Effect of antemortem electrical stunning on avian muscle

Bernard Davis Murphy

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Effect of ante-mortem electrical stunning on avian muscle

by

Bernard Davis Murphy

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Animal Science Co-majors: Poultry Products Technology Meat Science

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For the Graduate College

Iowa State University
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INTRODUCTION

The Humane Slaughter Act of 1958 requires United States abattoirs to practice humane handling and slaughtering methods in order to minimize animal perception of pain. Preslaughter stunning satisfies this requirement when it renders the animal insensible to pain prior to exsanguination. Approved methods of preslaughter stunning include captive bolt stunning, stun by gun shot, carbon dioxide immobilization, and electrical stunning. The last is the method of choice used to stun poultry.

Electrical stunning is the last processing step prior to exsanguination and may significantly affect the poultry carcass. Lee et al. (1979) showed that preslaughter electrical stunning alters both the time course of rigor and the development of tenderness in broiler breast muscle.

In view of these results, it seems possible that the poultry industry could influence the quality and functionality of muscle if the effects of antemortem electrical stunning were better understood. The purpose of this study was to identify some of the effects of antemortem electrical Stunning on the biochemical, physical, and functional properties of poultry muscle at different times postmortem.
LITERATURE REVIEW

Historical

One of the first reports of the use of electricity to stun fowl was written over 200 years ago. Benjamin Franklin (1749) wrote "A Turky is to be kill'd for our Dinner by the Electrical Shock."¹ He later reported his results in a letter to a friend (1750):

...we made several Experiments on Fowls this Winter; That we found two large thin glass Jars, gilt...were sufficient to kill common Hens outright; but the Turkies, tho' thrown into violent Convulsions, and then lying as dead for some Minutes, would recover in less than a quarter of an Hour. However, having added Mr. Kinnersley's Jarrs and mine together, in all 5, tho' not fully charg'd, we kill'd a Turky with them of about 10 lb. wt. and suppose they would have kill'd a much larger. I conceit that the Birds kill'd in this Manner eat uncommonly tender.²

Even though these comments are of historical interest only, they are the first documented use of electricity to stun fowl.

Mode of Action of Electrical Stunning

In a review article, Warrington (1974) noted that in the 1900s investigators thought electrical stunning caused cerebral vasoconstriction. They believed this led to cerebral anoxia, which in turn caused unconsciousness. Clark and Tweed


(1932), however, found electrical stunning caused an increase in blood pressure and therefore did not impair cerebral circulation. Similarly, Ivy and Barry (1932) showed that vasoconstriction, via vagal stimulation, failed to produce unconsciousness. The cerebral vasoconstriction theory was subsequently abandoned.

Croft (1952b) used electrocorticograms (ECoG) and observed the response of animals to pain stimuli in an attempt to determine the action of electrical stunning. Electrical stunning depressed the ECoG, while at the same time animals did not respond to pain stimuli. Within 8 minutes after the cessation of stunning, both the response to pain and the ECoG returned to normal. Similar ECoG activity was observed in chemically anesthetized human patients (Martin et al., 1959). Croft (1952a) concluded that electrical stunning severely alters brain activity, rendering an animal insensible to pain.

Richards and Sykes (1967) questioned the use of ECoG to determine insensitivity. They argued that an animal may appear to be insensible by not being able to respond to pain, when indeed it could feel it. This seems unlikely due to the similarity of the ECoG of stunned animals and chemically anesthetized surgery patients (Martin et al., 1959).

Furthermore, Warrington (1974) described human volunteers who, after electrical stunning and recovery, reported total loss of consciousness.
Breazile et al. (1963) used conditioning to determine consciousness in electrically stunned sheep. The sheep were conditioned by exposing them to a nonpainful stimulus prior to the administration of a painful stimulus. These workers used clicking sounds and electrical shock to the hind limb as the nonpainful and painful stimuli, respectively. Conditioning was complete when the sheep responded to the clicks, in anticipation of the painful shock, whether the shock was given or not. At this point, the sheep perceived the clicks as the painful stimulus. The conditioned sheep were then stunned electrically. After stunning, the sheep were exposed to the clicking sounds. There was no response. Breazile et al. (1963) concluded that the sheep were unconscious since they did not respond to the clicks. Between 3 and 10 minutes after stunning, the sheep began to show the conditioned response when presented with the clicks, indicating consciousness had been regained.

In an effort to determine if electrical stunning was painful, Leach et al. (1980) also conditioned sheep to respond to nonpainful stimuli. On 11 consecutive days, the sheep were exposed to a light source for 5 seconds prior to electrical stunning. Each time a stress response was observed after stunning. On the 12th day, the sheep were exposed to the light source only. No stress response occurred. Leach et al. (1980) reasoned that since the conditioned animals did not
exhibit a stress response, the sheep were not anticipating a painful stimulus. Thus, Leach et al. (1980) concluded that electrical stunning was painless and therefore humane.

Antemortem Effects

The Meat and Poultry Inspection Regulations require that an animal, destined for human consumption, must die from loss of blood. This also means that an animal must be able to survive antemortem electrical stunning. It is therefore pertinent to evaluate the effects of electrical stunning on the vital functions of the animal.

Heart rate

In broiler chickens, electrical stunning consistently decelerated the heart rate. Richards and Sykes (1967) electrically stunned both anesthetized and unanesthetized fowl with an AC circuit set at 90 volts and 50 Hz. In anesthetized birds, the stun was administered across the head. In the unanesthetized birds, the stun was administered from head to feet. Both experimental groups experienced a 40% decrease in heart rate.

Kuenzel and Walther (1978) used anesthetized birds to compare the effect of three electrical circuits on heart rate. The AC (50 v, 60 Hz), DC (90 v), and high frequency AC (30 v, 480 Hz) circuits all caused heart rate decreases of 28, 48, and 47%, respectively. Within 75 seconds after the cessation
of stunning, the heart rates returned to 96% of the pre-stun rate. In a subsequent trial, two groups of birds were allowed to recover from anesthesia for either 6 hours or 5 days before being electrically stunned. This minimized the interaction between anesthesia and stunning. The AC circuit (50 v, 60 Hz) treatment caused the heart rate to be depressed by 55% in birds that had recovered for 6 hours and by 66% in the 5 day recovery group.

The effect of electrical stunning on the heart rate of sheep is the opposite of that reported in fowl. Leach and Warrington (1976) reported an increase in the heart rate of sheep from 103 beats per minute to 300 beats per minute. The heart rate then decreased, followed by tachycardia and fibrillation. These workers proposed that the latter increase in heart rate was due to anoxia and not the stun (90 v, 50 Hz).

Lambooy (1982) stunned sheep with 300 volts (1.5 amps, 50 Hz) or 600 volts (4 amps, 50 Hz). Thirty seconds after the cessation of stunning, the heart rate had increased 200 and 300%, respectively. Electrically stunned calves (100 v, .87 amps, 50 Hz) experienced a 30% increase in heart rate (Lambooy and Spanjaard, 1982).

**Blood pressure**

Clark and Tweed (1932) showed that electrical stunning increased the blood pressure of sheep between 100 and 200%. The same results were reported by Kirton et al. (1978).
Richards and Sykes (1967) observed no significant effect of electrical stunning on the blood pressure of broilers. Using anesthetized birds, Kuenzel and Walther (1978) elicited blood pressure increases of 19.6, 14.4, and 10.8% immediately after electrical stunning with AC, DC, and high frequency AC circuits previously described. Likewise, conscious birds experienced a 14 to 18% increase in blood pressure.

Respiration rate

Warrington (1974) reported that some early results mentioned apnea during and after stunning. This was also reported in chickens by Rose (1939). However, Richards and Sykes (1967) did not observe this in broilers. They reported pre-stun and post-stun respiration rates of 41 and 28 breaths per minute, respectively. The same results were reported for both anesthetized and unanesthetized birds.

As part of a comparison of AC, DC, and high frequency AC circuits, Kuenzel and Walther (1978) recorded respiration rates. The three circuits had the same effect, a decrease of about 76% in the respiration rate. Within 75 seconds after stunning, the rates were 82% of normal.

Exsanguination

In the poultry industry, the primary interest in ante-mortem electrical stunning has been in its effect on blood loss. Rose (1939) visually evaluated blood loss in chickens.
He maintained that stunning by passing a current through the head resulted in a better bleed out, since the heart was not in the current path, as in the case of head to foot stunning. However, this treatment difference was not found by Fricker and Muller (1981). Rose (1939) also reported that neither method resulted in better bleed out than the conventional brain stick method.

Kotula and Helbacka (1966a) supported this opinion. In their study, stunned birds lost 44.48% of the total blood volume, compared to 51.06% lost by the nonstunned group. However, in a later study, there was no difference (Kotula and Helbacka, 1966b).

Several studies agreed with the latter report of Kotula and Helbacka (1966b). Wilson and Brunson (1968) recorded a blood loss of 3.2% of body weight for both stunned and non-stunned boilers. Likewise, when turkeys were allowed to bleed to completion, there were no differences observed (Mountney et al., 1956). The latter group of workers proposed that electrical stunning increased the rate of bleed out but not the total amount of blood lost.

Kotula and Helbacka (1966a) studied the distribution of residual blood in broiler carcasses after slaughter. They used iodinated human serum albumin as a tracer. The retail carcasses of stunned birds contained significantly more residual blood than the nonstunned controls. There was no difference in the percentage of blood located in the offal.
Pollard et al. (1973) subjectively evaluated pre-slaughter stunning, post-slaughter stunning, and a combination of the two. The post-slaughter stunning and the combination stunning resulted in more complete blood loss but also caused more wing tip and tail hemorrhaging. The viscera of stunned groups contained more blood, which is contrary to the results of Kotula and Helbacka (1966a). Finally, Pollard et al. (1973) reported that pre-slaughter stunning decreased skin discoloration of fowl without affecting the blood loss.

Kuenzel and Ingling (1977) compared plate stunning to brine stunning and AC circuits to DC circuits. The plate stunner consisted of a metal plate with current passing through it. As the bird passed over the plate, its head contacted the plate allowing the current to pass through the head to the feet. In the brine stunner, there was a concentrated saline solution flowing over the charged plate. The saline solution promoted better contact between the bird and the stunner. Kuenzel and Ingling (1977) considered the brine stunner superior because of better bird-to-stunner contact and more consistent blood loss. The DC voltages all gave comparable blood losses of about 39%. None of the DC voltages caused cardiac arrest or fibrillation. These maladies occurred when the AC voltages were greater than 85 volts with the brine stunner and 100 volts with the plate stunner. Fifty volts AC resulted in 42.4% blood loss, which was significantly
more than the 37.0% lost with the DC circuit at 90 volts. For maximum bleed out and minimum electrical hazard, these authors recommended stunning at 50 volts AC.

In a subsequent trial, Kuenzel et al. (1978) expanded the evaluation of stunning to include variable frequencies. Based on the previous results, they used only brine stunners. The DC peak voltages ranged from 50 to 100 volts. Within each setting, frequencies from 30 to 960 Hz were evaluated. The maximum bleed out occurred at a peak voltage of 100 volts and a frequency of 480 Hz. However, the blood loss at these settings was not different than the blood loss at the previously recommended 50 volts AC setting.

Postmortem Changes in Muscle

Chemical changes

Immediately after exsanguination, the blood supply to skeletal muscles begins to diminish. As a result, the oxygen supply to the tissue is eliminated and tissue stores of oxygen are depleted. Energy (ATP), for cell homeostasis, is then produced via anaerobic glycolysis instead of the more efficient aerobic pathways. Since neither the circulatory system nor the aerobic cycles are functioning, glycogen is rapidly depleted, lactic acid accumulates, and muscle pH drops (Bate-Smith and Bendall, 1949; de Fremery, 1966; Bendall, 1979). Once glycogen is depleted, creatine phosphate begins to decrease, followed by the disappearance of ATP (de Fremery and
Physical changes

As rigor proceeds, the once flaccid muscles become rigid and the carcass stiffens (Bendall, 1960). In an attempt to explain this transition, Jungk et al. (1967) and Busch et al. (1967, 1972) studied the tendency of freshly excised muscle to shorten. By monitoring the development of isometric tension, these workers were able to demonstrate an increase in muscle tension to a maximum, followed by a gradual decline. The same tension development pattern was later reported in poultry (Khan, 1974). The time course of tension development correlated with the time course of the onset, peak, and resolution of rigor in intact muscles. These results showed that the onset, peak, and resolution of rigor were due to tension developed by intact opposing muscles pulling on the carcass skeletal system (Goll et al., 1970).

Tenderness changes

Khan (1974) showed a significant correlation between maximum isometric tension and force required to shear cooked poultry breast muscle. However, before this correlation had been shown, the variation in tenderness during rigor had been recognized in poultry (Lowe, 1948; Pool et al., 1959; Klose et al., 1971, 1972). Dodge and Stadelman (1960) reported that
the development of tenderness in turkey coincided with the drop in pH. de Fremery and Pool (1960) showed that treatments that caused rapid postmortem decreases in pH, CP, ATP, or glycogen also caused decreased tenderness. They reported that mechanical defeathering, exhaustive electrical stimulation, electron radiation, storage temperatures above 30°C, and thawing of pre-rigor frozen muscle all caused tenderness to decrease.

Since rigor mortis, chemical changes and tenderness had been shown to be correlated, many workers tried to control tenderness by altering the time course of rigor. de Fremery and Pool (1963) injected broilers with adrenaline to deplete glycogen or iodobromate to inhibit glycolysis. Both groups of treated birds had a higher pH than the controls (6.6 vs. 5.8). During postmortem storage at 4°C, the treated muscles reached maximum tenderness sooner than the controls. Pool (1963) also injected birds with epinepherine. Glycogen was depleted quickly, as was ATP. The postmortem drop in pH was minimal and the breast muscles were tender immediately after death. In chemically anesthetized broilers, de Fremery (1965) reported the postmortem decline of CP and ATP was less than in the conscious birds. The muscles of the anesthetized birds were also more tender after 24 hours of aging. de Fremery (1965) proposed that the time course of rigor had been altered in the treated birds.
A similar effect was noted in broilers that were electrically stunned prior to slaughter. Lee et al. (1979) reported that the pH, CP, and ATP levels were higher in electrically stunned birds than in nonstunned birds. This apparent delay of rigor and slowing of glycolysis resulted in the breast muscle of stunned birds being more tender after 24 hours of aging. Lee et al. (1979) suggested that electrical stunning slowed the breakdown of glycogen and maintained higher levels of CP and ATP until the carcass temperature decreased. This in turn meant that nonstunned control birds experienced rapid postmortem glycolysis at body temperature, possible heat shortening, and severe isometric tension development, all of which cause decrease tenderness (Khan, 1971; Lee and Rickansrud, 1978; Khan, 1974).

Tenderness has also been increased by postmortem treatments. Peterson (1977) injected polyphosphates into broiler breast muscle 20 minutes after exsanguination. This treatment resulted in a significantly higher muscle pH. At 1 hour postmortem, injected muscles and noninjected controls were removed from the carcass. The muscles of a second noninjected control group remained attached to the carcass. The three groups were aged for 24 hours at 4°C. After aging, the excised polyphosphate treated muscles were more tender than excised control muscles and as tender as the intact controls. In a later study, Peterson and Lilyblade (1979)
injected a carbonate buffer into broiler breast muscle immediately after exsanguination. The pH was significantly higher in treated muscles. The carbonate treated muscles and non-treated controls were removed from the carcass 20 minutes after exsanguination. A second untreated control muscle group was left attached to the carcass. After aging for 5 hours at 4°C, the excised treated muscles were more tender than the excised nontreated controls. The 5-hour aged, treated muscles were also more tender than the intact controls aged for 24 hours. These results support the conclusion of de Fremery and Pool (1960), that slowing the postmortem pH drop yields more tender muscle.

Morphological changes

Sarcomere length Hay et al. (1973b) did not observe a change in the sarcomere lengths of postmortem muscle. Hegarty et al. (1973) also reported no changes when fixed myofibrils were used for sarcomere length determination. However, when unfixed myofibrils were evaluated, the sarcomere lengths decreased then increased at times that coincided with the onset and resolution of rigor, respectively. Johnson and Bowers (1976) reported the same pattern of sarcomere length changes in turkey breast muscle.

Ultrastructural changes Hay et al. (1973b) and Johnson and Bowers (1976) monitored ultrastructural changes in avian muscle with electron microscopy. Both groups observed
the typical banding pattern of skeletal muscle, as described by Huxley (1953). Hay et al. (1973b) attributed the narrow I bands to contraction taking place at the onset of rigor. Johnson and Bowers (1976) reported more severe narrowing of the I band and attributed it to inadequate muscle sample restraint during fixation (Dutson, 1974). Johnson and Bowers (1976) also noted that subtle changes had occurred by 3 hours postmortem. The H zone was indistinct and the junction of the A and I bands was not well-defined. The sarcomere alignment had decreased and more intercellular space separated the cells. The Z lines were more diffuse. At 48 hours postmortem, the H zone and M line were indistinguishable. The Z lines had lost lateral attachment with parallel fibers resulting in minimal sarcomere alignment. The Z lines were also more diffuse and sometimes absent.

Takahashi et al. (1967) monitored ultrastructural changes in myofibrils. He reported extensive fragmentation of myofibrils prepared from muscle aged for 48 hours. The myofibrils were fragmented into segments of four sarcomeres or less. Yamamoto et al. (1974) observed similar fragmentation in myofibrils prepared from muscle aged for 24 hours. Both studies reported the myofibrils had broken at the Z line. Fukazawa et al. (1969) inhibited myofibrillar fragmentation by treating live birds with insulin prior to exsanguination, thus
slowing the muscle pH decline. Fukazawa et al. (1969) also reported the Z lines remained intact after fragmentation.

Electrophoretic changes

Sender (1971) and Scopes and Penny (1971) were among the first to demonstrate that sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) could detect individual myofibrillar proteins. Hay et al. (1973a) used SDS-PAGE to monitor changes in myofibrillar proteins of chicken breast and leg muscles. These workers noted a 44,000 dalton protein band in the gels of myofibrils prepared from breast muscle soon after exsanguination. The 44,000 dalton band was less visible at 3 hours postmortem and had disappeared by 48 hours. Hay et al. (1973a) did not notice any myofibrillar or sarcoplasmic protein bands increasing or decreasing in intensity as the 44,000 dalton band faded. The 44,000 dalton band was not seen in gels of leg myofibrils. Accordingly, Hay et al. (1973a) proposed that the protein may be part of the M line that disappears from the breast muscle sarcomere during aging. The M line remains intact in leg muscle sarcomeres (Hay et al., 1973b).

Hay et al. (1973a) also observed a 30,000 dalton protein band in gels of myofibrils prepared from aged breast muscle and leg muscle. Yamamoto et al. (1974) observed a similar molecular weight protein band in breast muscle. Hay et al. (1973a) proposed that the band was a result of myosin
breakdown. However, more recent reports showed that the appearance of the 30,000 dalton band coincides with the proteolytic breakdown of Troponin-t by a calcium activated protease (Olson et al., 1977).

**Enzymatic ATPase changes**

Wu and Sayre (1971) prepared myosin from chicken breast and leg muscles. They observed no changes in the Ca^{++}-ATPase activity during 24 hours of storage. Throughout aging, the breast muscle myosin activity was always higher than the activity of the leg muscle myosin.

Hay et al. (1972) prepared actomyosin from chicken breast and leg muscles. They noted an increase in Mg^{++}-ATPase activity for both muscles during rigor mortis. The Ca^{++}-ATPase activity of leg muscle actomyosin did not change over 7 days of muscle storage. The breast muscle actomyosin activity decreased at 3 hours postmortem, then increased to the pre-rigor level. The Ca^{++}-ATPase activity was higher in the breast muscle actomyosin than in the leg muscle actomyosin. The opposite was true for the Mg^{++}-ATPase activity.

Hay et al. (1973b) prepared myofibrils from chicken breast and leg muscles. Aging up to 7 days had no effect on the Ca^{++}-ATPase or Mg^{++}-ATPase activities. The breast muscle myofibrils consistently exhibited higher activity.
Functional Property Changes of Muscle

Water holding capacity

In muscle, water is associated with myofibrillar proteins. The association is due to the attraction between polar water molecules and the charged side groups of the proteins. At physiological pH, the net charge of the myofibrillar proteins is negative. Since the negatively charged side groups repel one another, the lattice structure of the proteins is more open allowing space for water molecules to fill. Hydrogen bonding between the charged side groups and water further strengthens the attraction. As muscle enters rigor, the pH drops and some of the negatively charged side groups are titrated, thereby decreasing the concentration of negative charges. At the isoelectric point of muscle (pH 5.0-5.4), the net protein charge is zero due to the equal concentrations of positively and negatively charged side groups. Since the attraction between opposite charges is strong, the proteins are pulled together and the lattice space available for water decreases. Consequently, the water holding capacity is at a minimum. As rigor proceeds and the muscle pH increases, the protein-protein attraction decreases and water holding capacity increases slightly (Hamm, 1960).

Early work by Marsh (1952a, b) demonstrated the effect of ATP on the water holding capacity of muscle. Prior to the onset of rigor, there is enough ATP to prevent permanent
crossbridging between actin and myosin. However, at the onset of rigor, the ATP concentration drops rapidly. The result is increased crossbridging and minimal space within the protein matrix for water to bind (Marsh, 1981).

It has also been reported that the sarcoplasmic reticulum and mitochondria lose their ability to sequester calcium during rigor (Goll et al., 1971; Marsh, 1977; Whiting, 1980). The free cytoplasmic calcium stimulated actin–myosin cross-bridging and bound negatively charged protein side groups. These occurrences decreased the space within the protein matrix and decreased the net negative charge of the muscle proteins. The result is decreased water holding capacity.

Extractable protein

In the past, the extractability of myofibrillar protein has been studied in order to determine the changes that may occur in muscle during rigor mortis. Khan and van den Berg (1964) extracted protein from chicken breast muscles. They reported a minimum in protein extractability at 5 hours postmortem and a maximum at 36 hours. McIntosh (1967) published similar results. Khan and van den Berg (1964) attributed the protein extraction differences to changes in the myofibrillar proteins.

Sayre (1968) used high ionic strength (\(7/2=1.0\)) phosphate buffer to extract chicken breast muscle that had been stored for up to 24 hours at 0°C. Myosin extraction declined rapidly
in muscle that had been stored between 0 and 3 hours. The decline was more gradual between 3 and 24 hours. Sayre (1968) did not detect appreciable amounts of actomyosin until the muscle had been stored for at least 6 hours. Between 6 and 24 hours, the increase in the extractability of actomyosin was equal to the decrease in myosin extractability. Sayre (1968) concluded that the decrease in myosin extraction was due to the depletion of ATP and the formation of rigor crossbridges. He also proposed that the increase of actomyosin in the buffer extract indicated that the actin filaments were either fragmenting or detaching from the Z lines of the myofibrils.

Ma and Addis (1973) researched the effect of glycolysis rate on protein extraction. The rate of glycolysis had no effect. This was not surprising since their treatments did not significantly affect either the pH or ATP levels. There was a difference in protein extraction between muscles aged for 0 or 24 hours with the 0-hour aged group having higher levels of extractable protein. Ma and Addis (1973) also reported a significant correlation \( r=-.58 \) between protein extractability and the shear value.

**Emulsion stability**

Emulsion stability is the ability of an emulsion to retain fat and water during thermal processing. Since myofibrillar proteins are primarily responsible for water retention and fat emulsification, muscle conditions that affect
these proteins will also affect the emulsion characteristics. Acton and Saffle (1969) made emulsions with pre-rigor or post-rigor muscle and found more stable emulsions when pre-rigor muscle was used. Therefore, any treatment that prolongs the pre-rigor state of muscle should also increase the stability of an emulsion made with that treated muscle. According to the results of Lee et al. (1979), antemortem electrical stunning delays the onset of rigor or prolongs the pre-rigor condition of poultry muscle. Thus, an emulsion made with the muscle of an electrically stunned bird should be more stable than an emulsion made with the muscle of a nonstunned bird.

The purpose of this thesis research was to determine the effects of antemortem electrical stunning on poultry muscle at different times postmortem. If the results of Lee et al. (1979) were duplicated, then electrical stunning would be evaluated as a method to consistently delay the onset of rigor and enhance the functional properties of avian muscle. At the same time, the means by which electrical stunning altered muscle metabolism would be investigated.
EXPERIMENTAL PROCEDURES

Source and Handling of Turkeys

Nicholas male turkeys with an average weight of 13.6 kg were used in this study. Turkeys were obtained from either the Iowa State University Poultry Research Center or a local producer. Prior to being used, the turkeys were held in floor pens for at least 1 week. During this holding period, feed and water were available ad libitum. Turkeys were transported to the Poultry Abbatoir in the Iowa State University Meat Laboratory 16 hours prior to slaughter.

Processing

Live turkeys were weighed prior to slaughter. After weighing, the turkeys were hung by both feet from overhead poultry shackles. A Cervin Automated Systems knife stunner (Model FS) was used for the electrical stunning treatments. Stunning was accomplished by placing the charged knife in contact with the head of the bird for 5 seconds and allowing current to pass through the turkey's body from head to feet. Nonstunned birds were handled in the same manner except the knife was not charged. All of the birds were then exsanguinated by severing the jugular vein and carotid artery on one side of the neck. Turkeys were allowed to bleed for 90 seconds. Subsequent to bleeding, carcasses were scalded in an Ashley Sure-Scald (Model SS-36) for 1.75 minutes at 63°C.
Defeathering was done in an Ashley Sure-Pick (Model SP-30) for 30 seconds. After defeathering, carcasses were eviscerated and cleaned according to USDA procedures. Finally, carcasses were chilled in ice slush for up to 24 hours.

Stunning

The Cervin stunner contained an 800 volt storage cell. Output voltage was constant and stunning current was regulated by varying resistance in the stunner circuit. As the resistance was increased, stunning current decreased in accordance with Ohm's Law. Actual stunning current was measured with a cathode ray oscilloscope (Techtronix 502-A) placed in series with the stunned turkeys.

Cardiac Function

Cardiac function (electrocardiogram-EKG) was monitored with the cathode ray oscilloscope. Standard bipolar lead II was used to detect fibrillation or cardiac failure after electrical stunning. Stainless steel 28-guage needles were used as electrodes and inserted under the skin of the right and left wings and the left leg. The electrodes were disconnected from the oscilloscope during the administration of the stunning current. Immediately after the 5-second stun, the electrodes were reconnected and the EKG trace observed. The EKG was used to determine the greatest stunning current that would not cause fibrillation or cardiac failure. This current
should cause maximum animal immobilization with minimal effect on the heart. Forty-six turkeys ranging in live weight from 9.5 to 20.0 kg were used to determine the optimum stunning current.

Blood Loss

Turkey carcasses were weighed after a 90-second bleed time and blood loss was calculated as follows:

\[
\text{Blood loss (\%)} = \left( \frac{\text{live body weight} - \text{weight after bleed out}}{\text{live body weight}} \right) \times 100
\]

Electron Microscopy

In order to determine if antemortem electrical stunning had an effect on postmortem muscle ultrastructure, samples of the Biceps femoris and Pectoralis major were collected 0, 4, 8, and 24 hours postmortem from each of four stunned and four nonstunned turkeys. Samples were removed from muscles at a depth of 1 cm to avoid collecting tissue that may have been heat damaged during scalding. In situ muscle strips 4 cm long and 0.2 cm in diameter were tied to wooden applicator sticks to minimize shortening after excision. The restrained muscle strips were excised and immediately placed in fresh 2.5% glutaraldehyde (4°C) and phosphate buffer (0.15M, pH 7.2) and held 12-24 hours at 4°C. After fixation, the muscle strips were separated from the applicator sticks and cut into 1 mm³ pieces. Fixation in glutaraldehyde was followed by three
phosphate buffer rinses, 10 minutes each, and post-fixation with 1% osmium tetroxide in the same buffer at 4°C for 2 hours. Samples were washed twice in double distilled water for 30 minutes followed by a dehydration series of graded ethanol and infiltration with propylene oxide. Specimens were then infiltrated with epoxy resin (Medcast) according to the manufacturer's instructions for a "hard block," cast in aluminum weighing dishes, and held overnight in a 60°C oven.

Thick longitudinal sections (2 μm) were cut with glass knives on a Reichert OM-U2 ultramicrotome. Sections were collected with stick applicators and floated on a water droplet on a glass slide. Glass slides were placed on a hot plate to adhere sections to the slides and to remove wrinkles. Sections were stained with toluidine blue 0 and studied using phase contrast optics, to make sure muscle sections were properly oriented longitudinally.

Sections of approximately 70-85 nm were cut with a DuPont diamond knife on a Reichert OM-U2 ultramicrotome, spread with chloroform fumes, and collected on 200 mesh copper grid. Sections were stained with aqueous uranyl acetate (Watson, 1958) followed by lead acetate (Reynolds, 1963). All observations were made on a Hitachi HU-11C transmission electron microscope at either 50 or 75 kV accelerating voltage. Transmission micrographs were taken on DuPont Cronar Ortho S Litho 3 1/4 x 4 inch sheet film.
Muscle Metabolite Analysis

Muscle samples were taken from the B. femoris and P. major of 24 stunned and 24 nonstunned turkeys at 0, 4, 8, and 24 hours postmortem. Samples were frozen in liquid nitrogen, pulverized, and stored at -100°C until analyzed.

Glycogen

Muscle glycogen content was determined according to Lo et al. (1970). Only 0- and 4-hour samples were assayed since in preliminary studies glycogen was not detected in samples aged for more than 4 hours.

pH

Muscle samples were homogenized with 10 volumes of .15 M KCl, 5 mM iodoacetate (pH 7.0) for 30 seconds. The homogenate was filtered through Whatman #4 filter paper. The pH of the clear filtrate was measured with a Corning Model 125 pH meter.

ATP

Muscle samples were extracted according to Lee et al. (1979). ATP was determined according to Jaworek et al. (1974).

R ratio

The R ratio was determined according to Honikel and Fischer (1977). The absorbance ratio $A_{250}/A_{260}$ of the perchloric acid extract of muscle was used to monitor rigor
in postmortem muscle. During rigor, the concentration of adenosine decreases and that of inosine increases. These nucleotides are extractable with perchloric acid and absorb maximally at the wavelengths 260 and 250 nm, respectively. Therefore, the increase in the ratio $A_{250}/A_{260}$ can be used to monitor rigor.

**Myofibrillar Analysis**

**Myofibril preparation**

Myofibrils were prepared from the *B. femoris* and *P. major* of six stunned and six nonstunned turkeys at 0, 4, 8, and 24 hours postmortem according to the scheme outlined in Figure 1. Freshly excised samples were scissor minced and homogenized with 10 volumes of myofibrillar preparative solution (MPS) containing 100 mM KCl, 50 mM Tris-Acetate (pH 6.8), 2 mM MgCl$_2$, 2 mM EGTA, and 1 mM NaN$_3$. Homogenization was for 15 seconds at 4°C in a Waring Blender$^\circ$. The homogenate was centrifuged at 1000xg for 10 minutes at 4°C and the supernatant discarded. The pellet was homogenized with 6 volumes of MPS for 10 seconds followed by centrifugation at 1000xg for 10 minutes. The resulting pellet was homogenized with 8 volumes of MPS for 10 seconds. This homogenate was strained through a nylon food strainer to remove connective tissue and the filtrate centrifuged at 1000xg for 10 minutes. The pellet was homogenized with 8 volumes of MPS and strained again. After centrifuging this filtrate at 1000xg for 10 minutes, the
Minced muscle

1) Homogenize with 10 volumes of MPS for 15 sec
2) Centrifuge homogenate at 1000xg for 10 minutes

Supernatant
Discard

Pellet
1) Homogenize with 6 volumes of MPS for 10 sec
2) Centrifuge at 1000xg for 10 minutes

Supernatant
Discard

Pellet
1) Homogenize with 8 volumes of MPS for 10 sec
2) Strain through nylon food strainer
3) Centrifuge filtrate at 1000xg for 10 minutes

Supernatant
Discard

Pellet
1) Repeat homogenization, straining and centrifugation

Supernatant
Discard

Pellet
1) Homogenize with 6 volumes of MPS containing 1% Triton X-100
2) Centrifuge at 1000xg for 10 minutes

Supernatant
Discard

Pellet
1) Repeat homogenization with 1% Triton X-100 and centrifugation

Supernatant
Discard

Pellet
1) Wash in 6 volumes of 500 mM KCl by stirring vigorously
2) Centrifuge at 1000xg for 10 minutes
3) Repeat steps 1 and 2 four times, discard supernatant each time
4) Suspend final pellet in 2 volumes of 50 mM KCl and determine protein content

Figure 1. Preparation of myofibrils
remaining pellet was homogenized with 6 volumes of MPS and 1% Triton X-100 and the homogenate centrifuged at 1000xg for 10 minutes. Homogenization with 1% Triton X-100 and centrifugation were repeated. The remaining pellet was washed four times with 6 volumes of 50 mM KCl. The final pellet was suspended in 2 volumes of 50 mM KCl and the protein content determined (Gornall et al., 1949). All homogenization and centrifugation was carried out at 4°C.

Myofibrillar ATPase

A 2.40 ml volume of myofibrils in 50 mM KCl (1 mg/ml) was added to a test tube that contained 2.50 ml of buffer made up of 50 mM KCl, 40 mM Tris-Acetate (pH 6.8) and 4 mM MgCl₂. The tube was incubated for 5 minutes in a 25°C water bath with constant stirring. The assay was initiated by adding 0.10 ml of 0.10 M Tris-ATP to the assay tube. Inorganic phosphate was assayed at 1 minute intervals for 4 minutes according to Lin and Morales (1977). Absorbance was read at 400 nm (Lecocq and Inesi, 1966) on a Beckman Acta C-III spectrophotometer. Each assay was done in triplicate.

Myofibrillar sulfhydryl group analysis

Sulfhydryl groups were determined according to Sedlack and Lindsay (1968) with modifications suggested by Hay et al. (1972).
**Myofibrillar extractable protein**

Extractability of protein was done according to Sung et al. (1976).

**Electrophoresis**

SDS-PAGE was done according to Laemmli (1970). An outline of the modified procedure used in this study is in Appendix 1.

**Extractable protein**

Extractable protein was determined according to Saffle and Galbreath (1964). Muscle samples were homogenized with 100 volumes of unbuffered 0.6 M NaCl for 15 seconds. The homogenate was then centrifuged for 15 minutes at 5000xg. Protein content was determined according to Gornall et al. (1949).

**Emulsion Properties**

Turkey breast muscle samples (2-2.5 kg) were taken from 24 stunned and 24 nonstunned turkeys at 0, 4, 8, and 24 hours postmortem. These samples were preblended with 3.5% NaCl by weight and 10% water by weight. The preblend was chopped for 15 seconds in a Hobart Lab Chopper (Model 8418ID). Next, the preblends were packaged in polyethylene bags and frozen at -20°C. At time of emulsion manufacturing, preblends were tempered for 12 hours at 4°C. After tempering, the preblends were chopped for 1 minute. At this time, turkey fat and water
were added at levels of 35% and 20% of meat weight, respectively. Chopping continued until emulsions reached 10°C.

**Emulsion stability**

Emulsion stability was evaluated according to Townsend et al. (1968).

**Proximate analysis**

Moisture, fat, and protein of uncooked and cooked emulsions were determined according to AOAC (1980) methods 24003, 24005, and 23009, respectively.

**Study Design**

This study was designed as a 2x2x4 factorial. Main effects were stunning treatment (electrical stunning vs. nonstunning), muscle type (B. femoris vs. P. major), and time postmortem (0, 4, 8, and 24 hours). Statistical analysis was done using SAS (Barr et al., 1979).
RESULTS AND DISCUSSION

Stunning

In the setting range of 1 to 9 on the Cervin electrical stunner, 7 resulted in optimum antemortem stunning. Lower settings (1-6) caused violent preslaughter struggling. Higher settings (8 and 9) caused cardiac fibrillation and cardiac failure. The current produced with the stunner set at 7 was approximately 250 mA. Stunning with this current for 5 seconds caused immobility and decreased death struggle during exsanguination. However, in accordance with the Humane Slaughter Act, nonexsanguinated stunned turkeys were able to recover from the stun.

The 250 mA current was similar to that used to properly stun heavy fowl (Fricker and Muller, 1981). However, it was approximately three times greater than current reported to have killed broilers (Rose, 1939). This difference was probably due to carcass weight differences between turkeys, heavy fowl, and broilers.

Rose (1939) concluded it was the stunning current experienced by animals, not stunning voltage, that determined the level of stun. However, published reports on stunning have included only voltage (Kuenzel and Ingling, 1977; Kuenzel and Walther, 1978) or no stunning criteria at all (Lee et al., 1979). Poultry researchers must realize that different experimental facilities and different animals have different
resistances. According to Ohm's Law (E=I/R), if resistance (R) changes so does current (E). Therefore, even if stunning voltage (I) remained constant between trials, the actual basis of stunning effects (current) may have changed. Until investigators measure and report stunning current it will not be possible to meaningfully compare antemortem electrical stunning results.

Blood Loss

Electrically stunned birds lost less (p<.01) blood than nonstunned birds (n=20). Blood losses (X+s.e.) were 2.9±.14 and 3.6±.09%, respectively. Weight of blood lost during exsanguination was positively correlated (r=.65, p<.01) with live body weight.

The negative effect of stunning on blood loss agrees with observations of Kotula and Helbacka (1966a). Pollard et al. (1973) reported stunning caused blood to be retained in the viscera. Numerically similar blood losses were reported by Wilson and Brunson (1968). However, these workers did not observe a difference between stunned and nonstunned groups. Since current used in the present study minimized cardiac dysfunction, no explanation for decreased blood loss can be suggested other than visceral retention reported by Pollard et al. (1973).
Muscle Ultrastructure

At 0, 4, 8, and 24 hours postmortem, samples of the *B. femoris* and *P. major* were taken from each of four electrically stunned and four nonstunned turkeys. Severe contraction was observed in the *B. femoris* and *P. major* of one of the electrically stunned turkeys (Figures 2 and 3). These electron-dense contraction bands were located at the Z lines. Traces of M lines were observed in *B. femoris*; however, no regular banding was seen in micrographs of the *P. major*. Similar contraction bands have been observed in electrically stimulated bovine muscle (Will et al., 1980). Hoyle et al. (1965) reported contraction bands in barnacle muscle and attributed them to contractile proteins concentrating at the Z lines in response to supercontraction evoked by nonneural stimuli.

When severe contraction did not occur, the structure of the *P. major* and *B. femoris* did not appear to be affected by electrical stunning (Figures 4 through 19). Normal sarcomere shortening appeared to occur during postmortem aging. Slight Z line degradation was also observed in samples from muscle aged for 24 hours. At 24 hours, Z lines appeared to be less aligned in electrically stunned muscle samples.

The 24-hour changes in muscle have been described in broiler (Hay et al., 1973b) and turkey (Johnson and Bowers, 1976). However, stunning-related differences in sarcomere length and muscle structure have not been previously reported.
Figure 2. Contraction bands in electrically stunned *P. major*  

Figure 3. Contraction bands in electrically stunned *B. femoris*
Figure 4. Electrically stunned *P. major* at 0 hours postmortem

Figure 5. Nonstunned *P. major* at 0 hours postmortem
Figure 6. Electrically stunned *P. major* at 4 hours postmortem

Figure 7. Nonstunned *P. major* at 4 hours postmortem
Figure 8. Electrically stunned *P. major* at 8 hours postmortem

Figure 9. Nonstunned *P. major* at 8 hours postmortem
Figure 10. Electrically stunned *P. major* at 24 hours postmortem

Figure 11. Nonstunned *P. major* at 24 hours postmortem
Figure 12. Electrically stunned *B. femoris*  
Figure 13. Nonstunned *B. femoris* at 0 hours postmortem
Figure 14. Electrically stunned B. femoris Figure 15. Nonstunned B. femoris at 4 hours postmortem
Figure 16. Electrically stunned B. femoris Figure 17. Nonstunned B. femoris at 8 hours postmortem
Figure 18. Electrically stunned *B. femoris* Figure 19. Nonstunned *B. femoris* at 24 hours postmortem
Postmortem sarcomere shortening in avian muscle is correlated with tension development during rigor (Hegarty et al., 1973; Johnson and Bowers, 1976). Figures 2 and 3 show the potential for severe contraction associated with electrical stunning.

Z line degradation and alignment decrease are due to the activity of CAF (Dayton et al., 1976; Robson and Huiatt, 1983). If there were differences related to stunning it might be due to increased CAF activity in stunned muscles. Dayton et al. (1976) reported maximum CAF activity at pH 7.5. In the present study and in the study of Lee et al. (1979), the pH of stunned *P. major* was significantly higher than that of non-stunned muscle. Therefore, a higher pH in stunned muscle might enhance CAF activity thus contributing to more extensive degradation of Z lines.

Parallel myofibril alignment and attachment have been attributed in part to the cytoskeletal protein desmin (Robson and Huiatt, 1983). Richardson et al. (1981) located desmin at the periphery of the Z disc. Schreiner (1982) then observed desmin filaments connecting adjacent myofibrils at the Z line. O'Shea et al. (1979) had previously reported that CAF readily degraded desmin. Therefore, enhanced CAF activity possibly caused by a higher pH might also result in more decreased myofibril attachment and alignment in stunned muscle.
Postmortem Muscle Metabolite Analysis

The postmortem metabolite results are summarized in Table 1. These data support the conclusion of Lee et al. (1979) that electrical stunning delays glycolysis and the onset of rigor in postmortem poultry muscle. However, any difference caused by electrical stunning is not apparent after 4 hours postmortem.

Glycogen

At 0 hours postmortem, glycogen content of the B. femoris was greater (p<.01) than that of the P. major. Glycogen was not detected in either muscle at 4 hours postmortem. Electrically stunned muscle contained numerically less glycogen, but the difference was not significant.

Rapid depletion of glycogen in avian muscle is well established (Shrimpton, 1960; Wiskus et al., 1975) as is the fast glycolytic rate of the P. major. Since 95% of fibers in the P. major are white compared to 30% in the B. femoris, glycogen is more rapidly metabolized in the P. major.

Ma and Addis (1973) described the difficulty of quantifying glycogen in postmortem avian muscle due to the rapid rate of postmortem glycolysis. This could have caused the large variability within the stunning treatment observed in the present study.
Table 1. Effect of muscle type, stunning treatment, or time postmortem on muscle metabolite levels

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Muscle type</th>
<th>Stunning treatment</th>
<th>Time postmortem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BF</td>
<td>PM</td>
<td>ST</td>
</tr>
<tr>
<td>Glycogen</td>
<td>3.24₁</td>
<td>1.40²</td>
<td>1.93₁</td>
</tr>
<tr>
<td></td>
<td>(.42)</td>
<td>(.29)</td>
<td>(.37)</td>
</tr>
<tr>
<td>pH</td>
<td>5.96₁</td>
<td>5.80²</td>
<td>5.90₁</td>
</tr>
<tr>
<td>ATP</td>
<td>1.55₁</td>
<td>2.16²</td>
<td>2.18₁</td>
</tr>
<tr>
<td></td>
<td>(.16)</td>
<td>(.22)</td>
<td>(.22)</td>
</tr>
<tr>
<td>R value</td>
<td>1.03₁</td>
<td>1.11²</td>
<td>1.04₁</td>
</tr>
<tr>
<td></td>
<td>(.02)</td>
<td>(.02)</td>
<td>(.02)</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different (p<0.05). Values in parentheses are standard errors.

BF=B. femoris; PM=P. major (n=48).
ST=stunned; NS=nonstunned (n=48).
Hours (n=24).
mg glycogen/gm muscle.
µmoles ATP/gm muscle.
Muscle pH was positively correlated \( r = 0.49, p < 0.05 \) with muscle glycogen.

Muscle type significantly \( p < 0.01 \) affected postmortem muscle pH (Table 1). As expected, pH of the B. femoris was higher than that of the P. major. A significant \( p < 0.01 \) interaction between stunning treatment and time postmortem indicated that at 0 hours postmortem pH of stunned muscle was greater \( p < 0.05 \) than that of nonstunned muscle. This difference was not observed at 4, 8, or 24 hours postmortem (Table 2). Difference between muscle types was expected due to the higher proportion of anaerobic white fibers in the P. major (Ma and Addis, 1973). A similar interaction between stunning and time postmortem was reported in broilers (Lee et al., 1979). At 0 hours postmortem, stunned and nonstunned muscle pH were 6.15 and 5.92, respectively. At 4 and 24 hours postmortem, the pH of stunned and nonstunned muscles were not different. Lee et al. (1979) suggested that decreased glycogen breakdown was responsible for initially higher muscle pH.

ATP

The P. major had a higher \( p < 0.01 \) ATP content than the B. femoris. Electrical stunning resulted in increased \( p < 0.01 \) muscle ATP. As postmortem time increased, ATP concentration decreased. ATP was not detected at 8 or 24 hours postmortem.
Table 2. Effect of stunning treatment and time postmortem interaction on muscle pH

<table>
<thead>
<tr>
<th>Stunning treatment</th>
<th>Time postmortem</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunned</td>
<td></td>
<td>6.29\textsuperscript{1}</td>
<td>5.93\textsuperscript{1}</td>
<td>5.81\textsuperscript{1}</td>
<td>5.74\textsuperscript{1}</td>
</tr>
<tr>
<td>Nonstunned</td>
<td></td>
<td>6.09\textsuperscript{2}</td>
<td>5.86\textsuperscript{1}</td>
<td>5.78\textsuperscript{1}</td>
<td>5.75\textsuperscript{1}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Means in the same column with different superscripts are significantly different (p<0.05).

Muscle ATP difference is not readily explained. Muscles with faster glycolysis rates are reported to have less ATP (Fischer and Hamm, 1980). Secondly, ATP is correlated with pH (Lee et al., 1979; Fischer and Hamm, 1980). Lee et al. (1979) also reported decreased ATP hydrolysis in electrically stunned broilers. Again, their conclusion was delay of rigor but no mechanism was discussed.

The effect of electrical stunning was also evaluated at 0 and 4 hours separately (Table 3). The results indicate that the higher ATP content in stunned muscle occurs immediately after exsanguination and does not continue past 4 hours. This trend is similar to that described for pH in Table 2.

R value

The R value difference between muscles reflected the higher initial ATP content of the P. major. Since the P.
Table 3. Effect of stunning on ATP concentration\(^a\) at 0 and 4 hours postmortem\(^b\)

<table>
<thead>
<tr>
<th>Stunning treatment</th>
<th>Time postmortem(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stunned</td>
<td>3.34(^1)</td>
</tr>
<tr>
<td></td>
<td>(2.6)</td>
</tr>
<tr>
<td>Nonstunned</td>
<td>2.56(^2)</td>
</tr>
<tr>
<td></td>
<td>(.25)</td>
</tr>
</tbody>
</table>

\(^a\) moles ATP/gm muscle.
\(^b\) Means in the same column with different superscripts are significantly different (p<0.05). Values in parentheses are standard errors.
\(^c\) Hours.

major is primarily glycolytic, the greater R value was expected as a result of more adenosine metabolized to inosine. The R value also indicated that electrical stunning decreased the catabolism of adenosine. The R value was lower in stunned muscles at 0 and 4 hours postmortem only. Again, this trend is similar to that reported for pH and ATP concentration of postmortem muscle (Table 4).

The R value is the ratio of inosine nucleotides to adenosine nucleotides in muscle. As rigor proceeds, the ratio increases as adenosine is metabolized to inosine. The R value has been used to monitor time course of rigor in swine (Honikel and Fischer, 1977), poultry, and beef (Khan and Frey,
Table 4. Effect of stunning treatment on R value\(^a\) of muscle at different times postmortem\(^b\)

<table>
<thead>
<tr>
<th>Stunning treatment</th>
<th>Time postmortem (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stunned</td>
<td>0.90(^1)</td>
</tr>
<tr>
<td></td>
<td>(.02)</td>
</tr>
<tr>
<td>Nonstunned</td>
<td>0.96(^2)</td>
</tr>
<tr>
<td></td>
<td>(.03)</td>
</tr>
</tbody>
</table>

\(^a\)\(^250/\(^260)^*\)
\(^b\)Means in the same column with different superscripts are significantly different (\(p<0.05\)). Values in parentheses are standard errors.
\(^c\)Hours.

1971). Honikel and Fischer (1977) have successfully used the R value to detect stress-susceptible pigs.

**Myofibrillar Properties**

In the previous section, stunning was shown to delay the onset of rigor in avian muscle. This portion of the study was concerned with the effect of stunning on myofibrils.

Myofibrillar properties are presented in Table 5. No enzymatic or conformational changes could be attributed to electrical stunning.

**Mg\(^{++}\)-ATPase activity**

Mg\(^{++}\)-ATPase activity of the **P. major** was greater (\(p<0.05\)) than that of the **B. femoris** (Table 5). However, neither
Table 5. Effect of muscle type, stunning treatment, or time postmortem on myofibrillar properties

<table>
<thead>
<tr>
<th>Properties</th>
<th>Muscle type</th>
<th>Stunning treatment</th>
<th>Time postmortem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BF</td>
<td>PM</td>
<td>ST</td>
</tr>
<tr>
<td>Mg$^{++}$-ATPase$^e$</td>
<td>.180$^1$</td>
<td>.230$^2$</td>
<td>.209$^1$</td>
</tr>
<tr>
<td></td>
<td>(.006)</td>
<td>(.007)</td>
<td>(.009)</td>
</tr>
<tr>
<td>Sulfhydryl$^f$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.80$^1$</td>
<td>8.02$^1$</td>
<td>7.83$^1$</td>
</tr>
<tr>
<td></td>
<td>(.22)</td>
<td>(.26)</td>
<td>(.22)</td>
</tr>
<tr>
<td>Exposed</td>
<td>4.52$^1$</td>
<td>4.61$^1$</td>
<td>4.59$^1$</td>
</tr>
<tr>
<td></td>
<td>(.09)</td>
<td>(.09)</td>
<td>(.08)</td>
</tr>
<tr>
<td>Extractable protein$^g$</td>
<td>64.5$^1$</td>
<td>69.3$^2$</td>
<td>68.4$^1$</td>
</tr>
<tr>
<td></td>
<td>(1.1)</td>
<td>(1.2)</td>
<td>(1.2)</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different (p<0.05). Values in parentheses are standard errors.

BF=B. femoris; PM=P. major (n=48).
ST=stunned; NS=nonstunned (n=48).
Hours (n=24).
Umoles Pi/minute/mg myofibril.
Moles (-SH)/1x10^5 gm myofibril.
Percent of total myofibrillar protein.
electrical stunning nor postmortem aging had an effect on enzymatic activity. Hay et al. (1973b) reported breast muscle ATPase to be greater than that of leg muscle. These workers aged muscle for 7 days and observed no change in ATPase activity during that time.

The myofibrillar preparation used in this study required 4 hours and was quite rigorous. Enzyme activity differences that may have been present in intact muscle could have been minimized during preparation. Mabuchi and Streter (1980) described an ATPase procedure using cryostat sections of intact muscle. They reported increased activity compared to myofibril preparations. Perhaps this procedure would be more accurate in detecting differences in postmortem muscle.

Sulfhydryl groups

Neither muscle type, electrical stunning, nor postmortem aging caused changes in the concentration of total or exposed sulfhydryl groups. These results indicated that no major conformational changes were detected in electrically stunned muscle. Caldwell and Lineweaver (1969) monitored sulfhydryl group concentration in poultry muscle during postmortem aging and observed no changes.

Extractable protein

Myofibrillar proteins were extracted with Hasselbach-Schneider solution at 4°C. More protein was extracted from
the *P. major* myofibrils than from the *B. femoris* myofibrils. Stunning also increased protein extraction. Postmortem aging had no effect.

Increased protein extraction has been attributed to increased Z line degradation and muscle cell disruption (Sayre, 1968). Cheng and Parrish (1979) also reported that increased protein extraction from bovine muscle may have been caused by CAF activity on Z lines. If electrical stunning were to be associated with increased Z line degradation in the *P. major*, this may be the basis for increased protein extraction in stunned muscle.

**Electrophoresis**

Myofibrillar proteins were separated using SDS-PAGE. Gels were examined to determine if electrical stunning caused differences in protein solubility or degradation (Figures 20 and 21). Gels of stunned and nonstunned myofibrils were not different. Protein bands corresponding to myosin heavy chains, α-actinin, actin, and tropomyosin were present in all gels. Troponin-t was located immediately anodic to tropomyosin. These banding patterns were similar to those described by Hay et al. (1973a). However, these workers also reported the appearance of a 30,000 dalton band. Olson et al. (1977) had previously attributed the 30,000 dalton band to the degradation of troponin-t. Yamamoto et al. (1979) also reported a 30,000 dalton band in gels of avian myofibrils.
Figure 20. Electrophoretic gel of myofibrils prepared from stunned and nonstunned *P. major* at 0, 4, 8, and 24 hours postmortem
Myosin -

α-Actinin -

Actin -

Tropomyosin -

Stunned

Time
0 4 8 24

Non-stunned

Time
0 4 8 24
Figure 21. Electrophoretic gel of myofibrils prepared from stunned and nonstunned \textit{B. femoris} at 0, 4, 8, and 24 hours postmortem
However, Hay et al. (1973a) used broiler muscle aged for 72 hours and Yamamoto et al. (1979) incubated muscle homogenates at 25°C. Neither of these conditions was duplicated in the present study.

**Functional Properties**

The objective of the third portion of this study was to measure the effects of electrical stunning on emulsion properties. Due to the small quantity of B. femoris only P. major muscle was used for emulsion manufacturing. Since stunned muscle initially had higher pH, more ATP, and extractable protein than nonstunned muscle, use of the former might result in improved emulsion binding and stability.

**Extractable protein**

Electrical stunning significantly (p<.01) increased percent extractable protein at 0 and 4 hours postmortem (Table 6). This result was in agreement with myofibrillar protein extraction response to stunning (Table 5). Since more protein was extracted, a more stable emulsion should result.

**pH**

Electrical stunning did not affect emulsion pH of raw or cooked emulsions (Tables 7 and 8). As postmortem time increased, uncooked emulsion pH decreased (p<0.05). The pH of cooked emulsions manufactured with muscle aged for 0 or 4 hours was not different. However, the pH of these cooked
Table 6. Effect of stunning treatment and postmortem time interaction on percent extractable protein in the *P. major*.  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunned</td>
<td>51.1^1</td>
<td>46.8^1</td>
<td>34.6^1</td>
<td>30.0^1</td>
<td>40.8^1</td>
</tr>
<tr>
<td></td>
<td>(1.3)</td>
<td>(1.8)</td>
<td>1.2)</td>
<td>(.09)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Nonstunned</td>
<td>42.7^2</td>
<td>38.2^2</td>
<td>34.0^1</td>
<td>30.0^1</td>
<td>36.4^2</td>
</tr>
<tr>
<td></td>
<td>(1.8)</td>
<td>(1.9)</td>
<td>(0.9)</td>
<td>(0.9)</td>
<td>(1.4)</td>
</tr>
</tbody>
</table>

^aMeans in the same column with different superscripts are significantly different (p<0.05). Values in parentheses are standard errors.  
^bHours.

Table 7. Effect of stunning treatment and postmortem time on pH of uncooked emulsions

<table>
<thead>
<tr>
<th>Treatment^b</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunned</td>
<td>6.24</td>
<td>6.15</td>
<td>6.07</td>
<td>6.02</td>
<td>6.11</td>
</tr>
<tr>
<td>Nonstunned</td>
<td>6.21</td>
<td>6.12</td>
<td>6.03</td>
<td>6.05</td>
<td>6.10</td>
</tr>
<tr>
<td>X^c</td>
<td>6.22^1</td>
<td>6.14^1,2</td>
<td>6.05^2,3</td>
<td>5.98^3</td>
<td></td>
</tr>
</tbody>
</table>

^aHours.  
^bTreatment differences were not significantly different (p<0.05).  
^cMeans in this row with different superscripts are significantly different (p<0.05).
Table 8. Effect of stunning treatment and postmortem time on pH of cooked emulsions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Postmortem timea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Stunned</td>
<td>6.19</td>
</tr>
<tr>
<td>Nonstunned</td>
<td>6.13</td>
</tr>
<tr>
<td>(\bar{X})^c</td>
<td>6.16^1</td>
</tr>
</tbody>
</table>

^aHours.
^bTreatment differences were not significantly different (p<0.05).
^cMeans in this row with different superscripts are significantly different.

emulsions was greater (p<0.05) than that of emulsions manufactured with muscle aged 8 or 24 hours. The pH of cooked emulsions was less than that of uncooked emulsions (p<0.05). The overall pH values were 6.10 and 6.07 for raw and cooked emulsions, respectively.

Emulsion stability

Emulsion stability was not affected by electrical stunning (Table 9). Emulsion stability was at a minimum when muscle aged for 8 hours was used. However, the stability was not different than that of emulsions manufactured with muscle aged for 24 hours. Proximate analysis of uncooked and cooked
Table 9. Effect of stunning treatment or time postmortem on emulsion stability\(^{a,b}\)

| Stunning treatment\(^{c}\) | Time postmortem\(^{d}\) |  \\
|--------------------------|----------------------| \\
| ST | NS | 0 | 4 | 8 | 24 |
| 2.10\(^{1}\) | 2.17\(^{1}\) | 1.36\(^{1}\) | 1.40\(^{1}\) | 3.30\(^{2}\) | 2.46\(^{1,2}\) |
| (.46) | (.39) | (.36) | (.21) | (.91) | (.43) |

- Means with different superscripts are significantly different (p<0.05). Values in parentheses are standard errors.
- \(^{b}\) mls of water and fat released.
- \(^{c}\) ST = stunned; NS = nonstunned.
- \(^{d}\) Hours.

Emulsions (Table 10) did not indicate differences in emulsion composition associated with stunning or postmortem age.

These results agree with those of Kardouche and Stadelman (1978). These workers reported no difference in cooked yield or moisture and fat retention between loaves made with pre-rigor and post-rigor turkey muscle. Wardlow et al. (1973) observed similar results with broiler muscle. On the contrary, Nixon and Miller (1967) reported higher yields in turkey roll manufactured with hot boned pre-rigor turkey muscle. Similar to the present results, Froning and Neelakantan (1971) reported higher emulsion pH values for emulsions made with muscle aged 30 minutes as opposed to 24 hours. However, these authors also reported increased emulsion stability in pre-rigor product. In a later study,
Table 10. Effect of stunning treatment or time postmortem of the *P. major* on the composition of emulsions

<table>
<thead>
<tr>
<th></th>
<th>Stunning treatment</th>
<th>Time postmortem</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>64.9</td>
<td>64.5</td>
<td>64.4</td>
</tr>
<tr>
<td>Cooked</td>
<td>61.3</td>
<td>61.4</td>
<td>61.0</td>
</tr>
<tr>
<td>Difference</td>
<td>-3.6</td>
<td>-3.1</td>
<td>-3.4</td>
</tr>
<tr>
<td>Fat (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>18.7</td>
<td>19.3</td>
<td>19.0</td>
</tr>
<tr>
<td>Cooked</td>
<td>21.3</td>
<td>21.3</td>
<td>21.4</td>
</tr>
<tr>
<td>Difference</td>
<td>+2.6</td>
<td>+2.0</td>
<td>+2.4</td>
</tr>
<tr>
<td>Protein (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>15.0</td>
<td>14.9</td>
<td>15.2</td>
</tr>
<tr>
<td>Cooked</td>
<td>16.1</td>
<td>16.0</td>
<td>16.5</td>
</tr>
<tr>
<td>Difference</td>
<td>+1.1</td>
<td>+1.1</td>
<td>+1.3</td>
</tr>
</tbody>
</table>

aNone of the differences are significant (p<0.05).

bHours.
Froning et al. (1978) measured the water holding capacity of muscles with significantly different pH values (6.32 vs. 6.17). No difference was observed.
SUMMARY

Antemortem electrical stunning did not affect the normal postmortem changes in the *P. major* and *B. femoris*. No stunning-associated differences in myofibrillar physiology were detected. Therefore, no basis for the delay of glycolysis in stunned muscle could be proposed. However, metabolite results similar to those associated with stunning have been reported by other researchers. Ma and Addis (1973) reported a significantly higher pH and ATP level in muscle from turkeys that had been physically restrained during exsanguination. Similar differences were presented by Froning and Babji (1975) for anesthetized turkeys. All three treatments (electrical stunning, physical restraint, and anesthetization) minimized the death struggle. Therefore, perhaps the basis of the metabolite differences is reduced muscle activity immediately after death. If no death struggle occurs, then no muscle contraction is occurring. Therefore, ATP is not utilized as fast and glycolysis is delayed.

None of the effects of antemortem electrical stunning were retained for emulsion manufacturing. Therefore, antemortem electrical stunning does not offer any advantage that is not already available through the use of pre-rigor muscle.
CONCLUSIONS

1. A stunning current of approximately 250 mA administered for 5 seconds from the head to the feet of young male turkeys was optimum for immobilization prior to exsanguination.

2. Electrical stunning decreased blood loss.

3. Volume of blood lost is positively correlated to live body weight.

4. Electrical stunning did not affect the normal structural changes in postmortem muscle (B. femoris, P. major).

5. Electrical stunning did not affect the glycogen content of postmortem muscle.

6. Electrical stunning increased postmortem muscle pH and ATP content immediately after processing. Stunning did not affect these parameters after 4 hours postmortem.

7. The metabolism of adenosine was delayed immediately after processing by electrical stunning. After 4 hours post-mortem, the effect was not observed.

8. Electrical stunning did not affect myofibrillar enzyme activity, conformation or protein solubility.

9. Electrical stunning increased extractable myofibrillar protein.

10. Electrical stunning did not affect emulsion stability or composition.


ACKNOWLEDGMENTS

An individual's success is always due to the support and help of many people. During my six-year course of study and research, I have been fortunate to have those people also become my friends. This thesis is a testimony to those friendships.

During that time, many faces and names have slipped from my memory. At the same time, there are those special ones that never faded. Dee James, the undergraduate now graduate student with dreams of living with the land. She did my dirty work so I could concentrate on moving forward. She also babysat. Bob Hasiak my advisor allowed me to experience teaching and try to pass some of my thoughts and experiences to future graduates. Kay Litzel, the section secretary and source of endless energy, who's dedication to efficiency and success was an example to work by. Joe Sebranek, a committee member who openly and honestly discussed any topic any time, helped me plan my future. Joey Wayne Lusby, friend, neighbor, fellow graduate student, and realist. That friendship began my first day at I.S.U. and grew stronger every day. It had to, we were the only people in Iowa who knew what a Razorback was.

It is customary to finish with the phrase, last but certainly not least. However, last and least will never be associated with my family. My parents have prayed for this
moment. They have done without so that I might have. They have worried quietly and encouraged me readily. Sometimes they have even been irritating. Mom and Dad have never stopped believing in me. With support like that I can't fail. Valerie Petulla Murphy has accompanied me for 12 years. Valerie was the instigator of this scholastic endeavor. Her plans called for three years in school then employment. I agreed but still took six years. I have frustrated, infuriated, and disappointed her during these six years. However, she has never hesitated to love me. If Valerie had not been with me I would never have succeeded.

I have learned a lot. I am satisfied with the results. I would do it all over again. I am glad I don't have to.
APPENDIX

SDS-PAGE Procedure

I. Reagent Preparation

1) Acryl-Bis-30% (w/v) solution

Dissolve 30 g acrylamide and 0.8 g N\textsubscript{1}\textsubscript{N}-methylene-bis-acrylamide in distilled water, then bring volume to 100 ml. Store in dark bottle and refrigerate for up to 6 months.

2) Tris-HCl buffer - pH 8.9, 1.5 M solution

Dissolve 18.17 g Tris in 80 ml of distilled water, adjust to pH 8.9 with HCl and bring volume up to 100 ml.

3) SDS - 20%

20 g SDS (w/v) in 100 ml of distilled water. Allow to stand overnight until bubbling stops, then bring volume to 100 ml.

4) 0.2 M EDTA, pH 7.0

0.1 Mole EDTA in 400 ml of distilled water, add 10-11 g NaOH, adjust to pH 7.0 with 5 N NaOH. Bring volume to 500 ml, able to be refrigerated for up to 1 month.

5) Temed

Ready to use N,N,N\textsubscript{1}\textsubscript{N}^-Tetramethylenediamine.

6) Ammonium Persulfate - 10% (w/v)

Dissolve 2 oz ammonium persulfate in 2.2 ml of distilled water. This should be prepared fresh daily.

7) Tris-PO\textsubscript{4}-buffer, 0.5 M

Dissolve 6.06 g Tris in about 80 ml of distilled water, adjust to pH 6.7 with H\textsubscript{3}PO\textsubscript{4}, bring volume up to 100 ml.
8) Buffer Tris-Glycine

Dissolve 30 g Tris and 144 g glycine in distilled water and bring up to 1 liter, final pH 8.3-8.4.

9) Electrophoresis Buffer

Dilute 100 mls of #8 to 1 liter and dissolve 1 g of SDS powder in this.

10) MES, 1 M

19.52 g MES dissolved in 100 ml distilled water, adjust to pH 6.5 with 5 N NaOH solution.

11) Tracking dye solution

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M MES, pH 6.5</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>20% SDS</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.5 g</td>
</tr>
<tr>
<td>BP Blue</td>
<td>until dark blue color</td>
</tr>
<tr>
<td>H₂O</td>
<td>up to 10 ml</td>
</tr>
</tbody>
</table>

This solution can be maintained for up to 12 months if frozen.

12) Destain: Any volume

a) glacial acetic acid (CH₃COOH) 7 parts
b) methanol (CH₃OH) 40 parts
c) water (H₂O) 53 parts

13) Stain

Dissolve 2 g coomassie Brilliant Blue R in 1 liter of solution #12.

14) MCE – - mercaptoethanol – ready to use

15) HCl acid, concentrated

16) H₃PO₄ acid

17) NaOH, solid and 5N solution
II. Procedure for Gel Electrophoresis of Protein

1) Clean carefully all glassware and chamber, rinse glassware with C₂H₅OH (ethanol).

2) Clamp gel chamber securely, coat plastic strips with vaseline for best seal.

3) Prepare gel in following order: (for 8.0% polyacrylamide gel)
   a) 8.0 ml 30% Acryl-Bis
   b) 6 ml 1.5 M Tris-HCl (pH 8.9)
   c) 15.0 ml H₂O
   d) 0.15 ml 20% SDS
   e) 0.30 ml EDTA
   f) 0.02 ml TEMED
   g) 0.30 ml Ammonium pursulfate

   Immediately pour gel, remove bubbles, allow to set (about 30 minutes).

4) While 7.5% gel is setting, mix 4% gel in following order:
   a) 4 ml Acryl-Bis
   b) 3 ml 0.5 M Tris-PO₄ buffer (pH 6.7)
   c) 22 ml H₂O
   d) 0.15 ml 20% SDS
   e) 0.30 ml EDTA
   f) 0.02 ml TEMED
   g) 0.30 ml Ammonium pursulfate

5) Take out top steep only and fill chamber with 4% gel, remove bubbles, put comb strip in fluid 4% gel to make loading cells, allow gel to set.

6) Remove bottom strip and the top comb strip.

7) Clamp gel to chamber wall, using vaseline on the chamber wall to prohibit leaking of buffer between chamber wall and notched gel plate.

8) Put electrophoretic buffer in upper and lower reservoirs of set up.

9) Remove all air bubbles.

10) Load protein samples in loading cells at top.
11) Put negative polarity at top and positive polarity at bottom chamber.

12) Start current at 10 MA for 1 1/2 hours, then increase to 15 MA for duration of separation.

13) Stop separation when dye front is about 1 cm from the bottom of gel.

14) Put gel in stain bath for 1 hour.

15) Put gel in destain bath, 2 times, each time use a fresh destain bath. Allow to destain for 1 hour in each bath.

16) Developed gel can be stored in 7% CH$_3$COOH solution.