Influence of Preslaughter Stunning on Turkey Breast Muscle Quality

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ABSTRACT Pectoralis muscle quality was evaluated from 18-wk-old tom turkeys after electrical stun, carbon dioxide stun, or no stun methods were applied. Color was measured on raw muscle and cooked meat using a colorimeter. Muscle pH was measured 15 min postmortem (initial), 24 h post-mortem (final), and after cooking. The right Pectoralis muscle of each carcass was excised for m-calpain analysis within 4 min post-mortem. After 24 h of storage at 4 C, the left Pectoralis muscle was excised to determine cook loss and shear force measurements. No significant difference was found in initial muscle pH (15 min) from turkeys receiving electrical or carbon dioxide stunning, 6.36 ± 0.15 and 6.20 ± 0.14, respectively. However, initial muscle pH for birds that were not stunned (5.99 ± 0.08) was lower (P < 0.05) than the muscle pH of birds stunned using either of the two stunning methods. Stunning method had no effect on the final muscle pH, raw muscle color, cooked meat pH, cooked meat color, cook loss, or shear force. Cook loss was found to positively correlate with initial muscle lightness (r = 0.53), and cooked meat lightness (r = 0.48), but to negatively correlate with cooked meat yellowness (r = −0.48) and shear strength (r = −0.43). m-Calpain activity declined with the stunning methods in the following order: electrical > carbon dioxide > no stun. In addition, m-calpain activity was found to correlate with initial muscle pH (r = 0.95) and with cooked meat shear force (r = −0.43). The results of this study show that electrical stunning, carbon dioxide stunning, and no stunning methods provide comparable cooked turkey breast meat quality with no consistent differences after aging on the carcass for 24 h.

(Key words: muscle quality, turkey, calpain, stunning, carbon dioxide)


INTRODUCTION

Preslaughter immobilization of poultry is used to minimize distress and struggling in birds during exsanguination, and to shorten bleedout time, ensuring death prior to scalding (Murphy et al., 1988; Bilgili, 1992; Dickens and Lyon, 1993). The most common method of immobilization is electrical stunning because it is inexpensive, convenient, and safe (Bilgili, 1992; Fletcher, 1993). Because of its widespread use, numerous studies have investigated the effects of electrical stunning on post-mortem muscle and meat quality (Lee et al., 1979; Thomson et al., 1986; Kim et al., 1988; Murphy et al., 1988; Dickens and Lyon, 1993; Papinaho and Fletcher, 1995). Research has shown that under optimum conditions, electrical stunning not only renders the bird unconscious, but it also delays post-mortem muscle glycolysis and increases meat tenderness (Lee et al., 1979; Thomson et al., 1986; Kim et al., 1988; Murphy et al., 1988). However, carcass quality defects leading to downgrading have been reported with electrical stunning when the applied current is either too low, or too high (Lee et al., 1979; Wabeck, 1987; Murphy et al., 1988; Gregory and Wilkins, 1989; Dickens and Lyon, 1993).

Gregory and Wilkins (1989) studied noneviscerated turkey carcasses and found that turkeys stunned with low current (75 mA for 4 s) had a higher percentage of breast skin hemorrhages, engorged wing veins, and broken coracoids than turkeys stunned using higher current (150 mA for 4 s). Increasing the stunning current from 75 or 150 mA to 250 mA resulted in turkeys with a greater percentage of wing and breast muscle (Pectoralis minor) hemorrhaging (Gregory and Wilkins, 1989). In addition to muscle hemorrhaging, high electrical stunning current increases the incidence of broken keels, broken wings, engorged wing veins, red wing tips, and broken capillaries in the breast muscle (Wabeck, 1987; Bilgili, 1992; Gregory and Wilkins, 1993).

Because electrical stunning affects muscle quality, there has been considerable interest in developing alternate methods of immobilization (Fletcher, 1993). Fletcher (1993) suggested that, of the alternative stunning methods, gaseous stunning seems to be the only practical substitution for electrical stunning. Muscle

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Abbreviation Key: PSE = pale, soft, and exudative.
quality from birds stunned using gaseous stunning has been compared to muscle quality of birds stunned using electrical stunning (Kotula et al., 1957; Raj et al., 1990; Fleming et al., 1991; Raj and Gregory, 1991), or no stunning (Wilson and Brunson, 1968; Sams and Dzuik, 1995). Moreover, studies have compared the stunning effectiveness of various concentrations of gases (Fleming et al., 1991; Raj, 1994), and different types of gases on muscle quality (Raj, 1994; Poole and Fletcher, 1995). Stunning with CO₂ has been reported to result in a lower incidence of muscle hemorrhaging and broken bones (Mohan Raj et al., 1990), and to improve meat tenderness at levels of 45 and 40% CO₂, respectively (Mohan Raj et al., 1990; Fleming et al., 1991). Mohan Raj (1994, 1995) and Raj and Nute (1995) speculated that breast meat from gas-stunned poultry has improved muscle tenderness because gas-stunning causes an early onset of rigor mortis, and the activation of muscle proteinases, similar to electrical stimulation. Dransfield (1992) and Walker et al. (1995) supported this theory by demonstrating that electrically stimulated beef and broiler muscles, respectively had higher calpain levels than untreated muscles after aging. In addition, Dransfield (1992) found that 68% of the variation in toughness of beef *Pectoralis profundus* could be accounted for by variation in calpain activity. Because a direct relationship has not been established between type of stun, meat tenderness, and calpain activity, the present investigation was conducted using electrical stunning, CO₂ stunning, or no stunning methods on commercially reared turkeys.

**MATERIALS AND METHODS**

**Birds**

All of the turkeys used in this study were Nicholas × British United Turkey birds. On each of three processing days, six tom turkeys, 18-wk-old, were obtained from a local grower, and transported (<40 km) in a covered vehicle to a pilot plant processing facility. Birds were removed from the vehicle, weighed, and assigned to a stunning method.

**Stunning and Slaughter**

The stunning methods used were electrical stun, CO₂ stun, or no stun. Electrical stunning was conducted with an electrical knife that delivered an alternating current of 60 Hz from the neck to the shank on turkeys that were conventionally hung in grounded shackles. Birds were electrically stunned individually for 5 s at a setting of 7 (200 mA). Controlled atmosphere carbon dioxide stunning was conducted in an enclosed chamber (60.5 × 60.5 × 132 cm) using a 40 to 60% gradient of CO₂, continuously monitored with a CO₂ analyzer. Birds were placed in a conventional shackle, and transferred to the chamber that was preloaded with 40% CO₂. The CO₂ concentration was increased from 40 to 60% within 30 s after the transfer, and birds were held in 60% CO₂ for an additional 30 s. Immediately following electrical or CO₂ stunning, a single knife cut was made on the side of the neck, and the birds were bled for 90 s. Birds in the no stun group were slaughtered by a side neck cut, followed by exsanguination for 90 s.

Because calpain activity depends upon intramuscular pH, the time from exsanguination to enzyme extraction influences activity analyses. Therefore, calpain enzymes were extracted immediately post-mortem. To accomplish immediate extraction, none of the birds were scalded, picked, eviscerated, or chilled before sampling. Rather, immediately after exsanguination, the skin covering the right side was opened, the right *Pectoralis major* was excised, and pH, color, and calpain activity were determined. The left side of the breast remained intact. The whole carcass was placed in a plastic bag, and stored at 4 °C for 24 h before the left *Pectoralis* muscle was excised for cook loss and texture measurements. Scalding, feather removal, and carcass chilling affect the texture of meat by changing the rate of post-mortem rigor development; however, these standard commercial practices would have delayed the calpain extraction procedures, and thus were not performed in the present study.

**pH Measurement**

The pH of breast muscle was measured using the method of Jeacocke (1977) with modifications. Two grams of muscle were dispersed in 25 mL of 5 mM iodoacetate solution containing 150 mM potassium chloride. After two, 30-s bursts in a homogenizer, pH was measured on the slurry. One pH reading was taken per sample. Muscle pH was measured 15 min after exsanguination (initial), after a storage for 24 h (final), and after cooking (cooked). Initial, final, and cooked readings were averaged for each stunning method and for the 3 processing d, and the averages were reported.

**Color Measurements**

The C.I.E. (1978) L*, a*, and b* color values were measured on raw muscle immediately post-mortem and on cooked meat using a Minolta colorimeter. The colorimeter was standardized using white and pink ceramic tiles. Color of raw muscle was measured on the medial side of the *Pectoralis* muscle at approximately 1/3...
its length from the insertion. For cooked meat, cuts were made in the center of the muscle perpendicular to the fibers, and measurements were taken in the geometric center of the cut surface. One reading was taken for raw muscle (n = 18), and one reading was taken for cooked meat (n = 18). Cooked meat and raw muscle color values were reported as averages for the stunning methods.

**Calpain Extraction, Separation, and Assay**

m-Calpain activity was determined because it has been found to be more stable during isolation, purification, and storage that μ-calpain (Koohmaraie, 1988). m-Calpain was extracted and separated using the method of Koohmaraie (1990) with modifications. Seventy-five grams of breast muscle were removed from turkeys within 2 to 4 min after exsanguination. The muscle was trimmed of excess fat and connective tissue, manually diced and homogenized in 2.5 (vol/wt) of extraction buffer (50 mM Tris, 10 mM EDTA, 10 mM β-mercaptoethanol, pH 8.3). Homogenization was performed in a precooled blender9 using three 30-s bursts (one burst on medium and two bursts on high speed) with a 30-s resting period between each burst. After homogenization, the slurry was centrifuged10 at 25,000 × g and 4 C for 2 h. The supernatant was then filtered through cheese cloth and glass wool, adjusted to pH 7.5 with 1 N NaOH, and dialyzed11 for 24 h against 20 vol of dialysis buffer (40 mM, 5 mM EDTA, 10 mM β-mercaptoethanol, pH 7.5). After dialysis, the supernatant was removed from the dialysis tubing, centrifuged and filtered as before and loaded by gravity flow onto a DEAE-Sephacel column (26 mm i.d. and 40 cm length)12 that had been previously equilibrated with a minimum of 10 vol of elution buffer (50 mM Tris, 1 mM EDTA, 10 mM β-mercaptoethanol, pH 7.5). Columns with extracted proteins were washed again with a minimum of 5 column volumes to remove unbound proteins. m-Calpain was eluted from the column using 400 mM NaCl by collecting 50 fractions (5 mL). Column fractions were held at 4 C until they were assayed for activity.

Enzymatic activity of m-calpain was determined approximately 72 h after extraction according to the methods described by Koohmaraie (1990). An aliquot (0.5 mL) from each fraction with potential activity was screened for activity by mixing with 1.5 mL of casein substrate buffer (100 mM Tris, 1 mM NaN3, 5 mM CaCl2, 1 μL/mL β-mercaptoethanol, and 5 mg/mL of Hammerstein13 casein, pH 7.5). The reactions were incubated for 1 h at 25 C, and stopped by adding 2 mL of a 5% trichloroacetic acid solution. The denatured proteins were precipitated by centrifugation14 at 2,500 × g, and the soluble peptides were measured for absorbance15 (278 nm) as an indicator of proteolytic activity. Fractions containing calpain activity were pooled and assayed to determine the total activity of calpain present. One unit of calpain activity was defined as the amount of enzyme necessary to cause an increase of 1.0 optical density unit in 1 h at 25 C.

**Cook Loss and Texture**

Cook loss was measured on muscles excised after storage for 24 h (left Pectoralis muscle) at 4 C. Muscles were weighed, and a cooking thermometer was inserted into the center of each muscle. Muscles were vacuum sealed in cooking bags, tempered to 20 C for 1 h, and cooked to an endpoint temperature of 72 C by immersion in a 95 C water bath. When the endpoint temperature was reached, samples were removed from the water bath and immersed in an ice water bath. Samples were stored overnight at 4 C, before being removed from the bags and reweighed for cook loss determinations. Cook loss was reported as the percentage weight lost during cooking.

Texture of the cooked meat was measured as described in a previous study (Young et al., 1991). Briefly, two 1.9-cm-wide strips, beginning at the humoral insertion and ending at a point adjacent to the keel, were cut through the complete depth of each muscle. Force required to shear across the fibers in kilograms, was measured using a Warner-Bratzler shear device. Shear force was determined on adjacent duplicate samples.

**Statistical Analysis**

All data were analyzed using analysis of variance (SAS Institute, 1990). Means were separated using Tukey’s Studentized Range test at a significance level of P < 0.05. Correlation among variables was determined using the Pearson product-moment correlation coefficient (Sokal and Rohlf, 1969).

**RESULTS AND DISCUSSION**

Prior to processing and stunning method assignment, all of the turkeys were weighed to determine whether there was significant variation in bird size. No significant difference was found in body weight of turkeys assigned to the different stunning methods. On average, turkeys used in this study weighed approximately 14.0 kg.

The pH, color, and cook loss values measured for breast muscle and breast meat from turkeys stunned using the different methods are shown in Table 1. After 15 min post-mortem, breast muscles from electrical (6.36 ± 0.15) and CO2 (6.20 ± 0.14) stunned turkeys had the
highest pH values, followed by muscles from turkeys not exposed to stunning (5.99 ± 0.08). These data are similar to that of Mohan Raj et al. (1990), and Poole and Fletcher (1995). Sante et al. (1995) demonstrated that turkey breast muscle identified as pale, soft, and exudative (PSE) could obtain ultimate pH within 20 min post-mortem. These authors also suggested that the rate of pH decline was more significant in producing the PSE-associated characteristics than the ultimate meat pH (Sante et al., 1995). Although the meat from turkeys that were not stunned had a significantly lower pH 15 min after 4 h or more post-mortem, muscle pH was not affected by stunning method (Murphy, 1988) and broilers, respectively, to be redder (higher +a values) than breast muscle from CO2-stunned birds. The difference between the present results and those presented by Fleming et al. (1991) and Mohan Raj et al. (1990) may be due to variations in bleeding time. Fleming et al. (1991) does not report a bleeding time; however, Mohan Raj et al. (1990) indicated that process-
stunning, CO₂ stunning, and no stun methods provide
normal for broilers (Mohan Raj and Gregory, 1991). Cook loss was comparable to that previously reported as these muscles was not lower than normal (pH 5.8), and color category (high L value); however, the final pH of the muscles evaluated in this study fall into the PSE capacity, and therefore, exhibited the PSE condition. Using the guidelines of Barbut (1993), the color of all of muscle had an ultimate pH of 5.68, poor water-holding properties, and poor texture (Barbut, 1993; Barbut, 1997) reported similar findings in turkey breast muscle where raw muscle L values > 51 may be classified as PSE. In another study, Barbut (1993) reported that light-colored turkey breast muscle had an ultimate pH of 5.68, reduced water-holding properties, and poor texture (Barbut, 1993; Sosnicki, 1993; Sante et al., 1995). According to Barbut (1993), poultry muscle with lightness values (*L) greater than 51 may be classified as PSE. In another study, Barbut (1997) reported that light-colored turkey breast muscle had an ultimate pH of 5.68, poor water-holding capacity (r = 0.60). A strong correlation was observed between the pH of cooked meat and cooked meat lightness (r = 0.53), and cooked meat yellowness (r = 0.48) and cooked meat shear (r = 0.43). Barbut (1997) reported similar findings in turkey breast muscle where raw muscle L values correlated with texture (r = 0.61), and water-holding capacity (r = 0.60). A strong correlation was observed between the pH of cooked meat and cooked meat lightness (r = 0.60) and yellowness (r = 0.52). These relationships may be due to the PSE phenomenon, in which lighter colored muscles have been associated with rapid decline in post-mortem pH, reduced water-holding properties, and poor texture (Barbut, 1993; Sosnicki, 1993; Sante et al., 1995). According to Barbut (1993), poultry muscle with lightness values (*L) greater than 51 may be classified as PSE. In another study, Barbut (1997) reported that light-colored turkey breast muscle had an ultimate pH of 5.68, poor water-holding capacity, and therefore, exhibited the PSE condition. Using the guidelines of Barbut (1993), the color of all of the muscles evaluated in this study fall into the PSE color category (high L value); however, the final pH of these muscles was not lower than normal (pH 5.8), and cook loss was comparable to that previously reported as normal for broilers (Mohan Raj and Gregory, 1991).

The results of this study demonstrate that electrical stunning, CO₂ stunning, and no stun methods provide comparable cooked turkey breast meat quality with no consistent differences when aged on the carcass for 24 h. Moreover, these data suggest that when meat texture is altered because of stunning method, it may be more of a reflection of the effects on muscle pH, and thus proteolytic activity, rather than an activation of proteolytic enzymes.

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REFERENCES

| TABLE 2. Pearson product-moment correlation (r) coefficients (P < 0.05) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|               | Initial pH      | Initial L*      | Initial a*      | Initial b*      | Cooked pH      | Cooked L*      | Cooked a*      | Cooked b*      | Cooked Loss     | Shear            | m-calpain       |
| Shear          | -0.44           | NS              | NS              | NS              | 0.38           | 0.38           | NS             | -0.32          | 0.32            | 0.48             | -0.44           | 0.95            |
| Cooked b*      | -0.32           | -0.47           | NS              | NS              | -0.60          | NS             | -0.60          | NS             | 0.48            | -0.48            | NS              | 0.32            |
| Cook loss      | 0.32            | 0.53            | NS              | NS              | 0.48           | NS             | -0.48          | NS             | -0.43           | 0.43             | 0.33            |
| Initial L*     | NS              | -0.43           | 0.38            | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Initial a*     | NS              | -0.43           | 0.38            | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Initial b*     | NS              | 0.38            | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Cooked L*      | 0.38            | 0.46            | -0.44           | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Cooked a*      | NS              | NS              | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Cooked b*      | NS              | NS              | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Cooked Loss    | 0.32            | 0.48            | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| Shear          | -0.44           | 0.42            | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |
| m-calpain      | 0.95            | NS              | NS              | NS              | NS             | NS             | NS             | NS             | NS              | NS              | NS              |

1Color parameters are presented as C.I.E. L* = Lightness, a* = redness, b* = yellowness.
2NS represents correlation coefficients less than 0.30, or greater than ±0.30.


