ABSTRACT The objective of this study was to determine the relationship between meat quality and the protein content and composition of muscle exudate from broiler breast fillets. Deboned breast fillets (n = 48) were obtained from a commercial processing facility and segregated into 2 groups based on color (light and dark). Meat pH, color, moisture content, 3 measures of water-holding capacity (drip loss, salt-induced water uptake, cook loss), protein solubility, and the protein content of muscle exudates were determined in breast fillets. The protein composition of the muscle exudate was evaluated using SDS-PAGE analysis. Light breast fillets had lower meat pH (4 and 24 h postmortem) and higher L* (lightness) and b* (yellowness) values than dark fillets. Light breast fillets exhibited greater drip loss after 2 and 7 d of storage, lower salt-induced water uptake, and higher cook loss than dark fillets. Neither sarcoplasmic nor total protein solubility differed between light and dark fillets. Protein concentration of muscle exudates was greater in dark fillets and was negatively correlated to drip loss after 2 d of storage (r = −0.50) and salt-induced water uptake (r = 0.42). Electrophoretic protein banding patterns were similar between muscle exudates and sarcoplasmic protein extracts. Gel electrophoresis data from muscle exudates showed that the relative abundance of 4 bands corresponding to 225, 165, 90, and 71 kDa was higher in dark breast fillets. The relative abundance of 3 bands corresponding to 47, 43, and 39 kDa was higher in light breast fillets. Muscle pH and measurements of water-holding capacity were significantly correlated to the abundance of several individual protein bands within the protein profile of muscle exudates. Data from this study showed that protein differences in breast muscle exudates are related to meat pH, color, and water-holding capacity and suggest that muscle exudate could be a potential source of protein markers for fresh meat quality attributes in broiler fillets.

Key words: drip loss, exudate, meat quality, protein, sarcoplasmic

INTRODUCTION

Fluid lost from fresh meat through passive exudation is referred to as muscle exudate or drip. The amount of muscle exudate is an indicator of water-holding capacity (WHC), which refers to the ability of the uncooked meat to retain its inherent or added water through post-mortem processing and storage (Honikel and Hamm, 1994). Poultry meat with low WHC and high drip loss is undesirable for both processors and consumers. Excessive drip loss causes unappealing moisture accumulation in packaging, and results in a drier, less tender cooked product. For poultry processors, high drip loss diminishes product yield and meat with low WHC has reduced functionality in the production of further processed poultry products.

Drip formation is a complex phenomenon that is not fully understood. Fresh muscle is approximately 75% water by weight. Water in muscle is classified as bound, immobilized, or free water (Offer and Knight, 1988). Immobilized water makes up to 80% of the water in muscle and is held within the myofibrillar structure, between the myofibrils, and between the myofibrils and the sarcolemma (Offer and Cousins, 1992). Due to changes in muscle structure and pH that occur during the transformation of muscle to meat, immobilized water can escape from the muscle along with the free water as drip loss (Offer and Knight, 1988). The degradation of protein linkages between different structures within the muscle cell are also thought to influence drip loss (Huff-Lonergan and Lonergan, 2005).

In poultry, the relationship between the amount of drip and meat quality is well-established; however, little is known about the content and composition of muscle exudate from fresh poultry. A few studies in pork demonstrated that drip fluid contains water-soluble, sar-
coplasmonic proteins such as the glycolytic enzymes and myoglobin (Savage et al., 1990; Di Luca et al., 2011). Muscle drip is also thought to contain other sarcoplasmonic proteins, amino acids, and water-soluble vitamins. A negative correlation between the amount of drip loss and the protein concentration of the drip fluid has been observed in pork (Penny 1975, 1977; Savage et al., 1990). Furthermore, Di Luca et al. (2011) concluded that the composition of centrifugal drip fluid from pork muscle could be used as an indicator of meat aging and WHC. Although postmortem metabolism occurs much more rapidly in poultry than pork, it is hypothesized that the protein composition of muscle exudate from fresh poultry is similarly related to attributes of meat quality. Therefore, the objective of the current study was to determine the relationship between meat quality and the protein content and composition of muscle exudate collected from broiler breast fillets.

MATERIALS AND METHODS

Sample Collection

A total of 80 butterfly broiler breast fillets were collected on 2 separate trial days from a local processing plant, which used postmortem electrical stimulation to accelerate rigor onset. Samples were collected from a postchilling deboning line at approximately 2 h postmortem. Samples were placed on ice and transported to the Richard B. Russell Agricultural Research Center within 20 min for meat quality and biochemical measurements.

Sample Processing and Meat Quality Measurement

Right and left fillets were separated and weighed. Muscle pH at 4 h postmortem was measured with a Hach H280GB pH meter and a PH57-SS spear-shaped pH probe (Hach Inc., Loveland, CO) inserted into the thickest portion of the cranial end of the left fillet. Surface color [Commission Internationale d’Eclairage lightness (L*), redness (a*), yellowness (b*)] was measured with a Minolta Spectrophotometer CM-700d (Konica Minolta Inc., Ramsey, NJ) on the central portion of the ventral surface (bone side) of the left breast fillet. Each color measurement was the result of 3 averaged readings. For each trial, 24 fillets were selected based on L* values. The 12 samples with the highest L* values were designated as light and the 12 samples with the lowest L* values were designated as dark.

At approximately 6 h postmortem, the left fillet was trimmed of fat and connective tissue, and minced for 30 s in a food processor (HC306, Black & Decker Corp., Towson, MD). The muscle sample was packaged and stored at 4°C until 24 h for the determination of moisture, water-uptake, and cook loss. Moisture content was determined by drying 5 g of the minced muscle in an aluminum pan at 100°C for 18 h (AOAC, 1990). The minced muscle was used to measure salt-induced water uptake using a swelling/centrifugation method (Wardlaw et al., 1973). Duplicate 10-g samples of the minced muscle tissue were mixed with 15 mL of cold 0.6 M NaCl solution in 50-mL centrifuge tubes. Samples were vortex mixed for 1 min, stored at 4°C for 15 min, and then centrifuged at 7,000 × g for 15 min at 4°C. The remaining liquid was decanted and the sample reweighed. Salt-induced water uptake was expressed as the percentage of weight gained by the pellet [100 × (final weight – initial weight)/initial weight]. The pellet was then used to determine cook loss. Tubes (50 mL) containing pellets were placed in an 80°C water bath and pellets were heated to 78°C and the remaining liquid was decanted. Endpoint temperature was monitored with a Digi-Sense digital thermometer fitted with a Physitemp needle microprobe. Samples were weighed before and after heating, and cook loss was calculated as the weight lost during heating as a percentage of the initial weight [100 × (initial weight – final weight)/initial weight].

At approximately 8 h postmortem, a coring device was used to remove a 40-g muscle sample from the central portion of the right breast fillet for drip loss determination according to the modified procedure of Honikel and Hamm (1994). The core was placed on a mesh screen in a covered plastic container that was stored at 4°C. The sample was weighed after 48 and 168 h of storage. Drip loss was calculated as the weight lost as a percentage of the initial sample weight [100 × (drip loss weight/initial sample weight)]. Muscle exudates from drip loss samples were collected after 168 h of storage and diluted 10-fold in 1× PBS. Protein concentrations of the diluted samples were measured using a biuret assay with bovine serum albumin standards (Gornall et al., 1949). Diluted exudate samples were used for SDS-PAGE analysis.

The remaining portion of the right breast fillet was stored at 4°C until 24 h postmortem, minced with a knife, and then frozen at −20°C for subsequent pH measurement, protein solubility assays, and sarcoplasmonic protein extractions. To determine ultimate pH, duplicate 1-g samples of the tissue frozen at 24 h postmortem were homogenized with 10 mL of cold, deionized water with a tissue homogenizer (Powermax AHS 250, ProScientific Inc., Oxford, CT) and pH was measured in the slurry (Stewart et al., 1984).

Protein Solubility

Protein solubility was measured according to a modified procedure of Warner et al. (1997). To measure sarcoplasmonic protein solubility, duplicate 1-g muscle samples were homogenized with 10 mL of cold 25 mM potassium phosphate buffer (pH 7.2). Samples were placed on a rocker plate at 4°C for 20 h and then centrifuged at 2,600 × g for 30 min. The supernatant was
decanted and protein concentration was measured using the biuret assay with bovine serum albumin as a standard. Total protein solubility was similarly determined in a 1.1 M KI, 0.1 M potassium phosphate (pH 7.2) buffer.

**Sarcoplasmic Protein Extract**

Sarcoplasmic proteins from 24 h postmortem muscle samples were extracted according to a modified protocol from Pietrzak et al. (1997). Muscle samples (2 g) were homogenized with a tissue homogenizer in 20 mL of cold homogenization buffer (50 mM KCl, 20 mM Tris, pH 7.0, 2 mM EDTA, 4 mM MgCl₂, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 1% vol/vol Triton X-100). The homogenate was centrifuged at 10,000 × g for 10 min, and the supernatant was decanted and saved as a sarcoplasmic protein extract. The biuret assay was used to determine the protein concentration of the extracts.

**SDS-PAGE Analysis**

Muscle exudate samples (diluted in 1× PBS) and sarcoplasmic protein extracts were diluted to 2 mg/mL in sample buffer [8 M urea, 2 M thiourea, 3% SDS (wt/vol), 75 mM dithiothreitol, 25 mM Tris-HCl (pH 6.8), 0.004% bromophenol blue] and denatured for 3 min in boiling water (modified from Yates and Greaser, 1983). Denatured samples were loaded (20 μg of protein/lane) onto precast 4 to 20% Tris-glycine polyacrylamide resolving gels. Gels were run on the Novex Midi Gel System (Life Technologies Corp., Carlsbad, CA) at 4°C using a constant 200 V in reservoir buffer described by Laemmli (1970). Gels were stained in a solution of 0.1% (wt/vol) Coomassie brilliant blue R-250, 40% methanol, and 7% acetic acid for 2 h and destained in a solution of 40% methanol and 7% acetic acid. Images were captured using a FluorChem M imaging system (ProteinSimple Inc., Santa Clara, CA) and AlphaView software (v 3.4, ProteinSimple Inc.) was used to quantify protein band densities. Individual protein band densities were expressed relative to the total protein density for the entire lane.

**Statistical Analysis**

Data were analyzed as a one-way ANOVA using the PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The model included color group (light and dark) as a fixed effect. Correlation coefficients between meat quality and protein data were determined using the PROC CORR procedure of SAS.

**RESULTS AND DISCUSSION**

To investigate potential relationships between the protein characteristics of muscle exudate and broiler fillet quality, the first step of this experiment was to obtain breast fillets that varied widely in meat quality. Previous research has consistently shown that breast meat color is closely related to various measures of meat quality (Allen et al., 1997, 1998; Fletcher, 1999; Qiao et al., 2001, 2002). Therefore, samples in the current study were segregated into 2 groups based on L* values. Table 1 shows the meat quality characteristics of the dark and light breast fillets. As expected, light-colored fillets had on average higher L* and b* values and lower a* values than dark fillets. Light fillets had lower pH at both 4 and 24 h postmortem than dark fillets. The pH decline from 4 to 24 h postmortem was greater in light versus dark fillets (0.22 and 0.05 pH units, respectively), indicating that postmortem metabolism and pH decline were more extended in light samples.

Water-holding capacity of the fillets was determined by 3 different methods (drip loss, salt-induced water uptake, cook loss) that indicate different aspects of WHC. Drip loss is an indicator of purge or exudate accumulation during raw meat storage, and results from gravimetric action on the free and immobilized water within the muscle tissue. Drip loss is influenced by postmortem muscle pH and temperature, the degree of muscle shortening (Honikel et al., 1986), and the

<table>
<thead>
<tr>
<th>Trait</th>
<th>Dark</th>
<th>Light</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH4h</td>
<td>6.30a</td>
<td>6.04b</td>
<td>0.04</td>
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</tr>
<tr>
<td>pH24h</td>
<td>6.25a</td>
<td>5.82b</td>
<td>0.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>pH24h-24h</td>
<td>0.05b</td>
<td>0.22a</td>
<td>0.04</td>
<td>0.0002</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>74.6b</td>
<td>75.9a</td>
<td>0.1</td>
<td>0.0456</td>
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<tr>
<td>Drip loss2d (%)</td>
<td>0.48b</td>
<td>2.42a</td>
<td>0.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Drip loss7d (%)</td>
<td>1.54b</td>
<td>4.82a</td>
<td>0.20</td>
<td>0.0001</td>
</tr>
<tr>
<td>Drip loss7d-2d (%)</td>
<td>1.06b</td>
<td>2.40a</td>
<td>0.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>Water uptake (%)</td>
<td>96.0b</td>
<td>24.9b</td>
<td>4.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cook loss (%)</td>
<td>16.0b</td>
<td>22.8a</td>
<td>0.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>L*</td>
<td>45.5b</td>
<td>62.5a</td>
<td>0.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>a*</td>
<td>1.2a</td>
<td>0.3b</td>
<td>0.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>b*</td>
<td>9.4b</td>
<td>13.6a</td>
<td>0.4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

a,bMeans within a row lacking a common superscript differ (P < 0.05).

n = 24. Lightness (L*), a* (redness), b* (yellowness).
degradation of intermediate filament and cytoskeletal proteins (Huff-Lonergan and Lonergan, 2005). In the current study, light fillets exhibited greater drip loss after 2 and 7 d of storage and a greater increase in drip loss percentage between the 2 storage times than dark fillets. Salt-induced water uptake is indicative of meat’s ability to absorb and retain added salt water, which is an important trait for the production of moisture-enhanced poultry products. In the current study, salt-induced water uptake was almost 4-fold greater in dark than in light fillets. Cook loss represents moisture and fat loss due to the denaturation of proteins and disintegration of membranes upon heating and is a good indicator of production yields in cooked meat products. Light fillets in the current study exhibited a greater cook loss than dark fillets. Although greater moisture content is generally associated with elevated muscle pH, the light-colored fillets in the current study showed a greater moisture content than dark fillets. These results disagreed with the findings of Boulianne and King (1995), who reported that dark and pale breast meat did not differ in moisture content, but were similar to the findings of Qiao et al. (2001).

Overall, data from this study indicated that segregation of the breast fillets by L* values yielded populations of fillets that varied widely in quality. These results confirm numerous past reports showing that light-colored fillets have lower muscle pH and diminished WHC (Fletcher 1999; Allen et al., 1997, 1998; Qiao et al., 2001, 2002). Likewise, the current study found that L* value was highly correlated (P < 0.0001) to ultimate pH (r = −0.83), drip loss after 48 h (r = 0.84), salt-induced water uptake (r = −0.78), and cook loss (r = 0.77; full correlation data not shown). Thus, it was concluded that the variation in the quality traits of the breast fillets used in this study was sufficient to uncover potential relationships between muscle exudate protein characteristics and meat quality.

In the current study, the protein composition of the muscle exudates was measured using SDS-PAGE analysis (Figure 1). To account for slight variations in protein loads across lanes, staining intensities of individual protein bands were quantified and expressed relative to the sum total of all band intensities within the lane. Due to protein differences in Coomassie dye binding, direct comparisons in protein band abundance were only made within the same protein band across samples but not between different proteins within the same lane. With one-dimensional SDS-PAGE analysis, it is likely that proteins and protein fragments of similar molecular weight comigrated and resulted in bands representing multiple proteins. Where possible, the predominant protein within each band was identified based on molecular weight, abundance, and comparisons to past research that identified electrophoretic protein bands using purified sarcoplasmic protein standards and mass spectrometry analysis (Savage et al., 1990; Huang et al., 2011; Di Luca et al., 2011; Zapata et al., 2012).

Gel electrophoresis was also used to evaluate the profile of sarcoplasmic protein extracts obtained from muscle at 24 h postmortem prior to storage and exudate collection (Figure 1). Evaluation of gels indicated that the protein banding patterns of the muscle exudates and the sarcoplasmic protein extracts were qualitatively similar. This observation confirms that muscle exudates from breast fillets are composed primarily of the water-soluble, sarcoplasmic proteins within the muscle. Furthermore, because the pectoralis muscle of modern-day broilers is composed almost entirely of glycolytic type IIB muscle fibers (Sams and Janky, 1990), it is not surprising that the muscle exudates contain predominantly glycolytic enzymes.

From the SDS-PAGE protein profiles of the exudates, 16 protein bands within each lane were quantified (Figure 1). The relative abundance of the protein bands in muscle exudate from dark and light breast fillets is shown in Table 2. In general, protein bands larger than...
67 kDa had a greater relative abundance in dark fillets, and bands less than 61 kDa had a greater relative abundance in light fillets. Four bands corresponding to 225, 165, 90 (glycogen phosphorylase), and 71 kDa exhibited greater relative abundance in dark breast fillets. The relative abundance of 3 bands, corresponding to 47 (enolase), 43 (creatine kinase), and 39 (aldolase) kDa, was greater in light breast fillets. Differences in the protein profiles of the exudates from dark and light fillets are likely due to both variations in the in situ expression of sarcoplasmic proteins in the breast muscle and the cumulative effects of postmortem protein degradation and denaturation.

The most prominent differences between the muscle exudates of the light and dark fillets were observed in protein bands related to energy metabolism. The decreased abundance of the glycogen phosphorylase band (90 kDa) in the exudate of the light-colored fillets was likely due to denaturation and loss of protein solubility. Glycogen phosphorylase is particularly susceptible to protein denaturation at pH and temperature conditions within postmortem muscle (Fischer et al., 1979). Denaturation of glycogen phosphorylase in pale muscle with poor WHC has been documented in turkey (Pietrzak et al., 1997) and pork (Warner et al., 1997; Joo et al., 1999). Using extracted sarcoplasmic and myofibrillar proteins, Pietrzak et al. (1997) demonstrated that denaturation of glycogen phosphorylase decreases the solubility of the normally water-soluble protein and causes it to adhere to the myofibrils during subcellular fractionation procedures. Thus, increased protein denaturation and diminished water solubility could have caused the decreased abundance of the glycogen phosphorylase band in the exudate of the light fillets observed in the current study.

In addition to early postmortem alterations in protein solubility, differences in the abundance of glycogen phosphorylase and other enzymes in the exudate of light and dark fillets could have been influenced by postmortem proteolysis during the 7 d of 4°C storage. There is little data available on the influence of extended postmortem aging on sarcoplasmic proteins in broiler muscles. In beef and pork muscle, however, numerous studies have observed aging-related changes in the abundance of various metabolic enzymes (glycogen phosphorylase, enolase, creatine kinase, glyceraldehyde phosphate dehydrogenase, lactate dehydrogenase) in the sarcoplasmic fraction of muscle using both 1- and 2-dimensional electrophoresis (Okayama et al., 1992; Lametsch et al., 2002; Okumura et al., 2003; Bowker et al., 2008; Laville et al., 2009; Di Luca et al., 2011). In these studies, the impact of aging was observed by both the disappearance of the intact protein and the appearance of degradation products. Furthermore, it has been shown that glycogen phosphorylase and creatine kinase are calpain substrates in skeletal muscle (Purintrapiban et al., 2001). Aging-related proteolysis also occurs in broiler breast muscle (Lee et al., 2008; Li et al., 2012), but at a much earlier time frame than in red meat. Thus, it is reasonable to assume that differences in the protein profiles of the muscle exudates in the current study could be partially due to variations in aging-related degradation of sarcoplasmic proteins.

Based on the size of the 225 and 165 kDa bands and their relatively low overall abundance (<2%), these bands are thought to be of myofibrillar origin. They are likely soluble breakdown products of myofibrillar proteins from aging-related proteolysis that took place in the muscle samples over the 7 d of storage. This is supported by the observation that these bands were

<table>
<thead>
<tr>
<th>Protein band3 (kDa)</th>
<th>Dark</th>
<th>Light</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>225</td>
<td>1.34</td>
<td>0.73</td>
<td>0.13</td>
<td>0.0024</td>
</tr>
<tr>
<td>165</td>
<td>1.97</td>
<td>1.20</td>
<td>0.13</td>
<td>0.0003</td>
</tr>
<tr>
<td>90 (GP)</td>
<td>8.85</td>
<td>6.09</td>
<td>0.38</td>
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</tr>
<tr>
<td>71</td>
<td>2.24</td>
<td>1.77</td>
<td>0.15</td>
<td>0.0313</td>
</tr>
<tr>
<td>67</td>
<td>2.34</td>
<td>2.32</td>
<td>0.19</td>
<td>0.9276</td>
</tr>
<tr>
<td>61 (PGM)</td>
<td>2.84</td>
<td>3.33</td>
<td>0.23</td>
<td>0.1556</td>
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<tr>
<td>60 (PK)</td>
<td>11.35</td>
<td>11.53</td>
<td>0.42</td>
<td>0.7733</td>
</tr>
<tr>
<td>58 (PGI)</td>
<td>6.33</td>
<td>6.50</td>
<td>0.40</td>
<td>0.6636</td>
</tr>
<tr>
<td>47 (EN)</td>
<td>12.10</td>
<td>13.47</td>
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<td>0.0002</td>
</tr>
<tr>
<td>43 (CK)</td>
<td>8.05</td>
<td>9.19</td>
<td>0.27</td>
<td>0.0063</td>
</tr>
<tr>
<td>39 (ALD)</td>
<td>8.89</td>
<td>10.02</td>
<td>0.36</td>
<td>0.0381</td>
</tr>
<tr>
<td>36 (GAPDH)</td>
<td>11.59</td>
<td>11.90</td>
<td>0.49</td>
<td>0.6798</td>
</tr>
<tr>
<td>34 (LDH)</td>
<td>4.91</td>
<td>5.34</td>
<td>0.31</td>
<td>0.3550</td>
</tr>
<tr>
<td>25 (PGAM)</td>
<td>5.96</td>
<td>5.77</td>
<td>0.22</td>
<td>0.5644</td>
</tr>
<tr>
<td>23 (TPI)</td>
<td>5.44</td>
<td>5.74</td>
<td>0.27</td>
<td>0.4596</td>
</tr>
<tr>
<td>21</td>
<td>2.58</td>
<td>2.15</td>
<td>0.23</td>
<td>0.2181</td>
</tr>
</tbody>
</table>

a,bMeans within a row lacking a common superscript differ (P < 0.05).

1Data expressed as individual protein band abundance as a percentage of total protein abundance in the entire lane.

n = 24.

3GP, glycogen phosphorylase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphogluucose isomerase; EN, enolase; CK, creatine kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase.
less prominent or absent in the sarcoplasmic proteins extracted from the muscles at 24 h postmortem (Figure 1). Similarly, several past studies have reported that high molecular weight bands of myofibrillar origin accumulate with aging in the centrifugal drip fluid of intact beef and pork (Pospiech et al., 2000; Kolczak et al., 2003) and in sarcoplasmic extracts from ground beef (Xiong and Anglemier, 1989). The accumulation of more myofibrillar degradation products in muscle exudates from dark fillets than white fillets is consistent with the theory that increased protein degradation with postmortem storage leads to improved WHC (Huff-Lonergan and Lonergan, 2005).

In addition to the protein profiles of the exudates being different between light and dark fillets, the amount of protein in the exudates also varied between the 2 groups. Protein concentration (mg/mL) of the muscle exudate in dark fillets was greater than light fillets (Table 3). Due to the increased drip loss of light fillets, however, the total amount of protein lost (mg of protein per 100 g of muscle) due to drip was greater in light than in dark fillets after 2 and 7 d of storage. In the current study protein concentration of muscle exudate was negatively correlated to drip loss after 2 and 7 d of storage, which supports previous research on drip protein content in pork muscle (Penny, 1977; Savage et al., 1990). One possible explanation for the inverse relationship between the amount of exudate and the protein concentration of the exudate is the effect of dilution. With a dilution effect, as more water is released from the tissue and the drip loss increases, the concentration of the protein in the exudate diminishes. If the differences in the protein concentration of the exudate were due to dilution effects, then the SDS-PAGE protein profiles of the light and dark fillets would have been similar due to the fact that exudates from both groups were adjusted to equivalent protein concentrations for SDS-PAGE analysis. As previously discussed, the protein profiles of the exudates from light and dark fillets varied, indicating that differences were not explained simply by a dilution effect. Thus, differences in the protein profiles of the exudate of dark and light fillets suggest that protein denaturation may play a role in the inverse relationship between the amount of exudate and the protein concentration of the exudate in poultry meat.

Protein solubility is often used as a measure of protein denaturation. In this study, sarcoplasmic and total protein solubility did not differ between light and dark fillets (Table 3). This would suggest that differences in the degree of protein denaturation did not influence quality in this study. In a study comparing normal and pale broiler breast fillets, Van Laack et al. (2000) observed that both sarcoplasmic and total protein solubility were lower in pale fillets. Similar observations have been made in pork longissimus muscle (Warner et al., 1997), and this is consistent with the idea that light color and poor WHC are closely associated with excessive protein denaturation. Comparing the current results to Van Laack et al. (2000), it was noted that total protein solubility was comparable between the studies (~195 vs. ~215 mg of protein/g of tissue) but that sarcoplasmic solubility was much higher in the current study (~84 vs. 47 mg of protein/g of tissue). This was likely due to the fact that muscle samples were frozen before solubility assays in the current study, but not in Van Laack et al. (2000). This is supported by data showing that freezing of turkey muscles results in greater sarcoplasmic protein solubility measurements (Chan et al., 2011). Regardless of this difference, however, neither study observed a strong relationship between total protein solubility and measures of WHC. Thus, similar to Van Laack et al. (2000), data from the current study suggest that myofibrillar protein denaturation in poultry does not play as big of a role in the low WHC of pale meat as it does in pork.

Table 4 shows the correlations between meat quality traits and the protein profiles of the muscle exudates. Although significant correlations are not always indicative of a cause and effect relationship, most of the meat quality traits in this study were related to the relative abundance of multiple bands within the protein profile of the muscle exudates. Muscle pH values at 4 and 24 h postmortem were related to 10 and 8 protein bands, respectively. Interestingly, the amount of drip loss after 2 d of storage was correlated to the relative abundance of 7 protein bands, whereas drip loss after 7 d was correlated to the abundance of only 2 bands. This suggests that the total amount of drip loss likely reached its maximum and leveled off between 2 and 7 d of storage. Salt-induced water uptake was correlated to 3 protein

### Table 3. Biochemical traits of dark and light breast fillets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Dark</th>
<th>Light</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarc solubility</td>
<td>85.9</td>
<td>83.7</td>
<td>2.2</td>
<td>0.4856</td>
</tr>
<tr>
<td>Total solubility</td>
<td>195.8</td>
<td>194.5</td>
<td>4.4</td>
<td>0.8379</td>
</tr>
<tr>
<td>Drip protein concentration (mg/mL)</td>
<td>192.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total amount of protein lost&lt;sup&gt;1&lt;/sup&gt; (mg of protein per 100 g of muscle)</td>
<td>112.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>362.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>7 d storage</td>
<td>357.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>722.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row lacking a common superscript differ (P < 0.05).
<sup>1</sup>n = 24.
<sup>2</sup>Sarcoplastic protein solubility (Sarc) and total protein solubility are expressed as milligrams of protein per gram of tissue.
<sup>3</sup>Total amount of protein lost during drip collection for 2 and 7 d. Calculated based on drip loss percentage and the protein concentration of the drip.
Table 4. Correlation coefficients (r) between meat quality traits and protein profiles of muscle exudates in broiler breast fillets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Drip loss</th>
<th>Water uptake</th>
<th>Cook loss</th>
<th>pH 4h</th>
<th>pH 24h</th>
<th>Sarc2</th>
<th>Total3</th>
<th>Drip loss 2d</th>
<th>Drip loss 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drip protein</td>
<td>0.37*</td>
<td>0.22</td>
<td>0.38</td>
<td>0.57***</td>
<td>0.55***</td>
<td>0.32*</td>
<td>0.09</td>
<td>0.46**</td>
<td>0.57***</td>
</tr>
<tr>
<td>225 kDa</td>
<td>0.27</td>
<td>0.30*</td>
<td>0.30</td>
<td>0.55***</td>
<td>0.56***</td>
<td>0.04</td>
<td>0.06</td>
<td>0.38*</td>
<td>0.36*</td>
</tr>
<tr>
<td>165 kDa</td>
<td>0.59***</td>
<td>0.32*</td>
<td>0.27</td>
<td>0.30</td>
<td>0.04</td>
<td>0.25</td>
<td>0.23</td>
<td>0.55***</td>
<td>0.59***</td>
</tr>
<tr>
<td>90 kDa (GP)</td>
<td>0.60***</td>
<td>0.16</td>
<td>0.48**</td>
<td>0.38*</td>
<td>0.35*</td>
<td>0.32*</td>
<td>0.24</td>
<td>0.30</td>
<td>0.38*</td>
</tr>
<tr>
<td>71 kDa</td>
<td>0.34*</td>
<td>0.26</td>
<td>0.18</td>
<td>0.32</td>
<td>0.22</td>
<td>0.19</td>
<td>0.04</td>
<td>0.28</td>
<td>0.10</td>
</tr>
<tr>
<td>67 kDa</td>
<td>0.22</td>
<td>0.04</td>
<td>0.09</td>
<td>0.06</td>
<td>0.14</td>
<td>0.34*</td>
<td>0.07</td>
<td>0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>61 kDa (PGM)</td>
<td>0.29</td>
<td>0.37*</td>
<td>0.18</td>
<td>0.33*</td>
<td>0.27</td>
<td>0.30</td>
<td>0.41*</td>
<td>0.48**</td>
<td>0.15</td>
</tr>
<tr>
<td>60 kDa (PK)</td>
<td>0.22</td>
<td>0.29</td>
<td>0.08</td>
<td>0.38*</td>
<td>0.10</td>
<td>0.04</td>
<td>0.26</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>58 kDa (PGI)</td>
<td>0.27</td>
<td>0.21</td>
<td>0.27</td>
<td>0.11</td>
<td>0.07</td>
<td>0.03</td>
<td>0.12</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>47 kDa (EN)</td>
<td>0.57***</td>
<td>0.37*</td>
<td>0.43**</td>
<td>0.37*</td>
<td>0.51***</td>
<td>0.47**</td>
<td>0.33*</td>
<td>0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>43 kDa (CK)</td>
<td>0.68***</td>
<td>0.05</td>
<td>0.44**</td>
<td>0.03</td>
<td>0.28</td>
<td>0.19</td>
<td>0.04</td>
<td>0.10</td>
<td>0.27</td>
</tr>
<tr>
<td>39 kDa (ALD)</td>
<td>0.60***</td>
<td>0.34*</td>
<td>0.24</td>
<td>0.34*</td>
<td>0.04</td>
<td>0.05</td>
<td>0.21</td>
<td>0.37*</td>
<td>0.48**</td>
</tr>
<tr>
<td>36 kDa (GAPDH)</td>
<td>0.43**</td>
<td>0.51***</td>
<td>0.03</td>
<td>0.54***</td>
<td>0.20</td>
<td>0.17</td>
<td>0.36*</td>
<td>0.47**</td>
<td>0.63***</td>
</tr>
<tr>
<td>34 kDa (LDH)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.21</td>
<td>0.04</td>
<td>0.08</td>
<td>0.04</td>
<td>0.12</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>25 kDa (PGAM)</td>
<td>0.53***</td>
<td>0.39*</td>
<td>0.21</td>
<td>0.36*</td>
<td>0.01</td>
<td>0.12</td>
<td>0.39*</td>
<td>0.48**</td>
<td>0.08</td>
</tr>
<tr>
<td>23 kDa (TPI)</td>
<td>0.52***</td>
<td>0.43**</td>
<td>0.12</td>
<td>0.41*</td>
<td>0.06</td>
<td>0.18</td>
<td>0.42**</td>
<td>0.55***</td>
<td>0.58***</td>
</tr>
<tr>
<td>21 kDa</td>
<td>0.60***</td>
<td>0.30</td>
<td>0.26</td>
<td>0.26</td>
<td>0.03</td>
<td>0.04</td>
<td>0.31</td>
<td>0.36*</td>
<td>0.56***</td>
</tr>
</tbody>
</table>
| GP, glycogen phosphorylase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase.

In conclusion, data from this study support the hypothesis that protein differences in exudates from broiler breast fillets are related to meat quality attributes. Muscle exudates from breast fillets were found to contain primarily sarcoplasmic proteins. Due to their biological functions, it is unlikely that variations in sarcoplasmic proteins directly influence the integrity of the muscle structure to affect quality. However, the differences observed in the exudates from light and dark fillets and the correlations with meat quality traits in this study suggest that protein characteristics of muscle exudates may serve as good indicators of underlying muscle changes that have a direct impact on muscle ultrastructure and meat quality. To fully decipher the denaturation and proteolytic alterations that occur in sarcoplasmic proteins, further research is needed to confirm the identities and origins of the proteins involved. Establishing such relationships will provide further insight into the possible mechanistic link between sarcoplasmic protein profile changes and meat quality. The aqueous nature of muscle exudate and the observations made in this study suggest that muscle exudates might be a potential source for protein markers that can be used in the development of rapid, noninvasive techniques for measuring and predicting poultry meat quality. Additional research, however, is needed to identify proteins changes within muscle exudates, to determine the time postmortem at which they occur, and to establish the strength of these relationships and predictive capabilities of these protein changes across a broad spectrum of breast fillets.

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