INTERACTIVE EFFECTS OF LOW VOLTAGE ELECTRICAL STIMULATION AND LEG RESTRAINT ON MEAT QUALITY OF CHAROLAIS CROSSBRED STEER CARCASSES

The meat quality of the longissimus muscle of 12 Charolais crossbred steers that were electrically stimulated on randomly selected sides approximately 1 h postmortem was investigated. Temperature and pH measurements were taken on each side at 5 min prior and 1, 4, 24 and 48 h subsequent to stimulation and analyses performed following 7-d ageing for soluble, insoluble and total collagen and muscle protein solubility. Although there were no significant differences between the treatments for pH and temperature decline and collagen and protein characteristics, the unrestrained, stimulated sides had significantly lower shear force values than restrained, stimulated sides.

Key words: Collagen solubility, electrical stimulation, beef, Charolais

[Interaction d'un courant électrique à faible tension et des appareils de contention des pattes sur la qualité de la viande des bouvillons Charolais hybrides.]
Titre abrégé: Stimulation électrique des carcasses de Charolais.
On a évalué la qualité de la viande du longissimus chez 12 bouvillons Charolais hybrides dont on avait stimulé un côté (choisi au hasard) de la carcasse au moyen d'une décharge électrique, environ une heure après l'abattage. On a mesuré la température et le pH de chaque côté 5 minutes avant la stimulation de même que 1, 4, 24 et 48 h par la suite. On a également déterminé la concentration de collagène soluble, insoluble et total et la solubilité des protéines du muscle après 7 sept jours de rassissement. Bien qu'on n'ait noté aucune différence significative dans les deux traitements pour le pH et la température de même que les propriétés du collagène et des protéines, le côté non attaché et stimulé affichait un coefficient de cisaillement significativement plus faible que le côté stimulé, mais attaché.

Mots clés: Solubilité du collagène, stimulation électrique, boeuf, Charolais

Electrical stimulation has been cited to improve meat tenderness by accelerating muscle metabolism after slaughter, by releasing calcium-dependent proteases (CDP) and lysosomal enzymes and by damaging the muscle ultrastructure (Pearson and Dutson 1985), as well as by increasing the solubility of collagen (Judge et al. 1981). High-voltage electrical stimulation has produced the most dramatic and consistent improvements in tenderness; however, low voltages achieve the same results provided application is immediately after death (Bendall 1980). Carcasses exposed to low voltage electrical stimulation have shown differences in tenderness between the sides within a carcass (Solomon 1986), but the mechanism by which this occurs is unclear. Little research has been conducted to investigate the effect of hindleg restraint in intact carcasses stimulated with low voltages. This study was designed to test the effects of low voltage electrical stimulation and hindleg restraint on postmortem meat tenderness, postmortem glycolytic rate, collagen and protein solubilities.

Twelve 16-mo-old Charolais crossbred steers from the Elora Beef herd at the University of Guelph, which had been finished to a fat depth between 5 and 9 mm (Grade A1) on a corn-corn silage diet and weighed approximately 558 kg ($\pm$10.8 kg SEM), were fasted overnight and slaughtered conventionally in the abattoir at the University of Guelph. The leg used to suspend the carcass as it was bled was noted. Prior to electrical stimulation, the temperatures of the longissimus muscle of control and stimulated sides were taken at the 10th rib site with a digital display thermometer fitted with a steel probe (Sybron Corporation, Arden, NC).

Each randomly selected carcass side received five 12-s pulses, spaced by 3-s intervals, resulting in 1 min of stimulation, approximately 1 h postmortem (63.4 min, SEM 2.13). Stimulation was employed unconventionally late in the slaughter procedure in order to use the opposing side as the control. The electrical stimulation unit used was a 115 V, 0.25 amp, 60 Hz, alternating current BV-80 model (Jarvis Products Corporation, Middleton, CT). The ground was placed directly into the 12th rib site of the longissimus muscle, and the electrode inserted at the 8th rib site. Both electrodes were placed 6 cm into the longissimus muscle and 8 cm from the medial carcass split. Immediately after electrical stimulation, approximately 10 g of prerigor longissimus muscle was removed surgically from the 10th rib site for collagen analysis from each side and the pH of each muscle measured with a glass probe (Metrohm Herisau, Switzerland). Each prerigor sample was trimmed of fat and epimysium, frozen in liquid nitrogen, vacuum packaged and stored at −80°C. Measurements of pH and temperature at the 10th rib were recorded from each side 1, 4, 24 and 48 h and 7 d poststimulation. Seven days postmortem, the longissimus muscle from the 9th to the 12th ribs was excised, vacuum packed and frozen at −30°C, to be analyzed for shear force and collagen characteristics.

Hunterlab colour reflectance was measured from 2.2-cm-thick slices from the 12th rib site of the longissimus muscle at ribbing (24 h) and 7 d postmortem after 30 min bloom time at room temperature with the Spectrogard Colour System (Pacific Scientific, Silver Spring, MD).

Shear force was performed on longissimus muscle steaks cut to a 2-cm thickness from the 10th rib site of each frozen longissimus muscle and thawed overnight at 2°C. The steaks were broiled to an internal temperature of 73°C, as determined by a digital display thermometer fitted with a steel probe (Sybron Corporation, Arden, NC) and were allowed to cool to room temperature for 4 h. Three cores per steak, each core 2 cm in diameter, were removed parallel to the muscle fibres, subjected to a Warner-Bratzler shear force test and the peak force recorded.

For collagen and protein solubility analyses, a steak was cut from each frozen longissimus muscle at the 9th rib, thawed at 2°C, trimmed of all visible fat and epimysium and ground through a 2-mm plate. Samples were freeze-dried and powdered at full speed in a glass Waring blender (Waring Products Division, New Hartford, CT).

Heat-soluble collagen was extracted from the powdered meat samples in a 50-mL centrifuge tube according to Hill (1966). One millilitre of the resulting supernatant and 0.1 g of the residue were each hydrolyzed at 110°C for 6 h in 6 N HCl in a capped 20-mL glass tube. A time test done to determine the maximum recovery of hydroxyproline for soluble and insoluble collagen samples revealed that 6 h of hydrolysis was optimum (18.43 mg collagen/g dry muscle at 6 h vs. 14.28, 14.95, 13.35 mg collagen/g dry muscle at 3, 12 and 24 h respectively, SEM± 0.45) and that denaturation of hydroxyproline in the insoluble collagen occurred during unnecessarily long hydrolysis (15.32 mg insoluble collagen/g dry muscle at 6 h vs. 11.91, 11.77, 10.15 mg insoluble collagen/g dry tissue at 3, 12, and 24 h, respectively, SEM±0.43). Hydroxyproline was measured using the spectrophotometric method of Bergman and Loxley (1963), with the absorbance of the tubes read within 4 h at 558 nm (LKB Biochrom Ultrospec II, Cambridge, England). Hydroxyproline concentration was converted to soluble and insoluble collagen concentrations using 7.52 and 7.25, respectively (Cross et al. 1973).

Protein solubilities were determined on powdered meat samples by the methods outlined by Link et al. (1970). Briefly, two 1-g powdered meat samples were extracted for sarcoplasmic and total soluble protein with 30 mL of 0.03 M KH_2PO_4, pH 7.4 and 1.1 M KI, buffered to pH 7.4 with KH_2PO_4, respectively. Sarcoplasmic protein was extracted with two 3-h extractions and total protein with two 3-h extractions and one 2-h extraction. All extractions were performed at 2°C. Protein concentrations of the extracts

were determined with a biuret reagent (Layne 1957) from absorbances measured on a spectrophotometer (LKB Biochrom Ultrospec II, Cambridge, England) at 540 μm after 30 min incubation at 25°C. Myofibrillar protein content was calculated as the difference between the total soluble and sarcoplasmic protein fraction.

Total fat and protein contents of each sample were determined by ether extraction and micro-Kjeldahl analysis, respectively (Association of Official Analytical Chemists 1975). Protein (N×6.25) was used to express collagen and protein solubilities on a per-gram protein (N×6.25) basis.

Data were analyzed by analysis of variance and least square analysis using the General Linear Models procedure of the Statistical Analysis System (Statistical Analysis System Institute, Inc. 1982) in a 2×2 factorial to test for significant effects of electrical stimulation and restraint and their interactions. Data were tested using least square means comparisons for main effects of stimulation (control or electrically stimulated) and leg restraint (unrestrained or restrained during exsanguination). Means of significant interactions were compared with least square means comparisons, with differences considered significant at the $P<0.05$ level. Data involving prerigor and postrigor or hourly sampling postmortem had time included as a variable in the model. Two muscle samples from the prerigor collagen data were removed from the analysis because the values were greater than three standard deviations from the means. These samples were probably incorrectly taken from the spinalis dorsi, the muscle which overlays the longissimus muscle.

There were no significant differences in pH between the treatments prior to and at 1, 4, 24 and 48 h poststimulation ($P>0.05$) (data not shown), indicating that electrical stimulation did not increase postmortem muscle metabolism. Successful application of low voltage electrical stimulation depends upon employment very early postmortem because the low resistance of the live, active nervous system is the focal pathway of the low voltage electricity (Bendall 1980). There was, however, a significant interaction between stimulation and the side from which the animal was suspended during bleeding (restrained) for muscle temperature 1 h poststimulation (Table 1). The sides of the carcasses that were not electrically stimulated and were used to suspend the animal during bleeding (control, restrained) had a significantly lower temperature 1 h poststimulation than the unstimulated sides that did not suspend the animal (control, unrestrained) (Table 1). There was, however, no effect of leg restraint on the stimulated sides. The mechanism by which the muscle temperature was elevated in the control sides and not the stimulated sides is unclear; however, stimulation may have increased the muscle temperature of the restrained leg by inciting fibre contraction similar to that of the movements of the unrestrained leg. Although the longissimus muscle temperatures of the restrained and unrestrained sides of the control group were significantly different statistically, the absolute difference (2°C) appeared not to have been physiologically significant.

There were no significant differences between the treatments for longissimus muscle colour after 2 d or 7 d of aging (Hunter L values 28.95 and 31.52 vs. 29.42 and 30.99, control vs. stimulated, SEM ±0.61 and ±0.70, d 2 and 7, respectively).

There was a significant interaction between electrical stimulation and leg restraint for shear force ($P<0.05$) (Table 1). Unrestrained, stimulated sides had lower shear force values for the longissimus muscle than restrained, stimulated sides (Table 1). The decrease in shear force value of meat that has been electrically stimulated has been attributed to muscle damage (Takahashi et al. 1987). In the present experiment, some muscle fibres may have been in rigor at the time of stimulation and therefore may have been disrupted by the muscle movement resulting from the response of other muscle fibres to electrical stimulation. Glycolytic capacities vary with fibre type in the longissimus muscle. Currie and Wolfe (1979) showed that, at high temperatures, initiation of rigor was pH dependent, and that shortening

Table 1. Effects of electrical stimulation and leg restraint on temperature, shear force, collagen and protein solubilities of the longissimus muscle

Muscle characteristics	Treatment					
	Control		Stimulated			
	Rest[z]	Unrest.	Rest.	Unrest.	SEM[y]	
Sample size	7	5	5	7	5	7
Temperature (°C) 1 h poststimulation	33.3*a*	35.3*b*	35.1*b*	33.9*ab*	0.35	0.35
Shear force (kg)	4.35*ab*	4.37*ab*	4.82*b*	4.02*a*	0.27	0.23
Soluble collagen prerigor (g 100 g^{-1})[x]	14.64	17.34	16.63	17.34	3.41	4.18
Soluble collagen postrigor (g 100 g^{-1})	11.79	12.63	15.66	11.71	2.94	2.49
Sarcoplasmic protein (g 100 g^{-1})[w]	23	25	22	24	2.0	1.0
Myofibrillar protein (g 100 g^{-1})[w]	35	39	38	39	6.0	5.0
Total protein (g 100 g^{-1})[w]	57	63	61	63	6.0	6.0

[z] Rest. = restrained. Unrest. = unrestrained.
[y] SEM, standard error of mean for sample sizes $n = 5$ and 7.
[x] $n = 4$ and 6 instead of 5 and 7.
[w] g soluble protein/100 g total protein (N × 6.25).
a,b Values with different letters within rows are significantly different ($P<0.05$).

of muscle began between a pH of 6.15 and 6.35 under the restraint of a light load. The mean pH of the longissimus muscles in our experiment 1 h postmortem was 6.3. The significant decrease in shear force may have been a result of a compounding of the damage that occurred from unrestrained, reflex movement during exsanguination with that which occurred during stimulation. Collagen and protein solubilities did not explain the significant differences in tenderness of the cooked meat as indicated by shear force values (Table 1).

Although an 0.8-kg difference in shear force may be statistically significant, this difference may not be detectable by consumer or trained taste panels. Nonetheless, the leg by which an animal is suspended during exsanguination should be considered as a variable in the statistical analysis of low voltage electrical stimulation data because of its significant effect on shear force.

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H. L. BRUCE[1], C. R. JONES[2], and R. O. BALL[1,3]

[1]*Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1;* [2]*Agri-Services Laboratory Inc., Kitchener, Ontario, Canada N2G 4R2; and* [3]*Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Ontario, Canada M5S 1A8.* *Received 24 Feb. 1990, accepted 7 Aug. 1990.*

Reprint requests should be addressed to R.O. Ball