaining 105 carcasses, the data were analyzed using the MIXED procedure of SAS software (SAS Institute Inc., Cary, NC). For temperature and pH decline, the model included ES and chilling treatments, postmortem time and interactions as fixed effects with hot carcass weight and GR as covariates. Slaughter day was included as a random effect. Observations of post-mortem time were considered as repeated measures. For color, cooking loss, shear force and MFI, ES and chilling treatments, aging time (3 or 8 d) and interactions were included in the model as fixed effects with hot carcass weight and GR as covariates. Slaughter day was included as a random effect. The effect of aging time was treated as a repeated measure since both racks of each carcass were allocated to aging times. For sarcomere length, ES and chilling treatments and interactions were included in the model as fixed effects with hot carcass weight and GR as covariates. Slaughter day was included as random effect. In all cases, non-significant interactions and covariates were sequentially excluded from the model. When significant interactions were found, the slice option of SAS was used to perform an analysis of simple effects.

Temperature at pH 6.0 was determined for each carcasses using Matlab software (MathWork, Natick, MA). A spline interpolation was used to estimate the time at pH 6.0 and then this time was used to estimate the temperature at pH 6.0 for each carcass. Carcasses were grouped in different classes according to their temperature at pH 6.0 for distribution determination: $T \leq 10°C$; $10°C < T < 35°C$ (with a subclass of $18°C < T < 25°C$)

and T $\geq$ 35°C. The relation between temperature at pH 6.0 (linear and quadratic) and shear force, sarcomere length and MFI was also tested using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with slaughter day included as random effect. Based on the graphical exploration of the data, a 5°C threshold was used to test the effect of carcasses reaching pH 6.0 below and over 5°C. Then, the effect of temperature at pH 6.0 (linear and quadratic) was tested on the subset reaching pH 6.0 over 5°C.

RESULTS AND DISCUSSION

Carcasses

Although there were some differences in carcass traits between slaughter dates, carcass traits did not differ among ES and chilling treatments either within a slaughter date or for the entire project ($n = 105$, $P > 0.5$, data not shown). Hot carcass weight, conformation and fat cover measured at the GR site were, respectively, 22.4 $\pm$ 1.9 kg, 3.3 $\pm$ 0.4 and 11.2 $\pm$ 3.0 mm (means $\pm$ SD).

Temperature and pH Decline

As expected, within chilling procedures temperature decline was the same for ES and NES carcasses ($P = 0.691$). Therefore, the data from ES and NES carcasses are combined in Fig. 1a. A time $\times$ chilling interaction was observed ($P < 0.001$) as slow-chilled carcasses were warmer during the first 12 h, but had similar temperatures to normally chilled carcasses thereafter.

Irrespective of treatment, an important proportion of carcasses (16.7%) had a pHu higher than 5.8 and were removed from pH decline analysis. Color differences in terms of L^*, a^* and b^* confirmed that this pHu was high enough to induce DFD-like defects (Pouliot et al. 2012b). The incidence of these high pHu carcasses was not related to post-slaughter treatments applied in this study, slaughter dates or transport distance (data not

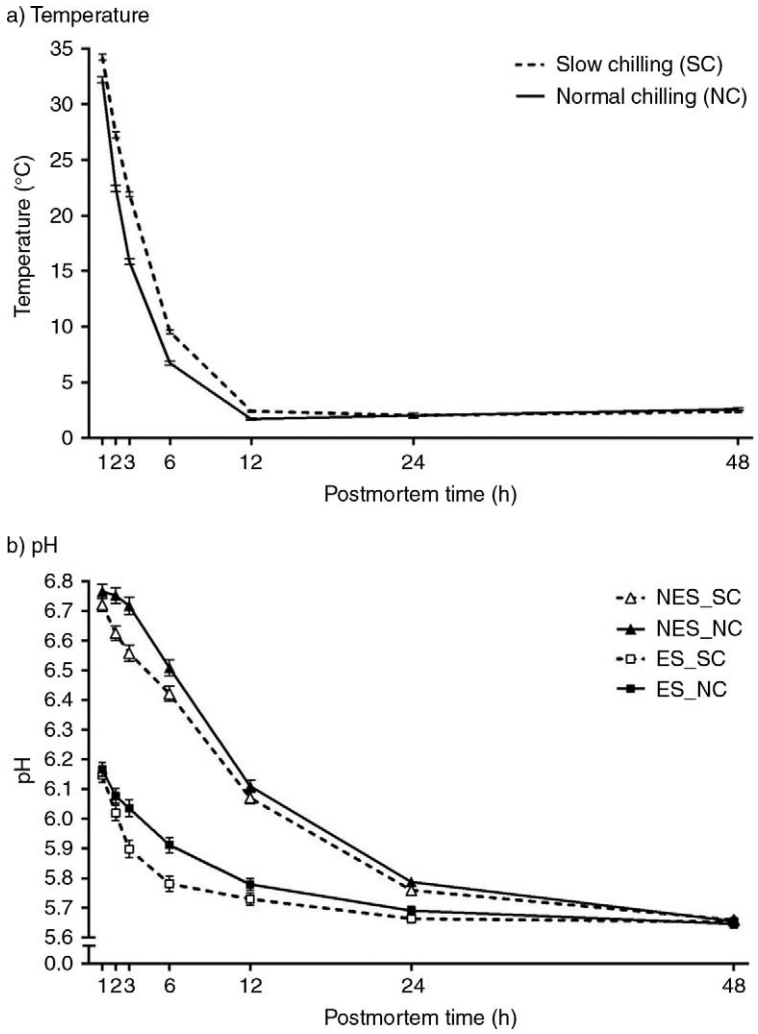

Fig. 1. Temperature (a) and pH (b) decline of lamb carcasses ($n = 105$) following electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling.

shown). To our knowledge, this is the first report indicating that the incidence of pH-related DFD-like condition could be important in Quebec, which suggests that some pre-slaughter practices are not optimal for lamb meat quality. Further investigations are needed to determine the real incidence of this condition across abattoir and season and to identify the pre-slaughter practices implicated.

At 45 min post-mortem, pH values for longissimus from ES carcasses averaged 6.16, which was 0.59 pH unit lower than values for longissimus from NES carcasses (Fig. 1b). This response to low-voltage ES is in accordance with previous results obtained with the same ES system (Pouliot et al. 2012a), although only half the ES time was required in the present study (30 s vs. 60 s). Genetic and pre-slaughter factors could be responsible for the variation in response to ES between the two studies as they have an impact on fibre type, metabolism and energy status. According to Pearce et al. (2010), optimal settings for the same ES systems differ among abattoirs and between slaughter dates. The authors recommended that optimal settings must be determined for each abattoir and that in-house testing must be done on a regular basis to ensure that ES systems work optimally.

A time × stimulation interaction for pH ($P < 0.001$; Fig. 1b) was observed due to the lower pH of ES carcasses throughout the first 24 h postmortem compared with NES carcasses ($P < 0.001$), with no differences in pHu ($P = 0.738$). Irrespective of ES treatment, a time × chilling interaction was also found ($P < 0.001$), owing to the faster pH decline for SC carcasses between 2 and 12 h ($P < 0.05$). This result can be explained by a faster rate of glycolysis and pH decline under warmer conditions (Marsh 1954; Jeacocke 1977). There were no differences thereafter ($P > 0.05$).

Grouping of carcasses based on their temperature at pH 6.0 showed that almost all NES carcasses, under both chilling procedures, reached pH 6.0 below 10°C (Table 1), making them susceptible to cold-shortening (Honikel 2004). In fact, these NES carcasses reached 10°C at pH 6.4–6.5, indicating that there is still a significant amount of ATP in the muscle at a temperature where calcium sequestration is reduced. Comparable results have been reported by Pouliot et al. (2012a).

Table 1. Carcass distribution into pH-temperature groups according to electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling

	NES		ES	
Temperature at pH 6.0	NC	SC	NC	SC
≤10°C	24	27	8	1
>10°C and <35°C	0	1	18	20
(>18°C and <25°C)	*(0)*	*(0)*	*(9)*	*(6)*
≥35°C	0	0	1	5

Some ES carcasses were below 10°C at pH 6.0, indicating that ES can reduce the risk of cold-shortening, but not completely prevent it. Moreover, the distribution of ES carcasses between the pH–temperature groups showed an important variation in response to ES in the lamb population (Table 1). Some carcasses reached pH 6.0 above 35°C, indicating such fast post-mortem metabolism could potentially induce defects as PSE-like condition (Simmons et al. 2008), rigor shortening or poor aging due to a fast autolysis of calpains (Simmons et al. 1996; Hwang and Thompson 2001b). This over-responsiveness to ES with its consequent steep pH drop caused these carcasses to almost reach a pH of 6.0 at 45 min post-mortem (individual data not shown). This phenomenon appeared more important in slow chilling conditions probably owing to a faster glycolysis at warmer carcass temperature (Marsh 1954; Jeacocke 1977). These observations indicate that response variability to ES between animals and chilling temperatures is relatively important and call for a better mastery of this technology. Important individual variation in response to low-voltage ES has also been reported in beef by Hollung et al. (2007). Notwithstanding, temperature at pH 6.0 was higher for ES_SC carcasses than ES_NC carcasses (25.6 vs. 15.8°C; $P < 0.001$) and both of them had higher temperature at pH 6.0 than NES (2.4 and 1.4°C for SC and NC respectively; $P = 0.581$). Almost 72% (38/53) of the ES carcasses in both chilling procedures in our study reached pH 6.0 between 10 and 35°C (Table 1), a window of carcass temperature where most defects are prevented according to Thompson (2002). However, only 28% (15/53; Table 1) of ES carcasses were in the pH–temperature window recommended by the Australian Sheep Meat Quality program (pH 6.0 at 18–25°C), which is between the 43 and 20% of ES carcasses reported by Australian studies using a different stimulation system and protocol (Pearce et al. 2006; Toohey et al. 2008). In the study by Toohey et al. (2008), 68% of ES carcasses reached pH 6.0 at or over 18°C compared with 25% for NES. In the present study, 62% of ES carcasses reached pH 6.0 at or over 18°C, but no NES carcasses did (data not shown). A few years ago, the MSA recommendations changed to a new target window of 18–35°C (Pearce et al. 2010).

Color

Electrical stimulation affected meat color after 3 and 8 d of aging (Table 2) according to the higher L^*, a^* and b^* values for ES meat compared with NES meat ($P \leq 0.037$). Similar results have been reported in a previous study in Quebec using the same ES system (Pouliot et al. 2012a) and in other lamb studies using high- or low-voltage systems (Riley et al. 1981; Kerth et al. 1999; Warner et al. 2005). Aging also increased L^* and a^* values (Table 2). In fact, there were trends for chilling × aging interactions for a^* and b^* parameters ($P = 0.063$ and $P = 0.064$), where aging had significant effects in the SC condition ($P < 0.05$), but not in the NC

Table 2. Color parameters of lamb longissimus thoracis aged for 3 or 8 d following electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling

	Aging 3 d				Aging 8 d								
	NES		ES		NES		ES			*P* value[z]			
	NC	SC	NC	SC	NC	SC	NC	SC	SEM	A	S	C	C × A
*L**	37.7	37.5	39.0	39.1	38.1	38.1	39.1	39.7	0.6	0.024	0.035	0.869	
*a**	12.2	12.5	13.6	13.2	12.5	13.0	13.6	14.1	0.4	0.008	0.006	0.488	0.063
*b**	7.3	7.4	8.4	8.1	7.4	7.6	8.2	8.5	0.4	0.366	0.037	0.840	0.064

[z]A, aging; S, stimulation; C, chilling.

condition ($P > 0.5$). The effect of ES and aging on meat color could be due to a difference in blooming, a term used to describe the oxygenation of the purple myoglobin pigment to the bright red oxymyoglobin pigment. It has been shown that oxygen consumption rates for muscle decrease with aging time (Atkinson 1969; McKenna et al. 2005) due to a decrease in mitochondrial activity (Bendall and Taylor 1972) and that blooming is faster in aged meat than fresh meat (Young and West 2001). According to Ledward (1992), ES can also damage the enzyme systems responsible for oxygen consumption. As samples were vacuum-packaged and only exposed to oxygen for 30 min prior to color measurements, the oxygenation time was the same for each sample. It is possible that ES and aged meat consumed less oxygen during the oxygenation period, which was therefore available for blooming and may explain color differences. Such an explanation, however, does not rule out any potential physico-chemical effect that faster pH decline at high temperature in ES carcasses can have on protein denaturation and hence color differences. The potential role of protein denaturation in color differences observed in this study is supported by the fact that aging seems to only have an impact on *a** and *b** in the SC condition, where pH fall was also faster and temperature fall slower.

Cooking Loss and Tenderness

Cooking losses were not affected by ES, chilling or aging treatments (Table 3). The absence of an aging effect differs with our previous results where cooking losses were reduced by aging time (Pouliot et al. 2012a). This difference could be due to different cooking methods resulting overall in more important cooking losses in the present study (around 30%) compared with the previous one (around 20%), albeit in the range reported by other studies (Safari et al. 2002; Hopkins et al. 2006). Cooking methods have been shown to influence cooking loss in beef (Panea et al. 2008).

Shear force values, however, were improved ($P < 0.001$) by both ES and aging as expected, although chilling had no effect (Table 3). A significant stimulation × aging interaction ($P = 0.019$) indicates that aging from 3 to 8 d had a more important effect on shear force values of NES compared with ES carcasses (respectively, −1.80 and −1.31 kg improvement), which is probably due to the fact that ES meat was already tender at 3 d and improved less thereafter. This is in accordance with the MFI results where a trend for a stimulation × aging interaction ($P = 0.097$; Table 3) indicates that the difference between ES and NES was more important at 3 d ($P = 0.001$), than at 8 d ($P = 0.155$). However, at 8 d ES meat was still more tender than NES. In fact, ES meat aged for 3 d had the same shear force value as NES meat aged for 8 d; these findings are supported by our previous study (Pouliot et al. 2012a) where, however, ES did not affect MFI values. The literature is not clear on the effect of ES on MFI in lamb. Past studies did not report any effect of ES on MFI (Kerth et al. 1999; Martin et al. 2006; Toohey et al. 2008), while

Table 3. Tenderness parameters of lamb longissimus thoracis aged for 3 or 8 d following electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling

	Aging 3 d				Aging 8 d									
	NES		ES		NES		ES			*P* value[z]				
	NC	SC	NC	SC	NC	SC	NC	SC	SEM	A	S	C	A × S	S × C
Cooking loss (%)	30.3	29.2	29.3	30.2	29.9	30.2	29.3	29.9	0.6	0.793	0.653	0.759		
Shear Force (kg)	5.49	5.72	4.03	4.24	3.56	4.06	2.77	2.87	0.31	<0.001	<0.001	0.400	0.019	
Sarcomere length (μm)[y]	1.60	1.69	1.73	1.73					0.02		<0.001	0.061		0.087
MFI[x]	69.4	62.8	73.8	75.6	96.7	95.1	98.0	101.0	2.7	<0.001	0.004	0.675	0.097	

[z]A, aging; S, stimulation; C, chilling.
[y]Sarcomere length were measured on 48 h samples.
[x]MFI, myofibrillar fragmentation index.

other studies found higher MFI following ES (Kadim et al. 2009; Abbasvali et al. 2012). Differential response to ES between studies may have arisen from inherent experimental conditions. It has been proposed that ES can improve tenderness due to an acceleration of aging and proteolysis (Hwang et al. 2003; Simmons et al. 2008). According to Simmons et al. (2008), stimulation could accelerate onset of rigor mortis and allow tenderization to begin sooner, when carcass temperature is higher, giving a head start to ES meat in term of tenderization. Indeed, faster proteolysis of cytoskeletal and myofibrillar proteins following ES have been reported (Uytterhaegen et al. 1992; Ho et al. 1996), which supports this assumption. Effects of ES on calpain activity have been reported (Dransfield et al. 1992; Uytterhaegen et al. 1992; Lee et al. 2000; Hwang and Thompson 2001a,b) and, more recently, a study on the proteome changes between ES and NES samples also suggested an acceleration of proteolysis following ES (Bjarnadóttir et al. 2011).

Electrical stimulation can also enhance meat tenderness by preventing or reducing the incidence of cold shortening, as was originally intended (Chrystall and Devine 1985). According to the trend for stimulation × chilling interaction ($P = 0.087$), ES meat had longer sarcomeres compared with the NES group, but the difference was found to be significant for the NC conditions only (NC: $P < 0.001$; SC: $P = 0.167$) with the shortest sarcomeres measured in NES carcasses chilled conventionally (Table 3). In fact, 50% of NES_NC and 25% of NES_SC had sarcomeres that were smaller than the shortest length measured in ES carcasses under both chilling conditions (data not shown). These results indicate that NES carcasses were subjected to a cold-induced contraction, at least in the so-called NC conditions of the abattoir. At first sight, these measurements suggest that SC allowed for longer sarcomeres in NES carcasses. However, the temperature and pH data showed rather that NES_SC carcasses were as much in the cold shortening risk zone as NES_NC, namely under 10°C, when ATP was still available for contraction, as indicated by their pH decline curve (Fig. 1). In fact, pH of these NES_SC carcasses was over 6.4 when their temperature reached 10°C and they reached pH 6.0 at 2.5°C, well under a temperature zone where cold shortening is prevented (Devine et al. 2004; Honikel 2004). In spite of the tendency for NES_SC meat to have longer sarcomeres compared with those from NES_NC, these two treatments were not different in terms of shear force values. Such results are difficult to explain. Overall, sarcomeres were short in this study compared with those measured in Warner et al. (2005), but are comparable to those obtained in a previous study conducted under similar conditions (Pouliot et al. 2012a). In the study reported herein, a wide range in sarcomere lengths was observed for both ES and NES meat (Fig. 2). Although there was no correlation between sarcomere length and WBSF ($P > 0.5$, data not shown), a curvilinear relation between sarcomere length and WBSF at 3 d of aging was observed for NES meat ($R^2 = 0.19$; $P = 0.031$), but not in the case of ES meat ($R^2 = 0.05$; $P = 0.221$, Fig. 2). In their classic work, conducted on pre-rigor excised muscle, Marsh and Leet (1966) found that tenderness was not affected by muscle contraction until a decrease of 20% of the initial muscle length, at which point tenderness decreases as shortening progresses to 40%, and then started to increase thereafter as shortening progresses further due to structural damage (Marsh et al. 1974). However, their data set showed an almost threefold range of shear force values in the 25–35% shortening window, indicating that for a similar shortening, muscles can present a wide range of shear force. Similarly, in the present study, the toughest NES muscle had sarcomeres between 1.60 and 1.70 μm, an area with a threefold range of shear force values (Fig. 2). Even if it is generally well accepted that shortened muscles are tougher than stretched muscles, the evidence came from

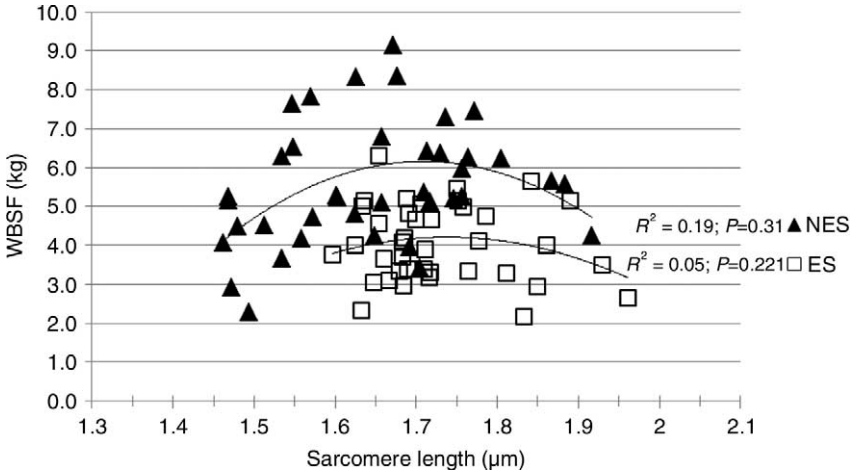

Fig. 2. Relationship between Warner Bratzler shear force at 3 d of aging and sarcomere length following electrical stimulation (ES) or not (NES).

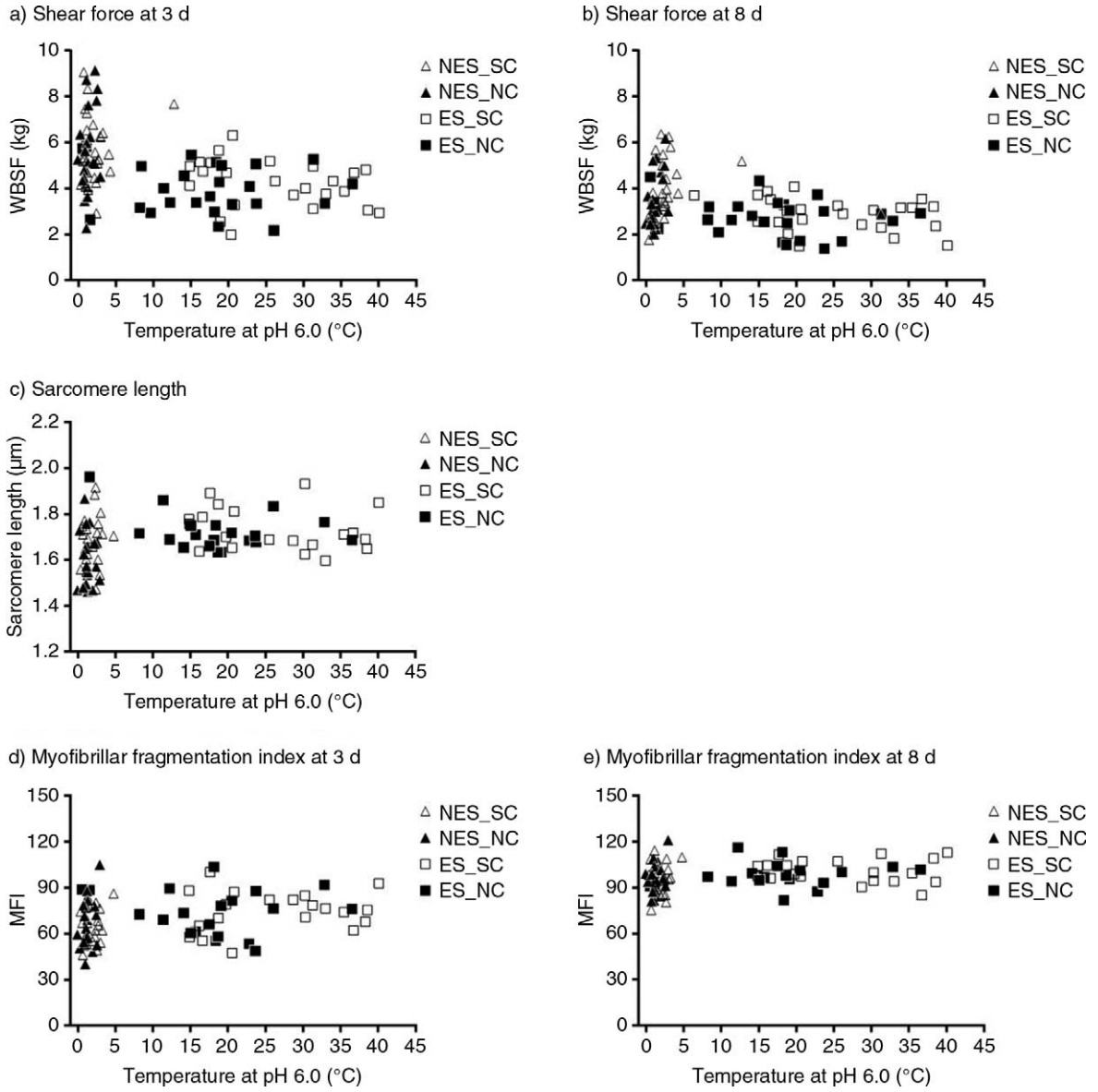

Fig. 3. Relationships between temperature at pH 6 and Warner Bratzler shear force at 3 d (a) and 8 d (b), sarcomere length (c) and myofibrillar fragmentation index at 3 d (d) and 8 d (e) following electrical stimulation (ES) or not (NES) and normal (NC) or slow (SC) chilling.

studies comparing a large range of shortening and stretching on excised muscles (Marsh and Leet 1966; Herring et al. 1967). Shortening of skeletally restrained muscles from hung carcasses could be less important (Locker 1960; McCrae et al. 1971) and result in more subtle and complex impacts on shear force than shortening of excised muscles. At the same sarcomere length, ES meat was more tender than NES meat (Fig. 2), which suggests that the enhancement of tenderness following ES is more complex than only reducing cold shortening. In fact, a substantial proportion of ES meat was tender despite having sarcomere lengths associated with tough meat for NES carcasses. The higher MFI observed for ES meat could explain, in part, this observation or at least indicate the importance of both parameters for meat tenderness.

Based on the 5-kg shear force value threshold for consumer acceptability reported in the literature (Shorthose et al. 1986; Safari et al. 2002), only 35% of NES meat was acceptable at 3 d compared with 76% for ES (data not shown). After 8 d of aging, 100% of ES meat was below the 5-kg threshold compared with 79% of NES meat (data not shown). Even if more NES meat had acceptable shear force values than in a previous study (Pouliot et al. 2012a), too many pieces presented unacceptable shear forces, especially after 3 d of aging. It appears therefore that electrical stimulation is efficient

in reducing negative eating experiences related to tough meat after 3 d of aging, which is representative of the aging period used in Quebec, and could even offer some kind of guarantee for tenderness when combine with 8 d of aging.

Beyond the effects of ES and chilling on meat quality improvement, the second goal of this study was to validate if the pH–temperature window proposed for optimal lamb meat quality in Australia also applies to carcasses from heavy lambs such as those produced in Quebec. For that purpose, the ES × chilling factorial design used in this study provided us with a wide range of temperature decline and glycolysis rates ensuing different muscle temperatures at pH 6.0. As seen in Fig. 3, despite the large range in temperature at pH 6.0 and shear force, no relationship is apparent between the two parameters, except for the obvious difference between carcasses reaching pH 6.0 either below or above 5°C ($P < 0.001$). Carcasses reaching pH 6.0 below 5°C produced the toughest meat, but presented a large range of shear force values over a very narrow window of temperature. Many carcasses in this group were as tender as others reaching pH 6.0 at higher temperatures. Above 5°C, no effect of temperature at pH 6.0 was observable on shear force values ($P = 0.576$).

A similar pattern was also obtained with sarcomere measurements, with a wide range in length occurring especially at temperatures below 5°C, where the shortest sarcomeres were found along with sarcomeres having the same length as those from carcasses reaching pH 6.0 at a more favorable temperature (Fig. 3). Therefore, below 5°C, some carcasses are at risk of cold shortening and meat is more prone to be tough, although a substantial proportion of carcasses could also provide tender meat in these conditions, which illustrates the importance of individual variations on tenderness and the multiplicity of factors involved in its development. However, sarcomeres were shorter for carcasses that reached pH 6.0 below 5°C than over it ($P = 0.011$).

The same conclusions applied to MFI, where smaller average MFI were obtained below 5°C ($P = 0.017$; Fig. 3) altogether, with an absence of any relationship over this temperature at pH 6.0 ($P = 0.800$). In fact, above 5°C, there were no relationships existing between temperature at pH 6.0, WBSF, sarcomere length and MFI ($P > 0.5$), although meat was more tender and had longer sarcomeres and higher MFI than below 5°C. As carcasses reaching pH 6.0 above 5°C were in major part ES carcasses, it is impossible to conclude altogether if resulting tenderness is caused by higher temperature at pH 6.0 (Devine et al. 2004; Honikel 2004), some kind of indirect effect of ES mediating pH fall, or even to a direct, but not measured, effect of ES on the onset of aging or on the fibre structure (Savell et al. 1978; Takahashi et al. 1984, 1987). As aging starts near or at rigor according to Devine and Graafhuis (1995), absence of the expected relationship between temperature at pH 6.0 and MFI does not allow us to support the assumption that ES accelerates proteolysis due, in part, to higher temperature (Simmons et al. 2008).

Considering MFI was higher for ES meat and was negatively correlated to shear force at both 3 and 8 d (respectively, $r = -0.36$ and $r = -0.28$; $P < 0.018$), it appears possible that ES promoted proteolysis independently of temperature, simply due to an early onset of aging and calpains activation. Overall, our results do not support the Australian recommendation with respect to attaining a temperature window at pH 6.0 for optimum eating quality of lamb (Thompson et al. 2005; Pearce et al. 2010). It is important to note that their recommendation was based on eating quality, not on shear force value.

CONCLUSIONS AND IMPLICATIONS

Tenderness is not optimal for heavy lamb carcasses from Quebec under current post-slaughter conditions. This study showed that ES enhances lamb tenderness at 3 and 8 d of aging, but the mechanisms still remain to be elucidated. Sarcomere length seems to play a role, but the important variation between carcasses under the same conditions (stimulation and chilling) and the weak relationship with shear force indicate that its role is not as important as thought. Myofibrillar fragmentation index indicates that proteolysis was faster for ES carcasses and correlated to tenderness. However, the results do not indicate there is an optimum window of temperature at pH 6.0 for tenderness, suggesting that ES may act in part independently of temperature. Electrical stimulation combined with 8 d of aging could offer some kind of guarantee for meat tenderness of heavy lambs, but protocols must be adjusted based on response measurements in abattoirs.

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