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The effects of low voltage electrical stimulation on donkey meat

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ABSTRACT

The effects of early post-mortem low voltage electrical stimulation (28 V, 60 Hz) on biochemical changes and on final tenderness in *Longissimus Thoracis et Lumborum* (LTL) muscle from donkey carcasses was studied. ES significantly accelerated the glycolytic process only during the first hours post-mortem, with a faster pH fall at 1, 3 and 6 h post-mortem, and a reduction in ATP content at 3 and 6 h post-mortem. The activities of μ-calpain and calpastatin were reduced ($P < 0.05$) by the treatment in the first 6 h post-mortem. Tenderness was improved ($P < 0.05$) at 4 and 7 days post-mortem by the application of ES. Muscle colour (L^*) was lighter ($P < 0.05$) in stimulated carcasses.

1. Introduction

The domestic equid species (horse and donkey) belong to the taxonomic order *Perissodactyla*, family *Equidae* and genus *Equus*. Donkeys are said to have originated in north-east Africa and then spread to other parts of the world. The world donkey population is about 41 million; half are found in Asia, just over one quarter in Africa and the rest mainly in Latin America (Kugler, Grunenfelder, & Broxham, 2008). Unlike cattle, buffaloes or camels, which are multi-use animals usually kept for their milk and meat as well as for work, donkeys are not sold for their meat, which is generally associated with poor social classes, famine and periods of short food supply (Belaunzaran, Bessa, Lavín, Mantecón, & Aldai, 2015).

Tenderness is generally judged as the most important quality attribute of fresh meat: a number of procedures (suspending via the pelvic bone, mechanical restraint of muscles, conditioning, delaying chilling, use of tropical plant or fungal enzymes, etc.) have been developed for increasing meat tenderness (Polidori, Kauffman, & Valfrè, 1996). All these procedures cause changes in meat tenderness via effects on the muscle fibres, on the connective tissue, or both. Initially, the connective tissue component of meat received the greatest attention; since 1960, the state of muscle contraction following rigor mortis has been the most intensively studied (Ouali, 1990).

The association between meat and electricity dates back to some of the earliest muscle physiology experiments (Bendall, 1980). Electrical stimulation has been extensively used since the 1950s to hasten *rigor mortis* and to modify steps of the glycolytic pathway (Hwang, Devine, & Hopkins, 2003). It is generally accepted that the accelerated post-mortem glycolysis induced by electrical stimulation can prevent the development of toughness caused by cold shortening (Fabiansson & Laser Reuterswärd, 1985; Marsh, Lochner, Takahashi, & Kragness, 1981). There is also strong support for stimulation increasing meat tenderness by accelerating post-mortem ageing, because rigor is achieved while temperatures are still high (Fabiansson & Libelius, 1985; Shaw et al., 2005; Hopkins, Jacob, Toheey, Pearce, Pethick & Richards, 2006). The voltage, the frequency, the duration of electrical stimulation and the method of application are important factors influencing the impact of electrical stimulation on pH fall and meat quality. High voltage electrical stimulation has been applied to achieve a very rapid pH decline (Smith, 1985). Medium voltage stimulation improved tenderness in lamb (Toohey, Hopkins, Stanley, & Nielsen, 2008) and in beef (Smulders, Eikelenboom, & van Logtestijn, 1986). Other researchers have also demonstrated a similar effect from low voltage electrical stimulation on pH decline and improvement of meat tenderness in beef (Eikelemboom, Smulders, & Ruderus, 1985) and in lamb (Polidori, Lee, Kauffman, & Marsh, 1999). In a review published by Hwang et al. (2003) it is stated that "although the pH falls are lower with low-voltage stimulation, it achieves the same rate of pH fall as high-voltages systems". The choice of the appropriate voltage (Hopkins, Toohey, Pearce, & Richards, 2008) must also satisfy safety concerns under commercial conditions.

The objective of the present study was to determine whether a low voltage (28 V peak) early post-mortem electrical stimulation, designed to accelerate glycolysis and proteolysis, could significantly improve the tenderness of donkey meat obtained from males slaughtered at 8 months of age.

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2. Materials and methods

2.1. Slaughter procedure

The study, conducted in compliance with the requirements of the Animal Ethics Committee of the University of Camerino, involved 16 entire male donkeys crossbred foals (Martina Franca × Ragusana), slaughtered at 8 months of age and at a mean final body weight of 106 ± 22 kg. Animals were slaughtered according to European Union Regulations (Council Directive 93/119 EEC) at an approved abattoir. All procedures involving animals were conducted according to the Italian law on animal welfare in scientific experiments. Following the slaughtering order, the first eight carcasses were stimulated immediately after bleeding, within 5 min of slaughter. Stimulation was applied via a rectal probe and a clip in the nostrils, using commercially available equipment (Mitab, Simrishamm, Sweden); the electrodes were turned on for 1 min, using a fixed output of 28 V peak, 60 Hz. During stimulation carcasses were suspended by the Achilles tendon. Immediately after stimulation carcasses were transferred to the dressing chain and then were put in a cold room at a temperature of ±2 °C (air velocity 0.5 m s^{-1}).

The effect of stimulation on post-mortem glycolysis was monitored by measuring the pH and the temperature values in triplicate at 1, 3, 6, 10 and 24 h post-mortem, inserting a thermocouple probe and a spear glass pH meter probe (Portamess Knick mod. 910, Elektronische Meβgeräte GmbH & Co., Berlin, Germany) into the *Longissimus Thoracis et Lumborum* (LTL) 2.5 cm below the dorsal surface adjacent to the thirteen vertebra. The final values were taken as the mean of readings from three different sites within the LTL.

2.2. Samples collection

Samples (20 g) for adenosine triphosphate (ATP) determination were removed from the LTL at 3 h (between the 12th and the 13th rib interface), 6 h (between the 10th and 11th ribs), 10 h (between the 8th and 9th ribs) and 24 h (between the 6th and 7th ribs) after stimulation. Samples were immediately frozen in liquid nitrogen, then were stored at −80 °C until assayed, using the enzyme method described by Bergmeyer, Gawehn, and Grassl (1974).

Samples for sarcomere length determinations were prepared 48 h after slaughter: cubes (20 × 20 × 20 mm) were removed from LTL between the 10th and the 11th rib interface. Sarcomere length was measured by laser diffraction using an electro optical division laser (Koolmes, Korteknie & Smulders, 1986). A thin (<1 mm) slice of muscle was cut with a scalpel and placed between two glass microscope slides before being read through a laser diffraction machine. The mean sarcomere length was the average of 10 fibres.

The muscle colour parameters were measured 48 h after slaughter using a Minolta CR-200 colorimeter (Minolta Camera Co., Osaka, Japan), with the Hunter-Lab method. A freshly cut surface of LTL was exposed to the air at ambient temperature for 30 min. After placing the measuring lens on the meat surface, it was turned through 0, 45 and 90° (clockwise) to obtain three different reflectance measurements that were later averaged. Using these spectra, the D65 Illuminant source, the CIE 1964 (10°) Standard Observer and an aperture size of 5.0 mm, measurements were taken with the camera set at maximum zoom, repeated 3 times in 3 different places, to determine L* (lightness), a* (redness) and b* (yellowness). The instrument was calibrated to a standard tile before analysis, according to the procedures described by Polidori, Pucciarelli, Ariani, Polzonetti, and Vincenzetti (2015).

The Ca^{2+} dependent proteases-I (μ-calpain), -II (m-calpain) and their inhibitor were prepared from 100 g of LTL collected at 3 h (between the 12th and the 13th rib interface), 6 h (between the 10th and 11th ribs), 10 h (between the 8th and 9th ribs) and 24 h (between the 6th and 7th ribs) after stimulation, according to Koohmaraie, Crouse, and Mersmann (1989). Samples were trimmed of fat and connective tissue, cut into 2 × 2 cm pieces, frozen in liquid N and stored at −70 °C for 14 days. Casein was used as a substrate for the determination of calpain and total caseinolytic activities, expressed as activity per g of muscle, according to Wheeler and Koohmaraie (1991). The assay medium I consisted of 100 mM Tris, 10 mM MCE, 5 mg/mL casein, 1 mM NaN_3, and 5 mM $CaCl_2$, with pH adjusted to 7.5 at 25 °C with 1 N acetic acid. Assay medium II containing the same components as assay medium I, except 10 mM EDTA was substituted for 5 mM $CaCl_2$, was used to determine Ca++-independent activity. An aliquot of sample fraction (1.0 mL) was incubated with 1.0 mL of assay medium I at 25 °C for 60 min to determine μ-calpain activity, and sample fraction (0.5 mL) was incubated with 1.5 mL of assay medium I at 25 °C for 60 min to determine m-calpain activity. Reactions were stopped by adding an equal volume of 5% trichloroacetic acid (TCA). Following centrifugation at 2000 × g_{max} for 30 min, the A_{278} of the supernatant fluid was determined. The A_{278} of the sample fractions in the presence of EDTA was subtracted from the A_{278} of sample fractions in the presence of $CaCl_2$ to determine Ca++-dependent proteolytic activity. One unit of calpain activity was defined as the amount of enzyme required to catalyze an increase of 1.0 absorbance unit at 278 nm in 60 min at 25 °C. Calpastatin activity was determined by incubating 1 mL of inhibitor and 1.5 mL of m-calpain (DEAE-Sephacel purified m-calpain with ≤0.45 units of activity) at 25 °C for 1 min before adding 1.5 mL assay medium I (Lee, Polidori, Kauffman, and Kim, 2000). Following incubation at 25 °C for 60 min, the reaction was stopped by adding TCA and then centrifuged at 2000 × g_{max} for 30 min. To produce the standard, the inhibitor fraction was replaced by the equal volume of equilibrating buffer. One unit of inhibitory activity was defined as the amount of the calpastatin that inhibited 1 unit of m-calpain activity.

2.3. Shear force determination

Samples weighing approximately 100 g for shear force determination were removed from LTL between the 12th and 13th rib interface. Samples were held in a vacuum bag in the usual cold room at 2 °C, and stored for 4 and 7 days post-slaughter before determination of shear force. Chops (each 2.5 cm thick) were roasted on a metal tray at an oven temperature of 80 °C to an internal temperature of 73 °C (monitored with thermocouples), according to the procedures of Riley, Savell, Smith, and Shelton (1981). Chops were cooled to room temperature (23 °C) for 30 min. From each sample, 8 cores (1.2 cm in diameter) were sheared with a Warner-Bratzler operating head mounted on an Instron apparatus 4411 (Instron, High Wycombe, UK) and crosshead speed set at 200 mm/min. Peak or maximum shear force across the fibres was expressed in N.

2.4. Statistical analysis

An analysis of variance was used to determine the significance of differences in values obtained in this study, using general linear model procedures of the statistical package of SAS (2001). In both stimulated and control group the trends in pH against temperature were modelled according to the procedure used by Hopkins, Ponnampalam, van de Ven, and Warner (2014). The model was used to estimate mean temperature at pH 6 (Temp@pH6) for each group (ES vs Non-ES). The statistical model for this analysis included an overall trend in pH with temperature decline across the carcasses. Significant differences between means were indicated when $P < 0.05$.

3. Results and discussion

The results of pH and temperature measurements obtained at 1, 3, 6, 10 and 24 h after slaughtering are shown in Table 1. Electrical stimulation caused a significant ($P < 0.05$) drop in pH compared to control group in the first 6 h. The values of temperature determined in both groups were not affected by the treatment. The rate of post-mortem