1995

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ABSTRACT This study was conducted to elucidate the mechanism of action of two selected rigor mortis-accelerating treatment systems employed in the prevention of the toughness associated with early-harvested (1 h post-mortem) broiler *Pectoralis* muscle. The treatments included 14 min of low voltage electrical stimulation (110 V, 1 A, pulsing 1 s on and 1 s off) combined with high temperature conditioning (39 C) and muscle tensioning (LV + HTC + MT); a 15-s high voltage stimulation (440 V, 1 A pulsing 2 s on and 1 s off) combined with muscle tensioning (HV + MT); and a control simulating commercial broiler processing practices. The rigor-accelerating treatments reduced pH and increased R-value (inosine:adenosine ratio) at 1 h post-mortem, but only the LV + HTC + MT treatment reduced sarcomere shortening. Both rigor treatments reduced the amount of measurable myofibrillar fragmentation. Cathepsin B and B + L activities were not affected by the rigor treatments. Calpain I activity was not detectable in any 24-h post-mortem sample. Calpain II activity at 24 h post-mortem was greater in muscles receiving HV + MT than from the LV + HTC + MT or control carcasses, but was reduced in all muscles by 24 h post-mortem. An SDS-PAGE indicated a 30-kDa polypeptide that was absent at death and appeared in control and LV + HTC + MT muscles but to a lesser extent in HV + MT muscles. These results suggested that the LV + HTC + MT treatment has a greater tenderizing effect than the HV + MT treatment because the former achieves a better balance between reduced sarcomere shortening and myofibrillar fragmentation.

(*Key words*: muscle, rigor mortis, post-mortem metabolism, aging, electrical stimulation)

1995 Poultry Science 74:176–186

INTRODUCTION

Storing intact carcasses at refrigerated temperatures prior to deboning or cooking, a process termed aging, has two phases. The first is rigor mortis development, in which post-mortem muscle cell metabolism gradually shifts from aerobic to anaerobic pathways, adenosine triphosphate (ATP) is depleted, and actomyosin is formed (Hamm, 1982). In broiler breast muscle this process requires 4 h and if meat is deboned prior to this time it is toughened (Stewart *et al.*, 1984; Lyon *et al.*, 1985; Dawson *et al.*, 1987).

The second phase, termed rigor mortis resolution, involves structural degradation of the myofibrillar protein matrix and results in improved meat tenderness (Lawrie, 1991). Although some controversy exists regarding their relative

Received for publication June 10, 1994.
Accepted for publication August 30, 1994.
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importance, two groups of endogenous proteases in skeletal muscle, the calpains and the cathepsins, have been implicated in this structural degradation (Ouali, 1990; Koohmaraie, 1992). Increasing ionic strength has also been implicated (Wu and Smith, 1987). The resolution phase of aging varies considerably due to different marketing situations and can be quite long. Although no change in shear value occurs upon deboning beyond the first 4 h of on-carcass aging in normal broiler marketing, Hirschler and Sams (1994) observed a significant decrease in shear value between 24 and 72 h of postexcision refrigerated aging. However, no effort was made to determine whether this decrease was perceptible to consumers. McKee and Sams (1994) later reported that this shear value improvement involved calpain activity against myofibrillar proteins.

Several researchers have reported the use of post-mortem electrical stimulation (ES) systems to accelerate rigor mortis development and prevent the toughening induced when meat is deboned prior to completion of this development (Li *et al.*, 1993). Walker *et al.* (1994) compared several ES systems that had been reported for use with broilers and determined two quite different systems to be the most effective based on reducing shear values. One system combined high voltage ES (HV) (630 V for 15 s) with muscle tensioning (MT) induced by wing restraint, whereas the other system combined low voltage ES (LV) (115 V for 14 min) with high temperature conditioning (HTC) and MT. However, the effects these two systems had on the structural and metabolic aspects of rigor mortis development in the *Pectoralis* were not determined.

Cross (1979) reviewed three theories by which post-mortem ES tenderizes red meat. First, ES accelerates ATP depletion, resulting in the prevention of cold shortening and improved tenderness. Secondly, ES hastens the decline of post-mortem pH while muscle temperatures are still high, enhancing the action of endogenous proteases responsible for tenderization during the aging process. Finally, ES tenderizes meat by inducing tearing of muscle fibers. These effects of ES are related to changes in the post-mortem metabolism and contractile activities of the muscle.

Several reports have been made on the effects of rigor mortis-accelerating treatments on the proteolytic mechanism of tenderization in red meat. Savell *et al.* (1977) postulated that ES increased the rate at which post-mortem pH declines, thereby enhancing the role of the acid proteases (cathepsins) in the tenderization process. Other researchers (Dutson *et al.*, 1980; Wu *et al.*, 1985) supported this theory. Ducastaing *et al.* (1985) contended that calpain I and to a lesser extent calpain II may be responsible, even with the rapid decline in pH that accompanied ES-treated bovine *Longissimus dorsi*. Because the activity of the calpains are optimized at around physiological pH, Ducastaing *et al.* (1985) postulated that their activities in the rapidly achieved acid post-mortem environment of ES-treated carcasses may be reduced. Koohmaraie *et al.* (1986) supported the findings of Ducastaing *et al.* (1985) because calpain I was found to retain up to 28% of its activity under acid conditions (pH 5.5 to 5.8) at refrigeration temperatures. This level of activity was found sufficient to reproduce the majority of the changes that are associated with post-mortem aging of beef carcasses (Koohmaraie *et al.*, 1986).

Although extensive research has been conducted on the specific changes occurring in mammalian meat species during post-mortem aging and the extent to which they are affected by rigor mortis-accelerating treatments, little corresponding research exists on these changes and treatment effects in avian tissue. This deficit is important considering that rigor mortis development (and presumably its resolution) proceeds at a rate at least threefold faster in broiler breast muscle than in beef (Hamm, 1982; Stewart *et al.*, 1984; Sams and Janky, 1991). Furthermore, Kang and Sams (1992) determined that the effects these ES systems have on rigor mortis development are different for avian white muscle fibers than for avian red muscle fibers. The objective of this study was to compare the two best tenderizing systems of Walker *et al.* (1994) (HV + MT and LV + HTC + MT) for their biochemical and structural effects on rigor mortis

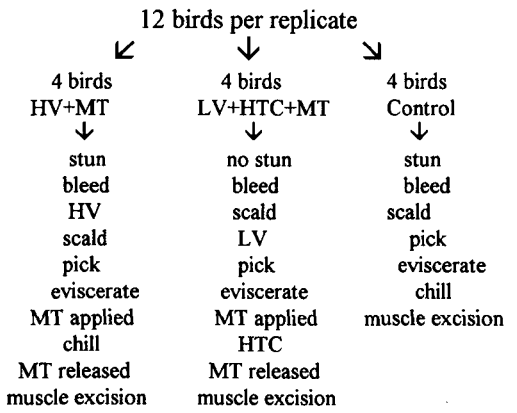

FIGURE 1. Flow diagram of the distribution of birds to each processing treatment system. HV = high voltage electrical stimulation, MT = muscle tensioning, LV = low voltage electrical stimulation, HTC = high temperature conditioning.

development and resolution in broiler *Pectoralis.*

MATERIALS AND METHODS

Experiment 1

Broilers were reared to 48 d of age in litter-covered floor pens and fed a corn and soybean meal-based diet (20% CP; 3,234 kcal ME/kg feed). Following a 12-h period of feed deprivation, 36 male broilers were cooped into three separate replicates (12 birds per replicate). The processing treatments are diagrammed in Figure 1. In each replicate, four birds were assigned to each of the three treatments (LV + HTC + MT, HV + MT, and control) prior to hanging on shackles. With the exception of those designated for the LV + HTC + MT treatment, all birds were stunned (110 V, 1A, 60 Hz for 5 s) using an electrified kill knife[6] on low setting. All voltages and amperages represent average values with a variation of ±1%. The absence of stunning in the LV + HTC + MT birds were in accordance with an earlier report by Walker *et al.* (1994), in which LV birds were left unstunned. All birds were then bled for 90 s through a single neck cut that severed the right carotid artery and the right jugular vein.

The carcasses assigned to the LV + HTC + MT treatment were removed from the processing line and subscalded (60 C, 45 s), then returned to the line (prior to picking) and electrically stimulated (110 V AC, 1 A, 60 Hz, 2 s on and 1 s off for 14 min). They were then picked in a rotary drum[7] for 25 s and manually eviscerated. Following evisceration, the LV carcasses were subjected to MT by binding the wings behind the back with nylon cable ties (Birkhold *et al.*, 1992) and then HTC by immersion in a 39 ± 1 C static, tap water bath for the remainder of 1 h post-mortem (45 min) (Walker *et al.*, 1994). The LV + HV + MT carcasses were not chilled prior to breast muscle excision. The HV carcasses were electrically stimulated with 440 V AC, 1 A, and 60 Hz pulsed 2 s on and 1 s off for five pulses. Along with the untreated controls, they were subscalded, picked, and eviscerated as previously described. The HV carcasses were subjected to the MT treatment, and, along with control carcasses, were chilled in a 2 C static tap water ice-slush for the remainder of 1 h post-mortem.

Using the method of Hamm (1981), both *Pectoralis* muscles were stripped from all 1 h post-mortem carcasses. Following the removal of the wing and skin, the anterior-most 2.5-cm of each muscle were discarded. A 1-cm-wide sample was cut parallel to the direction of the muscle fibers from the anterior end of both remaining muscles from each carcass. These samples were immediately placed in plastic bags, frozen in liquid nitrogen, and transferred to –75 C storage until later analysis (<1 mo). The sample from the right muscle of each carcass was used to measure the adenosine: inosine ratio (R-value) as an indication of ATP depletion (Thompson *et al.*, 1987). The sample from the left muscle was used to measure sarcomere length with laser diffraction (Sams *et al.*, 1990). Data were subjected to analysis of variance using replicate and treatment system as main effects and the residual mean square as the error term (SAS Institute, 1985). Means were separated with Duncan's multiple range test (SAS Institute, 1985). Because no

[6]Model S, Cervin Electrical Systems, Minneapolis, MN 55410.

[7]Model SP3055, Brower Corp., Haughton, IA 52631.

significant interaction between replicate and treatment system was detected, the data from the replicates were combined.

Experiment 2

Forty-eight male broilers were reared to 48 d and slaughtered in three replicates (16 birds each) using the same procedures and conditions as in Experiment 1. One modification from Experiment 1 was the inclusion of a 0-h post-mortem sampling point to provide data representing the control, untreated muscle condition at the time of death. Thus, four birds were assigned to each of four treatments (the 0-h control and the three treatments from Experiment 1).

For the 0-h treatment, the *Pectoralis* muscle was stripped from the carcass (hot boned) immediately after exsanguination (Hamm, 1981). Following wing and skin removal, the fillets from both sides of the 0-h carcasses were cut parallel to the fiber direction into four equally sized pieces that were placed in individual plastic bags, frozen in liquid nitrogen, and stored at –75 C until later analysis (<2 mo).

Following the removal of wing and skin, the posterior tip of all left side *Pectoralis* muscles were removed, immediately bagged, tagged, frozen in liquid nitrogen, and stored at –75 C. These samples were later analyzed for 1-h post-mortem pH values. The remains of all the left side *Pectoralis* and all those from the right sides were packed in ice and aged for the remainder of 24 h post-mortem. After aging, all right side *Pectoralis* were divided into four equally sized pieces and the remains of those from the left sides divided into three equally sized pieces. Each piece was individually bagged and tagged for analysis of the different biochemical and proteolytic variables to be analyzed. The variables were pH at 0, 1, and 24 h; myofibrillar fragmentation index at 0 and 24 h; activities of calpain I, calpain II, cathepsin B, and combined cathepsins B and L at 0 and 24 h; and the appearance of a 30-kDa polypeptide, reported to be the product of the degradation of troponin T by calpain (Macbride and Parrish, 1977) at 0 and 24 h.

Post-mortem pH at 0, 1, and 24 h were analyzed using the iodoacetate method as described by Sams and Janky (1986). Myofibrillar fragmentation at 0 and 24 h post-mortem were analyzed using the modified gravimetric method described by Sams *et al.* (1990). The activity of calpain I and II at 0 and 24 h post-mortem was measured using the ion-exchange chromatographic procedure and caseinolytic assay of Wheeler Koohmaraie (1990). Because the objective of this project was to compare relative treatment effects, a digitizing computer program[8] was used to determine the peak areas from the resulting elution profiles as an indication of relative enzyme activities. The activity of cathepsin B and cathepsins B + L were assayed using the fluorometric method of Koohmaraie and Kretchmar (1990) with N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin as the substrate for cathepsin B and N-CBZ-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin as the common substrate for cathepsins B and L. The myofibrillar protein extraction and SDS-PAGE procedures described by Macbride and Parrish (1977) were used to quantify the presence of the 30-kDa polypeptide reported to be the product of the activity of calpain on troponin T. Following the electrophoretic separation of 100 μg of extracted myofibrillar proteins in each lane of a 20% T acrylamide gel, gels were stained with a combination of Coomassie blue and silver stain.[9] Polypeptide components were identified using a set of standard SDS molecular weight markers.[10] The 30-kDa polypeptide bands were quantified using a laser densitometer,[11] and the relative amount of protein per band was estimated based on the total amount of protein loaded on each lane.

The data for each biochemical variable were subjected to ANOVA using treatment system as the main effect (SAS Institute, 1985). Because there was no significant interaction between replicates and treatment, the data from all replicates were pooled into a completely randomized design. Significance of differences among treatment means were tested with Duncan's multiple range test (SAS Institute, 1985).

[8]Java Scan, Jandel Scientific, Sansikits, CA 94965.
[9]Silver Stain Kit, BioRad, Hercules, CA 94547.
[10]Dalton Mark VII-L, Sigma, St. Louis, MO 63187.
[11]Model 2202 Ultroscan, LKB, Bromma, Sweden.

TABLE 1. R-value and sarcomere lengths (± SEM) for *Pectoralis* muscle from broiler carcasses treated with different rigor-accelerating systems

Treatment[1]	R-value[2]	Sarcomere length
		(μm)
Control	.84 ± .05[c]	1.67 ± .09[b]
HV + MT	1.0 ± .06[b]	1.42 ± .06[c]
LV + HTC + MT	1.22 ± .08[a]	2.02 ± .05[a]

[a-c]Means within a column with no common superscript differ significantly ($P \leq .05$).

[1]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[2]R-value = absorbance at 250 nm:absorbance at 260 nm.

RESULTS AND DISCUSSION

Experiment 1

The R-value is a measure of the inosine: adenosine ratio of the muscle cell, and its increase during post-mortem aging is an indication of the depletion of ATP that occurs during rigor mortis development (Calkins *et al.*, 1982). The R-value results presented in Table 1 indicate that both treatment systems accelerated ATP depletion by 1 h post-mortem compared with the control. Furthermore, the LV + HTC + MT treatment was significantly more effective in this acceleration than was the HV + MT treatment. Although this rigor-accelerating effect has been previously reported for HV + MT (Birkhold and Sams, 1993) and for a LV + HTC treatment (Sams, 1990), the LV + HTC + MT treatment has not previously been studied. The greater effect from the LV + HTC + MT treatment is consistent with the report of Thompson *et al.* (1987), who reported that LV operated more through an acceleration of metabolism whereas HV induced its effect through physical disruption, or tearing, of the myofibril.

Table 1 indicates that the sarcomeres in the LV + HTC + MT muscles were significantly longer than those of the control muscles, which were longer than those of the HV + MT muscles. The reduction in sarcomere shortening afforded with LV + HTC + MT was consistent with the reduction in ATP, the fuel for the contraction, observed in the R-value data for the LV + HTC + MT treatment. A similar effect was reported for LV + HTC by Sams (1990). The increased shortening with the HV + MT treatment was not expected as Birkhold *et al.* (1992), and Birkhold and Sams (1993) reported that this treatment resulted in longer sarcomeres. Birkhold and Sams (1991, 1993) observed physical tears in the myofibrils, which would allow unrestrained contraction. If such tears were sufficiently extensive in the HV + MT muscles of the present study, the HV + MT muscles could have more shortening by virtue of the loss of their passive restraint. Unfortunately, this cannot be verified from the current data.

Experiment 2

Table 2 presents mean pH values for all treatments and post-mortem sampling

TABLE 2. pH (± SEM) values of *Pectoralis* muscle from broiler carcasses treated with different rigor-accelerating systems

	Time of sample collection and freezing		
Treatment[1]	0 h PM[2]	1 h PM	24 h PM
Control	6.74 ± .09	6.57 ± .04[a]	6.06 ± .03
HV + MT	ND[3]	6.22 ± .03[b]	6.07 ± .03
LV + HTC + MT	ND	6.14 ± .03[b]	5.95 ± .04

[a,b]Means within a column with no common superscript differ significantly ($P \leq .05$).

[1]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[2]PM = post-mortem.

[3]ND = not determined.

times. Although not indicated in the table, the 0-h mean was significantly ($P < .05$) greater than the samples collected from control carcasses at 1 h. This represents the normal post-mortem pH decline that occurs as a result of lactic acid accumulation and has been previously reported (Stewart *et al.*, 1984). At 1 h post-mortem, the *Pectoralis* pH was significantly higher in the control muscles than in the two rigor-acceleration treatments, which were not different from each other. Post-mortem ES, with or without additional treatments, has been reported to accelerate post-mortem pH decline (Thompson *et al.*, 1987; Froning and Uijttenboogaart, 1988; Lyon *et al.*, 1989; Sams, 1990; Birkhold *et al.*, 1992; Lyon and Dickens, 1993). By 24 h post-mortem, all rigor-acceleration treatments yielded similar pH means. These results indicated that only the rate of achieving the ultimate pH value was accelerated by these treatments, not the ultimate pH value itself.

Table 3 presents the results for the myofibrillar fragmentation index, which is inversely related to the actual amount of fragmentation existing in the muscle. Consistent with the normal myofibrillar fragmentation that occurs during post-mortem aging, the index decreased in the control muscles from 0 to 24 h post-mortem. However, there was less of a decline in the muscles of the LV-treated carcasses and no decline in the muscles of the HV-treated carcasses. This inhibition of fragmentation by rigor mortis-accelerating treatments has been previously reported for broiler *Pectoralis* (Sams *et al.*, 1991; Birkhold and Sams, 1993) and was observed to result from the prevention of Z-line dissolution (Birkhold and Sams, 1991). These results suggest that if myofibrillar fragmentation is involved in the tenderization resulting from these rigor-accelerating treatments, another type of fragmentation than that measured by this index must be occurring. Using a different fragmentation measure, Takahashi *et al.* (1984) observed that HV at 60 Hz (as used in the present study) significantly increased the amount of myofiber fracturing compared to unstimulated muscle.

Residual cathepsin B and cathepsin B + L activities are reported in Table 4 and are inversely proportional to the expressed activity *in situ* because these proteases are autolytic when activated (Moeller *et al.*, 1976; Dutson *et al.*, 1980; Wu *et al.*, 1985). There was no difference in residual cathepsin B or cathepsin B + L activities between the control or either treatment after the 24-h post-mortem aging period. The lack of difference in cathepsin B or cathepsin B + L activities at 24 h post-mortem suggested these enzymes were not involved in the tenderizing effects of either rigor-accelerating treatment.

Although this conclusion agrees with that of Koohmaraie (1992) for the tenderization during normal aging, the case is less clear for the tenderness improvement resulting from ES. Etherington *et al.* (1990) reported that the activities of cathepsins B

TABLE 3. Fragmentation index[1] (± SEM) of *Pectoralis* muscle from broiler carcasses treated with different rigor-accelerating systems

Treatment[2]	Sample collect time	Sample freeze time	Fragmentation index
	(h PM[3])		
Control	0	0	76.34 ± 6.44[4]
Control	1	24	48.03 ± 1.64[c]
HV + MT	1	24	84.44 ± 11.79[a]
LV + HTC + MT	1	24	66.75 ± 6.12[b]

[a–c]Means within a column with no common superscript differ significantly ($P \leq .05$).

[1]Fragmentation index = (residue weight / wet sample weight) × 1,000. High index indicates low fragmentation.

[2]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[3]PM = post-mortem.

[4]Not included in statistical comparison of means.

TABLE 4. Cathepsin B and B + L activities (± SEM) of *Pectoralis* muscle from broiler carcasses treated with different rigor-accelerating treatments

Treatment[1]	Muscle harvest time	Sample freeze time	Cathepsin B	Cathepsin B + L
	(h PM[2])		(nmol product·min^{-1}·g tissue^{-1})	
Control	1	24	.17 ± .02	1.88 ± .11
HV + MT	1	24	.19 ± .02	1.98 ± .11
LV + HTC + MT	1	24	.18 ± .02	1.95 ± .08

[1]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[2]PM = post-mortem.

and L did not decline during 48 h post-mortem storage of chicken muscle subjected to post-mortem ES. Wu *et al.* (1985) and Uytterhaegen *et al.* (1992) reported that ES did not affect the total residual lysosomal enzyme activities of beef muscle. However, Dutson *et al.* (1980) reported that post-mortem ES of beef muscle significantly decreased the total residual lysosomal enzyme activity and further suggested that ES caused a shift in the distribution of free and membrane-bound lysosomal enzymes.

Several other factors may contribute to the conflicting reports of cathepsin activities in ES-treated muscle. Many of the previous studies used enzyme assay methods that have been determined (Koohmaraie and Kretchmar, 1990) to underestimate cathepsin activity by not removing the cathepsin inhibitor, cystatin, from the assay environment. Also, Takahashi *et al.* (1984, 1987) and Thompson *et al.* (1987) reported that the method of ES can greatly affect the tenderizing and biochemical effects in beef and poultry muscle, respectively. In addition, Kang and Sams (1992) reported that red and white muscle fibers responded differently to rigor mortis-accelerating treatments, further confounding the extrapolation of mammalian data to the avian data of the present study. Although the focus of most previous research has been the effects of ES, Moeller *et al.* (1976) observed a similar lack of effect of HTC on total residual lysosomal enzyme levels. The individual effect of MT on cathepsin activity is not known. Because combination treatment systems were used in the present study, the isolated effects of the individual treatments cannot be separated. In retrospect, it is difficult to assess the influence of differences in cathepsin assay procedures, ES techniques, or species on the previous ES reports or their comparisons to the present study.

Although substantial calpain I activity was observed in the untreated muscle at death, no calpain I activity was detected at 24 h post-mortem in samples from either rigor treatment or from the controls (data not shown). The absence of any residual calpain I activity suggested that the enzyme was completely autolysed over 24 h of aging in both treated and untreated samples. Ducastaing *et al.* (1985) also reported that the residual activity of calpain I was drastically reduced during 4 h of post-mortem aging of ES beef *Longissimus* muscle and was the likely candidate in the post-mortem proteolysis that resulted in tender meat. These authors postulated that ES caused an increase in the cytosolic level of Ca^{2+}, which activated the calpains.

By 24 h post-mortem, calpain II activity for both rigor treatments and the control carcasses was reduced compared with the calpain II activity present at death (Table 5). The untreated aged and LV-treated samples experienced more calpain II decline (89.4 and 84.4%, respectively) than those samples receiving the HV treatment (61.6%). The loss of calpain II activity in the control muscles was unexpected and cannot be readily explained. This result conflicts with reports by Vidalenc *et al.* (1983), Ducastaing *et al.* (1985), and Koohmaraie *et al.* (1987), who observed no appreciable loss in calpain II activity with normal post-mortem storage. These authors reported that intracellular free Ca^{2+} levels never get high enough to

TABLE 5. Elution profile peak areas for calpain II activity (mean ± SEM) remaining after 24 h refrigerated aging in broiler *Pectoralis* from control or rigor-accelerated carcasses

Treatment[1]	Muscle harvest time	Sample freeze time	Peak area
	(h PM[2])		(mm^2)
Control	0	0	137.3 ± 11.5[3]
Control	1	24	14.3 ± 1.8[b]
HV + MT	1	24	52.4 ± 4.4[a]
LV + HTC + MT	1	24	21.0 ± 4.8[b]

[a,b]Means within a column with different superscripts differ significantly ($P \leq .05$).

[1]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[2]PM = post-mortem.

[3]Not included in comparison of means.

activate calpain II, which requires millimolar concentrations of Ca^{2+} for activity. It is doubtful that incomplete recovery of the enzyme from the tissue and column or that improper assay conditions could be responsible for the decline in activity, because unaged control samples were analyzed in between the aged samples as standards to verify the procedures. The unaged samples consistently yielded the normal enzyme elution profiles observed in at-death chicken muscle (Birkhold and Sams, 1994). Because the objective of this study was to compare treatment effects on calpain activities, chromatogram peak areas were digitized as a measure of relative enzyme activity. Unfortunately, this prevents direct comparison with previous studies that either reported actual activity units or simply reported percent of maximal activity. Because the unaged muscles had normal activity, the lower activity in the aged muscle may have resulted from some extractability or stability factor yet to be determined in the more rapidly glycolyzing avian *Pectoralis*. This would be consistent with Etherington *et al.* (1987), who suggested that the uniquely rapid conditioning rate of chicken muscle may involve species-specific differences in protease release, activation, and substrate susceptibility. Etherington *et al.* (1990) later reported a 30% reduction in calpain II activity during storage of untreated chicken muscle.

The decline in calpain II activity that occurred during aging was less in the HV treatment than in the LV or control (Table 5). Birkhold and Sams (1993) previously suggested that the accelerated pH decline induced by HV reduced the activity of the calpain neutral proteases. The substantial decline in calpain II activity observed in the present study differs somewhat from the reports of Ducastaing *et al.* (1985) and Uytterhaegen *et al.* (1992), who reported smaller reductions in calpain II activity in ES-treated beef *Longissimus*. These authors suggested that the loss in activity occurred because of the high Ca^{2+} levels induced by ES. In the present study however, less of a decline was observed in the HV treatment than in the LV treatment or the control. This conflicts with Ducastaing *et al.* (1985) and Uytterhaegen *et al.* (1992), who reported that HV increased the decline in calpain II activity in beef *Longissimus* muscle compared with controls. The lack of a difference between the LV treatment and the controls was consistent with Koohmaraie *et al.* (1989), who reported that LV produced no significant change in calpain I or II activities from those of the control ovine muscles.

The greater overall loss in calpain II activity observed in the present study may be explained by differences in muscle fiber types. The intracellular Ca^{2+} flux from the sarcoplasmic reticulum induced by ES may have been greater in the chicken muscle of the present study than that in previous mammalian studies because the sarcoplasmic reticulum is more extensive in white fibers than in red fibers (Fawcett and Revel, 1961). Chicken *Pectoralis* has been reported to contain only white fibers (Smith and Fletcher, 1988; Sams and Janky, 1990; Ono *et al.*, 1993), whereas beef *Longissimus* has been

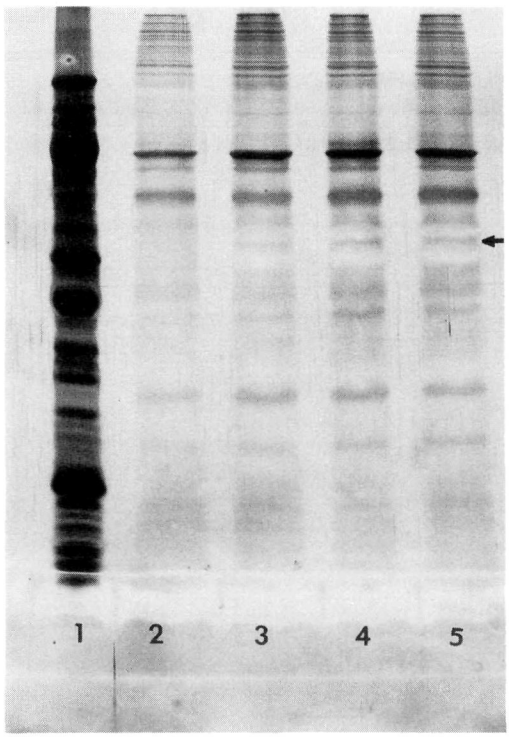

FIGURE 2. Silver-stained SDS-PAGE gel of myofibrillar proteins of post-mortem broiler *Pectoralis*. Lanes 1 to 5 represent standard molecular weight markers; control 0 h; high voltage electrical stimulation and muscle tensioning; low voltage electrical stimulation, high temperature conditioning, and muscle tensioning; and untreated aged, respectively. Arrow indicates location of 30-kDa polypeptide.

reported to contain 30 to 38% red fibers and 46 to 62% white fibers (Hunt and Hedrick, 1977; Rao and Gault, 1989). Another factor may be the use in the present study of additional treatments (MT and HTC), which have an unknown effect on calpain activity. The importance of these additional treatments and the effects of differences in ES techniques are emphasized by the differences between the two rigor treatments in the calpain II activities present after aging. It should be noted that it is difficult to clearly interpret the loss in calpain II activity in the 24-h rigor-accelerated samples in the present study, considering the unexpected loss of activity that also occurred in the control muscles during the 24-h aging period.

The SDS-PAGE analysis of the myofibrillar proteins indicated the presence of a 30-kDa polypeptide band in samples from aged muscles that was absent in samples analyzed at death (Figure 2). The presence of this polypeptide, thought to be the product of calpain activity (Macbride and Parrish, 1977), indicated the involvement of calpain-mediated proteolysis in the aging of these muscles. The densitometric estimates of the protein quantity in these 30-kDa polypeptide bands indicated that the HV + MT and LV + HTC + MT treatments produced less of this peptide than in the aged controls (Table 6). This result for the HV + MT treatment is consistent with the lower loss of calpain II occurring in the HV + MT muscles (Table 5). Uytterhaegen *et al.* (1992) reported that HV increased the production of the 30-kDa polypeptide in ES-treated beef muscle. However, these authors also reported that HV activated the calpains.

Both treatment systems accelerated the depletion of ATP. The previously observed tenderness improvement resulting from the LV + HTC + MT treatment (Walker *et al.*, 1994) involves the reduction of sarcomere shortening, an effect that is a prevention of toughening and not technically a tenderization process per se. The HV + MT treatment system reduced both the calpain activity expressed during aging (measured by residual activity and the 30-kDa product) and the associated myofibrillar fragmentation. This fragmentation inhibition effect seemed to be more pronounced in the HV + MT treatment, with the LV + HTC + MT treatment being closer to the controls in proteolytic evidence. Because the HV + MT treatment did not prevent sarcomere shortening and inhibited proteolytic myofibrillar disruption, its tenderness improvement must result from some factor not measured in this study. Birkhold and Sams (1993, 1994) speculated that, instead of Z-line dissolution, gross physical muscle tearing that is not measurable by the fragmentation index was the fragmentation through which HV + MT induced its tenderness effect. The LV + HTC + MT treatment yielded the lowest shear value in our previous study (Walker *et al.*, 1994) because it may have achieved a better balance between a reduction in shortening and sufficient myofibrillar fragmentation than the HV + MT treatment.

TABLE 6. Densitometric estimates (mean ± SEM) of a 30 kDa polypeptide appearance during post-mortem aging of *Pectoralis* muscle from broiler carcasses treated with different rigor-accelerating systems

Treatment[1]	Muscle harvest time	Sample freeze time	30 kDa
	(h PM[2])		(μg)
Control	0	0	0[3]
Control	1	24	.82 ± .30[a]
HV + MT	1	24	.16 ± .18[b]
LV + HTC + MT	1	24	.30 ± .12[b]

[a,b]Means within a column with different superscripts differ significantly ($P \leq .05$).

[1]HV = high voltage electrical stimulation; LV = low voltage electrical stimulation; HTC = high temperature conditioning; MT = muscle tensioning.

[2]PM = post-mortem.

[3]Not included in comparison of means.

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