

# EFFECT OF HIGH VOLTAGE ELECTRICAL STIMULATION ON BUFFALO MEAT CONDITIONING

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## ABSTRACT

The efficiency of high voltage electrical stimulation (700V, 1400V peak, pulses 1s on/1s off, 60Hz, 2A) on buffalo (*Bubalus bubalis*) carcass in muscle conditioning, during nine days storage period, was studied. Electrically stimulated (ES) muscles resulted in significantly more rapid pH fall as compared with controls ( $P<0.01$ ) and the difference at first hour *post mortem* was 0.75 value. The IMP/ATP ratio clearly indicates that the applied process reduced significantly ( $P<0.01$ ) time period necessary for the onset of *rigor mortis* in ES muscle. Myofibrillar fragmentation index differences between control and ES muscles increased throughout storage at 2°C being statistically significant ( $P<0.01$ ), and it was confirmed by slurry myofibrillar photomicrographies from 6th day of *post mortem* conditioning. On this storage time the SDS-electrophoretic patterns of myofibrillars proteins also indicated light weakening of Troponin T (37,000D) band. The rate of the myofibrillar degradation, hence the acceptable tenderness in buffalo muscle, can be predicted by high voltage electrical stimulated process.

Key Words: Meat - Buffalo - Conditioning - Electrical Stimulation - Carcass

## RESUMO

A eficiência da estimulação elétrica de alta voltagem (700V, 1400V(pico), pulsos 1 s<sup>-1</sup>, 60Hz, 2A) na carcaça de búfalo (*Bubalus bubalis*), com ênfase na maturação muscular durante um período de nove dias de armazenamento, foi estudada. Nos músculos eletricamente estimulados (ES) foi bastante significativa ( $P<0.01$ ) a queda de pH, em relação ao controle, sendo esta diferença de 0,75 na primeira hora do *post mortem*. A relação IMP/ATP indicou claramente que o estímulo aplicado reduziu significativamente ( $P<0.01$ ) o tempo necessário para o músculo ES atingir o *rigor mortis*. A diferença no índice de fragmentação miofibrilar entre os músculos ES e controle aumentou significativamente ( $P<0.01$ ) durante todo o tempo de estocagem, sendo esta confirmada pelas fotomicrografias das miofibrilas no 6º dia de maturação. Neste tempo de estocagem o

perfil eletroforético das miofibrilas em SDS, também indicaram um leve enfraquecimento na banda da Troponina T (PM 37000D). A velocidade de degradação miofibrilar, num amaciamento aceitável para o músculo búfalino, pode ser antecipada pelo processo de estimulação elétrica de alta voltagem.

Palavras-Chave: Carne - Búfalo - Maturação - Estimulação Elétrica - Carcaça

## INTRODUCTION

Buffaloes meat texture and palatability are comparable to beefs. The buffalo can reach an adequate slaughter weight at two years old and at this time period they have also similar size as bovine cattle. This allows use of the same line in abattoir to bovine and buffalo meat process, including electrical stimulation.

The mechanical and biochemical responses produced by electrical stimulation of muscle have widely been studied by physiologists and biochemists in investigations of contractile process. The acceleration of glycolysis has important practical implications in the meat industry because the reducing time needed for muscles to enter *rigor mortis* minimises the delay before chilling for freezing and the risk of toughening from cold and thaw shortening to be avoided.

Several groups of workers (CARSE, 1973; CHRYSTALL & HAGYARD, 1976; BENDALL, 1980; SMULDERS et al. 1986; KOH et al. 1987; CARBALLO et al. 1988) have substantiated the direct tenderising effect of electrical stimulation process on beef or ovine carcasses during slaughter.

The tenderising effect of electrical stimuli was attributed to the release of catheptic enzymes during the vigorous muscle contractions it induced (SPECHT, 1990; LAWRIE, 1985; McKEITH et al. 1981; DUTSON et al. 1980). However, the electrical stimulation efficiency is impedance dependent and it may differ between animals muscles (LAWRIE, 1985).

McKEITH *et al.* (1981) showed that high voltage (550V) is more effective and strength than low voltage to cross section on beef carcass sides. Therefore the septate carcasses increase intersection areas and gain electrical current resistance. Frequencies from 15 to 25 pulses.sec<sup>-1</sup> was adequate to drop the muscle pH and the physical manifestation of polarisation in form of twitch was absent with alternating pulses (SAVELL *et al.* 1978; CHRYSTALL & DEVINE, 1978; TAKAHASHI *et al.* 1987; HARRIS & SHORTHOSE, 1988).

This work studies the efficiency of high voltage electrical stimulation on buffalo carcass with emphasis on the effect on meat aging, during nine days of storage period, on certain biochemical characteristics.

## MATERIALS AND METHODS

### Muscles

*Longissimus dorsi thoracis* muscles were excised from carcasses, as soon as possible, after electrical stimuli, or from the non stimulated control. At first 24 hours samples were taken for the initial determination. The muscles were divided into two portions, vacuum packed and stored at 2°C to *post rigor* meat conditioning, for nine days. The packs were aleatory open at 1, 3, 6 e 9 days of ageing and samples were analysed for various parameters as described below.

### pH Measurements

For pH determination, samples were collected at 1, 2.5, 3.5, 4.5, 6, 8, 12 and 24 hours after animal death. All samples were immediately immersed in sodium iodoacetate to inhibit glycolysis (BENDALL, 1973) and pH was determined (pH meter Mod. 115 - Digimed, Brazil) after 10 seconds homogenisation (samples kept at 20°C with ice bath) in Ultraturrax (Mod. TE 102 - Tecnal, Brazil).

### IMP/ATP ratio

This evaluation was performed at the same time interval as for pH determination. Muscles samples were immediately frozen in liquid N<sub>2</sub> and ground in a mortar (with liquid N<sub>2</sub>) until complete desintegration. Nucleotides were extracted by sample homogenisation with 1M perchloric acid at 1:10 ratio (w/v). The suspension was then filtered and diluted with 0.1M phosphate buffer pH 7.0 and absorption at 250 and 260 nm was used to calculate IMP/ATP ratio (R Value) according to HONIKEL & FISCHER (1977).

### Myofibrillar Fragmentation Index (MFI)

Samples were collected at 1, 3, 6 and 9 days from meat matured at 2°C and frozen in liquid N<sub>2</sub> and kept for

further myofibril isolation. The isolation was performed according to DAVEY and GILBERT (1969), after thawing the samples, removal any visible fat and homogenisation in a Waring Blendor at 2°C in 0.1M KCl, 20 mM phosphate pH 7.0, 1 mM EDTA, 1 mM sodium azide at 1:10 (w/v) ratio. Samples were then centrifuged at 1,000 x G for 15 min at 4°C (Centrifugal Sorvall Mod. 1000 - Dupont Instruments, USA), supernatant discarded and sediment washed three times with the initial buffer. The washed myofibrils were suspended in 5 volumes of the initial buffer and protein was determined according to Lowry *et al.* (1951). An aliquot of 10 ml of this suspension with 4 mg.ml<sup>-1</sup> was homogenised for 20 sec at 2°C (Homogenizer PCU-Drehzahlregler kinematica GmbH, German) at speed 5. Turbidity measured at 540nm in the homogenised aliquot multiplied by factor 200 is the Myofibrillar Fragmentation Index (DAVEY and GILBERT, 1969).

### Microscopic Examination

All samples collected at 1, 3, 6 and 9 days from meat matured at 2°C, after myofibrillar extraction and turbidity measured for myofibrillar fragmentation index, were examined and photomicrographied at a magnification of 1875X (Microscope Nikon, Nippon - Microflex HFM-Labophot).

### Myofibrillar protein gel electrophoresis

From the matured muscles myofibrillar protein was extracted by an Ultraturrax homogeniser (Mod. TE 102, Tecnal, Brazil), under cold temperature (2°C), using buffer pH 7.0 in 1:20 (w/v) ratio, containing 20 mM imidazol, 0.6M KCl, EGTA 2mM, EDTA 2mM, NaN<sub>3</sub> 0.01%, MgSO<sub>4</sub> 1mM, ATP 5 mM, and centrifuged 10 000 x g<sub>max</sub> for 30 min. After 12 hour dialysis, protein was determined (LOWRY *et al.*, 1951). The SDS polyacrylamide gel electrophoresis was performed according to STEPHENS (1975), using 10% gels, containing acrilamide/bisacrilamide 30:0.8 (w/w), Tris-glycine, pH 8.6, Tris-HCl 3M, pH 8.8, dodecyl sodium sulfate 20% (w/v), ammonium persulfate 0.1gml<sup>-1</sup>. A 10µl aliquot was applied to each lane of slab gel, using 150V.

### Statistical Analysis

Experimental design adopted was of completely randomised blocks. The variance analysis was employed with Duncan test at 5 and 1%, according to YATES (1937), for mean values comparison.

## RESULTS AND DISCUSSION

Compared with the muscle control treatments (Figure 1), the high voltage electrical stimulation (HVES)

effectively reduces the *post mortem* pH values of buffalo carcass. Typical *rigor mortis* pHs are achieved sooner after slaughter on the stimulated muscles whereas on non stimulated these values are reached only after a 24 hour period. The muscles contractions are cytoplasmic ATP dependent and by electrical stimulation process, who makes successives and

intensives muscular movements of contractions, happening depletion in energy compounds and the anaerobic glycolysis result in remarkable fall in pH, when the muscle have glycogen reserve (CALKINS *et al.* 1983; FABIANSOON & LASER REUTERSWARD, 1985).

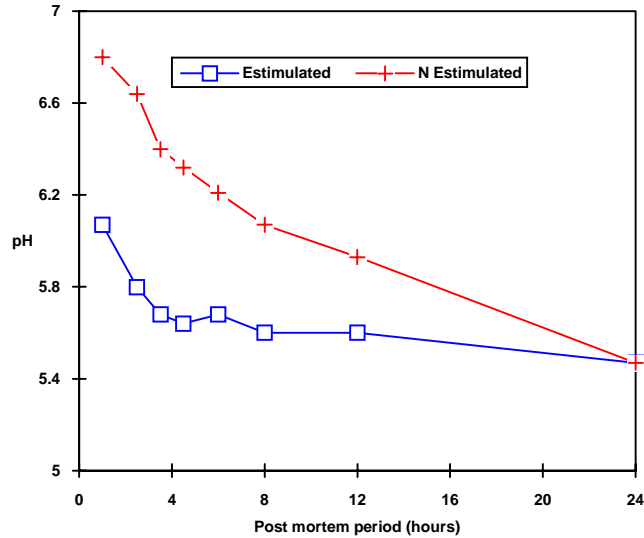


Fig. 1 *Post mortem* pH of *Longissimus dorsi thoracis* muscle of buffalo with electrical stimulation and non stimulated.

During electrical stimulation on bovine carcass the rate of underlying biochemical reactions, at first stage, increase 150-fold inducing 0.5 to 0.7 pH unit drop (CHRYSTALL & DEVINE, 1978). These results are similar to found for buffalo carcass pH difference between muscles wich high voltage electrical stimulated and control, and those values are 0.75, at first hour and near to 0.85 after 3.5 hour. Another indication of the *post*

*mortem* metabolism is the degradation of compounds rich in energy by the IMP/ATP ratio (HONIKEL & FISCHER, 1977; HONIKEL *et al.* 1981). Figure 2 clearly indicates that process applied reduced significantly the time necessary for the onset of the *rigor mortis* to be achieved in ES muscles compared to control.

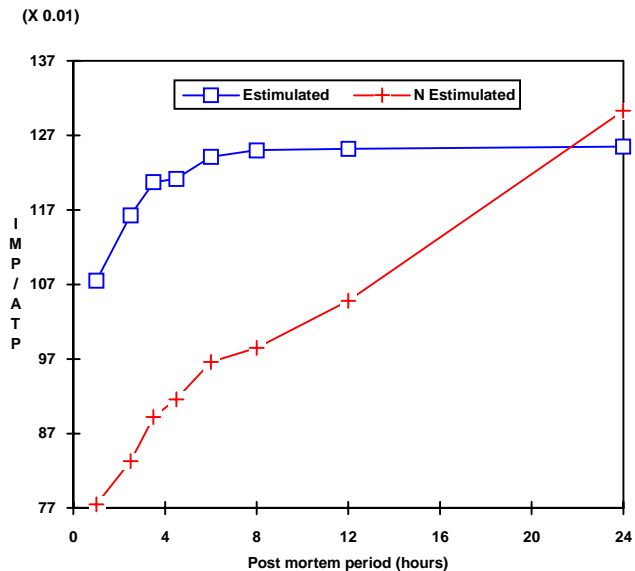


Fig. 2 IMP/ATP ratio of *Longissimus dorsi thoracis* muscle of buffalo with electrical stimulation and non stimulated.

Studies on bovine muscle extensibility indicated that *rigor mortis* occurs at IMP/ATP ratio 1.1 and pH 5.9 (BENDALL, 1973). Applying these criteria to buffalo meat it reaches this *rigor mortis* after 2 hour. There is no report in literature on buffalo meat ES but the present results are comparable to the majority of those obtained for beef and ovine carcass (MARSH *et al.*, 1987; HARRIS & SHORTHOSE, 1988; CARBALLO *et al.*, 1988; SMULDERS *et al.*, 1989).

The *post mortem* muscle tenderization is largely influenced by alterations in myofibrillar proteins. Proteolysis by *in situ* proteases of skeletal muscle has been a primary hypothesis to explain *post mortem* muscle tenderization. This evidence has clearly been demonstrated when Z-disk degradation, which occurs in myofibrils during *post mortem* aging, as consequence of myofibrils fragmentation into smaller segments (DAVEY & GILBERT, 1969; KOOHMARAIE *et al.* 1987). Figure 3

shows the myofibrillar fragmentation index (MFI) for the non stimulated and stimulated *Longissimus dorsi thoracis* muscles.

This index is an indirect measurement of fragmentation of sarcomer structure during conditioning of buffalo meat. No difference was found on experimental bases between muscles until the 3th day of maturation and both muscles increased their myofibrillar fragmentation index. Meanwhile, at 6th day of maturation those all electrical stimulated muscles increased significantly ( $P < 0.01$ ) this index compared to control. HAWRISH *et al.* (1987), using low voltage electrical stimulation process produced a slight but constant difference on behaviour of *Biceps femoris* and *Semimembranosus* bovine muscles stored at 2°C for 48 hour.

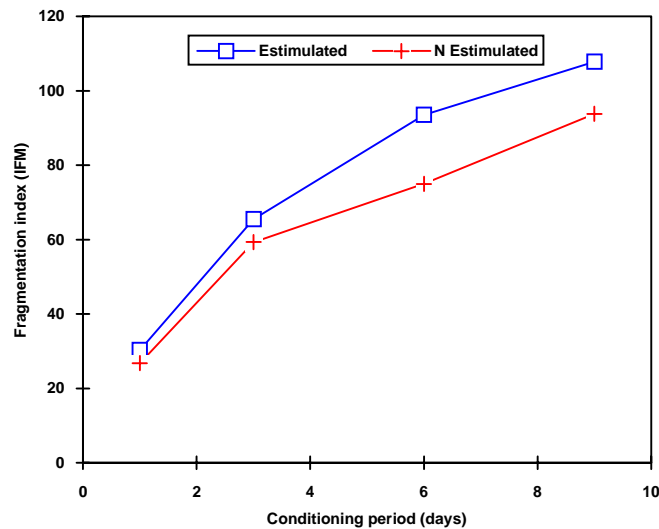


Fig. 3 Myofibrillar fragmentation index of *Longissimus dorsi thoracis* buffalo muscle during conditioning at 2°C with electrical stimulation and non stimulated.

There is no report on buffalo muscle aging in literature but the fragmentation index value 70, was found to the *Longissimus dorsi* muscle from bovine at 3th day of conditioning (OLSON *et al.* 1977), without stimulation process. Already KOOHMARAIE *et al.* (1987), showed during 6th day of maturation in the same muscle used on this experiment a fragmentation index value 71.2 with 90% of myofibrillar degradation. This skeletal protein degradation was monitored by photomicrographies (Fig. 4 and 5) of myofibrillar extract, increasing turbidity measure results as consequence of the acceleration buffalo muscle conditioning. It has been suggested by other reports (ASHGAR & HENRICKSON, 1982; WU *et al.* 1985) that electrical stimulation, besides

sarcomere structure physical disruption, is also responsible for the availability of proteolytic enzymes that may act earlier on Z disk rupture producing thus a softer meat. It was concluded (KOOHMARAIE *et al.* 1988) that *post mortem* damages in the presence of  $Ca^{++}$  chelants do not affect lysosomal enzyme activities and a calcium activated factor enzyme (calpains) are responsible for myofibrillar degradation. If the changes in myofibril were  $Ca^{++}$ -dependent, the increase of calpain proteases activity takes the principal role on proteolysis when the major concentration of this cations are induced by electrical stimulation allowing *post mortem* tenderization.

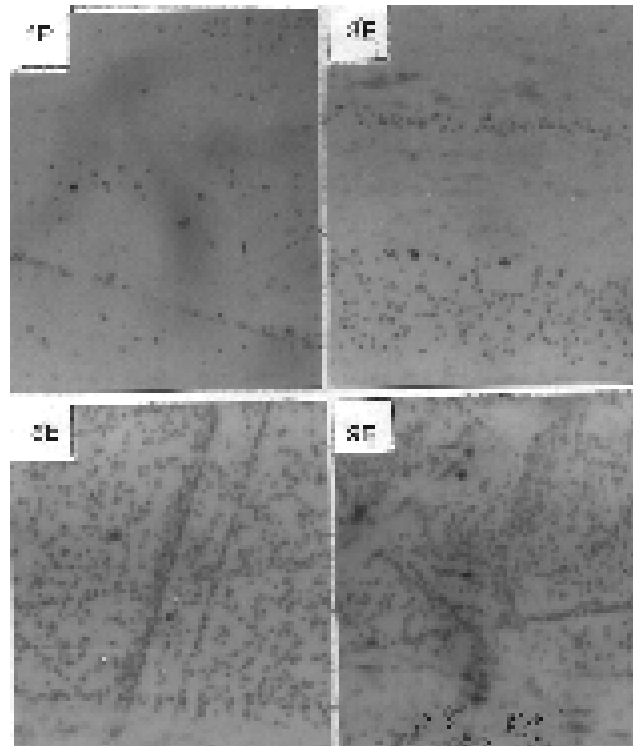


Fig. 4 Photomicrographies of myofibrillar proteins extracted of electrically stimulated *Longissimus dorsi thoracis* muscles of buffaloes matured for 9 days. The fragmentation index were 30.3; 65.5; 93.5 and 107.8 correspond to 1th, 3th, 6th and 9th days, respectively.

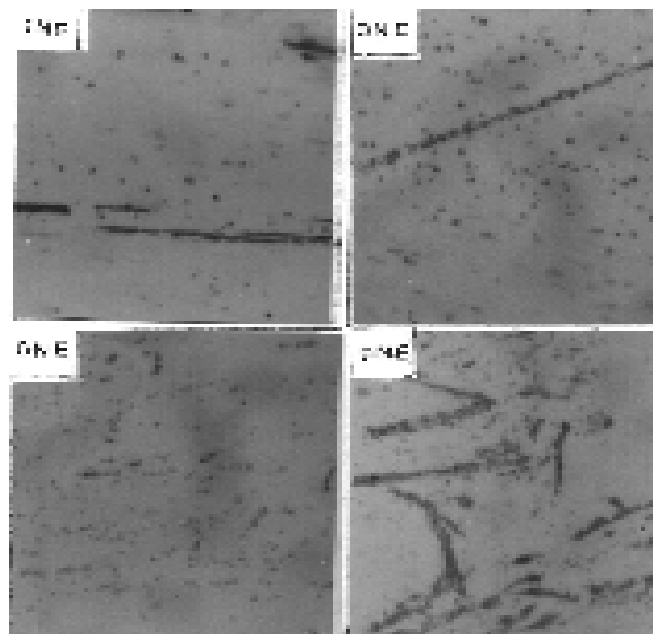


Fig. 5 Photomicrographies of myofibrillar proteins extracted of non electrically stimulated *Longissimus dorsi thoracis* muscles of buffaloes matured for 9 days. The fragmentation index obtained were 26.8; 59.3; 75.0 and 93.8 correspond to 1th, 3th, 6th and 9th days, respectively.

Muscle fragmentation was observed by polyacrilamide gel electrophoresis. Figures 6 shows the electrophoretic pattern of buffalo muscle myofibrillar protein during the maturation period. Troponin T band weakening and the appearance of a 30,000 dalton component is associated to the tenderness changes during beef meat conditioning (OLSON *et al.* 1977; GOLL *et al.* 1983; ETHERINGTON *et al.* 1987). No difference was found in the myofibrillar protein

electrophoretic pattern (Fig. 6-4.A and B), at first day of stored in 2°C. On stimulated muscles, it was observed (Fig 6-A2) the slow hydrolysis of Troponin T, wich is present up to the sixth day and increased in ninth day of aging. The time of myofibrillar protein hydrolysis on *post mortem* muscle storage differs between animals and the rate is pH, temperature and Ca<sup>++</sup> dependent (ETHERINGTON *et al.* 1987 and PENNY & FERGUSON-PRYCE, 1979).

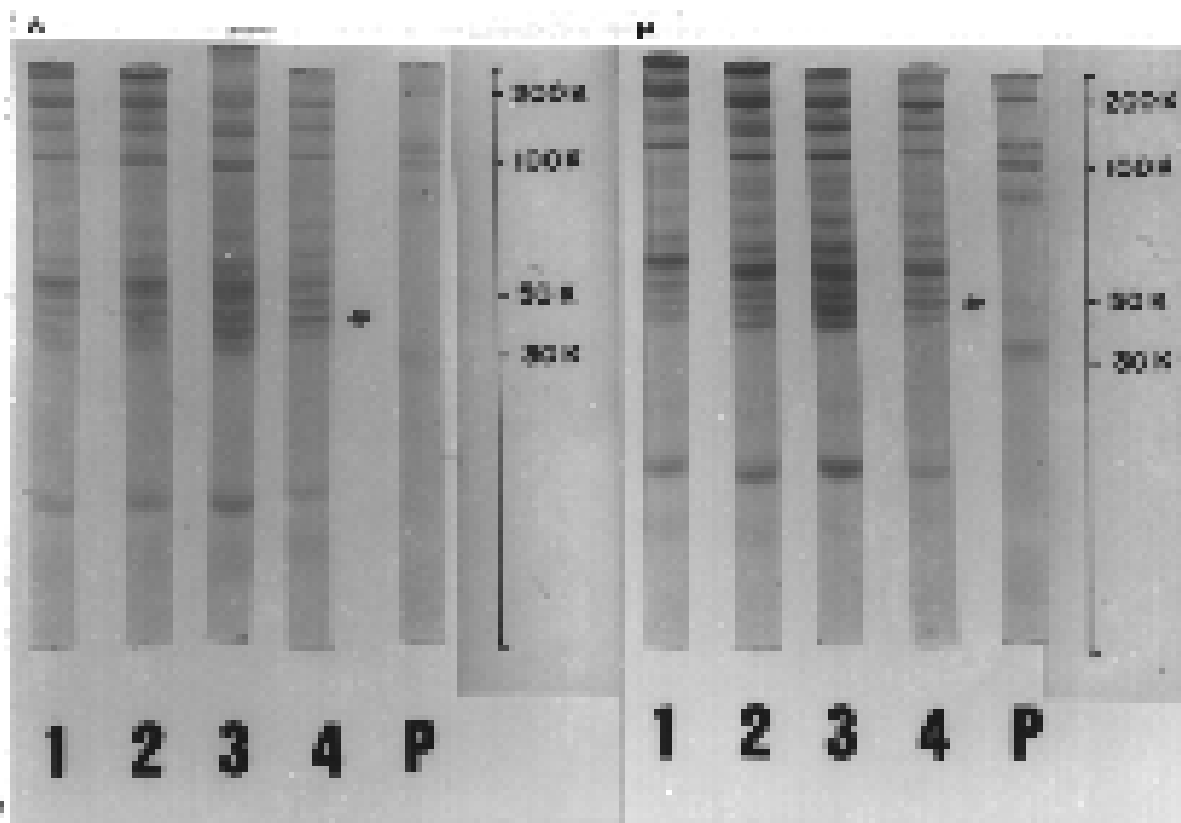


Fig. 6 (A,B) SDS-Polyacrylamide gel electrophoresis of myofibrillar protein extracted of *Longissimus dorsi thoracis* muscles of buffaloes, matured for 9 days. (A) Electrically stimulated (B) Non stimulated (P) standard protein mixture molecular weight, Numbers 1, 2, 3 and 4 correspond to 9th, 6th, 3th and 1th day of aging, respectively.

#### CONCLUSION

Results show that the rate of the myofibrillar degradation, hence the acceptable tenderness in muscle carcass, can be predicted by high voltage electrical stimulated process. The potential for the application of this technology is enormous for the acceleration of buffalo meat conditioning.

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