Effekt auf elektrische Stimulierung lysosomer Fermenttätigkeit, pH Abfall und Rindfleischmürbigkeit

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Der Mechanismus oder die biochemischen Änderungen, die die Erweichung von Fleisch durch elektrische Stimulierung hervorrufen, sind nicht ausreichend bekannt. Lysosome Fermente werden durch ein niedriges pH aktiviert und tragen teilweise zur Mürbigkeit durch Hydrolysierung der Bindegewebe (Beta-Glukorodinase) und der Eiweiss (Kathepsin) bei. Ein Versuch wurde unternommen, den Erweicherungsprozess zu erklären. Zwölf Stiere ähnlicher Herkunft wurden willkürlich zwei verschiedenen antemortem Behandlungen ausgesetzt (keim stress [Kontrolle] oder stress - 48 Stunden ohne Futter, 10-Min. Bewegung alle 3 Stunden während 15 Stunden und ununterbrochene 30-Min. Bewegung unmittelbar vor dem Schlachten). Eine Stunde post mortem wurden Teile beider Gruppen willkürlich zwei Behandlungen ausgesetzt - keime elektrische Stimulierung (Kontrolle) oder Stimulierung mit 1-Amp Strom (145-250 V) in 15 Sek. Pausen während 3 Min. (Wechselstrom - 60 Zykl.). Longissimus Proben wurden bei 0, 3, 6, 12, 24 und 48 Stunden entnommen, um das pH und die lysosome Fermenttätigkeit zu bestimmen, und bei 48 Stunden für sensorische Gruppenbewertung.

Der schnellste Abfall der pH-Werte wurde bei elektrisch stimulierten Leichen von Tieren beobachtet, die Stress nicht ausgesetzt wurden, während stimulierte oder unstimulierte Leichen den niedrigsten Abfall aufwiesen. Stimulierte und unstimulierte Leichen von Stress ausgesetzten Tieren erwiesen sich als weniger mürbe (Gruppenbewertung) als stimulierte Leichen von Tieren, die Stress nicht ausgesetzt waren (5,5, 5,3, 4,9, versus 6,0).

The effect of electrical stimulation on lysosomal enzyme activity, pH decline and beef tenderness

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The mechanism or biochemical changes responsible for meat tenderization by electrical stimulation are not completely known. Lysosomal enzymes are activated by low pH and partially contribute to tenderness by hydrolysing connective tissue (β-glucuronidase) and by hydrolysing proteins (cathepsins). An attempt was made to give an insight to this tenderization process. Twelve steers of similar breeding and management history were randomly assigned to two antemortem treatments (no stress {control} or stress--48 hr off feed, 10 min exercise every 3 hr for 15 hr and 30 min continuously just prior to slaughter). One hr postmortem, sides in each group were randomly assigned to two treatments--no electrical stimulation (control) or stimulation--with 1 amp current (145 to 250 volts) at 15 sec intervals for 3 min (AC - 60 cycle). Longissimus samples were removed at 0, 3, 6, 12, 24 and 48 hr for pH and lysosomal enzyme activity determination and at 48 hr for sensory panel evaluation.

Electrically stimulated carcasses that were not stressed had the most rapid rate of pH decline while stressed carcasses (stimulated and unstimulated) had the lowest. Stressed carcasses (stimulated and unstimulated) and control (unstimulated and unstressed) carcasses were less tender (panel ratings) than unstressed, stimulated carcasses (5.5, 5.3, 4.9 vs 6.0).

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Effets de la stimulation électrique sur l'activité des enzymes lysosomales, le fléchissement du pH et la tendreté du boeuf.

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Les changements mécaniques ou biochimiques qui assurent la tendreté de la viande par une stimulation électrique sont mal connus. Les enzymes lysosomales sont mises en marche par un pH faible et contribuent en partie à la tendreté en hydrolysant le tissu conjonctif (β - glucuronidase) et les protéines (cathepsines). On s'est efforcé de mieux connaître ce processus. Douze boeufs de même race et de même élevage furent choisis aléatoirement et affectés à deux traitements antemortem (sans stress et avec - contrôle) - 48 heures à jeun, 10 minutes d'exercice toutes les 3 h pendant 15 h. et 30 min. consécutivement avant abattage). Au postmortem d'une heure, les quartiers de chaque groupe furent affectés aléatoirement à deux traitements --- sans stimulation électrique (groupe témoin) ou avec stimulation - courant d'un ampère (145 à 250 volts) à intervalles de 15 secondes pendant 3 min. (alternatif-60 cycles). Des échantillons longissimus furent prélevés aux heures suivantes: 0, 3, 6, 12, 24 et 48, afin d'en observer l'activité des enzymes lysosomales et du pH, et à 48 heures aux fins d'évaluation sensorielle (sensory panel).

Les carcasses stimulées électriquement non soumises au stress présentent le taux de fléchissement du pH le plus rapide, alors que les carcasses soumises au stress (stimulé ou non-stimulé) possédaient le plus faible. Les carcasses soumises au stress (stimulé ou non-stimulé) et les carcasses témoins (de contrôle) étaient moins tendres (indices du panel) que les carcasses sans stresses et stimulées (5.5, 5.3, 4.9 contre 6.0).

Влияние лизосомной деятельности ферментов, снижения pH и мягкости говядины на электрическую стимуляцию

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Механизм или биохимические изменения, вызывающие размягчение мяса путем электрической стимуляции полностью не исследованы. Лизосомные ферменты активизируются низким pH и частично содействуют размягчению, гидролизируя соединительную ткань (бета-глюкоролиназ) и белки (катепсины). Была сделана попытка определить этот процесс размягчения. Двенадцать быков сходного разведения были подвергнуты на выборочной основе двум типам предсмертного обращения (отсутствие стресса /контроль/ или стресс -- 48 часов без корма, 10-минутное движение каждые 3 часа на протяжении 15 часов и непрерывное 30-минутное непосредственно перед убоем). Один час после убоя, стороны каждой группы подвергались, на выборочной основе, двум процессам -- отсутствие электрической стимуляции (контроль) или стимуляция током в 1 амп (145-250 в) по 15-секундным интервалам в течение 3 минут (переменный ток - 60 циклов). Образцы longissimus брались в 0, 3, 6, 12, 24 и 48 часов для определения pH и лизосомной деятельности ферментов, и после 48 часов для оценки сенсорной деятельности.

Электрически стимулированные туши, не подлежавшие стрессу, выявили наиболее скорый спад pH, тогда как туши животных, подвергнутых стрессу (со стимуляцией или без нее) выявили наиболее медленный спад pH. Туши животных под стрессом (со стимуляцией или без) и туши контрольных животных (со стимуляцией или без нее) оказались менее размягченными (оценка группы) чем стимулированные туши животных не подвергнутых стрессу (5,5, 5,3, 4,9 по сравнению с 6,0).

The effect of electrical stimulation on lysosomal enzyme activity, pH decline, and beef tenderness

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Introduction

Several studies on beef have suggested that electrical stimulation will accelerate post-mortem pH decline, hasten rigor development, and improve tenderness (Harsham and Deatherage, 1951; Chrystall and Hagyard, 1976; Smith et al., 1977; and Savell et al., 1977). Some investigators (Chrystall and Hagyard, 1976; and Davey et al., 1976) have attributed the improved tenderness effects of electrical stimulation to prevention of "cold shortening." However, recent studies have failed to show consistent differences in sarcomere length of stimulated and unstimulated carcasses (Savell et al., 1977 and Grusby et al., 1976). Several workers (Dutson et al., 1978; Smith et al.; 1977) have suggested that part of the tenderization may result from increased activity of the lysosomal enzymes in the treated carcasses. Lysosomal enzymes are activated by low muscle pH (Tappel, 1966) and may partially contribute to meat tenderness by hydrolysing connective tissue (β-glucuronidase) and/or proteins (cathepsins).

To obtain an insight into the tenderization process, we evaluated the effect of electrical stimulation on lysosomal enzyme activity in carcasses from stressed and unstressed steers.

Material and Methods

In a 2x2 factorial experiment, 12 steers of similar breeding and management history (USDA quality grade of low to average Good; USDA yield grade of 2.5 to 3.0 and 375-425 kg in weight) were randomly assigned to two antemortem treatment groups. One group of steers were not stressed (control) but allowed free access to feed and water prior to slaughter; the other group were taken off feed and water for 48 hr prior to slaughter and stressed by exercising for 10 min every 3 hr for a total of 15 hr and then continuously for 30 min just prior to slaughter. The stress treatment was designed to lower muscle glycogen level so that lactic acid level, also, would be lowered and the pH would remain high (Ashmore et al., 1971). The two treatments with and without stress were performed to provide two different rates of pH decline.

At 1 hr postmortem, sides from unstressed and stressed carcasses were randomly exposed to two treatments--no electrical shock and electrical shock. Metal pins serving as electrodes were placed in the round muscle near the Achilles' tendon and in the muscles between the scapula and the thoracic vertebrae. Electrical stimulation was administered for 15 sec at about 1 sec intervals. Stimulation was for a total of 3 min, with 1 amp current passing through the carcass (145-268 volts; AC, 60HZ).

Longissimus muscle samples were excised at 0, 3, 6, 12, 24, and 48 hr poststimulation and analyzed immediately for pH and lysosomal enzyme activity, with β-glucuronidase as a marker enzyme. After 48 hr of chill at 2-3°C, two 2.5 cm thick steaks were removed, wrapped in freezer paper, and stored at -28°C for subsequent palatability evaluation by a 10-member descriptive attribute panel as described by Cross et al. (1978).

For pH determination, 10g of sample was homogenized with 50 ml of cold 0.25 M sucrose containing 0.02 M KCl in a Sorvall Omnimixer for 20 sec at speed 6. The homogenate was filtered by two layers of cheese cloth, and pH of the filtrate was determined before adjusting to 7.3. Subsequently, the filtrate was processed to yield β-glucuronidase, which was then assayed according to Gianetto and deDuve (1955). The amount of soluble protein was determined by the modified biuret procedure (Gornall et al., 1949), and bovine albumin was used as standard.

Results and Discussion

Rate of pH decline is presented in figure 1. Electrically stimulated beef sides that were not stressed had the most rapid rate of decline ($P < 0.0001$) while the stressed sides (stimulated or unstimulated) had the slowest rate of pH decline. The nonstressed and unstimulated group (control) had a pH value of 6.50 at 1 hr post-mortem (i.e., 0 hr poststimulation). This value falls within the range 6.48-7.04 reported in the literature (Moeller et al., 1976; McCollum and Henrickson, 1977; Shaw and Walker, 1977; and Tarrant and Mothersill, 1977).

The pH value dropped to 5.45 within 6 hr poststimulation (7 hr post-mortem) in the nonstressed, stimulated group. Gilbert and Davey (1976) obtained a pH of 5.49 in 5 hr using a higher voltage (3600 V) than that which we used. At 48 hr poststimulation pH of the stressed carcasses were significantly higher ($P < 0.05$) (5.80 and 5.76, respectively, for unstimulated and stimulated carcasses) than those of the unstressed beef sides.

Davey et al. (1976) observed that tenderness is the palatability attribute most affected by electrical stimulation. Mean values for palatability and Instron shear force are presented in table 1. Carcasses from unstressed, stimulated animals were rated significantly more tender than unstressed, unstimulated carcasses. This difference was supported by signifciantly different Instron shear force values between the same groups. The unstressed, stimulated carcasses had the least variability about the means for tenderness and Instron shear force. The differences in tenderness were large enough to be of practical importance. Carcasses from stressed animals were intermediate to those from the unstressed animals in tenderness and were not significantly affected by electrical stimulation. Unstressed and stimulated carcasses were rated significantly lower than unstressed, unstimulated carcasses in detectable connective tissue. Data in table 1 suggest that electrical stimulation of stressed animals does little to enhance tenderness.

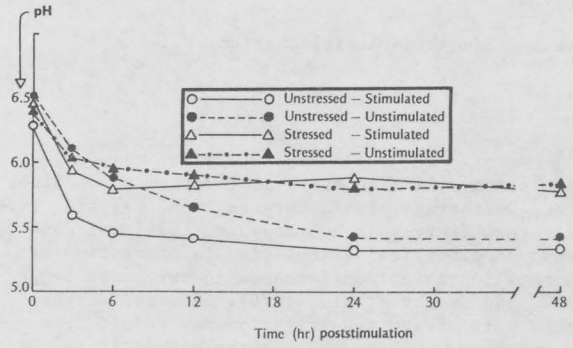

Fig. 1

The effect of electrical stimulation and stress treatment on the rate of pH decline.

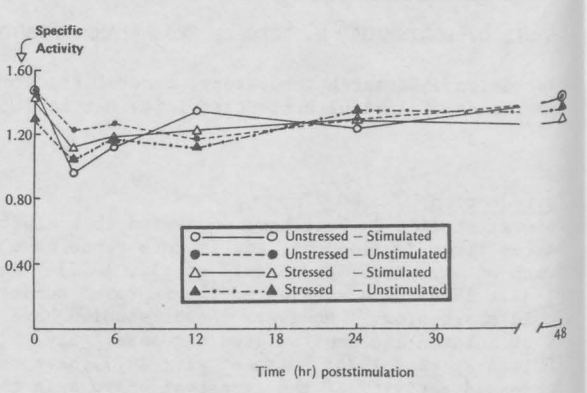

Fig. 2

The effect of electrical stimulation and stress treatment on the release of lysosomal enzyme (β- glucuronidase)

Table 1. Means and standard deviations of Instron and palatability ratings[1]

Treatment	n	Sensory Panel Ratings: Tenderness[2]	Juiciness[3]	Connective tissue amount[4]	Instron maximum shear force (kg)
Stressed and unstimulated	6	5.3(0.85)^{ab}	5.4(0.27)^{ac}	6.4(0.29)^{ab}	6.0(1.40)^{a}
Stressed and stimulated	6	5.5(0.77)^{ab}	4.9(0.67)^{b}	6.4(0.51)^{ab}	5.3(1.86)^{ab}
Unstressed and unstimulated	6	4.9(1.15)^{b}	5.8(0.38)^{a}	6.2(0.31)^{b}	5.9(1.51)^{a}
Unstressed and stimulated	6	6.0(0.40)^{a}	5.0(0.30)^{bc}	6.8(0.42)^{a}	5.0(0.92)^{b}

^{abc}Means in the same column with different superscripts are significantly different ($P < 0.05$).
[1]Means in the same column having different letters are significantly different ($P < 0.05$).
[2]Tenderness - 8 = extremely tender, 1 = extremely tough.
[3]Juiciness - 8 = extremely juicy, 1 = extremely dry.
[4]Connective tissue amount - 8 = none, 1 = abundant.

Analysis of variance revealed that there was no difference between treatments in the specific activity of lysosomal enzymes, as monitored with β-glucuronidase. Perhaps the number of observations used in this study was not sufficient to demonstrate any significant difference due to the stimulation treatment (figure 2). The free activity of lysosomal enzyme in all groups dropped within the first 3 hr and then started to increase. At 12 hr poststimulation activity was higher, but not significantly so, in the nonstressed, stimulated sides than in the nonstressed, unstimulated sides. The unstressed, stimulated sides (pH 5.60) tended to have the least amount of free activity at 3 hr poststimulation. The combined effects of low pH and high carcass temperature cause the disruption of the lysosomal membrane and the rapid release of acid hydrolases particularly β-glucuronidase into the muscle tissue (Moeller et al., 1976, 1977). The β-glucuronidase, so released, then undergo autolytic digestion. Apparently, the rate of autolysis was highest at 3 hr poststimulation. Dutson et al. (1978) observed significantly ($P < 0.05$) less total activity of both β-glucuronidase and cathepsin C in ovine muscle that had been electrically stimulated. The loss in activities suggests that a greater amount of autolysis had occurred in this muscle.

In conclusion, rate of pH decline was the most rapid for unstressed, electrically stimulated sides and slowest for the stressed sides (stimulated or unstimulated). The unstressed, stimulated sides were the most tender, and these sides had the least variability about the means for tenderness and Instron shear force. A low variability could be of practical importance to the industry and the consumer. The differences in lysosomal enzyme activity were not significant, however, there was a relationship with the rates of pH decline and the lysosomal enzyme activation. Our results do not conclusively establish a direct relationship between lysosomal enzyme activity and muscle tenderness. If no significant differences in tenderness had been detected, the role of lysosomal enzymes could have been ruled out; but since this was not the case, the possibility for their contribution to tenderness still exists.

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