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Carcass chilling method and electrical stimulation effects on meat quality and color in lamb

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ABSTRACT

Five treatments: Control (C), Pre-evisceration Electrical Stimulation (CES: 15 Hz, 700 mA, 500 μs pulse width, 45 s pulse duration), vascular Rinse & Chill® (RC), CES + RC (ESRC), and RC with ES after evisceration (RCES:15 Hz, 600 mA, 1000 μs, 45 s), were applied to 21 lambs each. After being excised from the carcass, muscles were vacuum packaged and aged (*Longissimus lumborum*, LL, 3 and 22 d postmortem; *Semimembranosus*, SM, 3 d postmortem). Temperature, pH, purge, cooking loss, color, Warner-Bratzler Shear Force (WBSF), and consumer sensory evaluations were determined. CES and ESRC resulted in the fastest drop in pH below 6 and ESRC had the lowest likelihood of cold shortening. Sensory tenderness in the ESRC LL was greater than C. No differences in WBSF were found among treatments. RC generally produced lamb with higher lightness (L^*). The rapid drop in pH likely was responsible for the increased purge and cooking loss observed. Color was affected by the order of ES and RC application.

1. Introduction

Electrical stimulation (ES) is widely recognized as a procedure to facilitate blood removal, accelerate pH decline, and improve tenderness through preventing cold-shortening. Currently, three different types of electrical stimulation (Adeyemi & Sazili, 2014) are being utilized in the industry: extra low-voltage electrical stimulation (ELVES), low voltage electrical stimulation (LVES), and high-voltage electrical stimulation (HVES). The ELVES is carried out at a voltage of less than 100 V while HVES is carried out at and over 110 V. Low voltage is sometimes referred to as medium-voltage and is carried out between 100 and 110 V. LVES is mostly used when there is a short delay time (10 to 20 min) between bleeding and stimulation. The timing of LVES is pertinent because the ability of the nervous system to conduct the stimulation deteriorates rapidly early postmortem whereas HVES provides direct muscle stimulation (Adeyemi & Sazili, 2014).

Advances in carcass chilling have the potential to improve meat quality and shelf life. Rinse & Chill® is a process applied to the carcass immediately after exsanguination and involves infusion of a chilled substrate solution into the vascular system, which facilitates the removal of blood. This process was developed with the aim of more rapidly chilling carcasses but also to improve tenderness and meat color (Dikeman et al., 2003). A more recent study showed the Rinse & Chill® process in bison carcasses reduced shear force by 24 % (Mickelson & Claus, 2020). Fowler et al. (2017) reported an 11 Newton reduction in shear force in addition to color improvements in Rinse & Chill® lamb carcasses. Both the Rinse & Chill® process and ES appear to produce similar quality improvements. However, the mechanisms by which Rinse & Chill® cause these meat quality effects are unknown. Also, how Rinse & Chill® would interact with ES is not well understood. The aim of our study was to determine the effects of Rinse & Chill® combined with different electrical stimulation applications on lamb meat quality properties.

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2. Materials and methods

2.1. Experimental design

Five treatments were randomly applied to 105 carcasses from eight-month-old lambs that had an average hot carcass weight of 23.4 kg (std. dev. = 2.34; $n = 21$ per treatment). Seven lambs were randomly selected for five treatments on each of three weeks for harvest on one day per week. Treatments were: control (C, no treatments applied), control electrical stimulation (CES applied before carcass evisceration: 15 Hz, 700 mA, 500 μs pulse width, 45 s pulse duration), Rinse & Chill® (RC), CES applied before RC (ESRC), and RC followed by ES applied after evisceration (at approx. 45 min PM; RCES: 15 Hz, 600 mA, 1000 μs, 45 s). The different stimulator parameters were used to address the different postmortem application times during harvest. The stimulation treatment order was rotated (before evisceration; after evisceration) every fifth animal. The carcasses were electrically stimulated with a constant current stimulator (model: Bleeder EBS-300 V, CPMS Electronic Stimulator, Applied Sorting Technologies Pty Ltd.; Bulleen, Victoria, Australia) by clipping one electrical lead to the base of the tail and the other electrical lead to the neck region. The RC process involved vascular rinsing of the circulatory system for approximately 35 s, early postmortem (PM) using a chilled (14 °C) isotonic substrate solution (98.5 % water; balance: glucose, polyphosphates, maltose; Fowler et al., 2017). Carcasses were then moved to the cooler (average chiller temperature: 5.5 °C). Carcass shrink percentage was determined (weight loss between hot carcass weight and 24 h chilled carcass weight divided by hot carcass weight times 100).

The *Longissimus lumborum* (LL) pH and temperature were determined using a dual pH meter and electrode with automatic temperature compensation (Ionode IJ44 electrode, WP-80 Waterproof pH-mV-Temperature Meter, TPS, Brendale, Australia) with the electrode inserted into the geometric center of the LL at 0.75, 1, 2, 3, 4, 5, 8, 12, and 24 h PM to obtain measurements. The pH meter was calibrated in advance against two pH standard buffers (4 and 7) that were stored in the carcass cooler. The likelihood of cold shortening was determined (pH > 6, temperature < 10 °C; Tornberg, 1996; Aberle et al., 2012). Both conditions had to be met on the same carcass in order to be considered at risk of causing cold shortening. At 24 h PM, both LL and *Semimembranosus* (SM) muscles were excised, individually vacuum packaged, and shipped via a temperature regulated van (2 °C) to the University of Melbourne. The vacuum packed LL muscles from a given carcass were randomly assigned to an aging period (3 or 22 days, 2 °C). The SM muscles were aged 22 days (2 °C). On day 3 PM, SM and LL were cut into chops (1.5 cm thick) and blocks (average:10 cm length, 2.5 cm height, 5 cm width), re-vacuum packaged (plastic pouch; gauge 70 μm; size 165 × 250 mm) and frozen at −18 °C prior to sensory analysis. Percentage purge was based on the total net weight of the meat before opening the bag, draining the fluid in the bag, then re-weighing the bag plus meat. Samples from both LL and SM for color measurement were cut into 15 mm thick chops, vacuum packaged and aged 6 days PM prior to simulated retail display. Chops were then taken out of their vacuum pack, allowed to bloom (oxygenate) for 30 mins and then placed on polystyrene trays, over-wrapped with Glad® wrap (low density polyethylene; oxygen transmission rate, OTR, at 23 °C, 0 % RH; OTR = 7000–8500 cc/m^2/24 h) for continuous display (3 °C; Model GM100LCASB, Bromic Refrigeration, Ingleburn, Sydney NSW) under LED lighting (18 watt, 60 lm). Color measurements were taken on 0 (after 30 min bloom), 1, 3, and 5 days of display and measurements were made with the over-wrap in place. Tray placement in the display unit was randomized and rotated once daily to ensure equivalent illumination for all trays. Samples to be cooked for Warner Bratzler Shear Force (WBSF) were cut into blocks (average:10 cm length, 2.5 cm height, 5 cm width) before vacuum packaging and freezing them at −18 °C. These samples were later trimmed to 65 g, while still frozen, as described below. Frozen WBSF samples were stored for a minimum of 30 days before testing. The remaining samples from each muscle and each carcass were cut into five 1.5 cm thick chops, vacuum packaged, and frozen (−18 °C) for sensory evaluation. The same procedure for WBSF, purge, and consumer sensory samples was repeated for LL muscles aged in vacuum bags for 22 d PM at 2 °C. Color during simulated display was not measured for 22 d aged LL but color measurements (L^*, a^*, b^*) were made after blooming for 30 min.

2.2. Color measurements

Color and spectrophotometry measurements were obtained for each LL and SM at every display interval. Meat surface color was measured once on each sample using a Hunter Lab MiniScan EZ (Model 45/0-L; aperture size = 25 mm, illuminant D65, standard observer 10^o, calibrated with black and white tiles; Hunter Associates Laboratory, Inc. Reston, VA). The machine provided L^*, a^* and b^* values as well as percentage reflectance over 400 to 700 nm in 10 nm intervals. Chroma C^* and Hue angle were calculated using a^* and b^* values according to the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012). The chemical states of myoglobin were estimated by the following reflectance (R) wavelength ratios: oxymyoglobin (OMb, %R610 nm/%R525nm), deoxymyoglobin (DMb, %R474nm/%R525nm) and metmyoglobin (MMb, %R572nm/%R525nm) also recommended by the American Meat Science Association Meat Color Measurement Guidelines (Hunt et al., 2012).

2.3. Warner-Bratzler shear force (WBSF)

For WBSF, frozen roasts were removed from vacuum bags, and while still frozen samples were cut out using a band saw, trimmed to 65 g, then re-vacuum packed. Samples were cooked (Julabo F-38 ME, Julabo USA, Inc., Allentown, PA) while still frozen, in their vacuum bags at 70 °C for 35 min. This cooking method has been used and described previously by Hopkins and Thompson (2001). The samples were then removed from the water bath and placed directly into an ice bath. There were 10 samples per cooking batch and the samples were randomized (two samples per treatment) for equal distribution across cooking batches. Samples were cooled for 30 mins in an ice bath, then bags were opened, and samples removed and weighed. Samples were then placed back in bags and stored in the chiller (2 °C) overnight. Cooking loss was determined by measuring the frozen weight of the samples compared to the cooked weight directly after ice bath chilling. After overnight storage at 2 °C, samples were placed out at room temperature for 45 min before cutting. Six samples of 1 cm^2 x ~ 4 cm long were cut from each sample parallel to the muscle fibers for instrumental tenderness determination (model: Lloyd LS5; AMETEK, INC., Largo, FL) with a Warner-Bratzler V-notched blade using a 5 kN load cell a shear rate of 300 mm/min. Samples were sheared perpendicular to the muscle fibers (Belk et al., 2015).

2.4. Consumer taste panel

Consumer taste panel testing was performed by a commercial company (Tastepoint Pty Ltd., Melbourne, VIC, Australia). Samples for the consumer taste panel were organized according to commercial sensory software. Samples representing all treatments, muscles, and aging were included in the selection process of chops a consumer evaluated. Each consumer (n = 540) evaluated six samples. The consumer panel was performed over 9 different days with 60 consumers per day. A Latin square (6 × 6) arrangement was used to balance sample comparisons along with the order presentation of the pair. Each consumer also received a mutton sample to serve as a warmup sample. For the consumer panel, out of the 21 carcasses used for each treatment, 12 randomly selected carcasses were used to obtain 3 d aged LL, 22 d aged LL, and 2 d aged SM muscles.

Samples were thawed for 24 h (2–5 °C) prior to transport to the

testing site. The meat temperature immediately pre-cooking was less than 10 °C. The grill (Clam shell unit, Silex Grills Australia Pty, Ltd., New South Wales, Australia) used to cook the samples was pre-heated (45 min). The top plate temperature was 180 °C and the bottom plate was 195 °C. Chops were loaded onto the grill and cooked for a total of 2 min and 15 s. The grill lid was closed at the 30 s mark. The samples were cut up and served to the consumer at 3 min and 45 s from the start of cooking. Endpoint temperature was estimated to be 65 °C based on previous data and was designed to cook the samples to a medium degree of doneness.

Consumers were recruited using organizations who wanted to raise funds and thus each organization that provided consumers was awarded 1000 (Australian) dollars for providing participants for the consumer panel. Four consumers were allocated to each table and a divider was used to ensure consumers at the same table could not see each other. Consumers were asked to score the chops using a 100 mm unstructured line for tenderness (not tender to very tender), juiciness (not juicy to very juicy), liking of flavor (dislike extremely to like extremely) and overall liking (dislike extremely to like extremely). Consumer meat quality scores were obtained following the MSA protocols described by Watson, Gee, et al. (2008). The analysis was performed on the clipped means calculated by removing the highest and lowest 2 scores for each trait (Watson, Polkinghorne, & Thompson, 2008).

2.5. Statistical analysis

For the statistical analysis, animal/carcass was the experimental unit. Calculated percentage carcass shrink values were converted to a decimal (dshrink) and then transformed using the ARSIN function (SAS Institute) on the square root of dshrink prior to performing the statistical analysis. This approach was used to standardize the variances associated with percentage values because without adjustment, the values would be close to the lower limit of the percentage scale (0 to 100 %). This transformation provided a more appropriate procedure to analyze differences in shrink due to treatment. This determination was used to assign differences to the percentage shrink means. Purge percentage was handled in the same manner. After statistical analysis, the percentage values were reported.

Statistical designs that only entailed the main effect (treatment) were analyzed as a completely randomized block design. Dependent variables analyzed in this manner included: hot carcass weight, carcass shrink, cold shortening likelihood (percentage), purge percentage (single time point), and re-bloom color. Carcass and harvest week were included in the model as the random effects. The likelihood of cold shortening was analyzed using the GLIMMIX procedure (SAS 9.1.3 Service Pack 3, SAS Institute Inc., Cary, NC, USA) associated with binomial counts. The binomial 1 was assigned for each condition: pH >6.0, temperature < 10 °C; the binomial 0 was assigned for each: pH ≤ 6.0, temperature ≥ 10 °C. The binomials (pH and temperature) for an individual carcass were multiplied together and when the product was 1, that carcass treatment was counted for the determination of the percentage of carcasses susceptible to cold shortening (likelihood CS %). Differences in the CS % treatment means were based on the GLIMMIX results. ARSIN of SAS was used to determine differences in percentage shrink and purge means.

A 5 × 2 factorial (treatment x muscle) design was used to statistically evaluate: ultimate pH, cook loss, and WBSF. A 5 × 2 factorial (treatment x day aged) design was used to statistically evaluate: purge percentage, cook loss, and WBSF. A 5 × 2 factorial (treatment x day aged) with a split plot factor of days displayed was used to statistically evaluate meat color (CIE $L^*a^*b^*$, chroma C^*, hue angle, reflectance estimators of the chemical states of myoglobin). The consumer panel results were analyzed for the main effects of carcass treatment and days aged (LL only). The main effects of carcass treatment and muscle (LL and SM) were analyzed on the 3 d aged meat associated with the consumer panel results. In the models, the random effects of carcass and harvest week were included.

The SAS MIXED procedure (SAS 9.1.3 Service Pack 3, SAS Institute Inc., Cary, NC, USA) was used to determine significance ($P < 0.05$) in the model. When significance was found Adjusted Tukey-Kramer was used for mean separation. Letter assignment to individual means to enable statistical comparisons was achieved using the pdmix800 macro (Saxton, 1998).

3. Results and discussion

3.1. Hot carcass weight and dressing percentage

Hot carcass weights were similar ($P > 0.05$) among the treatments and the treatments did not affect carcass shrink (Table 1). However, Moreira et al. (2018) found that vascular RC resulted in a greater dressing percentage for lean cows (51.3 vs 48.6 %, RC vs. C, respectively) but was not different for grain fed cows (61.6 versus 56.2 %). Similar dressing percentage findings were observed by Yancey et al. (2002) in Charolais cattle carcasses that were vascularly infused.

3.2. pH decline, cold shortening likelihood and purge

Normal physiological pH just prior to stunning and bleeding would likely be around 7.4 (Pearson & Young, 1989). For our study the earliest time the pH could be consistently measured was 0.75 h postmortem. The postmortem pH decline in the LL associated with CES and ESRC at 0.75 h started at a lower ($P < 0.05$) pH than all other treatments but all treatments were similar at 10 h PM (Fig. 1). In the case of CES and ESRC, since ES associated with these two treatments was applied immediately after exsanguination, the resulting stimulation of muscle contraction accelerated postmortem glycolysis producing the lower pH prior to the first available pH measurement. In addition, after 2 h PM, the pH of ESRC continued to decline more than CES. The additional decline in pH of ESRC was likely associated with the substrates included in the rinse solution. Although RC resulted in a more rapid decline in pH than C, by stimulating after RC (RCES) resulted in a similar pH decline to RC alone. This suggests that perhaps either RC reduced the amount of available energy for contraction or the effectiveness of contraction was reduced because of deterioration of the nervous system associated applying

Table 1
Least square means on the effects of lamb carcass treatment[1] on various physical and chemical dependent variables[2].

Treatment	H.C.W. (kg)	Shrink (%)	CS (%)	pHu	Purge LL (%)	Purge SM (%)
C	23.42	1.97	15.82^{ab}	5.61	2.83	1.25^{b}
CES	22.65	2.13	12.66^{b}	5.73	2.87	1.64^{a}
RC	23.48	2.37	18.98^{ab}	5.66	3.16	1.45^{ab}
RCES	23.68	2.29	21.76^{a}	5.65	3.01	1.33^{ab}
ESRC	23.76	2.34	3.75^{c}	5.61	3.45	1.72^{a}

$^{a-c}$Means within a column with unlike superscript letters are different ($P < 0.05$).
[1] Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before electrical stimulation, ESRC = Rinse & Chill® after electrical stimulation; Muscles: LL = *Longissimus lumborum*, SM = *Semimembranosus*.
[2] Dependent variables: H.C.W., hot carcass weight; Shrink, percentage carcass shrink, CS %, percentage of carcasses likelihood of exhibiting cold shortening; pHu, ultimate pH pooled across LL and SM aged 3 days; Purge LL, percentage purge for LL pooled over days 3 and 22 aged; Purge SM, percentage purge determined on SM aged 3 days. S.E: standard errors. H.W.C. (0.660) and pHu (0634). To determine differences in means for shrink (S.E., 0.00660; purge LL (S.E., 0.0064); purge SM (S.E., 0.00648), ARSIN of SAS Institute was used after which superscripts were assigned to percentage means if differences were found. Cold shortening percentage: Mean separation was performed using PROC GLIMMIX binomial count analysis (S.E. = 0.321) then converted to percentage means.

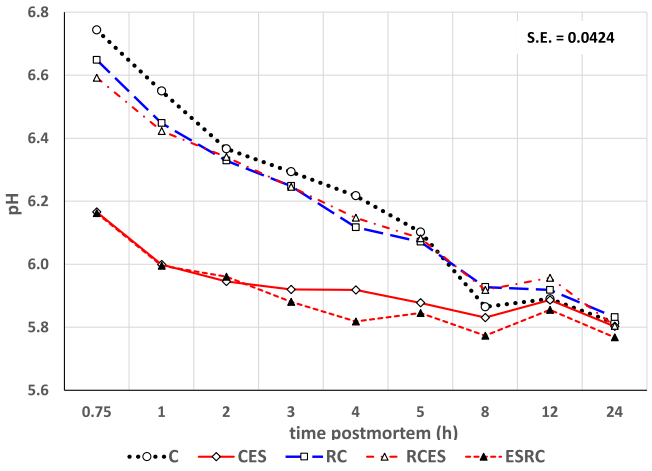

Fig. 1. Postmortem pH decline (Least Square Means) in the lamb *Longissimus lumborum* as affected by carcass treatment. Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®. S.E. = Standard error of difference of treatments on compared a given day. Tukey-Kramer average value 0.208.

stimulation after the carcass was eviscerated. In comparison, Fowler et al. (2017) found no RC effect on the rate of pH decline in lamb LL when assessed in relationship to carcass temperature; their study did not include ES. Although Fowler et al. (2017) did not indicate the exact time postmortem the first pH measurement was taken, their first value was 6.7–6.8. Minimal sarcomere shortening occurs around 12–15 °C (Locker & Hagyard, 1963; Tornberg, 1996). Temperatures below this range results in greater rigor toughness (Tornberg, 1996). In general when the pH is above 6 and the temperature is below 10 °C, this creates the conditions likely to result in cold shortening (Aberle et al., 2012). CES and ESRC had the smallest ($P < 0.05$) likelihood of cold shortening percentage compared to C, RC, and RCES in the LL (Table 1). Ultimate pH (pHu) was not affected ($P > 0.05$) by treatment. Purge percentage was not affected ($P > 0.05$) by treatment in the LL. CES and ESRC had greater ($P < 0.05$) purge percentages in the SM muscle than C. A potential reason why the SM was affected by the treatments and the LL was not, may have been associated with differences in the chilling rate of these two muscles. The SM within the hind leg of the lamb carcass is much thicker and would be expected to take longer to chill down. Delayed chilling and low pH create conditions known to cause denaturation of myofibrillar and sarcoplasmic proteins that affect water holding capacity.

3.3. Cooking loss

A treatment by muscle interaction ($P < 0.05$) in cooking loss was found (Table 2). However, carcass treatment did not affect ($P > 0.05$) cooking loss in the muscles only aged 3 d. The LL did have a greater ($P < 0.05$) cooking loss percentage than the SM, associated with CES and ESRC. These results correlate with the postmortem pH decline and purge results discussed earlier.

For the LL aged 3 and 22 d, there was not a treatment by age interaction ($P > 0.05$). The analysis of the aged LL (overall aged) determined that ESRC had the greater cooking loss than C and CES (Table 2). Moreira et al. (2018) also reported increased cooking losses in RC electrically stimulated carcasses from grain-finished cull cows. In contrast, Fowler et al. (2017) found no cooking loss treatment effect in lamb *m. longissimus thoracic et lumborum.* Cooking loss was lower for the 22 d aged LL (18.32 %) than the 3 d aged LL (20.88 %; standard error, 0.694).

Table 2
Least square means of cooking loss percentage for lamb carcass treatment, muscle, and days aged[1].

Treatment[2]	Muscle		Aged LL		
	LL	SM	Day 3	Day 22	Overall
C	20.00^{y}	19.68^{y}	20.00	17.26	18.63^{b}
CES	20.64^{y}	18.76^{z}	20.64	17.75	19.19^{b}
RC	20.63^{y}	20.58^{y}	20.62	18.74	19.68^{ab}
RCES	20.87^{y}	20.64^{y}	20.87	18.54	19.71^{ab}
ESRC	22.26^{y}	20.22^{z}	22.26	19.31	20.79^{a}
S.E.	0.711		0.664		0.469

$^{a-b}$Means within a column with unlike superscript letters are different ($P < 0.05$).
$^{y-z}$Means with unlike superscript letters between muscles within a given treatment are different ($P < 0.05$).
[1] Carcass treatment, muscle, days aged: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = ES after Rinse & Chill®, ESRC = CES before Rinse & Chill®; Muscle: LL = *Longissimus lumborum*, SM = *Semimembranosus*; muscle effect on 3 d aged LL and SM; day aged for LL.

3.4. Color display and rebloom

Type 3 Tests of fixed effects for instrumental color determinations are provided in Table 3. CIE L^* and CIE a^* were the only two dependent variables in which interactions were found between treatment and day, specifically in just the LL. A significant carcass treatment main effect was found for CIE L^* (SM), CIE b^* (LL, SM), Chroma C^* (SM), Hue (LL, SM), OMb (LL), and DMb (LL).

Fig. 2 shows the CIE L^* of the LL aged 6 days before being displayed 0, 1, 3, and 5 days. RCES was lighter ($P < 0.05$) than CES on all display days. In addition, RC was lighter ($P < 0.05$) through day 3 of display than CES. For the SM (Table 4), RC and RCES treatments produced lighter ($P < 0.05$) chops than CES and C. Fowler et al. (2017) also found that lamb LL was lighter with RC than controls. Farouk and Price (1994) found that infused (10 % live weight; solution containing maltose, glycerin, dextrose, and polyphosphates; with and without added calcium chloride) lamb carcasses were lighter than controls in both fresh and frozen samples. Mickelson and Claus (2020) also reported lighter color with RC bison. No main treatment effect was found for CIE a^* but a treatment by day interaction occurred (Fig. 3). No CIE a^* differences were detected in the SM (Table 4). Farouk and Price (1994) reported that the 10 % tenderizing blend (0.1 % maltose, 0.21 % glycerin, 0.23 % dextrose, 0.14 % tripolyphosphate; Farouk et al., 1992) combined with calcium chloride had less red color than the 10 % tenderizing blend by itself and controls at all times and storage conditions. Fowler et al. (2017) reported that RC had no impact on redness (CIE a^*) of the *m. longissimus thoracic et lumborum* and a critical consumer threshold for redness (CIE a^*) was not breached on average for RC or control LL. Khliji et al. (2010) found that the critical consumer threshold for CIE a^* was 9.5 for fresh lamb. As such all of the chops at the end of our display were still acceptable in color based on this CIE a^* threshold.

CIE b^* values (Table 4) for RCES were greater ($P < 0.05$) than CES and C in the LL. Additionally, RC was greater ($P < 0.05$, CIE b^*) than CES in the LL. In the SM, RC was more yellow than ESRC and C. Fowler et al. (2017), Mickelson and Claus (2020), and Farouk and Price (1994) all found that RC or infused carcasses had yellower outcomes for lamb and bison meat than controls.

Treatments did not alter ($P > 0.05$) chroma C^* in the LL (Table 4). However, in the SM, chroma C^* was greater ($P < 0.05$) in RC in comparison to C while the other treatments were intermediate. RC and RCES had greater hue angles than CES in the LL. Similarly, RC and RCES had greater ($P < 0.05$) hue angles than CES in the SM. In addition, RC had a greater ($P < 0.05$) hue angle than C in the SM.

RCES had less ($P < 0.05$) oxymyoglobin than CES but was not different than C in the LL. Both RCES and RC had less ($P < 0.05$; Table 5) deoxymyoglobin than CES. Treatments did not influence ($P > 0.05$) metmyoglobin in the LL or any other chemical state of myoglobin in the

Table 3
Type 3 tests of fixed effects associated with carcass treatment and display time on lamb for various meat color related dependent variables[1].

Independent Variable[2]	CIE L^*		CIE a^*		CIE b^*	
	LL	SM	LL	SM	LL	SM
trt	0.005	0.0002	0.892	0.372	<0.0001	<0.0001
day	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
trt x day	0.044	0.732	0.014	0.155	0.327	0.860
	Chroma C^*		Hue			
	LL	SM	LL	SM		
trt	0.182	0.040	<0.0001	0.003		
day	<0.0001	<0.0001	<0.0001	<0.0001		
trt x day	0.083	0.4325	0.0569	0.4815		
	OMb		DMb		MMb	
	LL	SM	LL	SM	LL	SM
trt	0.0049	0.3104	0.022	0.0723	0.2053	0.7204
day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
trt x day	0.0614	0.6339	0.6202	0.9995	0.0918	0.2587

[1] Dependent variables: CIE L^* (lightness), CIE a^* (redness), CIE b^* (yellowness), Chroma C^* (saturation), Hue (hue angle), OMb (oxymyoglobin), MMb (metmyoglobin), DMb (deoxymyoglobin); Muscles (LL, *Longissimus lumborum*; SM, *Semimembranosus*).
[2] Independent variables: trt = carcass treatment, day = days displayed.

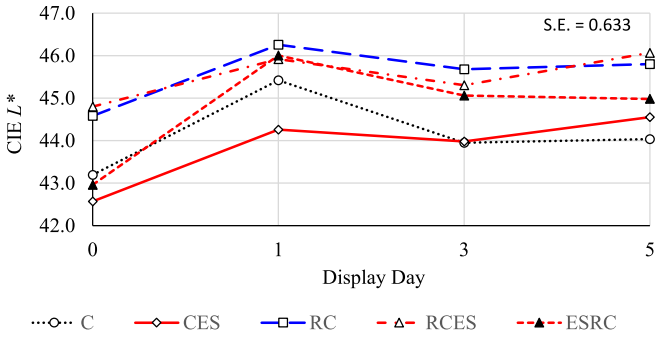

Fig. 2. Carcass treatment effects on CIE L^* of the lamb *Longissimus lumborum* (LL) during display (0, 1, 3, 5 d). Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®; LL was vacuum package aged 6 days before display. S.E. = standard error of difference (treatment x day).

SM. Mickelson and Claus (2020) showed that PVC wrapped RC steaks had a higher estimate of deoxymyoglobin than C on day 1, but no other differences were found on day 4 and 7. They did not find any differences for oxymyoglobin in PVC wrapped bison steaks. The authors did report that RC vacuum packaged bison steaks had a greater deoxymyoglobin content with less metmyoglobin content than C.

The only difference detected in rebloom was in the 22-day aged LL (bloom 30 min.). RCES was lighter (47.35; $P < 0.05$, S.E. = 0.849) than CES (44.14). C had a slightly greater (3.11; $P < 0.05$, S.E. = 0.084) oxymyoglobin than RC (2.71), RCES (2.69), and ESRC (2.83).

Determining the mechanisms as to how the treatments affect fresh meat color is very complexed because of numerous factors that could involve pH, pigment concentration, moisture content, intracellular versus extracellular fluids, lipid content, muscle redox conditions, mitochondria activity, and availability of oxygen. A more rapid pH decline early postmortem could be expected to result in lighter colored meat as a result of denaturation of myoglobin and reduced water holding capacity causing reflection of light from increased presence of extracellular moisture. CES and ESRC demonstrated a more rapid pH decline compared to C. In addition, the SM in these treatments exhibited greater purge than C. However, lightness in the SM was not influenced by greater purge.

In general it appears the application of RC resulted in meat being lighter in color. It is known that RC facilitates removal of more blood and accelerates postmortem glycolysis (Hwang et al., 2022a, 2022b). A reduction in the heme pigments by RC was demonstrated in lamb (Li et al., 2023) and in ground salted pork from sows (Erazo-Castrejón et al., 2019). Less residual hemoglobin in the muscle may have contributed to the lamb appearing lighter in this study. However, a reduction in heme pigments due to RC was not found in pork from 5-month old hogs (Kethavath et al., 2021) and was greater in RC meat from dairy cows (Kethavath et al., 2022) compared to controls.

Kethavath et al. (2021) proposed that the potential negative effects of a rapid drop in pH early postmortem in pork carcasses could be offset by more rapid chilling achieved by RC. They found that RC did not affect carcass shrink or expressible moisture. They did find that RC loin chops were lighter and had less deoxymyoglobin compared to the control. Compared to C, our treatments did not affect the chemical state of myoglobin. However, it is unknown why some differences in oxymyoglobin and deoxymyoglobin existed among the other treatments in the LL while no differences existed in the SM.

Table 4
Least square means of carcass treatment[1] effects on lamb muscles for various color dependent variables[2] determined under continuous lighting display.

	LL			SM				
Treatment	CIE b^*	Chroma	Hue	CIE L^*	CIE a^*	CIE b^*	Chroma	Hue
C	7.79^{bc}	15.76	29.97^{bc}	41.19^{b}	13.83	7.42^{c}	15.78^{b}	28.67^{bc}
CES	7.34^{c}	15.71	28.14^{c}	41.15^{b}	14.13	7.50^{bc}	16.10^{ab}	28.54^{c}
RC	8.43^{ab}	16.45	31.49^{ab}	42.70^{a}	14.57	8.59^{a}	17.03^{a}	31.28^{a}
RCES	8.66^{a}	16.41	32.59^{a}	42.91^{a}	13.89	8.13^{ab}	16.22^{ab}	30.98^{ab}
ESRC	8.05^{abc}	16.09	30.50^{abc}	41.80^{ab}	13.87	7.66^{bc}	15.93^{ab}	29.45^{abc}
S.E.	0.269	0.341	0.622	0.476	0.415	0.242	0.421	1.11

[a-c]Means within a column with unlike superscript letters are different ($P < 0.05$). S.E., standard error of the difference.
[1] Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®. Muscle: LL = *Longissimus lumborum,* SM = *Semimembranosus*.
[2] Dependent variables: CIE L^* (lightness), CIE a^* (redness), CIE b^* (yellowness), Chroma (Chroma C^*, saturation), Hue (hue angle).

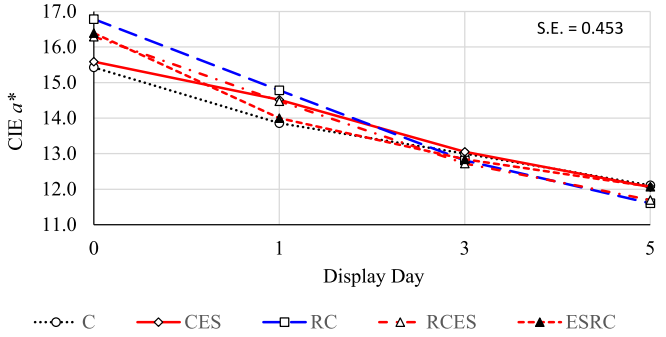

Fig. 3. Carcass treatment effects on CIE a^* of the lamb *Longissimus lumborum* (LL) during display. Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®, LL was vacuum package aged 6 days before display. S.E. = standard error of difference (treatment x day).

Table 5
Least square means of carcass treatment[1] effects on reflectance estimators of the chemical states of myoglobin[2] on lamb muscles under continuous lighting display.

	Oxymyoglobin		Deoxymyoglobin		Metmyoglobin	
Treatment	LL	SM	LL	SM	LL	SM
C	2.43^{ab}	2.55	1.10^{ab}	1.12	1.00	1.03
CES	2.53^{a}	2.65	1.12^{a}	1.11	0.95	1.00
RC	2.39^{ab}	2.51	1.08^{b}	1.09	0.98	1.03
RCES	2.28^{b}	2.47	1.09^{b}	1.10	1.02	1.04
ESRC	2.40^{ab}	2.54	1.10^{ab}	1.10	0.99	1.02
S.E.	0.064	0.087	0.012	0.012	0.030	0.031

$^{a-b}$Means within a column with unlike superscript letters are different ($P < 0.05$). S.E., standard error of the difference.

[1] Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®. Muscles: LL = *Longissimus lumborum*, SM = *Semimembranosus*.

[2] Chemical states of myoglobin: Reflectance (R) estimators of myoglobin chemical states: oxymyoglobin (% R610nm / % R525nm), deoxymyoglobin (% R474nm / % R525nm), metmyoglobin (% R572nm / % R525nm), larger values indicate more of that state.

3.5. Warner-Bratzler shear force

Figs. 4 and 5 illustrate the absence of treatment differences in WBSF ($P > 0.05$) in either the LL aged 3 and 22 d or SM aged 3 d. Similarly, in a study by Li et al. (2023), they evaluated the effects of Rinse and Chill on the tenderness of cooked lamb steaks and did not find a difference in

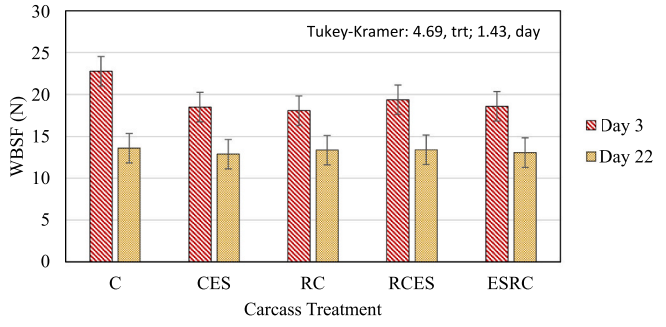

Fig. 4. Warner-Bratzler Shear Force peak (WBSF, Newtons) in the lamb *Longissimus lumborum* (LL) as affected by carcass treatment and days aged. Pr > F: Treatment (0.372, S.E. = 1.36), day ($P < 0.0001$, S.E. = 0.72) and trt x day (0.340, S.E. = 1.76). Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®, LL was aged 3 and 22 days.

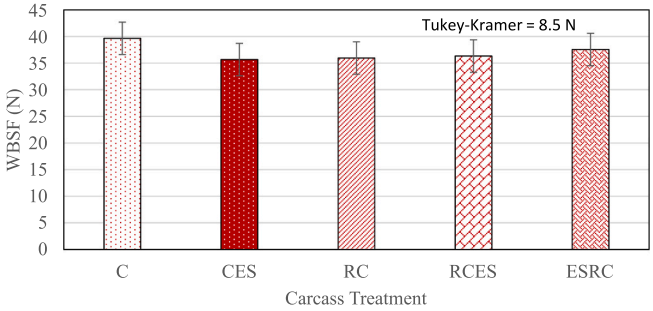

Fig. 5. Warner-Bratzler Shear Force peak (WBSF, Newtons) in the lamb *Semimembranosus* (SM, 3 d aged) as affected by carcass treatment ($P = 0.67$, Standard error of difference = 3.05 N). Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®, SM was aged 3 days.

WBSF or texture profile analysis hardness with control samples. Likewise when RC was applied to pork carcasses, the tenderness of the loin chops was comparable to the control chops (Kethavath et al., 2021). In contrast Mickelson and Claus (2020) found a 24 % decrease in WBSF for RC bison steaks and Kethavath et al. (2022) reported a 55 to 58 % reduction in WBSF in the LL from RC treated cull dairy cow carcasses. Fowler et al. (2017) also found an 11-N reduction which was a 34 % WBSF decrease in the RC lamb chops. Fowler et al. (2017) froze, cooked, and prepared their WBSF LL samples similar to the method used in this study. Their controls had a higher WBSF (32 N, 7 d post-mortem aged) compared to our controls (22.8 N, 3 d post-mortem aged). In our study WBSF values pooled across all carcass treatments for lamb aged 3 d was 19.5 N and for 22 d aged lamb was 13.2 N. Fowler et al. (2017) stated the absolute level of shear force even for the control samples demonstrated tenderness acceptability (below 49 N threshold; Hopkins et al., 2006). It is unknown why the lamb used in our study was more tender than Fowler et al. (2017). Nevertheless, this likely limited our ability to demonstrate tenderness differences among carcass treatments.

3.6. Consumer panel

The demographics of the consumer panel are found in Table 6. Consumers found that ESRC LL chops had greater ($P > 0.05$) tenderness than those from C (Table 7). Perhaps applying electrical stimulation before vascularly rinsing the carcass enabled the muscles to be more relaxed thereby facilitating more effective rinsing and distribution of the rinse solution. Rinse and Chill also facilitates a more rapid drop in pH and it has been suggested this application may enhance activation of the calpains (Hwang et al., 2022a; Hwang et al., 2022b). Among the other carcass treatments no differences in tenderness were found. Although consumers found a tenderness difference, WBSF did not support a difference in tenderness. In contrast to instrumental measurement of tenderness, a consumer's perception of tenderness can be very complex and influenced by various textural properties such as chewiness, impact of first bite impression, effects of residual connective tissue, and other sensory properties. In addition, differences in cookery and evaluation conditions can affect the assessment of tenderness. The WBSF samples

Table 6
Demographics of the consumer panel.

Age (years)	%	Gender	%	Frequency eat lamb	%
18–19	8.3	Male	47.4	Daily	0.6
20–25	16.5	Female	52.6	4–5 times a week	1.3
26–30	7.8			2–3 times a week	13.0
31–39	17.0			Weekly	28.7
40–60	40.0			Fortnightly	25.6
61–70	10.4			Monthly	29.1
				Never eat lamb	1.9

Table 7
Least square means of carcass treatment[1]2 effects and days aged for consumer taste test scores.

Muscle	Treatment or Days Aged	Tenderness	Juiciness	Flavor Liking	Overall Liking
LL	C	74.0[b]	70.0[a]	69.8[ab]	71.6[a]
LL	CES	79.2[ab]	71.3[a]	72.3[ab]	73.4[a]
LL	RC	78.7[ab]	69.7[a]	70.0[ab]	71.9[a]
LL	RCES	78.2[ab]	70.2[a]	69.3[b]	71.2[a]
LL	ESRC	79.8[a]	76.2[a]	75.0[a]	75.6[a]
S.E.		2.58	3.61	1.93	2.22
LL	3	75.9[b]	72.7[a]	71.9[a]	72.7[a]
LL	22	80.0[a]	70.3[a]	70.7[a]	72.9[a]
S.E.		2.32	3.38	1.60	1.93
SM	C	43.2[a]	56.8[a]	54.0[a]	51.4[a]
SM	CES	45.1[a]	56.4[a]	56.8[a]	52.9[a]
SM	RC	44.5[a]	57.3[a]	54.6[a]	52.0[a]
SM	RCES	43.4[a]	57.9[a]	54.2[a]	50.0[a]
SM	ESRC	45.4[a]	57.9[a]	54.6[a]	51.9[a]
S.E.		5.21	4.46	4.48	4.92

[a,b]Means within column of an individual analysis with unlike superscript letters are different ($P < 0.05$). Treatment means for the LL are pooled acrossed days aged as there was not a treatment by days aged interaction ($P > 0.05$). S.E., standard error of difference.

[1] Carcass treatment: C = control, CES = control electrical stimulation, RC = Rinse & Chill®, RCES = Rinse & Chill® before ES, ESRC = CES before Rinse & Chill®. Muscles: LL = *Longissimus lumborum*, SM = *Semimembranosus*. Days aged are postmortem. SM musles were aged 3 d.

[2] Taste test scores: Larger values are more favorable. Traits were based on a 100 mm unstructured line. The consumer response was measured on the line (0 to 100). Anchors for taste traits were: tenderness (not tender to very tender), juiciness (not juicy to very juicy), liking of flavor (dislike extremely to like extremely) and overall liking (dislike extremely to like extremely).

were water cooked (70 °C internal) starting from the frozen state and after being chilled overnight along with being set out at room temperature before shearing with controlled fiber orientation, In contrast the consumer samples were first thawed, then grilled (65 °C) and promptly served warm.

In the LL, juiciness was not affected ($P > 0.05$) by carcass treatment. For flavor liking in the LL, the only difference found ($P < 0.05$) between two carcass treatments was the preference for ESRC over RCES. When consumers rated their overall liking, no differences ($P > 0.05$) were found among the carcass treatments in the LL. Additional aging of the LL improved ($P < 0.05$) tenderness but did not influence any other consumer taste test traits. In the SM, which was only aged 3 day, consumers did not find ($P < 0.05$) any sensory trait differences among the carcass treatments.

4. Conclusion

Application of ES early postmortem with or without RC can dramatically accelerate postmortem glycolysis. RC generally produces lamb with a lighter color. The order in which RC is coupled with ES may impact various meat quality traits. Future research should evaluate the application of ES before RC on inherently less tender lamb and carcasses that are rapidly chilled since it appears this combination has the potential to reduce the likelihood of cold shortening.

Consumer consent

The consumer sensory tests were independently performed by Tastepoint Pty Ltd. (Melbourne, VIC, Australia). Tastepoint Pty Ltd. is a commercial consumer testing company.

CRediT authorship contribution statement

Maggie A. Mickelson: Writing – original draft, Visualization, Investigation, Data curation. **Robyn D. Warner:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Rod J. Polkinghorne:** Writing – review & editing, Formal analysis. **Dennis L. Seman:** Formal analysis. **Peter M. Crump:** Formal analysis. **James R. Claus:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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