

Effect of spaghetti meat abnormality on broiler chicken breast meat composition and technological quality¹

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ABSTRACT The effects of spaghetti meat (SM) myopathy and sampling location on chicken breast meat physical traits, composition, and protein functionality were investigated using 30 normal (N) and 30 SM boneless fillets. Weight, drip loss, pH, and color traits were determined on intact fillets. Proximate composition, water holding capacity, mineral profile, SDS-PAGE, myofibrillar, and sarcoplasmic protein solubility, and emulsifying properties were assessed on both the superficial (S) and deep (D) layers of the breasts. SM fillets were heavier ($P < 0.0001$) and exhibited greater drip loss ($P = 0.0131$) and higher b^* index on the skin side of the muscle ($P < 0.0001$). Muscle condition by layer interaction effect revealed that the superficial portion of SM fillets (SM-S) exhibited the highest moisture ($P = 0.0003$) and fat contents ($P = 0.0011$) coupled with the lowest protein ($P < 0.0001$) and ash contents ($P = 0.0458$). Total and soluble collagen amounts were higher in N-S and SM-S groups compared with N-D and SM-D ($P < 0.0001$).

SM-S group exhibited the highest calcium ($P = 0.0035$) and sodium ($P < 0.0001$) levels. Overall, the myopathy had only minor impacts on protein profiles, while the muscle layer exerted a more remarkable effect. SM fillets exhibited higher pH but a lower myofibrillar protein solubility ($P < 0.0001$). Salt-induced water uptake, cooking loss, and final yield values suggested a potential impairment of water-holding capacity in SM-affected meat. Sarcoplasmic and myofibrillar emulsion activity indexes were similar between the 2 muscle conditions, but the stability of the emulsions was lower in SM meat. Overall, significant layer and muscle condition by layer effects were not observed in the functional properties of the breast meat. SM exerted a profound and negative impact on breast meat composition that led to detrimental consequences on functionality traits. Given the fundamental role of protein quality for meat processing, these data suggest that a further step toward the understanding of this myopathy should be the investigation of intrinsic protein characteristics.

Key words: chicken breast, spaghetti meat, proximate composition, protein functionality

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INTRODUCTION

Spaghetti meat (SM) is an emerging muscular abnormality affecting broiler chicken *Pectoralis major* muscle, whose onset and morphological characteristics were first described by Bilgili in 2015. Distinguishing macroscopic traits of affected breasts include the overall impaired muscle integrity and a stringy, soft consistency of the

ventral-cranial portion of the *Pectoralis major* because of the poor cohesion of the muscular fiber bundles. The altered histological features, which are primarily observed on the superficial layer of the fillet, partly overlap those of white striping and wooden breast, respectively, described by Kuttappan et al. (2013) and Sihvo et al. (2014); extensive myodegeneration coupled with regeneration, loss of the normal tissue architecture, necrosis and lysis of fibers, adipose tissue infiltration, inflammatory cells infiltration, and edema. However, a peculiar trait of SM not detected in other emerging myopathies is a progressive rarefaction of the endomysial and perimysial connective tissue that compromises the fiber bundles cohesion, coupled with a loose connective tissue deposition (Baldi et al., 2018). Accordingly, Bilgili (2015) speculated that the impaired muscle structural integrity was ascribable to the immaturity of this newly deposited collagen. As the visual acceptability of affected meat is compromised, processors either discard

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or use the meat to manufacture further-processed products rather than using breast fillets in fresh retail (Petracci et al., 2019; Soglia et al., 2019). Therefore, it is crucial to understand the impact of this myopathy on breast meat quality and the suitability of SM fillets to be included in the formulation of meat products. To this respect, the first studies available (Baldi et al. 2018; 2019) indicated poor meat quality traits in affected fillets, with the impairment observed mainly on the superficial portion of the muscle. Given the limited data available on SM and the importance of this issue for the poultry meat sector, the objective of the present study was to investigate in greater detail the effects of the SM myopathy on chicken breast meat composition and functionality attributes. Thus, aspects not yet evaluated were assessed (mineral profile, salt-induced water uptake, cook loss, final yield, myofibrillar and sarcoplasmic protein solubility, and emulsifying properties) to provide additional insights into breast fillet properties that may influence quality and processing traits. The effect of measurement location was also considered, as SM lesions distribution displayed intrafillet variations (Baldi et al., 2018).

MATERIALS AND METHODS

Sample Collection and Preparation

A total of 60 skinless boneless breast fillets (*Pectoralis major*) were obtained from a commercial broiler processing plant during 2 sampling repetitions; each time, 15 spaghetti meat (SM) affected and 15 unaffected (normal, N) samples were collected 3 h postmortem, packed in resealable bags on ice and transported to the U.S. National Poultry Research Center (Athens, GA). The selected SM samples exhibited a mushy and stringy consistency, because of muscle fiber bundle separation, especially in the ventral-cranial portion of the fillets (Bilgili, 2015; Baldi et al., 2018). Breasts exhibiting the white striping and/or wooden breast conditions were excluded from selection in this study. At 6 to 8 h postmortem, fillets were trimmed, weighed, and $L^*a^*b^*$ color values (CIE 1976) were measured on the cranial, medial, and caudal portions of both skin and bone sides of the *Pectoralis major* muscles with a Konica Minolta CM-700d spectrophotometer (Konica Minolta, Ramsey, NJ). The color values reported for each side represent an average of the 3 measurements. To quantify the drip loss according to the method of Honikel (1998), all intact fillets were suspended in sealable, water-impermeable plastic bags, stored overnight at 4°C, and reweighed at 24 h postmortem. After drip loss determination, ultimate pH was measured in the superficial and deep layers of the cranial end of the *Pectoralis major* muscles with a Hach H160 portable pH-meter (Loveland, CO) equipped with a spear-tipped probe and calibrated through the two-point calibration method. Thereafter, the caudal portions of the fillets were removed, and the cranial-middle portions were horizontally split into superficial (S) and deep (D) layers of

equal thickness. All 4 types of samples (N-S, N-D, SM-S, and SM-D) from each fillet were then individually chopped in a food processor.

Breast Meat Composition

Proximate Composition Five samples per group per repetition were used for proximate composition and mineral profile analysis. Moisture and fat were determined in duplicate on fresh samples at 24 h postmortem using a rapid microwave moisture analyzer and a NMR fat analyzer (CEM Smart ProFat 6 and Oracle systems, CEM Corporation, Matthews, NC). The remaining portions of the chopped samples were then frozen at -80°C, freeze-dried, and utilized for total ash quantification (AOAC 1990) and mineral profile analysis. Protein content was calculated by difference, based on the proportion of moisture, fat, and total ash in the sample.

Mineral Profile For each sample, pellets (60 mg) were prepared from lyophilized powder using a 13.0 mm diameter stainless-steel die (Pike Technologies, Madison, WI) and compressing the pellets to 10 mPa using a laboratory press (Columbia International, Irmo, SC). The resultant pellets were analyzed for Na, K, Ca, and Mg contents through the laser-induced breakdown spectroscopy, using a RT100-HP laser-induced breakdown spectrometer (Applied Spectra, Fremont, CA). Plasma was induced using a Nd:YAG laser with <5 ms pulses at the fourth harmonic (266 nm) and a repetition rate of 2 Hz. Pulse energy was 24 mJ focused onto a 100 µm spot size. A gate delay of 0.2 µs was followed by acquisition of the emitted light in a 1.0 ms window, allowing for minimization of background continuum radiation, while retaining suitable signal from atomic emission lines. Emitted light was collected with a Czerny-Turner monochromator coupled with an intensified charge-coupled detector, allowing simultaneous detection of all atomic emission lines. Spectral acquisition was performed in an air atmosphere. Each sample spectrum was averaged over 50 shots and obtained by moving the sample in a raster pattern that was 1 mm × 1 mm. A total of 10 spectra were obtained from different locations on each pellet. All spectra were normalized to the C line at 247.9 nm, and the resultant peak heights of Mg (279.6 nm), Ca (422.7 nm), Na (589.0 nm), and K (766.5 nm) were obtained.

Collagen Content Five samples per group per repetition were frozen in liquid nitrogen and ground into powder for the determination of soluble and insoluble collagen content according to the procedure described by Hill (1966) with modifications. Quadruplicate 3 g meat samples were added to 16 mL of ¼ strength Ringers solution, heated at 77°C for 70 min in a water bath, centrifuged 10 min at room temperature at 3,600 rpm, and decanted through Whatman 09-795 paper filter. For each sample, 2 aliquots were designated for soluble collagen determination: residues were added to 8 mL of ¼ strength Ringers solution, centrifuged at the same conditions, and added to 25 mL of concentrated HCl.

For the insoluble collagen determination, meat residues were added to 25 mL of 6 N HCl. Both soluble and insoluble sample solutions were autoclaved for 18 h at 121°C, 18–20 psi, their pH adjusted to 6.00, diluted respectively to 250 and 500 mL and filtered through Whatman 09-795 paper filter. Spectrophotometric determination of collagen was carried out according to the procedures of Bergman and Loxley (1963) and Cross et al. (1973). Thus, 2 mL of isopropanol, 1 mL of an oxidant solution (7% w/v chloramine T, 1 volume, and acetate/citrate buffer, pH 6.0, 3 volumes), and 4 mL of Ehrlich's reagent were pipetted to each sample. Tubes were incubated at 60°C for 25 min and cooled. Spectrophotometric determination of the hydroxyproline content was carried out at 558 nm using a Jasco V-630 spectrophotometer (Jasco, Easton, MD); the following equations were used to convert μg of hydroxyproline/mL of solution to mg of collagen/g of meat:

$$\begin{aligned} & [(\mu\text{g} / \text{ml}) \times \text{dilution factor} \times \text{constant}] / \text{sample wt} \\ & \times 1000 = \text{mg collagen} / \text{g of meat} \end{aligned}$$

With 250 and 500 used as dilution factors and 7.52 and 7.25 used as constants for soluble and insoluble collagen, respectively. Total soluble was reported as the sum of the soluble and insoluble collagen contents.

Protein Composition and Functionality

Five samples per group per repetition were utilized to determine muscle protein profiles and functionality attributes. SDS-PAGE, solubility, and emulsifying properties were determined on meat stored under vacuum at -20°C , while water holding capacity traits (salt-induced water uptake, cook loss, and final yield) were assessed on fresh meat.

Protein Isolation and SDS-PAGE Analysis

Sarcoplasmic and myofibrillar protein fractions were isolated from 2 g of chopped meat according to the procedures of Pietrzak et al. (1997). A biuret assay was performed to determine the protein concentration of the 2 fractions. Samples were diluted to 2 mg/mL in sample buffer (8 mol urea, 2 mol thiourea, 3% SDS (wt/vol), 75 mmol DTT, 25 mmol Tris-HCl (pH 6.8), 0.004% bromophenol blue) and denatured for 3 min in boiling water. Denatured protein samples (15 μg protein/lane) and a broad range molecular weight standard (5 to 250 kDa, Thermo Scientific PageRuler Broad Range Unstained Protein Ladders, Waltham, MA) were loaded onto Novex precast 4–20% tris-glycine polyacrylamide gels (Life Technologies Corp., Carlsbad, CA) and ran at 4°C at a constant voltage. Gradient gels (4–20%) were utilized to allow for a broader range of proteins to be analyzed, and equal protein loads ensured that differences were because of actual variations in the protein profiles. Gels were then stained (Coomassie brilliant blue R-250) and destained. The densities of 13 sarcoplasmic and 17 myofibrillar protein bands were quantified by Alpha View software (v 3.4, ProteinSimple Inc., Santa Clara, CA); the relative abundance of each individual

protein band was expressed as a percentage of the total protein abundance of all bands in the lane.

Water Holding Capacity Traits Salt-induced water uptake and pellet cook loss were determined at 48 h post-mortem on triplicate 5 g samples of freshly minced meat, according to the procedures of Bowker and Zhuang (2016). Meat was weighed into 50 mL centrifuge tubes along with 7.5 mL of cold 0.6 mol NaCl buffer. Tubes were vortexed for 30 s, stored at 4°C for 15 min, and then centrifuged at $3,000 \times g$ for 15 min at 4°C. The resulting supernatant was discarded, and salt-induced water uptake was computed according to the formula:

$$\begin{aligned} & [100 \times (\text{swollen pellet wt} - \text{initial meat sample wt}) \\ & / \text{initial meat sample wt}] \end{aligned}$$

Subsequently, tubes were put in an 80°C water bath for 20 min. Liquid was then removed, and cook loss and final yield were determined according to the following formulas, respectively:

$$\begin{aligned} & [100 \times (\text{swollen pellet wt} - \text{cooked pellet wt}) \\ & / \text{swollen pellet wt}] \end{aligned}$$

$$[100 \times (\text{cooked pellet wt} / \text{initial meat sample wt})]$$

Protein Solubility Protein solubility was measured following the procedures of Bowker and Zhuang (2016). For sarcoplasmic protein solubility, triplicate 1 g meat samples were added with 10 mL of cold 25 mmol potassium phosphate buffer (pH 7.2) and homogenized for 10 s at 18,000 rpm with a VWR AHS 250 homogenizer (VWR International, Radnor, PA). Tubes were then vortexed and centrifuged at $2,600 \times g$ for 30 min at 4°C. The extract was filtered through 4 layers of cheesecloth, and its protein concentration was measured using a biuret assay with a BSA standard curve. Using a similar procedure, total protein solubility was measured with 0.55 mol KI, 0.05 mol potassium phosphate buffer (pH 7.2). Myofibrillar solubility was calculated as the difference between total and sarcoplasmic protein solubility.

Emulsion Activity Index and Emulsion Stability Index

Sarcoplasmic and myofibrillar emulsion activity index (EAI) and emulsion stability index (ESI) were assessed on 2 g of meat per sample, according to the method of Chan et al. (2011) as modified Bowker and Zhuang (2016). Meat was homogenized 3×30 s at 18,000 rpm in 40 mL of cold 25 mmol potassium phosphate buffer (pH 7.2) and centrifuged at $15,250 \times g$ for 15 min at 4°C. The supernatant was filtered through Whatman no. 1 filter paper and collected as the sarcoplasmic protein fraction. The remaining pellet was homogenized in 40 mL of 0.55 mol KI, 0.05 mol potassium phosphate buffer (pH 7.2), centrifuged, and filtered at the same conditions previously described to obtain the myofibrillar proteins fraction. Protein concentrations were measured with a biuret assay using a BSA standard

curve. Aliquots of the extracts were adjusted to 2.0 mg/mL with their respective buffers to a final volume of 3.6 mL. The obtained solutions were mixed with corn oil in a 3:1 volume ratio and homogenized at 14,000 rpm for 1 min. Immediately, triplicate emulsion aliquots of 35 μ L were diluted to 3.5 mL in 0.1% SDS buffer; emulsion turbidity absorbance was read immediately after preparation (A_0) and after 10 min (A_{10}) at 500 nm on a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific). EAI and ESI were then calculated according to the equations of Selmane et al. (2008):

$$EAI = 2.33 \times A_0$$

$$ESI = 10 \times [A_0 / (A_0 - A_{10})]$$

Statistical Analysis

Statistical analysis was carried out using a mixed model (PROC MIXED) of SAS (version 9.3, SAS Institute Inc., Cary, NC). A one-way ANOVA was used to evaluate the effects of muscle condition (M: N and SM) on weight, color, and drip loss, with sampling repetition included as a random effect. A two-way ANOVA was used to analyze pH, composition, and protein functionality traits. Muscle condition, layer (L: S and D) and their interaction $M \times L$ were included as fixed effects and sampling repetition and breast sample included as random effects. SDS-PAGE data were analyzed using the same two-way ANOVA mixed model, with the inclusion of gel among the random effects. Means were separated using Bonferroni adjustments; $P < 0.05$ was assigned as significance level.

RESULTS AND DISCUSSION

Physical Traits

Breast weight, drip loss percentage, and color traits are reported in Table 1. In accordance with the previous study of Baldi et al. (2018), fillets affected by the SM myopathy were heavier ($P < 0.0001$) than unaffected fillets. Affected fillets displayed a greater drip loss

Table 1. Breast weight, drip loss, fillet skin, and bone sides color traits according to muscle condition (M - normal, N vs. spaghetti meat, SM).

Traits	Muscle condition (M)		SE	P-value
	N	SM		
Weight, g	483 ^b	564 ^a	16	<0.0001
Drip loss, %	1.22 ^b	1.44 ^a	0.13	0.0131
L* skin side	61.6	61.3	1.3	0.7459
L* bone side	58.8	59.5	1.0	0.3464
a* skin side	-1.18	-0.92	0.43	0.1157
a* bone side	-0.85	-0.80	0.11	0.7365
b* skin side	10.2 ^b	12.9 ^a	2.0	<0.0001
b* bone side	10.9	11.7	0.4	0.0668

^{a,b}Means within the same row followed by different superscripts differ $P < 0.05$.

($P = 0.0131$) than normal ones. This phenomenon could be attributed to the compromised structural integrity of the muscle tissue observed with the SM condition, as well as to the edema of the interstitium associated with the other histological lesions. The results obtained for color traits indicated that neither breast lightness (L^*) nor redness (a^*) were impacted by the pathological condition. Differently, the b^* values measured on the skin side of SM fillets were higher than those detected on the corresponding side of the N fillets ($P < 0.0001$). In addition, even though the discrepancies were not significant between the 2 muscle conditions, the bone surface of affected fillets was tendentially more yellow than its unaffected counterpart ($P = 0.0668$). Increased yellowness corroborates the histological observation of Baldi et al. (2018), who found adipose tissue accumulation among muscle fibers occurring in SM insulted fillets. As fat possess a yellowish color, it might have directly contributed to the increased b^* values (Dalle Zotte et al., 2017).

Breast Meat Composition

The effects of the SM myopathy on breast meat composition (Table 2) were not uniform throughout the *Pectoralis major* muscle. Significant muscle condition by layer interaction effects were observed for moisture, protein, fat, and ash content. Consistent with the macroscopic characteristics of the fillets, the effects of the SM on muscle composition were more profound in the superficial portion of the muscle. In normal fillets, the proximate composition was similar between the superficial and deep layers of the muscle. In SM fillets, however, the superficial portion of the muscle was significantly different than the deep portion. Proximate composition was similar between the deep layers of normal and SM fillets. However, the superficial portion of SM fillets exhibited greater moisture ($P = 0.0003$) and fat contents ($P = 0.0011$) and lower protein ($P < 0.0001$) and ash contents ($P = 0.0458$) than the superficial portions of normal fillets.

This impairment in meat composition is consistent with histological traits, such as tissue myodegeneration, necrosis and lysis of cells, poor fibers uniformity, and fat deposition, observed in SM fillets, which are similar to those reported for white striping (Kuttappan et al., 2013; Ferreira et al., 2014; Radaelli et al., 2017; Baldi et al., 2018) and wooden breast (Sihvo et al., 2014, 2017; de Brot et al., 2016; Soglia et al., 2016a; Radaelli et al., 2017). The reduced protein content in SM fillets may potentially be because of the greater myofibrillar protein degradation that is observed with the structural breakdown of muscle fibers resulting from tissue degenerative processes (Harris et al., 2003) and increased enzyme efflux because of a compromised sarcolemma integrity (Mitchell, 1999; Sandercock and Mitchell, 2003). The greater moisture content detected in SM samples might reflect a reduction of the myowater fraction retained within the myofibrillar matrix. This phenomenon, ascribable to changes in muscle microstructure and structural proteins intrinsic

Table 2. Breast meat composition according to muscle condition (M - normal, N; spaghetti meat, SM) and muscle layer (L - superficial, S; deep, D).

Traits	Normal		SM		SE	P-value		
	S	D	S	D		M	L	M × L
Moisture, %	75.6 ^b	75.6 ^b	76.9 ^a	76.0 ^b	0.2	0.0172	0.0002	0.0003
Protein, %	22.5 ^a	22.2 ^a	20.3 ^b	21.6 ^a	0.3	0.0002	<0.0001	<0.0001
Fat, %	1.20 ^b	1.01 ^b	1.80 ^a	1.22 ^b	0.28	0.0679	<0.0001	0.0011
Ash, %	1.13 ^a	1.16 ^a	1.01 ^b	1.13 ^a	0.06	0.0650	0.0004	0.0458
Total collagen ¹	5.10 ^a	4.42 ^b	5.15 ^a	4.31 ^b	0.20	0.8944	<0.0001	0.4809
Soluble collagen ¹	1.39 ^a	1.14 ^{b,c}	1.29 ^{a,b}	1.04 ^c	0.10	0.2735	<0.0001	0.9710

^{a,b,c}Means within the same row followed by different superscripts differ $P < 0.05$.

¹mg/g of muscle tissue.

features (Bertram et al., 2003), was observed in SM (Baldi et al., 2018) and wooden breast fillets through low-field nuclear magnetic resonance T₂ relaxation analyses (Soglia et al., 2016b; Tasoniero et al., 2017).

Table 2 also displays the total and soluble collagen content in normal and SM fillets. Interestingly, the lack of significance observed for the muscle condition effect indicated that SM and normal breast muscles possessed similar amounts of total and soluble collagen. Muscle layer, on the contrary, had a remarkable effect on these traits. The superficial portion of both normal and SM fillets displayed greater levels of total (S = 5.13 mg vs. D = 4.36 mg; $P < 0.0001$) and soluble collagen (S = 1.34 mg vs. D = 1.09 mg; $P < 0.0001$) than their deep counterparts. Despite the endomysial and perimysial connective tissue rarefaction that has been histologically observed in SM fillets (Baldi et al., 2018), neither the current nor previous data (Baldi et al., 2018, 2019) found differences in total collagen with the SM myopathy. Additionally, it emerged that soluble collagen content did not differ between affected and unaffected fillets. This finding seems to be in contrast with a lower content of hydroxylysylpyridinoline, a non-reducible cross-link of muscular collagen, observed in the superficial portion of SM breasts by Baldi et al. (2019).

Overall, the muscle composition data in the current study confirms the previous findings of Baldi et al. (2018; 2019), who also indicated an altered proximate composition in SM fillets, as well as a strong sampling position effect. Interestingly, the current results seem to suggest that even breast fillets that do not exhibit the SM condition also possess uneven collagen distribution throughout the muscle.

Mineral Profile

The emission line intensities of magnesium, calcium, sodium, and potassium were discriminated based on their relative proportions and their peak height values were reported in Table 3. Overall mineral profile differences because of the SM myopathy were more pronounced in the superficial portion of the muscle. Like the proximate composition, the Ca²⁺ ($P = 0.035$) and Na⁺ ($P < 0.0001$) levels in the tissue exhibited significant muscle condition by layer interaction effects. The greatest emission intensities for Ca²⁺ and Na⁺ were observed in the SM-S samples, whereas SM-D and the 2 normal groups exhibited similar intensities for these minerals. No differences were detected in Mg⁺ levels. A muscle layer effect was observed for K⁺ levels (S = 20.4 vs. D = 19.8; $P = 0.0272$). Sandercock and Mitchell (2003; 2004) and Sandercock et al. (2009) investigated the role of an altered cation regulation on the pathogenesis of skeletal muscle damage in broiler chickens. These authors hypothesized that a greater sodium level in the muscle was a triggering factor for the imbalanced Ca²⁺ intake into the muscle fibers, which in turn was responsible for the altered cell membrane integrity through the activation of Ca²⁺-dependent proteases and lipases. To date, SM etiology is still obscure, and the possible participation of defective cation regulation as a pathological mechanism needs further investigation. A connection between cation homeostasis disturbances and cell injury development has already been suggested for other emerging myopathies (Mutryn et al., 2015; Petracci et al., 2015; Soglia et al., 2016a; Tasoniero et al., 2017).

Table 3. Breast meat mineral profile according to muscle condition (M - normal, N; spaghetti meat, SM) and muscle layer (L - superficial, S; deep, D). Emission line intensities, based on peak height, of magnesium (Mg), calcium (Ca), sodium (Na), and potassium (K) lines normalized to the carbon emission line at 247.9 nm.

Traits	Normal		SM		SE	P-value		
	S	D	S	D		M	L	M × L
Mg (279.6 nm)	2.20	2.17	2.22	2.22	0.02	0.0997	0.5483	0.5258
Ca (422.7 nm)	1.61 ^b	1.61 ^b	1.74 ^a	1.55 ^b	0.03	0.2650	0.0027	0.0035
Na (589.0 nm)	16.9 ^b	16.6 ^b	19.2 ^a	17.0 ^b	0.24	<0.0001	<0.0001	<0.0001
K (766.5 nm)	20.3	19.8	20.5	19.9	0.27	0.4851	0.0272	0.7541

^{a,b}Means within the same row followed by different superscripts differ $P < 0.05$.

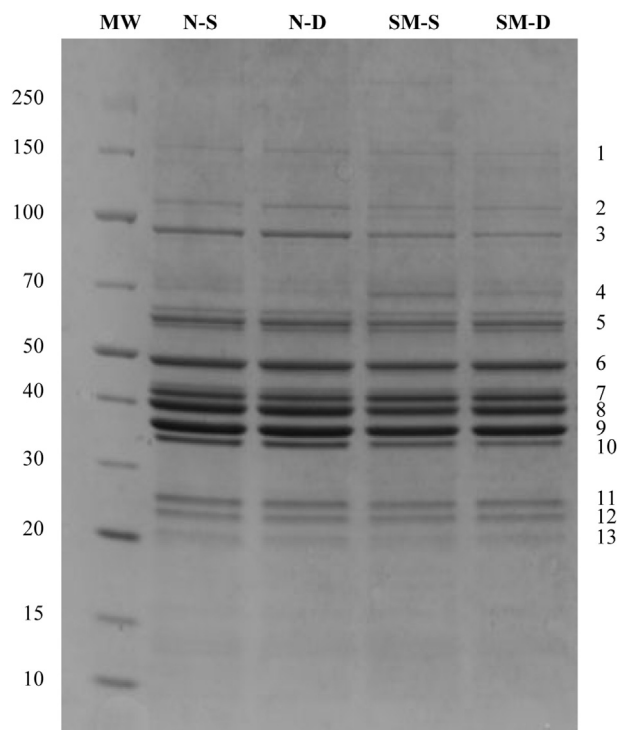


Figure 1. Representative SDS-PAGE protein profiles of the sarcoplasmic fractions from the superficial (S) and deep (D) layers of normal (N) and spaghetti meat (SM) broiler breast fillets. MW, molecular weight marker (kDa).

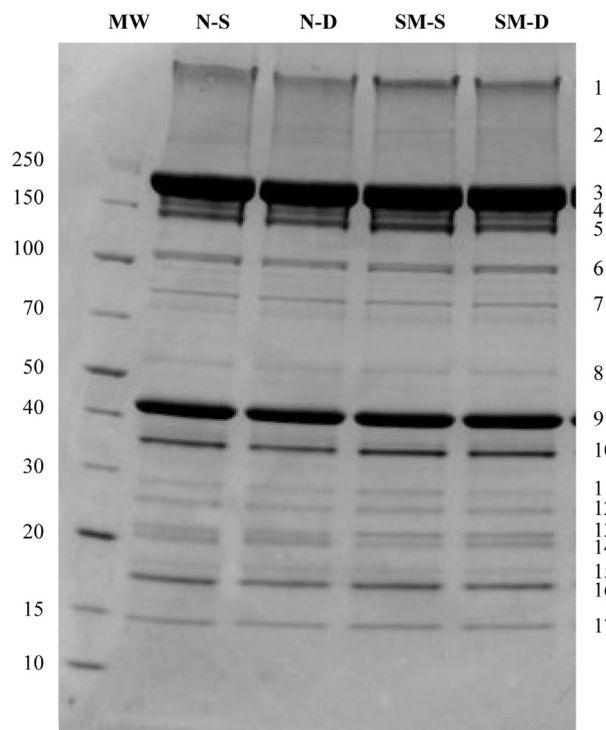


Figure 2. Representative SDS-PAGE protein profiles of the myofibrillar fractions from the superficial (S) and deep (D) layers of normal (N) and spaghetti meat (SM) broiler breast fillets. MW, molecular weight marker (kDa).

Electrophoretic Analysis

To evaluate the specific effects of the SM myopathy on muscle protein composition, sarcoplasmic and myofibrillar protein fractions were isolated, and the extracts were adjusted to equal concentrations and then analyzed using SDS-PAGE (Figures 1 and 2). While it is likely that bands represented multiple proteins or protein fragments of similar molecular weight, where possible the identity of the predominant protein within each band was assigned based on molecular weight, relative abundance, and comparisons to previous research on muscle protein profiles. Thirteen electrophoretic protein bands were quantified in the sarcoplasmic fraction, consisting primarily of glycolytic enzymes (Figure 1 and Table 4). Differences in the sarcoplasmic protein profiles were minor, which suggests that the SM myopathy does not cause a large shift in the metabolic profile of the muscle. Layer exerted a stronger influence on the sarcoplasmic protein profile than muscle condition (Table 4). The superficial portions of the fillets had a greater relative abundance of a band corresponding to 158 kDa ($P < 0.05$), while the deep portions exhibited a greater abundance of proteins corresponding to 110 kDa and 23 kDa ($P < 0.05$). It is possible that high molecular weight bands (>150 kDa) found in the sarcoplasmic protein fraction of the muscle are breakdown products of larger structural proteins indicating increased postmortem proteolysis. However, further work is needed to confirm this hypothesis. An unknown 67 kDa protein

band exhibited a significant ($P < 0.01$) muscle condition by layer interaction. The abundance of this protein was greater in the superficial layer than the deep layer in SM fillets (SM-S 1.78% vs. SM-D 1.26%, $P < 0.001$). In normal fillets, however, the abundance of the 67 kDa was similar between the superficial and deep layers (N-S 1.17% vs. N-D 1.00%, $P > 0.05$). Seventeen electrophoretic protein bands were quantified in the myofibrillar fraction, consisting primarily of contractile and cytoskeletal proteins (Figure 2 and Table 5). Like the sarcoplasmic protein fractions, differences in myofibrillar protein compositions were minor with muscle layer having a greater impact than the SM condition. In both normal and SM fillets, the protein band corresponding to nebulin was less abundant in the superficial layer ($P < 0.01$), whereas a 28–30 kDa band was more abundant in the superficial layer ($P < 0.001$). These observations could suggest that more extensive postmortem proteolysis occurs in the superficial layers of both normal and SM fillets than in the deep layers. The relative abundance of an 80 kDa protein band was greater in normal fillets compared with SM fillets. Significant interaction effects were observed for the bands corresponding to α -actinin and MLC-3. In SM fillets, the relative abundance of the α -actinin was less in the superficial layer compared with the deep layer (SM-S 2.32% vs. SM-D 2.63%, $P < 0.05$). However, in normal fillets the abundance of the α -actinin band was similar between the layers (N-S 2.62% vs. N-D 2.51%, $P > 0.05$). It is well established that the myodegeneration associated with white striping

Table 4. Relative abundance¹ of SDS-PAGE protein bands from the sarcoplasmic fraction of broiler breast filets according to muscle condition (M - normal, N; spaghetti meat, SM) and muscle layer (L - superficial, S; deep, D).

Band	Approximate molecular size and identification	Muscle condition (M)			Layer (L)			P-value		
		N	SM	SE	S	D	SE	M	L	M × L
1	158 kDa	1.13	1.02	0.20	1.14 ^a	1.01 ^b	0.20	0.1777	0.0345	0.5491
2	110 kDa	1.81	1.54	0.25	1.55 ^b	1.80 ^a	0.25	0.0757	0.0183	0.8865
3	90 kDa, GP	5.99	6.04	0.83	5.96	6.06	0.78	0.9306	0.6393	0.0917
4	67 kDa	1.06 ^b	1.52 ^a	0.42	1.45 ^a	1.13 ^b	0.41	0.0200	<0.0001	0.0045
5	58-61 kDa, PGM-PK-PGI	8.38	8.38	0.94	8.18	8.59	0.94	0.9965	0.0643	0.8766
6	47 kDa, EN	14.4	14.2	1.36	14.4	14.2	1.35	0.7205	0.3094	0.1384
7	43 kDa, CK-PGAK	12.2	12.3	1.2	12.2	12.3	1.1	0.9361	0.6493	0.2124
8	39 kDa, ALD	17.7	17.8	1.4	18.0	17.6	1.4	0.8060	0.0647	0.3937
9	36 kDa, GAPDH	20.0	20.6	1.8	20.4	20.1	1.8	0.1193	0.2635	0.6562
10	34 kDa, LDH	7.87	7.78	0.42	7.99	7.67	0.41	0.6834	0.0589	0.9620
11	25 kDa, PGAM	4.63	4.42	0.29	4.39	4.66	0.29	0.2663	0.0525	0.4573
12	23 kDa, TPI	3.40	3.24	0.23	3.13 ^b	3.51 ^a	0.21	0.4843	0.0132	0.4511
13	21 kDa	0.98	0.90	0.12	0.85	1.03	0.11	0.5486	0.0585	0.7058

^{a,b}Means within the same row and effect followed by different superscripts differ $P < 0.05$.

Abbreviations: ALD, aldolase; CK, creatine kinase; EN, enolase; GAPDH, glyceraldehyde phosphate dehydrogenase; GP, glycogen phosphorylase; LDH, lactate dehydrogenase; PGAK, phosphoglycerate kinase; PGAM, phosphoglycerate mutase; PGI, phosphoglucose isomerase; PGM, phosphoglucumutase; PK, pyruvate kinase; TPI, triosephosphate isomerase.

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

(Mudalal et al., 2014; Petracci et al., 2014; Bowker and Zhuang, 2016) and wooden breast (Soglia et al., 2016a) results in diminished total protein content and altered protein profiles. Similarly, the proximate composition and electrophoretic data in the current study together suggest that the myodegeneration associated with the SM myopathy diminishes the total amount of muscle protein and to a lesser extent alters the composition of the muscle proteome. The minor, yet significant, differences observed in the electrophoretic protein profiles suggest that further research to elucidate the SM effects on inherent properties of the muscle proteins (i.e., degrees of oxidation, denaturation, and degradation) is warranted.

Breast Meat Functionality

The results obtained for pH and protein functionality traits are reported in Table 6. Similar to wooden breast and white striping, filets affected by the SM condition exhibited a higher pH ($P < 0.0001$) than the unaffected filets. Previously, metabolomic studies conducted on wooden breast (Abasht et al., 2016; Zambonelli et al., 2016) and white striping (Alnahhas et al., 2016) indicated that a reduced muscle glycogen content as well as an altered glucose metabolism might explain the altered pH observed in filets affected by those myopathies. As the metabolic features of SM have not been investigated yet, future research should elucidate the

Table 5. Relative abundance¹ of SDS-PAGE protein bands from the myofibrillar fraction of broiler breast filets according to muscle condition (M - normal, N; spaghetti meat, SM) and muscle layer (L - superficial, S; deep, D).

Band	Approximate molecular size and identification	Muscle condition (M)			Layer (L)			P-value		
		N	SM	SE	S	D	SE	M	L	M × L
1	Titin	3.37	3.33	1.16	3.34	3.36	1.16	0.8574	0.8952	0.1689
2	Nebulin	1.03	0.99	0.25	0.88 ^b	1.14 ^a	0.25	0.6738	0.0049	0.3262
3	220 kDa, MyHC	40.0	40.0	2.0	39.9	40.0	2.0	0.9435	0.7992	0.8391
4	165 kDa, myomesin	6.26	6.09	0.53	6.41	5.94	0.52	0.5710	0.0957	0.8433
5	150 kDa, MyBPC	4.85	4.80	1.85	4.96	4.69	1.85	0.8195	0.1004	0.5912
6	105 kDa, α -actinin	2.57	2.48	0.96	2.47	2.57	0.96	0.3153	0.2721	0.0230
7	80 kDa	1.11 ^a	0.92 ^b	0.16	1.02	1.01	0.16	0.0003	0.8270	0.1085
8	53 kDa, desmin	0.76	0.75	0.08	0.73	0.78	0.08	0.8887	0.4611	0.2054
9	42 kDa, actin	23.1	23.3	1.1	23.1	23.1	1.1	0.5982	0.7057	0.9452
10	38 kDa, TnT	5.37	5.64	0.23	5.58	5.42	0.22	0.2115	0.3914	0.9053
11	28-30 kDa	0.21	0.20	0.08	0.25 ^a	0.16 ^b	0.08	0.8623	0.0007	0.1073
12	25 kDa	0.65	0.55	0.21	0.64	0.56	0.21	0.0918	0.1738	0.8195
13	22 kDa, MLC-1	1.44	1.57	0.26	1.45	1.56	0.26	0.3038	0.3720	0.2926
14	21 kDa, TnI	1.49	1.42	0.52	1.41	1.49	0.52	0.5501	0.4881	0.6691
15	18 kDa, TnC	0.93	0.95	0.18	0.90	0.99	0.17	0.8493	0.2092	0.3045
16	17 kDa, MLC-2	4.38	4.41	0.59	4.52	4.27	0.59	0.8649	0.1883	0.2902
17	15 kDa, MLC-3	1.92	1.96	0.27	1.89	1.99	0.27	0.7771	0.3592	0.0061

^{a,b}Means within the same row and effect followed by different superscripts differ $P < 0.05$.

Abbreviations: MLC-1, myosin light chain 1; MLC-2, myosin light chain 2; MLC-3, myosin light chain 3; MyBPC; myosin binding protein C; MyHC, myosin heavy chain; TnC, troponin C; TnI, troponin I; TnT, troponin T.

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

Table 6. Breast pH and protein functionality traits according to muscle condition (M - normal, N; spaghetti meat, SM) and muscle layer (L - superficial, S; deep, D).

Traits	Muscle condition (M)			Layer (L)			P-value		
	N	SM	SE	S	D	SE	M	L	M × L
pH	5.84 ^b	5.99 ^a	0.03	5.92	5.90	0.03	<0.0001	0.2224	0.5596
Protein solubility ¹									
Myofibrillar proteins	143 ^a	124 ^b	4.3	135	132	3.9	<0.0001	0.2488	0.2495
Sarcoplasmic proteins	85.1	82.1	2.9	82.3 ^b	84.9 ^a	2.7	0.1729	0.0001	0.7351
Salt-induced H ₂ O uptake, %	67.6	53.7	7.3	60.3	61.0	5.3	0.1864	0.8032	0.8186
Cook loss, %	25.0	26.1	1.2	25.0	26.1	0.9	0.5182	0.0990	0.0901
Final yield, %	126	114	7	121	120	5	0.2246	0.6105	0.6918
Emulsion activity index									
Myofibrillar proteins	0.78	0.79	0.02	0.79	0.78	0.02	0.5242	0.9274	0.7298
Sarcoplasmic proteins	0.81	0.77	0.02	0.81	0.77	0.02	0.2635	0.2106	0.8642
Emulsion stability index									
Myofibrillar proteins	16.6 ^a	15.4 ^b	1.0	16.0	16.0	1.0	0.0271	0.9496	0.0052
Sarcoplasmic proteins	18.3 ^a	16.9 ^b	1.1	17.3	17.8	1.1	0.0243	0.1738	0.7383

^{a,b}Means within the same row and effect followed by different superscripts differ $P < 0.05$.

¹mg protein/g of muscle tissue.

energetic status of the affected muscle and its role on the pathogenesis of this defect.

Typically, greater pH values are associated with better water holding capacity in meat. However, the values obtained for salt-induced water uptake and final yield denoted a conspicuous reduction of water holding capacity in affected meat, although the discrepancies between SM and N were not statistically significant. This phenomenon might be partially explained by a lower total protein content resulting from myodegeneration. Because most of the water in muscle tissue is typically held within the myofibrillar structure, with fewer myofibrils (as suggested by less muscle protein) there is simply a lower potential for the muscle to bind water. On the other hand, a poorer water retention ability could also be explained by diminished muscle protein functionality due to inherent structural or chemical changes within the proteins themselves. Protein solubility was defined by Xiong (2004) as the amount of protein that dissolves into an aqueous solution under specific extraction conditions. As solubility reflects the equilibrium between protein and water, it is a useful indicator of meat protein functionality. In this study the SM myopathy reduced ($P < 0.0001$) myofibrillar protein solubility (Table 6). The impact of the SM myopathy on sarcoplasmic protein solubility was not statistically significant; however, sarcoplasmic protein solubility was lower in the superficial layers of the muscles compared to the deep layers ($P < 0.0001$). Decreased protein solubility is often attributed to protein denaturation and associated with decreased meat water-holding capacity and meat functionality. However, Baldi et al. (2019) reported that protein denaturation patterns for normal and SM fillets analyzed with differential scanning calorimetry were similar. This would suggest that the reduced protein solubility measured in the current study may be predominantly a function of reduced muscle protein in the tissue. Adjusting protein solubility values for initial muscle protein content, however, did not fully account for reduced protein solubility in SM fillets.

The ability of protein to form a stable emulsion can also be used as an indicator of protein functionality. In this study, the emulsifying properties of myofibrillar and sarcoplasmic protein extracts were assessed through the determination of the emulsion activity index (EAI) and emulsion stability index (ESI). The EAI measures the ability of proteins to rapidly adsorb at the fat-water interface during emulsion formation due to their hydrophobic and hydrophilic groups (Xiong 2004; Chan et al., 2011). The ESI reflects the degree of fat globules stabilization reached through both protein-protein and protein-fat interactions (Barbut, 1995) by measuring the rate of emulsion destabilization over time (Chan et al., 2011). It was observed that normal and SM fillets exhibited similar EAI values for both the sarcoplasmic and myofibrillar protein extracts. With regards to the ESI, there was a significant muscle condition by layer interaction effect with the least stable myofibrillar protein emulsion produced by the SM-S group (SM-S = 14.6; SM-D = 16.1; N-D = 15.8; N-S = 17.4; $P = 0.0052$). The stability of the emulsions formed by the sarcoplasmic protein extracts were lower ($P = 0.0243$) in SM fillets compared to normal fillets. Because the protein extracts in this study were adjusted to similar concentrations prior to measuring the EAI and ESI, changes in these values due to the SM myopathy were attributed directly to changes in the intrinsic properties of the proteins rather than the reduced protein content of the muscle.

CONCLUSIONS

As breast meat with the SM condition is likely destined for use in further processed meat products, it is vital to understand the impact of the myopathy on meat composition and functionality. The findings of the current study demonstrated that the SM condition negatively impacted breast meat proximate composition, particularly with regards to protein content. As the superficial layer of affected fillets was characterized

by a reduction in total protein as well as increased levels of calcium and sodium, these data further support the hypothesis that the myopathic insult is unevenly distributed throughout the breast muscle. It was also demonstrated that the SM myopathy impaired meat functionality traits: altered pH, potentially decreased water holding capacity, inferior protein solubility, and emulsion stability. Overall, data suggested that the reduction in muscle protein content was likely the cause of reduced meat functionality. However, minor shifts in the muscle protein profiles of SM filets and alterations in protein solubility and emulsification properties suggest that the effects of the myopathy on the integrity and intrinsic properties of muscle proteins (i.e., degree of denaturation, degradation, and oxidation) merits further investigation because of the importance of meat protein quality for processed products.

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