Histology, composition, and quality traits of chicken *Pectoralis major* muscle affected by wooden breast abnormality

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ABSTRACT Only a few years ago, the poultry industry began to face a recent abnormality in breast meat, known as wooden breast, which frequently overlaps with white striping. This study aimed to assess the impact of wooden breast abnormality on quality traits of meat. For this purpose, 32 normal (NRM), 32 wooden (WB), and 32 wooden and white-striped (WB/WS) *Pectoralis major* muscles were selected from the same flock of heavy broilers (males, Ross 708, weighing around 3.7 kg) in the deboning area of a commercial processing plant at 3 h postmortem and used to assess histology, proximate (moisture, protein, fat, ash, and collagen) and mineral composition (Mg, K, P, Na and Ca), sarcoplasmic and myofibrillar protein patterns, and technological traits of breast meat. Compared to the normal group, WB/WS fillets showed more severe histological lesions characterized by fiber degeneration, fibrosis, and lipidosis, coupled with a significantly harder texture. With regard to proximate and mineral composition, abnormal samples exhibited significantly ($P < 0.001$) higher moisture, fat, and collagen contents coupled with lower ($P < 0.001$) amounts of protein and ash. Furthermore, increased calcium (131 vs. 84 mg kg^{-1}; $P < 0.05$) and sodium (741 vs. 393 mg kg^{-1}; $P < 0.001$) levels were found in WB/WS meat samples. The SDS-PAGE analysis revealed a significantly lower amount of calcium-ATPase (SERCA, 114 kDa), responsible for the translocation of Ca ions across the membrane, in normal breasts compared to abnormal ones. As for meat quality traits, fillets affected by wooden abnormality exhibited significantly ($P < 0.001$) higher ultimate pH and lower water-holding/water-binding capacity. In particular, compared to normal, abnormal samples showed reduced marinade uptake coupled with increased drip loss and cooking losses as well. In conclusion, this study revealed that meat affected by wooden breast or both wooden breast and white striping abnormalities exhibit poorer nutritional value, harder texture, and impaired water-holding capacity.

Key words: chicken breast meat, wooden breast, white striping, composition, calcium

2016 Poultry Science 95:651–659
http://dx.doi.org/10.3382/ps/pev353

INTRODUCTION

The production of poultry meat has dramatically increased in the last few decades. The majority of this increase has been rendered possible by genetic selection of animals for quantitative traits, which has tremendously augmented the growth rate of muscle by inducing hypertrophy in existing fibers due to fusion of satellite cells (Dransfield and Sosnicki, 1999; Picard et al., 2002; Scheuermann et al., 2004).

The increase in growth rates and breast yield due to genetic progress has obviously put more stress on broilers and induced the manifestation of several ante- and postmortem histological and biochemical alterations in muscle tissue (Sandercock et al., 2009; Petracci and Cavani, 2012; Petracci et al., 2015). In particular, some of these alterations are attributed to homeostatic dysregulation, which leads to cellular dysfunction (MacRae et al., 2006; Sandercock et al., 2006). It has been hypothesized that both growth- and stress-related myopathies may occur as a consequence of disruption in homeostasis of intracellular cations (calcium and sodium) (Sandercock et al., 2009). Accordingly, an increase of sodium in muscle cells can increase the calcium concentration, activating phospholipase A2 and other proteases (i.e., calpains), leading to membrane dysfunction and loss of creatine kinase (Sandercock and Mitchell, 2003, 2004).

© 2015 Poultry Science Association Inc.
Received March 25, 2015.
Accepted October 6, 2015.
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At present, recent breast muscle myopathies or abnormalities that heavily affect the poultry industry are white striping (**WS**), white striations parallel to muscle fibers mainly on the ventral surface (Kuttappan et al., 2012a), and wooden breast (**WB**), visually hard, out-bulging and pale areas, that may occur separately or together (Sihvo et al., 2014). It was recently estimated that up to 40% of medium (2.2 to 3.0 kg) and heavy (>3.0 kg) broiler chickens raised under commercial conditions are affected by different levels of white striping (Lorenzi et al., 2014), while wooden breast abnormality affects mainly the heavy ones (personal communication). Because of their unsightly visual appearance, WS and WB fillets, especially in severe cases, are normally downgraded and transformed into processed meat products leading to economic losses for the poultry industry (Petracci et al., 2015). Previous observations have revealed that the histopathological changes in WS and WB muscles show similar features such as an increase in degenerative and atrophic fibers associated with loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, interstitial inflammation, and fibrosis (Kuttappan et al., 2013; Sihvo et al., 2014). These myopathies may have substantial implications on meat quality. WS already has been the subject of several studies that have investigated its consequences on consumer acceptability (Kuttappan et al., 2012a), histological traits (Kuttappan et al., 2013), chemical composition (Kuttappan et al., 2012b; Petracci et al., 2014), protein profile (Mudalal et al., 2014), and processing abilities (Petracci et al., 2013). On the other hand, only 2 studies have evaluated the technological traits (Mudalal et al., 2015) and suitability for further processing (Puolanne and Ruusunen, 2014) of breast muscles affected by WB. In addition, there are no data regarding mineral composition that would assess the homeostatic status of breast muscle tissue in both breast abnormalities.

Therefore, the aim of this study was to evaluate the impact of WB abnormality with or without WS on muscle histology and chemical composition with special emphasis on mineral levels and protein profile, which may affect the texture and water-holding capacity (**WHC**) of meat.

MATERIALS AND METHODS

Sample Selection and Preparation

According to the presence or absence of WB and wooden and white-striped (**WB/WS**) muscle abnormalities, 96 boneless, skinless, *Pectoralis major* muscles were selected, in 2 replicates, from 52-day-old male Ross 708 broilers (3.7 kg live weight) in the deboning area of a commercial processing plant at 3 h postmortem. Consistent with the criteria proposed by Kuttappan et al. (2012a) and Sihvo et al. (2014), breast fillets were then graded into 3 classes (Only severe cases of WB and WB/WS were included in the study) as follows:

— Normal (NRM): fillets free of hardened areas and white striations;
— Wooden Breast (WB): fillets with diffuse, hardened areas and pale ridge-like bulges at the caudal end;
— Wooden Breast and White Striping (WB/WS): fillets affected by both WB and white striations.

Histological evaluations were performed on 3 samples/group for each replication for a total of 18 samples. Approximately one cm^3 from the cranial part of each *Pectoralis major* muscle (from the skin surface) was immediately removed and fixed in 10% formalin buffer for 24 h at room temperature. Specimens were oriented for transverse fiber sectioning, dehydrated in a graded series of ethanol, and embedded in paraffin. From each sample, serial transverse sections (6 μm thick) were obtained, mounted on polylysine-coated slides, and stained with Masson's trichrome. For each section of muscle, the presence of abnormal fibers (giant fibers, fibers with hyaline degeneration, and damaged fibers with round profile) in 10 primary myofiber fascicles (**PMF**) were assessed, and the levels of myodegeneration were graded according the same criteria adopted in our previous study (Mazzoni et al., 2015):

Score F1 – mild: abnormal fibers ranging from 2 to 4 for each PMF (Figure 1A);

Score F2 – moderate: abnormal fibers ranging from 5 to 10 for each PMF (Figure 1B);

Score F3 – severe: abnormal fibers represent the majority of the fibers for each PMF (Figure 1C).

Subsequently, all breast muscles (16/group/replication) were packaged and transported under refrigerated conditions to the laboratory. At 24 h postmortem, *Pectoralis major* muscles were trimmed from superficial fat and connective tissues and used to determine pH, compression test, drip loss, cooking loss, marination performance, and textural profile. The remaining parts of raw breast fillet were finely ground with a blender for one min at 1 to 4°C to obtain a homogenous ground meat mass and stored at –20°C for further analysis (proximate composition, mineral composition, and SDS-PAGE analysis).

Chemical Composition (Proximate, Mineral, and Collagen)

Proximate chemical composition (moisture, protein, lipid, and ash) of breast meat was determined in each sample using official methods (AOAC, 1990). The moisture content was estimated by weight difference before and after drying 5 g of ground meat in a conventional oven at 100 to 102°C for 16 h. Total nitrogen content

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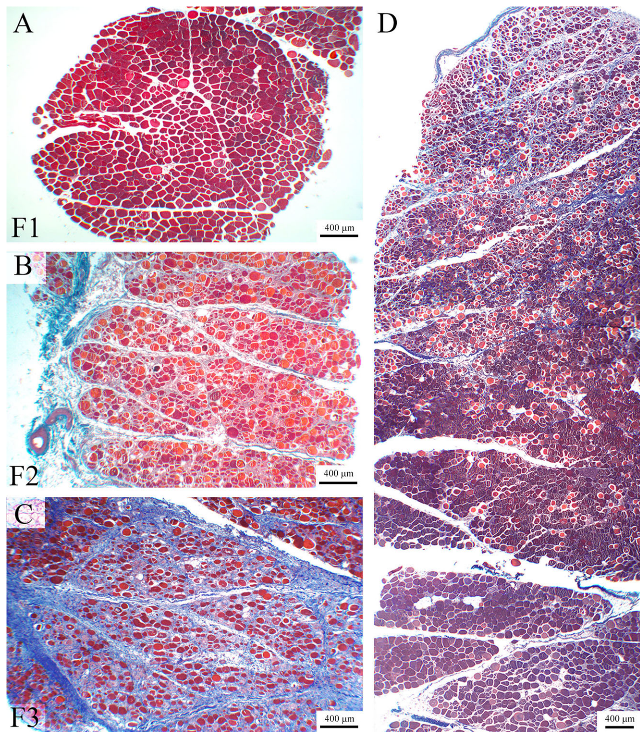

Figure 1. Images of mild (A), moderate (B), and severe (C) samples in the histological scale (ranging F1 to F3) used to score the levels of myodegeneration (Masson's Trichrome). A = The polygonal muscle fibers in an F1 score are well packaged and relatively of the same size. B = The fibers show different diameter and the perimysial connective tissue is thickened. C = The number of muscle fibers is reduced; variably sized muscle fibers are rounded and separated or replaced by a loose or more organized connective tissue. In (D) a sample is represented (score F3) with gradual progression of the histopathological lesions. From the surface (upper part image) up within the muscle (lower part of the image) the histological lesions are gradually disappearing (Masson's Trichrome). Score F1 – mild: abnormal fibers ranging from 2 to 4 for each PMF (Figure 1A); Score F2 – moderate: abnormal fibers ranging from 5 to 10 for each PMF (Figure 1B); Score F3 – severe: abnormal fibers represent the majority of the fibers for each PMF (Figure 1C).

was measured applying the Kjeldahl method to calculate total crude protein content, while intramuscular fat content was estimated by diethyl ether extraction using the Soxhlet method. Ash content was determined by weight difference before and after turning 5 g of ground meat sample to ashes in a muffle furnace (525°C), and collagen was calculated from hydroxyproline content determined according to the colorimetric method proposed by Kolar (1990).

Elemental analysis (Ca, Na, P, K, and Mg) was performed using the Inductively Coupled Plasma Optical Emission Spectrometry method (**ICP-OES**) after microwave digestion of 3.0 g of meat. Samples were digested by microwave using a Milestone ETHOS ONE oven (Milestone, Sorisole, Italy) using 4 mL nitric acid and 1 mL hydrogen peroxide. All reagents were from Merck (Darmstadt, Germany); acids were of Suprapur grade. Elements were quantified by ICP-OES using a Perkin Elmer Optima 2100 DV instrument, coupled with a CETAC U5000AT+ ultrasound nebulizer for mercury. Two blanks were run during each set of analysis to check for chemical purity, and accuracy of the method was verified with reference materials (CRM GBW 09101, human hair control, Shanghai Institute of Nuclear Research Academia Sinica; CRM 201505 and 201605 Trace Element Whole Blood, Seronorm, Billingsad, Norway). All values of the reference materials were within certified limits. Instrumental detection limits were expressed as wet weight (**w.w.**). Concentrations of elements in tissues were expressed as mg kg^{-1} breast muscle wet weight.

Electrophoresis Analysis

The extraction of sarcoplasmic and myofibrillar protein fractions was carried out in triplicate on 12 samples (6/each replication) according to the procedure described by Liu et al. (2014) and, in order to get a clear background on SDS-PAGE, potential interfering substances were removed using the ReadyPrep^{TM} 2-D cleanup kit (BioRad). Protein concentration was measured on the 36 extracts by Bradford Assay (1976) and the results were expressed as an average of 2 replicates. SDS-PAGE analysis was carried out to determine the molecular weight (**MW**) and relative abundance of sarcoplasmic and myofibrillar proteins. Before loading, samples were mixed 1:1 (v/v) with standard sample buffer (pH 6.8) containing 8 M urea, 2 M thiourea, 3% (wt/v) SDS, 75 mM DL-dithiothreitol (DTT), and 25 mM Tris-HCl and heated for 5 min at 100°C (Fritz et al., 1989). A Mini-Protean TGX any kDa Stain-Free Gel (BioRad) was used to determine the relative abundance and MW of sarcoplasmic proteins (4 μg proteins/well). At the same time, in order to accurately quantify the bands for myofibrillar proteins with both low and high abundance, the samples were run twice at different protein loads on gels with 2 different polyacrylamide concentrations. Thus, 6 and 22 μg of the extracted samples were respectively loaded in 7.5% (to quantify the bands of high abundance) and 12% (to quantify the bands of low abundance) Mini-Protean TGX Stain-Free Gel (BioRad), selected according to their migration charts. Gels were settled in a Mini-PROTEAN® Tetra Cell (BioRad) and run for one h at 100 V with a Tris-Gly running buffer containing 50 mM Tris, 0.384 M glycine, and 0.1% (wt/v) SDS (Laemmli, 1970) and acquired for analysis with ChemiDoc^{TM} MP System (BioRad).

Protein MWs were determined by comparing their relative mobility against those of reference proteins included in the Precision plus Standard protein, all blue pre-stained (BioRad). Proteins corresponding to each band were identified by comparison with Mass Spectrometry characterized proteins, available from the literature (Lan et al., 1995; Huang et al., 2011; Zapata et al., 2012).

For quantification of proteins, band fluorescence was analyzed by Image Lab Rev 4.0 software. A calibration

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curve (R^2 value from 0.98 to 0.99) was made for each gel by loading and analyzing different amounts of bovine serum albumin (**BSA**, and, in particular, standard set from BioRad including BSA at 7 different concentrations, from 2 mg/mL to 0.125 mg/mL). The amount of sample protein, whose bands had fluorescence intensity within the range of the calibration curve, was expressed as relative abundance (%).

Technological Traits (pH, Drip and Cooking Losses, and Marinade Performance)

A cross-sectional thin slice was cut from the cranial part of each *Pectoralis major* muscle and used to assess ultimate pH, which was determined by homogenizing 2.5 g of meat in iodoacetate solution (Jeacocke, 1977). In addition, a parallelepiped meat cut (8 × 4 × 3 cm) weighing about 80 g was excised from the cranial part of each fillet and used to determine drip (of refrigerated storage) and cooking losses (in a water bath at 80°C for 45 min) using the same procedures described in our previous study (Petracci et al., 2013). A second parallelepiped meat cut (8 × 4 × 2 cm) weighing about 60 g was excised from the middle part of each fillet, individually labeled and tumbled with a 15% (wt/wt) brine solution containing sodium tripolyphosphate (2.3%) and sodium chloride (7.6%) and subsequently cooked in a water bath at 80°C for 25 min. Marinade uptake and cooking losses were calculated for each sample (Petracci et al., 2013).

Textural Traits

A raw cylindrical meat cut 2.5 cm diameter × 1 cm height and weighing about 5 g was excised from the caudal part of each fillet. It was compressed to 40% of the initial height using a 25 kg loading cell connected to a 50 mm DIA cylinder aluminum probe using a TA.HDi heavy duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). The test speed of the probe was 1 mm/sec, while the pre- and post-test speeds were both 3 mm/sec. The compression value was recorded as the maximum force needed to compress 40% of the initial height of the sample and expressed in kg.

In addition, a cylindrical meat cut (3 cm diameter × 0.8 cm height) was excised from the 8 × 4 × 3 cm sample (designed for cooking loss determination) and used to assess Texture Profile Analysis (**TPA**) consisting in a double compression of the sample up to 50% of its initial height. The test was run in a TA.HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) equipped with a 50 kg loading cell using a 5 cm-diameter cylindrical probe. Hardness, cohesiveness, springiness, gumminess, and chewiness were calculated from each texture profile (De Campos et al., 2008; Lyon et al., 2010).

Statistical Analysis

The results were statistically evaluated with the ANOVA option of the GLM procedure present in SAS software (1988). The main effects of meat abnormality (NRM, WB, and WB/WS) and replication, as well as the interaction term on meat quality traits, were evaluated. Means were separated using Tukey's HSD test (multiple range test) of the GLM procedure (SAS Institute, 1988) and, according to the not significant *P*-values found, the interaction terms were not considered.

RESULTS AND DISCUSSION

Histological Analysis

In pectoral muscle, overall, histological observations showed correlations with the gross lesions used as selection criteria during sampling. Indeed, all NRM breasts not showing either hardened area or white striations on the surface had myofibers with a normal profile and endo- and perimysial connective tissues without remarkable alterations: PMF showed few abnormal fibers (Figure 1A, F1). On the other hand, muscle fibers with an abnormal polygonal profile (rounded fibers) were found in correspondence to diffuse hardened areas in WB breasts. These fibers showed a different cross-sectional area and nuclear internalization (Figure 1B, F2 score): a large cross-sectional area of the fibers and small ones were concomitantly detected. Similarly, Sihvo et al. (2014) described myodegeneration accompanied by rounded fibers (reduced in number) and central nuclei in wooden breast muscles of broilers. In addition, the same authors observed diffuse interstitial thickening with variable amounts of loose connective tissue, granulation tissue and fibrosis in the areas affected by lesions. In the present study, proliferation and thickening of the perimysial network (fibrosis) was also observed, which appeared to separate muscle fibers. In addition, an increase in intramuscular fat was observed. In some cases, multifocal degenerative aspects of some fibers were identified together with inflammatory cell infiltration (Figure 1C). Finally, samples from WB/WS fillets exhibited profound degenerative myopathic lesions together with replacement of chronically damaged muscle with adipocytes and fibrosis of muscle tissue in agreement with the observations of Kuttappan et al. (2013) in WS breast samples. In the present study, microscopic observations showed complete reorganization of skeletal muscle structure (F3 score) characterized by replacement of muscle fibers with boundless proliferation of peri- and endo-mysial connective tissue stained by Masson's trichrome. As a result of the severe fibrosis, connective tissue was the most abundant tissue in PMF. Fibers appeared to be decreased both in number and cross-sectional area dimension and showed a rounded profile. Many degenerate and/or necrotic fibers were accompanied by an interstitial inflammatory

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Table 1. Effect of wooden breast (WB) and wooden breast/white striping (WB/WS) on composition of raw meat (n = 32/group).

Parameter	Category			SEM	Probability
	Normal	WB	WB/WS		
Moisture (%)	74.1^{b}	75.3^{a}	75.1^{a}	0.28	***
Protein (%)	22.8^{a}	21.4^{b}	20.4^{c}	0.11	***
Fat (%)	0.87^{c}	1.25^{b}	1.98^{a}	0.02	***
Ash (%)	1.37^{a}	1.26^{b}	1.21^{b}	0.03	***
Collagen (%)	1.09^{c}	1.18^{b}	1.26^{a}	0.28	***
Mg (mg/100g)	36.4	35.0	32.0	1.29	NS
K (mg/100g)	375.4	379.4	361.1	8.47	NS
P (mg/100g)	220.2	205.9	207.9	4.47	NS
Na (mg/100g)	39.3^{b}	63.7^{a}	74.1^{a}	4.51	***
Ca (mg/100g)	8.40^{b}	20.8^{a}	$13.6^{a,b}$	2.72	*

*** = $P \leq 0.001$; * = $P \leq 0.05$; NS = Not significant.

a–cMeans within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

infiltrate (Figure 1C) in agreement with previous findings (Kuttappan et al., 2013; Sihvo et al., 2014; Ferreira et al., 2014).

An interesting macroscopic appearance was observed in the majority of F3 samples, where there was a clear and gradual decrease of histopathological lesions from the surface towards the inside of the muscle; histological sections corresponding to these macroscopic lesions showed a gradual modification of the architecture of muscle tissue from external (surface muscle) to internal (about 1 cm deep) (Figure 1D).

Chemical Composition (Proximate, Mineral, and Collagen)

The incidence of WB and WB/WS abnormalities significantly affect the proximate and mineral compositions of breast meat (Table 1). In particular, compared to WB, fillets affected by both abnormalities exhibited the greater changes in the chemical composition and the differences were more pronounced when the wooden breast abnormality occurs together with white striations. Although no significant differences were found between WB and WB/WS samples, the latter exhibited higher moisture and lower ash content in comparison with NRM. Moreover, a significant ($P < 0.001$) decrease in protein content was found going from NRM to WB/WS breasts, whereas fat and collagen levels revealed an opposite trend. Similar effects on the chemical composition were previously found in WS meat by Kuttappan et al. (2012b), Petracci et al. (2014) and Mudalal et al. (2014). Overall, these differences are likely due to progressive myodegeneration and regeneration of muscle tissue as well as to the deposition of a variable amount of interstitial connective tissue or fibrosis (Figures 1C and D). Hence, myodegeneration of muscle fibers may lead to a decrease in protein content, while fibrosis likely results in an increase in collagen tissue. Indeed, Velleman and Clark (2015) found an increase of extracellular matrix glycosaminoglycans content in WB muscles. Moreover, replacement of degenerated muscle fibers by adipose tissues through lipidosis also may increase the fat content. The increase in moisture content may be explained by the occurrence of moderate to severe edema (fluid accumulation) as a result of inflammatory processes (Sihvo et al., 2014).

Regarding the mineral content, there were no significant differences in the amounts of Mg, K, and P. However, compared to NRM, WB/WS and WB fillets had significantly higher amounts of Na and Ca content, the latter exhibiting the highest value in WB fillets. In agreement with previous studies, the increased intracellular calcium level can be considered as a common trigger of necrosis in all cells in general and for myofibers, in particular, since they store high levels of calcium ions within their sarcoplasmic reticulum. In particular, as a result of the extracellular calcium influx and the release of the intracellular stores of calcium from the damaged sarcolemma structure and sarcoplasmic reticulum, myofibers can undergo calcium-induced necrosis (Zachary and McGavin, 2012). Furthermore, considering its key role as a signaling molecule, it is very important for cells to regulate the intracellular calcium concentrations (Carafoli, 1991; Carafoli and Stauffer, 1994). In particular, in a previous study McLennan (2000) pointed out that prolonged-high intracellular calcium concentrations (above 10 μM) exert a detrimental effect on cells and induce apoptosis. So then, the overall increase in Na and Ca may explain the presence of histological lesions such as multifocal degeneration and necrosis (Figure 1C). In agreement with Sandercock and Mitchell (2004) it is likely that initial alteration in cellular Na homeostasis may play a key role in the development of wooden breast muscle abnormality. In particular, the increase in the Na level in WB and WB/WS samples may lead to higher Ca uptake, resulting in the development of muscle damage as proposed by Sandercock and Mitchell (2004). It was also found that increased calcium and sodium levels might activate specific enzymes, such as phospholipase A2, that are involved in membrane damage. The unbalance in ion levels underlying these muscle myopathies in poultry is also consistent with the mechanisms leading to Duchenne muscular dystrophy in mammals (Wallace and McNally, 2009). In this case, previous findings on patients affected by Duchenne muscular dystrophy (Robert et al., 2001) and mdx mice (De Backer et al., 2002) revealed that the alteration of membrane is combined with an abnormal intracellular calcium concentration and a deregulated calcium-responsive pathway that exert a detrimental effect on cellular functions, inducing diseases or apoptosis (McLennan, 2000). Furthermore, elevated calcium has been causally linked to a higher calcium-dependent protease activity (Iwata et al., 2003) and abnormal mitochondrial function (Millay et al., 2008).

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Table 2. Effect of wooden breast (WB) and wooden breast/white striping (WB/WS) abnormalities on the relative abundance (%) of myofibrillar proteins in raw meat (n = 6/group).

Protein	Gel%	MW (kDa)	Category			SEM	Prob.
			Normal	WB	WB/WS		
1. LC3	12	16	14.1	14.1	15.8	0.69	NS
2. LC2	12	19	1.2	1.1	1.7	0.22	NS
3. LC1	12	27.5	12.5[a]	9.3[b]	8.4[b]	0.59	*
4. 30 kDa troponin T fragment	12	29	4.1[b]	4.9[a]	5.0[a]	0.17	*
5. Troponin T	12	34	4.7	5.2	4.6	0.18	NS
6. Actin	7.5	42	34.1	34.4	34.6	0.99	NS
7. Desmin	12	53	5.2	6.6	6.3	0.29	NS
8. 70 kDa MHC fragment	12	70	4.9[b]	6.7[a]	6.5[a]	0.33	*
9. MHC	7.5	220	15.9	15.6	15.4	0.99	NS

* = $P \leq 0.05$; NS = Not significant.

MHC = Myosin heavy chain; LC = Myosin light chain.

[a–b]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

Table 3. Effect of wooden breast (WB) and wooden breast/white striping (WB/WS) abnormalities on the relative abundance (%) of sarcoplasmic proteins in raw meat (n = 6/group).

Protein	Molecular weight (kDa)	Category			SEM	Prob.
		Normal	WB	WB/WS		
1. PGAM	25	7.2[a]	6.3[b]	6.2[b]	0.16	*
2. TPI1	26.4	7.3	7.3	6.8	0.18	NS
3. Carbonic anhydrase	31.8	9.6	9.9	9.0	0.31	NS
4. LDH	34	18.6[b]	21.2[a]	22.3[a]	0.47	*
5. GAP	36	11.2[b]	13.3[a]	12.8[a]	0.35	*
6. ALD	39	7.1[b]	8.6[a]	9.8[a]	0.36	*
7. CK	43	10.9[a]	9.5[b]	9.6[b]	0.18	***
8. PGI	58	7.7[a]	7.3[a]	5.4[b]	0.38	*
9. PK	68	5.6[a]	4.5[b]	4.7[b]	0.19	*
10. GP	90	13.6[a]	11.7[b]	14.4[a]	0.41	*
11. Calcium ATPase	114	n.d[b].	3.3[a]	2.7[a]	0.33	***

*** = $P \leq 0.001$; * = $P \leq 0.05$; NS = Not significant; n.d. = Not detected.

[a–c]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

PGAM = Phophoglycerate mutase; TP1 = Triosephosphate isomerase 1; LDH = Lactate dehydrogenase; GAP = Glyceraldehyde dehydrogenase; ALD = Aldolase; CK = Creatine kinase; PGI = Phosphoglucose isomerase; PK = Pyruvate kinase; GP = Glycogen phosphorylase.

Electrophoretic Analysis

The results of SDS-PAGE quantification of sarcoplasmic and myofibrillar proteins obtained from NRM, WB, and WB/WS fillets were expressed as relative abundance to avoid small differences in protein loading (Tables 2 and 3). Moreover, in order to reduce the variability associated with differences between gels, calibration curves were created for each gel, loading increasing amounts of standard BSA. All the curves had a good linearity ($R^2 > 0.98$) and any band above or below the linear range of the densitometer was not considered for quantification. Overall, the incidence of WB and WB/WS exerted a significant effect on sarcoplasmic and myofibrillar protein patterns. For myofibrillar proteins, it was possible to estimate the relative abundance of 9 bands with MWs ranging from 16 to 220 kDa (Table 2). It is interesting to note that 6 out of 9 of these proteins did not show any significant difference between NRM and affected samples. On the other hand, WB and WS/WB samples exhibited different amounts ($P < 0.05$) of LC1 slow-twitch light chain myosin (27.5 kDa) and 30 kDa-troponin T fragment. In particular, both WB and WB/WS revealed a lower relative abundance of LC1, with respect to NRM samples, that might be due to under-expression or increased degradation. At the same time, the affected samples exhibited higher relative abundance of the 30 kDa-fragment resulting from the proteolytic degradation of troponin T. In addition, although no significant differences were found among NRM, WB, and WB/WS samples concerning the MHC content, the latter exhibited a higher amount of the 70 kDa-fragment resulting from the degradation of MHC. This finding supports the atypical actin:myosin heavy chain ratio found within this study and underlines the higher proneness of MHC to proteolysis in WB and WB/WS fillets.

For sarcoplasmic proteins, it was possible to estimate the relative abundance of 11 bands having MWs from 25 to 114 kDa. Overall, the findings revealed that most of the enzymes involved in glycolytic-gluconeogenesis pathways significantly differ in normal and affected samples. In particular, the WB and WB/WS groups exhibited similar and significantly higher amounts of

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lactate dehydrogenase (34 kDa), glyceraldehyde dehydrogenase (36 kDa), and aldolase (39 kDa). On the other hand, compared to NRM, WB and WB/WS samples exhibited significantly lower levels, respectively, of glycogen phosphorylase and phosphoglucose isomerase. These differences suggest an impaired ability of muscle cells to satisfy the energy demand of the tissue in pathological conditions, leading so far to the occurrence of wooden breast abnormality. Furthermore, WB and WB/WS samples exhibited significantly lower pyruvate kinase (68 kDa) and creatine kinase (43 kDa) levels, the latter being used as an indicator of muscle damage. In particular, according to Mitchell (1999), the lower creatine kinase levels can be considered as a consequence of the impaired muscle contractile and metabolic functions given by increased calcium concentration that induces cellular breakdown with the subsequent loss of the intracellular constituents. Furthermore, in agreement with Sandercock and Mitchell (2004), the altered Na homeostasis may play a key role in the development of wooden breast muscle abnormality. In particular, the interaction between a higher Na level and Ca uptake in WB and WB/WS samples leads to the development of muscle damage resulting in the leaking and loss of various enzymes such as creatine kinase. In addition, compared to NRM, WB and WB/WS samples exhibited significantly higher relative abundance of calcium ATPase (SERCA, 114 kDa), which catalyzes the hydrolysis of ATP coupled with the transport of calcium ions across the membrane (Periasamy and Kalyanasundaram, 2007). This outcome agrees with Mutryn et al. (2015) who recently found that ATPase, Ca transporting, cardiac muscle, slow twitch 2 (ATP2A2) gene is upregulated in birds affected by WB abnormality. Indeed, this gene encodes for SERCA isoform 2a and 2b. Higher concentration of SERCA in WB samples may likely occur in response to increased amounts of intracellular calcium. Mutryn et al. (2015) found that parvalbumin is also overexpressed in WB birds. This protein acts as a calcium-binder and exerts an essential role in regulating the calcium concentrations within muscle cells. Thus, it is possible that its up-regulation occurs as a compensatory effect to avoid an excessive calcium concentration that has been found to occur in other muscle disorders such as Duchenne dystrophy.

Technological Traits

The effects of wooden abnormality on pH, drip and cooking losses, and marination performances are shown in Table 4 (Mudalal et al., 2015). Overall, fillets affected by both abnormalities (WB/WS) had significantly higher ($P < 0.001$) ultimate pH values than normal and wooden fillets, which did not differ from each other. In addition, there was a general decrease in the ability to retain liquid during refrigerated storage (drip losses) and cooking as well as pick-up of marinade solutions (marinade uptake)

Table 4. Effect of wooden breast (WB) and wooden breast/white striping (WB/WS) on pH and water holding ability of non-marinated and marinated meat (n = 32/group) (Mudalal et al., 2015). SEM = Standard Error of Means.

	Category				
Parameter	Normal	WB	WB/WS	SEM	Prob.
Non-marinated meat					
Ultimate pH	5.82^{b}	5.87^{b}	6.05^{a}	0.02	***
Drip loss (%)	0.93^{b}	1.19^{a}	1.03^{b}	0.03	*
Cooking loss (%)	21.6^{b}	28.0^{a}	29.5^{a}	0.4	***
Marinated meat					
Uptake (%)	13.15^{a}	6.94^{b}	6.24^{b}	0.32	***
Cooking loss (%)	15.3^{c}	17.4^{b}	18.7^{a}	0.3	***

*** = $P \leq 0.001$; * = $P \leq 0.05$.

[a–c]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

in WB and WB/WS fillets. Higher pH may be related to altered glucose utilization in birds affected by WB that results in glycogen depletion. Indeed, Mutryn et al. (2015) found an up-regulation of asparagine synthetase and down-regulation of 6-phosphofructo-2-kinase, which have been associated with deficient glucose metabolism in birds affected by WB abnormality. Impairment of the water-holding/binding ability in WB/WS samples cannot be ascribed to differences in ultimate pH, which were higher than normal (i.e., higher pH is associated with higher WHC), but it can be attributed to the negative effects of the WB abnormality seen in histological analysis (Figure 1) characterized mainly by degeneration of muscle fibers accompanied by fibrosis and lipidosis. Alterations in fiber membrane integrity contribute to the loss of liquid during refrigerated storage and cooking. Moreover, degeneration of muscle fibers (Figure 1) may affect the ability of meat to bind water because it is well known that the majority of water (>85%) in the cell is held in myofibrils and that most water is retained (steric) by capillary forces generated by the arrangement of thick and thin filaments within the myofibril (Huff-Lonergan and Lonergan, 2005). This cytoarchitectural design may be disorganized due to the previously described histological changes. On the other hand, the change in the chemical composition is likely to play a major role in reduction of WHC. In fact, the decrease in muscle fiber number, which exerts a major role in binding water molecules during storage and processing, may contribute to this reduction (Mazzoni et al., 2015). Additionally, the increase in fat content (high hydrophobicity) may further reduce the ability of meat to bind water through its different distribution within the muscle structure.

Textural Traits

The results of the compression test and the texture profile analysis are shown in Table 5 (Mudalal et al., 2015). Both WB and WB/WS raw fillets exhibited significantly higher compression force than normal fillets (4.2 and 3.3 vs. 1.9 kg, respectively;

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Table 5. Effect of wooden breast (WB) and wooden breast/white striping (WB/WS) on the textural traits of raw and cooked meat (n = 32/group). SEM = Standard Error of Means.

Parameter	Category			SEM	Prob.
	Normal	WB	WB/WS		
Raw meat					
Compression force (kg)[1]	1.9[b]	4.2[a]	3.3[a]	0.23	***
Cooked meat					
Hardness (kg cm^{-2})	19.1[b]	22.1[a]	21.6[a]	0.53	*
Cohesiveness	2.80	2.87	2.93	0.065	NS
Gumminess (kg cm^{-2})	52.5[b]	63.3[a]	61.3[a]	2.0	*
Springiness (mm)	1.66[a]	1.66[a]	1.58[b]	0.017	*
Chewiness (kg mm)	89.3[a]	107.3[c]	97.8[b]	3.7	*

[1]Mudalal et al. (2015).

*** = $P \leq 0.001$; * = $P \leq 0.05$; NS = Not significant.

[a–c]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

$P < 0.001$). Moreover, the findings of TPA on cooked meat revealed significant ($P < 0.05$) higher hardness, gumminess, springiness, and chewiness in the WB/WS and WB groups that did not differ one from the other except for the latter's exhibiting higher springiness and chewiness values.

The instrumental tests conducted confirm that WB fillets are characterized by a very noticeable hardness in raw meat as shown by the dramatic increase in compression force values (Mudalal et al., 2015). Therefore, instrumental compression tests can be helpful to objectively establish the presence of the wooden abnormality in raw breast fillets. In addition, it was found that the textural traits of cooked meat are dramatically modified in WB fillets, which were harder, gummier, and less elastic (lower springiness) and, as a consequence, more energy is needed to grind them (higher chewiness). In general, the changes in texture profile can be attributed to complex chemical changes that are more relevant to muscle fibers and connective tissues (Wattanachant et al., 2004). SDS-PAGE showed that there was a different pattern of myofibrillar proteins between normal and abnormal meat, which may indicate compositional changes. While the increase in the quantity of collagen (as a result of fibrosis) may contribute to textural changes, the structural changes in collagen also may have a role in these differences, although this has not been previously investigated. On the other hand, the increase in hardness, gumminess, and chewiness values in WB and WS/WB fillets can be explained by the higher cooking loss due to lower WHC (Table 4). This process is normally associated with shrinkage of muscle due to protein denaturation, thus increasing the packing density of fibers after cooking (Huff-Lonergan and Lonergan, 2005).

CONCLUSIONS

Overall, the findings of the present study reveal that WB and WB/WS breast meats exhibit different chemical composition, in comparison with NRM breast meat, since they are characterized by higher fat and collagen content coupled with a lower amount of proteins. In addition, as a consequence of their reduced WHC and impaired textural traits, the affected fillets show inferior processing attributes that can result in economic losses for the poultry industry. An increased Na level in WB and WB/WS samples, coupled with the overexpression of the fast-twitch skeletal muscle Calcium-ATPase, leads to higher cellular Ca uptake, which seems to play a key role in the development of muscle damage. A gradual decrease in the histopathological lesions was detected moving from the surface towards the deep portion of the muscle, with the first exhibiting profound degenerative myopathic lesions accompanied by the replacement of chronically damaged muscle with adipocytes and fibrosis. As a consequence of the impaired muscle structure, most of the enzymes involved in glycolytic-gluconeogenesis pathways significantly differ in normal and affected samples that, furthermore, reveal different myofibrillar protein patterns. In particular, the WB and WB/WS samples exhibit higher relative abundance of both the 30 kDa-fragment resulting from the proteolysis of troponin T and the 70 kDa fragment resulting from the degradation of MHC.

In conclusion, the outcomes of this study reveal that the occurrence of WB abnormality with or without the concomitant incidence of white striations exerts a detrimental effect on muscle histology, composition, and quality traits of the affected meat. Further studies are needed to investigate the causal event that represents the starting point for the development of the abnormality.

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