The Effect of Feed Withdrawal Duration on the Responsiveness of Broiler *Pectoralis* to Rigor Mortis Acceleration[1]

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ABSTRACT This experiment was conducted to examine the effect of the duration of preslaughter feed withdrawal on the responsiveness of chicken muscle tissue to rigor mortis acceleration. Rigor mortis was accelerated by combining electrical stimulation and high temperature conditioning immediately post-mortem. At 1 h post-mortem, one breast fillet from each carcass was removed, frozen, and analyzed for metabolic and structural characteristics. The other fillet was excised, aged for 23 h, cooked, and sheared. As duration of feed withdrawal increased, muscle glycogen decreased. Shear value and pH decreases were most pronounced after 5 h of feed withdrawal. The feed withdrawal period did not affect R value, fragmentation index, or sarcomere length. Rigor mortis acceleration resulted in longer sarcomeres and increases in R value and fragmentation index.

(*Key words*: broiler breast meat, rigor mortis, feed withdrawal, post-mortem metabolism, tenderness)

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INTRODUCTION

With increasing consumer demand for boneless poultry products, the broiler processing industry is considering the feasibility of early harvesting the meat. Early harvesting is removing the meat prior to the onset of rigor mortis. Unless the unacceptable toughness of early-harvested meat is eliminated, processors will be forced to maintain a minimum 4 h postchill aging period prior to carcass boning to allow the development of rigor mortis (Shelton, 1985). Hamm (1982) reported that the depletion of cellular supplies of adenosine triphosphate (ATP) through post-mortem metabolism is the biochemical event responsible for rigor mortis development. The author stated that muscle cells degrade glycogen in an attempt to maintain their cellular ATP level.

In addressing the toughness of early-harvested meat, many researchers have examined a variety of processing methods. One method investigated is post-mortem electrical stimulation. Electrical stimulation has been applied to chicken carcasses to hasten the onset of rigor mortis (Thompson *et al.*, 1987; Lyon *et al.*, 1989; Sams, 1990). With rigor mortis onset occurring earlier post-mortem, it has been possible to harvest the meat earlier in the processing scheme without inducing objectionable toughness.

Carcass temperature has been shown to have a marked effect on the rate of post-mortem glycolysis and rigor mortis development. Khan (1971) reported that high post-mortem temperatures accelerated glycolysis and toughened broiler breast meat, whereas low temperatures delayed glycolysis without toughening.

Clatfelter and Webb (1987) were issued a patent for a method to process broilers using a scheme that involved both electrical stimulation and high temperature conditioning to prevent the toughening effect

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of early harvesting of the meat. Electrical stimulation and high temperature conditioning were already in practice in the beef (Pearson and Dutson, 1985) and lamb (Carse, 1973) industries.

Feed withdrawal is a routinely employed production management technique (May and Lott, 1990). This practice of denying broilers access to feed 8 to 12 h prior to slaughter may impact the effectiveness of the rigor mortis acceleration treatment and the final product quality by decreasing the amount of glycogen available for depletion prior to rigor mortis development. Although both Wood and Richards (1975) and Rasmussen and Mast (1989) reported no significant effect of feed withdrawal duration on shear value of meat excised after at least 24 h of post-mortem aging, Lyon *et al.* (1991) observed that shear value of meat excised at 4 h post-mortem decreased with increasing the duration of feed withdrawal. The shear value difference observed in this latter study suggested that duration of feed withdrawal affects the responsiveness of broiler breast muscle to early post-mortem excision, possibly by altering glycolytic potential, as the authors also reported muscle pH differences between treatments at 2 h post-mortem.

To achieve the greatest effect from rigor mortis acceleration treatments, the effect of general husbandry practices such as feed withdrawal need to be investigated. Therefore, the objectives of this study were to determine the effects of feed withdrawal on the tenderness and post-mortem metabolism of breast muscle from carcasses receiving treatments that accelerate rigor mortis.

MATERIALS AND METHODS

In each of two 72-bird trials, male broilers of a commercial strain were reared using standard husbandry practices, fed a corn and soybean-based diet (20% CP, 3,234 kcal ME/kg), and killed at 8 wk of age. In each of eight nine-bird groups per trial, feed and water were withdrawn from one-third of the birds 10, 5, and 0 h prior to slaughter. From each withdrawal period, one bird was subjected to the rigor mortis acceleration treatment of electrical stimulation and high temperature conditioning, one bird was designated as an untreated control, and one bird served as a 0 h post-mortem (time of death) representative. This particular rigor mortis acceleration treatment was selected for study because it is patented and in commercial use (Clatfelter and Webb, 1987; Amey, 1988; Webb *et al.*, 1989) and because its mechanism is highly dependent on metabolic acceleration (Thompson *et al.*, 1987; Sams, 1990; Sams *et al.*, 1991). All nine birds were cooped together and represented a processing replicate. All replicates in a trial were processed on the same day, and the two trials were processed 1 wk apart.

By processing replicate, the broilers were killed by bleeding through a neck cut for 90 s. Ante-mortem stunning was not used, as Clatfelter and Webb (1987) reported that stunning interfered with the acceleration of rigor mortis development. However, after the completion of the present studies, Webb *et al.* (1989) were issued a patent using ante-mortem stunning with their electrical stimulation and high temperature conditioning processes. After bleeding, left breast fillets (*Pectoralis*) were harvested with the method of Hamm (1981) from one uneviscerated bird in each of the different withdrawal periods to provide baseline (0 h post-mortem) samples. Tenderness was not evaluated for baseline fillets.

The remaining six carcasses were scalded at 60 C for 45 s. Following scalding, one carcass from each withdrawal time was subjected to the rigor mortis acceleration treatment of electrical stimulation for 14 min followed by high temperature conditioning, both as described by Sams (1990). The last three carcasses not receiving the acceleration treatment served as the controls. For electrical stimulation, an electric current [110 V alternating current (AC), 1 A] was pulsed (2 s on and 1 s off) for 14 min through the carcass immediately after bleeding by placing the charged stun knife blade against the cervical region of the spine inside the neck cut made for bleeding. After electrical stimulation, the feathers were removed from all of the accelerated and control carcasses using a

rotary drum picker[3] for 25 s. The six carcasses were eviscerated by hand and conditioned for 45 min. The carcasses receiving electrical stimulation were conditioned in a warm water bath at 39 C (high temperature conditioning) and unstimulated control carcasses were conditioned in a water-ice slush (Sams, 1990). This arrangement resulted in three carcasses per trial (one from each withdrawal period) having received the rigor mortis acceleration of electrical stimulation followed by high temperature conditioning, and three other carcasses (one from each withdrawal period) served as untreated controls. Immediately following the conditioning period, both left and right fillets were harvested from the carcasses (Hamm, 1981). Right fillets were packed in ice and stored for 24 h at 4 C prior to cooking and shearing according to the procedures described by Sams (1990).

Immediately after excision the left fillets from all nine carcasses in each processing replicate were cut parallel to the muscle fibers into four pieces of approximately equal size, packaged in plastic bags, frozen in liquid nitrogen, and stored at –75 C until analyzed. Evidence reported by Papa and Fletcher (1988) indicated that rigor mortis development varied with the location on the breast fillet. To lessen the possibility of fillet location affecting the results, each variable was evaluated from the same position on all fillets. The samples were numbered 1, 2, 3, and 4 for their respective cranial to caudal location in the muscle. Sample 1 was analyzed for gravimetric fragmentation index using the oven-dried method of Sams *et al.* (1991). Sample 2 was analyzed for R value (absorbance at 250 nm:absorbance at 260 nm) as described by Thompson *et al.* (1987). The glycogen content of Sample 3 was determined with an ultraviolet-method starch biochemical analysis kit.[4] Sample 4 was used to measure both sarcomere length (Sams *et al.*, 1990) and pH (Sams and Janky, 1986). Following the 5-s homogenization at 11,500 rpm in a Virtis homogenizer[5] to separate fibers and the subsequent sampling of these fibers for sarcomere length determination, homogenization was continued for an additional 25 s, and the pH of resulting slurry was measured with a pH meter[6] and combination electrode.

Shear value data were analyzed as a 3 × 2 factorial design (three withdrawal durations by two post-mortem treatments), whereas all other variables were analyzed as a 3 × 3 factorial design [three withdrawal durations by three post-mortem treatments (0 h post-mortem baseline, 1 h post-mortem control, 1 h post-mortem accelerated)], all by analysis of variance with the SAS® using Duncan's multiple range test to separate means (SAS Institute, 1985). Residual mean square was used as the error term, and, because no significant interaction between trial and treatment was detected, the data from the two trials were pooled.

RESULTS AND DISCUSSION

The effects of feed withdrawal duration and rigor mortis acceleration on shear value of broiler breast fillets are presented in Table 1. There was a significant decrease in the shear value of early-harvested *Pectoralis* when the carcasses were subjected to the rigor mortis acceleration treatments of electrical stimulation and high temperature conditioning following 5 or 10 h of feed withdrawal. These data are consistent with the observations of Sams (1990). Although all observed shear value means would be considered tough by consumers according to Lyon and Lyon (1990), the relative differences between the means can still be used to study the effects of feed withdrawal on the effectiveness of the rigor mortis acceleration treatments. The length of the feed withdrawal period did not significantly affect the tenderness of fillets from control carcasses. However, when the rigor mortis acceleration treatment was employed, the tenderizing effectiveness of this treatment was improved when the birds were deprived of feed for 5 h. The

[3]Model SP30SS, Brower, Houghton, IA 52631.
[4]Boehringer Mannheim Biochemicals, Indianapolis, IN 46250.
[5]Model 23, The Virtis Co., Gardiner, NY 12525.
[6]Model 240, Corning Science Products, Corning, NY 14831.

TABLE 1. The effect of feed withdrawal duration on the shear value means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration and harvested at 1 h post-mortem

Rigor mortis acceleration treatment	Feed withdrawal duration		
	10 h	5 h	0 h
	(kg/g of meat)		
None	16.83 ± .90[x]	16.55 ± 1.12[x]	15.49 ± 1.05
ES + HTC[2]	13.47 ± .78[a,y]	11.83 ± .63[b,y]	13.72 ± .62[a]

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

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

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

5-h period may have sufficiently reduced the glycogen content of the cells, expediting the depletion of remaining cellular glycogen by the rigor mortis acceleration treatments. The fact that the 10-h withdrawal did not yield the same tenderizing improvement as the 5-h withdrawal was unexpected and cannot be readily explained. The prolonged 10-h withdrawal may have been long enough to induce alternate mechanisms of energy production such as fatty acid oxidation by the bird prior to death. Because fatty acid oxidation is much more efficient at producing ATP than is glycolysis (Stryer, 1981), even a brief period of induction of the oxidation system may have compensated for the losses in glycogen-derived ATP resulting from feed withdrawal.

An indication of rigor mortis development in muscle tissue is pH. During post-mortem glycolysis, there is a build up of lactic acid and a decrease in pH (Stewart *et al.*, 1984). At death (0 h post-mortem), no significant difference ($P > .05$) in pH values was observed between the feed withdrawal periods (Table 2). This was expected because homeostatic mechanisms maintained the physiological muscle pH. The control samples and the accelerated samples all have significantly lower pH than the baseline samples due to post-mortem glycolysis (Stewart *et al.*, 1984). In comparing the control samples and the accelerated samples within each of the feed withdrawal times, the pH means of the accelerated samples were lower than the controls only for the 5-h withdrawal period. Lower pH indicates acceleration of rigor mortis through the electrical stimulation and high temperature conditioning treatment. The pH of 1 h post-mortem fillets from control and accelerated carcasses had a similar trend to that observed for shear value. Five hours of feed withdrawal improved the effectiveness of the

TABLE 2. The effect of feed withdrawal duration on pH means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration

Post-mortem fillet harvest time	Rigor mortis acceleration treatment	Feed withdrawal duration		
		10 h	5 h	0 h
0 h	None	6.33 ± .08[a]	6.28 ± .06[a]	6.21 ± .05[a]
1 h	None	5.93 ± .04[b]	6.00 ± .06[b]	5.94 ± .06[b]
1 h	ES + HTC[2]	5.86 ± .06[b]	5.78 ± .03[c]	5.81 ± .06[b]

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

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

TABLE 3. The effect of feed withdrawal duration on glycogen concentration means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration

Post-mortem fillet harvest time	Rigor mortis acceleration treatment	Feed withdrawal duration		
		10 h	5 h	0 h
0 h	None	3.08 ± .40^{b,x}	4.39 ± .36^{ab,x}	4.72 ± .48^{a,x}
1 h	None	2.94 ± .34^{xy}	3.31 ± .45^{y}	3.34 ± .41^{y}
1 h	ES + HTC[2]	1.80 ± .19^{y}	2.61 ± .23^{y}	2.69 ± .45^{y}

^{a,b}Means within a row with no common superscripts differ significantly ($P \leq .05$).

^{x,y}Means within a column with no common superscripts differ significantly ($P \leq .05$).

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

rigor mortis acceleration by manifesting a significant pH difference between control and accelerated means that did not occur when feed withdrawal was not employed. However, this difference was not detected when feed withdrawal was extended to 10 h.

Levels of the primary storage form of muscle cell energy, glycogen, are presented in Table 3. During feed withdrawal, the body utilizes glycogen to maintain energy homeostasis (Stryer, 1981). At the point of death (0 h post-mortem), the utilization of glycogen by the tissues is depicted by the significant differences between the sample from birds that ate (0 h) and samples from birds that did not eat for 10 h. Very little glycogen was utilized during the 1st h post-mortem by the control muscles in the 10-h feed withdrawal treatment, possibly because there was less initial (0 h post-mortem) glycogen available in this withdrawal treatment. This was contrasted by the significant reduction in glycogen concentration manifested by the rigor mortis acceleration treatment in the 10-h feed withdrawal treatment. In the 0-h and 5-h withdrawal treatments, glycogen concentrations did not significantly differ between the 1-h accelerated and 1-h control or between the 0-h controls. Thus, the extended (10-h) feed withdrawal improved the ability of the rigor mortis acceleration treatment to deplete cellular glycogen supplies by reducing the glycogen concentration at 0 h post-mortem.

The R value is used to determine the state of rigor mortis (Calkins *et al.*, 1982). Increasing R values indicate advancing rigor mortis development, finally reaching a plateau with maximum rigor mortis development. The R value means for the different feed withdrawal durations were not significantly different ($P > .05$) at 0 h post-mortem (Table 4). These samples

TABLE 4. The effect of feed withdrawal duration on the R value means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration

Post-mortem fillet harvest time	Rigor mortis acceleration treatment	Feed withdrawal duration		
		10 h	5 h	0 h
0 h	None	.97 ± .04^{c}	1.00 ± .04^{c}	1.02 ± .04^{b}
1 h	None	1.10 ± .03^{b}	1.12 ± .04^{b}	1.09 ± .03^{b}
1 h	ES + HTC[2]	1.40 ± .03^{a}	1.36 ± .03^{a}	1.37 ± .04^{a}

$^{a-c}$Means within a column with no common superscripts differ significantly ($P \leq .05$).

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

TABLE 5. The effect of feed withdrawal duration on the sarcomere length means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration

Post-mortem fillet harvest time	Rigor mortis acceleration treatment	Feed withdrawal duration		
		10 h	5 h	0 h
0 h	None	1.05 ± .04[c]	1.06 ± .04[c]	1.08 ± .03[c]
1 h	None	1.43 ± .05[b]	1.34 ± .08[b]	1.36 ± .03[b]
1 h	ES + HTC[2]	1.72 ± .04[a]	1.77 ± .05[a]	1.81 ± .03[a]

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

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

were harvested immediately after death and sufficient time had not elapsed for a significant R value difference to manifest. The lack of a difference in R value at death may support the involvement of ATP not derived from glycogen, as previously mentioned for the shear value results. The rigor mortis acceleration treatment significantly increased R value compared with the control samples, indicating that the combined electrical stimulation and high temperature conditioning hastened the onset of rigor mortis, consistent with previously reported results (Sams, 1990).

The prevention of sarcomere shortening has been reported as one of the mechanisms by which electrical stimulation treatment of carcasses prevents toughness development in early-harvested broiler *Pectoralis* (Sams, 1990). Also, conditioning of broiler carcasses near physiological temperature has been shown to result in longer sarcomeres (Bilgili *et al.*, 1989). Feed withdrawal duration had no significant effect ($P > .05$) on the ability of the rigor mortis acceleration treatment to prevent sarcomere shortening (Table 5), consistent with a similar lack of feed withdrawal effect observed for R value. The observed differences in shear value between 0 and 5 h of feed withdrawal, in the presence of no sarcomere length effect, is consistent with the report of Sams (1990) that prevention of sarcomere shortening is not the only mechanism by which electrical stimulation and high temperature conditioning improve the tenderness of early-harvested chicken meat. Sarcomeres from samples that were subjected to electrical stimulation and high temperature conditioning were significantly longer than sarcomeres from control or baseline samples for all feed withdrawal durations (Table 5).

Fragmentation index is inversely related to myofibrillar fragmentation, with a high fragmentation index value indicating little fragmentation of the myofibrils (Thompson *et al.*, 1987). Myofibrillar fragmentation index has been reported to be related to reduced shear value of breast meat early harvested from broiler car-

TABLE 6. The effect of feed withdrawal duration on fragmentation index means[1] (± SEM) of broiler breast fillets subjected to rigor mortis acceleration

Post-mortem fillet harvest time	Rigor mortis acceleration treatment	Feed withdrawal duration		
		10 h	5 h	0 h
0 h	None	105.10 ± 13.20	83.53 ± 13.83[c]	108.75 ± 11.78[b]
1 h	None	125.03 ± 10.52	118.92 ± 9.54[b]	115.08 ± 8.42[b]
1 h	ES + HTC[2]	134.28 ± 9.52	148.99 ± 9.15[a]	146.63 ± 8.72[a]

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

[1]n = 16 per mean.

[2]ES = electrical stimulation; HTC = high temperature conditioning.

casses subjected to post-mortem electrical stimulation and high temperature conditioning (Sams, 1990). The fragmentation index data (Table 6) indicates there was no significant ($P > .05$) effect of feed withdrawal duration. However, the effect of the rigor mortis acceleration treatment on myofibrillar fragmentation varied with duration of feed withdrawal. Within feed withdrawal treatment, the greatest fragmentation occurred in the baseline samples and the least fragmentation occurred in the accelerated samples, similar to the report of Sams *et al.* (1991). These authors suggested that rigor mortis acceleration treatment may prevent myofibrillar fragmentation by increasing the rates of post-mortem metabolism and pH decline. The pH decline may inhibit the calcium-dependent proteases, which require neutral pH. The suggestion that the myofibrillar fragmentation inhibition by electrical stimulation and high temperature conditioning is pH-mediated is consistent with the results of the present experiment. The degree of fragmentation inhibition caused by the acceleration treatment increases from 0 to 5 h of feed withdrawal and then decreases back to the 0-h level by 10-h of withdrawal (Table 6). This trend is similar to the pH trends in Table 2, taking into account the inverse nature of the fragmentation index.

This study indicated that feed withdrawal from broilers prior to slaughter reduces the energy stores in the muscle tissue that could be used during post-mortem metabolism. This reduction enhances the efficacy of the rigor mortis acceleration treatment in the prevention of toughness development in broiler breast fillets, especially at the 5-h withdrawal time. These results suggest that it may be advantageous to control the feed withdrawal duration when rigor mortis acceleration techniques such as electrical stimulation and high temperature conditioning are employed.

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