PROCESSING AND PRODUCTS

Effect of breast myopathies on quality and microbial shelf life of broiler meat

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ABSTRACT To evaluate the impact of emerging myopathies on meat quality and microbial shelf life, 48 normal, 48 white striped (**WS**), and 48 wooden breasts (**WB**) were stored for 11 d at 4°C aerobically and analyzed at 24, 72, 120, 168, 216, and 264 h post-mortem. Normal breasts showed lower ($P < 0.001$) redness index (−0.88 vs. −0.41 and −0.43) and cooking losses (22.0 vs. 23.8 vs. 26.9%) than those of WS and WB meat. Normal and WS breasts exhibited higher protein content than that in WB meat (23.9 and 23.2 vs. 21.4%; $P < 0.001$). Normal meat also had a lower ether extract content than that in WB meat (1.09 vs. 1.88%; $P < 0.001$), with intermediate values for WS meat. Normal breasts exhibited higher saturated fatty acid (**FA**) rate (31.3 vs. 28.0% of total FA on average) and lower unsaturated FA rate (68.7 vs. 72.0%) than those in WS and WB meat ($P < 0.001$). Differences were mainly due to polyunsaturated FA (30.5% in normal vs. 35.3 and 35.4% in WS and WB meat; $P < 0.001$). Normal breasts had higher initial total viable count (**TVC**) and a shorter TVC lag phase than those of WS and WB meat (46.3 vs. 85.2 and 77.8 h). The microbial shelf life threshold (7 $\log_{10}$ CFU TVC/g) was achieved first in normal (130 h) and then in WS (149 h) and WB (192 h) meat. TVC and *Pseudomonas* spp. counts were significantly higher in normal than those in the affected breasts between 72 and 216 h of storage. *Enterobacteriaceae* spp. and lactic acid bacteria counts were significantly higher in normal meat, lower in WB meat, and intermediate in WS meat until 216 h. All differences in microbial targets across meat types disappeared by 264 h of storage. Further studies are necessary to elucidate the factors and the mechanisms that may modulate microbial growth and composition during storage in broiler breast meat affected by myopathies.

Key words: white striping, wooden breast, technological traits, fatty acid profile, microbial spoilage

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INTRODUCTION

The poultry market has evolved in the last 50 yr. The high consumer acceptability of poultry meat for its perceived healthy value, little culinary skill needed for preparation, and suitability for further processing have increased the poultry demand (Petracci et al., 2013a). This increase has thus driven producers to optimize farming techniques to produce more in less time (Kuttappan et al., 2016). Additionally, the increased consumer awareness of food safety has driven the poultry industry to guarantee high meat quality standards (Mead, 2004; Grashorn, 2017).

Moreover, the poultry industry is facing a rising occurrence of meat abnormalities due to both preslaughter and slaughtering factors (Kuttappan et al., 2016). Some of these defects have also been observed in other species (e.g., pale soft and exudative meat in pork) and are largely described in the literature. Other abnormalities are specific to poultry, such as white striping (**WS**) and wooden breast (**WB**) affecting the *Pectoralis major* muscle of broiler breast (Kuttappan et al., 2013 and 2016; Trocino et al., 2015; Tijare et al., 2016). These defects not only alter the meat visual appearance, but also modify the muscle chemical composition and histological traits; both WS and WB meat exhibit increased intramuscular fat content and decreased protein, provoking some dysfunction in muscle tissues, with the resultant degeneration of the myofibrillar structure (Mudalal et al.,

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2015; Radaelli et al., 2017; Soglia et al., 2017). Consequently, these myopathies may affect features influencing consumer judgment (texture, juiciness, tenderness, color, flavor) (Kuttappan et al., 2012b; Tasoniero et al., 2016) as well as the technological properties of meat (pH, water holding and binding capacity, texture) (Mudalal et al., 2015; Trocino et al., 2015; Soglia et al., 2016a).

Numerous studies discuss the mechanisms involved in meat changes due to WS and WB (Kuttappan et al., 2016). However, little is known about the quality of these meats during storage (Soglia et al., 2017; Sun et al., 2018). Additionally, the presence of pathogenic and spoilage microorganisms in abnormal meat has not been widely investigated, and to our knowledge, only one study has assessed microbial growth in WB meat (Dalgaard et al., 2018). This issue could be a significant concern to the poultry industry because of the possible effects on the microbial shelf life and food safety of meat both sold fresh and used for processing. Thus, the present study aimed at comparing technological, chemical, and microbiological traits as well as the microbial shelf life during storage for 11 d in normal, WS, and WB broiler breast meat.

MATERIALS AND METHODS

Sampling Procedure

At a commercial slaughterhouse, 144 carcasses were selected from a single batch of 10,401 male chickens, belonging to the Ross 308 genotype. The animals were slaughtered at 49 d of age and at an average slaughter body weight of 2,797 g. After processing in the chilling tunnel, a trained and skilled operator selected 48 normal breasts, 48 WS breasts, and 48 WB breasts, according to the criteria proposed by Kuttappan et al. (2012b) and Sihvo et al. (2014). The total time of sampling was about 30 minutes, and after collection the same procedures and materials were adopted for packaging, cold storage, and transport of samples. Skinless bone-in breasts were individually numbered and packaged under aerobic conditions using rigid trays and transported by a refrigerated truck at 4°C to the university laboratories within 24 h after slaughter. In the lab, the trays were stored in a refrigerated cabinet (Costan SpA, Belluno, Italy) for 264 h (corresponding to 11 d). The storage setting was designed to reproduce the standard refrigeration conditions during sale (packaging and exposure to light). Exposure to light (OSRAM L36W/76–1) was set from 8:00 to 20:00 at an average temperature of 4 ± 1°C.

Breasts were sampled at 24, 72, 120, 168, 216, and 264 h (corresponding to 1, 3, 5, 7, 9, and 11 d, respectively) after slaughter to examine meat technological, chemical, and microbiological traits. At each sampling day, 24 breasts were processed (8 normal, 8 WS, and 8 WB). First, the presence of WS and WB was confirmed via gross examination. Then, breasts were dissected into the right and left *Pectoralis major* using sterile tools. The right *Pectoralis major* muscles were processed for microbiological analyses. The left ones were used for all other analyses.

Technological Analyses

On each sampling day, the left *Pectoralis major* muscles were submitted to meat quality analyses according to the procedures described by Petracci and Baéza (2011). The pH was measured in triplicate on the muscle ventral side with a pH-meter equipped with a specific electrode for meat penetration and a probe with an automatic temperature control (portable Sension+, Hach, Geneva, Switzerland). The $L^*a^*b^*$ color indices were measured in triplicate on the ventral side of the same muscles, by a Minolta CM-600d colorimeter (Minolta Corp., Ramsey, NJ, USA) using illuminant source D65 (observer 10°, daily light 6,500 K^*).

Thereafter, a parallelepiped meat portion (8 × 4 × 3 cm), parallel to the muscle fiber direction, was separated from the cranial side of the *Pectoralis major* and put under a vacuum at 30% of absolute vacuum by ORVED VM 53 equipment in plastic bags for the vacuum (95 g, 14 × 22 cm) (ORVED SPA, Musile di Piave, Italy) and stored at −18°C until meat analyses. Thawing and cooking losses were measured in this cut according to Petracci and Baéza (2011). After thawing, the meat portion was put into plastic bags and cooked in a water bath for 45 min until an internal temperature of 80°C was achieved. After a 40-min cooling, a further parallelepiped meat portion (4 × 2 × 1 cm) was separated from the original one. Using this latter section, the maximum shear force was measured with an LS5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK) using the Allo-Kramer probe (10 blades; load cell: 500 kg; distance between the blades: 5 mm; blade thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal et al., 2015).

Microbiological Analyses

On each sampling day, a total of 15 right *Pectoralis major* muscles (5 normal, 5 WS, and 5 WB) were submitted to microbiological analyses. A total of 10 representative sub-samples (about 2 cm long × 1 cm deep × 2 cm wide) were aseptically excised along each breast to obtain a representative sample of 25 g per each breast. Then, the sample was homogenized in a sterile stomacher bag with 225 mL of buffered peptone water followed by serial dilutions. The total viable count (**TVC**) was evaluated on Plate Count Agar (Biokar Diagnostics, Beauvais, France) incubated at 30°C for 72 h. The contamination provided by *Enterobacteriaceae* was assessed by Violet Red Bile Glucose Agar (Biokar Diagnostics, Beauvais, France) incubated at 37°C for 24 h. Lactic acid bacteria (**LAB**) were analyzed on De Man, Rogosa, and Sharpe Agar (Biokar Diagnostics, Beauvais, France) in anaerobic conditions at 30°C for 48 h. The *Pseudomonas* spp. count was

evaluated on *Pseudomonas* Agar Base supplemented with cetrimide, fucidine, and cephaloridine at 25°C for 48 h (Oxoid Ltd, Basingstoke, Hampshire, UK). The count of H_2S-producing bacteria (putative *Shewanella* spp.) was carried out on iron agar (Lyngby, Laboratorios Conda, Torrejón de Ardoz, Spain) at 25°C for 48 h. Results are reported as $\log_{10}$ CFU/g meat.

Proximate Composition and Fatty Acid Profile Analyses

The *Pectoralis major* muscles sampled at 24, 72, and 264 h (corresponding to 1, 7, and 11 d of storage) were individually minced by Grindomix GM 200 (Retsch GmbH, Haan, Germany). An aliquot of fresh minced meat was analyzed for fatty acid (**FA**) composition; the remaining meat was freeze-dried, re-ground, and used to determine dry matter (934.01), ash (967.05), crude protein (2001.11), and ether extract (991.36) contents (AOAC, 2000).

Fat was extracted from fresh aliquots by accelerated solvent extraction (ASE®, Dionex, Sunnyvale, CA, USA, Application Note 334) using two extraction cycles with petroleum ether as a solvent at 125°C and 10.3 Mpa, a 6-min heating phase, and a 2-min extraction phase. Then, 10 mL of $NaSO_4$ (0.47% in H_2O) was added to extracted lipids. Samples were kept at 4°C for 30 min and supernatant (petroleum ether and lipids) was collected in another vial previously weighed. Dry evaporation in N_2 stream (Genevac EZ-2, SP Industries, Warminster, PA, USA) was applied; residual samples (extracted lipids in vials) were weighed before adding 2 mL of 2% H_2SO_4 in methanol (Christie, 1982). Vials were stored at 50°C in a heater overnight. Thereafter, the lipid rate was calculated; hexane (1 mL hexane/20 mg lipids) and potassium bicarbonate 2% (5 mL) were added. Samples were centrifuged, stored at 4°C for 30 min, and supernatant sampled to be analyzed by an Agilent 7820A Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), with a split to 92.199 mL/min and rate set at 65:1. Supelco OMEGAWAX-TM 250 (Sigma-Aldrich, St. Louis, MO, USA) (30 m × 0.25 mm internal diameter, 0.25 μm film thickness) was used with hydrogen as the carrier at 1.4 mL/min. The oven temperature was set at 50°C, held for 2 min, raised to 220°C at the rate of 4°C/min, and then held for 17 min. Both the injector and the detector temperatures were set at 250°C.

The FA was identified by comparing the retention time of the standard FA methyl esters (**FAMEs**) mixture (Supelco 37–component FAME Mix, 47,885–U). Individual FAMEs are expressed as the percentage of the total area of eluted FAMEs.

Statistical Analysis

The data from technological traits and proximate and FA composition were analyzed by ANOVA with meat type (normal, WS, and WB), storage time, and their interaction as the main effects. The PROC GLM of the Statistical Analysis System (SAS, 2013) was used for all analyses. Adjusted means were compared by Bonferroni-t test. Differences between means with $P \leq 0.05$ were accepted as statistically significant differences.

Extreme outliers were evaluated through a Boxplot in IBM SPSS Modeler V15.0.0 (International Business Machines Corporation, New Orchard Road, Armonk, New York, USA). A total of 4 data points were excluded (i.e., for *Pseudomonas* count: one normal sample at 72 h and one normal sample at 120 h; for LAB count: one WB sample at 168 h and one WS sample at 216 h).

Then, the DMFit program (DMT CO. LTD, Incheon, Korea) modeled the count of each bacteria group to calculate the following microbial growth parameters: initial value ($\log_{10}$ CFU/g), lag phase (h), maximum rate ($\log_{10}$ CFU/g/h), and final value ($\log_{10}$ CFU/g) (Baranyi and Roberts, 1994). The shelf life end was set at 7 $\log_{10}$ CFU/g for TVC and 7.3 $\log_{10}$ CFU/g for *Pseudomonas* (as specific spoilage organisms) (Raab et al., 2008; Rukchon et al., 2014).

The non-parametric combination (**NPC**) test was conducted with the free software NPC Test R10 (http://www.wiley.com/legacy/wileychi/pesarin/material.html). The partial and global P-values were calculated for microbial count profiles ($\log_{10}$ CFU/g) considering meat type and storage time. Time was also applied as a stratification block according to the NPC Test's C-sample procedure for dependent variables. Partial P-values were corrected for multiplicity, and the global P-values were obtained using the Tippet combining function.

Hierarchical clustering was applied as an agglomerative approach based on the full linkage method of PRIMER software. Non-metric multidimensional scaling plots visualized sample variability, i.e., dissimilarity between pairs of objects in a 2- or 3-dimensional space.

RESULTS AND DISCUSSION

Effect of Myopathies on Meat Quality Traits

In the present study, normal breasts were the lightest and WB meat was the heaviest (237 g vs. 312 g), whereas WS breasts showed intermediate values (280 g) ($P < 0.001$) (Table 1). Myopathy occurrence and degree have been positively associated with breast weight and thickness (Kuttappan et al., 2012a; Mudalal et al., 2015), high breast-yield genotypes, and older slaughtering age of the birds (Kuttappan et al., 2013 and 2017; Petracci et al. 2013b; Lorenzi et al., 2014; Trocino et al., 2015).

Overall, under our conditions, the presence of myopathies had little effect on the technological traits. Normal breasts showed a lower redness index than WS and WB meat (−0.88 vs. −0.41 and −0.43; $P <$ 0.001) and lower cooking losses (22.0 vs. 23.8 vs. 26.9%;

Table 1. Effect of myopathy occurrence and storage time on the technological traits of *Pectoralis major* in broiler chickens.

	Meat type[1] (M)			Storage time[2] (h) (H)						*P*-value			
	Normal	White striping	Wooden breast	24	72	120	168	216	264	M	H	M × H	RMSE[3]
Breasts (n)	48	48	48	24	24	24	24	24	24				
Weight (g)	237^{c}	280^{b}	312^{a}	277	284	272	272	270	282	<0.001	0.80	0.17	42.4
pH	6.00	6.02	6.05	6.03	6.00	6.03	6.02	6.00	6.05	0.14	0.56	0.80	0.112
L*	46.3	46.4	46.8	48.1^{a}	45.1^{b}	46.6^{a,b}	45.9^{b}	47.1^{a}	46.2^{a}	0.29	<0.001	0.54	1.84
a*	−0.88^{b}	−0.41^{a}	−0.43^{a}	−0.64	−0.64	−0.55	−0.57	−0.58	−0.47	<0.001	0.92	0.10	0.594
b*	7.28	7.79	7.77	8.28^{a}	7.64^{a,b}	7.41^{a,b}	6.98^{b}	7.41^{a,b}	7.95^{a,b}	0.11	0.02	0.89	1.327
Cooking losses[4] (%)	22.0^{c}	23.8^{b}	26.9^{a}	24.4^{b}	23.9^{b}	22.5^{c}	21.5^{c}	26.9^{a}	26.3^{a,b}	<0.001	<0.001	0.05	2.35
Shear force (kg/g)	3.74	3.80	3.39	5.08^{a}	4.15^{b}	2.83^{c}	3.39^{c}	3.50^{c}	2.94^{c}	0.12	<0.001	0.77	1.041

[1]Mean of the different storage times within each meat type.
[2]Mean of the different meat types within each storage time.
[3]RMSE, root mean square error; SEM is equal to RMSE/$\sqrt{n}$.
[4]Significant probability of the interaction Meat type × Storage time: Cooking losses, 21.0, 22.8, 22.4, 19.4, 24.7, and 23.8% in normal breasts at 24, 72, 120, 168, 216, and 264 post mortem; 22.8, 23.1, 22.3, 21.1, 26.2, and 27.2% in white striping breasts at 24, 72, 120, 168, 216, and 264 h post mortem; 29.2, 25.7, 24.7, 24.1, 29.6, and 27.8% in wooden breasts at 24, 72, 120, 168, 216, and 264 h post mortem.
[a,b,c]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 2. Effect of myopathy occurrence and storage time on the proximate composition of *Pectoralis major* in broiler chickens.

	Meat type[1] (M)			Storage time[2] (h) (H)			*P*-value			
	Normal	White striping	Wooden breast	24	168	264	M	H	M × H	SEM
Breasts (n)	24	24	24	24	24	24				
Water[3] (%)	73.4^{b}	73.6^{b}	75.0^{a}	74.8^{a}	73.8^{b}	73.4^{b}	<0.001	<0.001	<0.05	0.23
Crude protein (%)	23.9^{a}	23.2^{a}	21.4^{b}	22.2^{b}	22.6^{b}	23.6^{a}	<0.001	<0.001	0.22	0.23
Ether extract (%)	1.09^{b}	1.48^{a,b}	1.88^{a}	1.22^{b}	1.94^{a}	1.29^{b}	<0.001	<0.001	0.49	0.128
Ash (%)	1.20^{a}	1.20^{a}	1.14^{b}	1.17^{b}	1.16^{b}	1.21^{a}	<0.001	<0.001	0.18	0.010

[1]Mean of the different storage times within each meat type.
[2]Mean of the different meat types within each storage time.
[3]Significant probability of the interaction Meat type × Storage time: Water, 74.0, 73.0, and 73.2% in normal breasts at 24, 168, and 264 h post mortem; 73.8, 73.6, and 73.3% in white striping breasts at 24, 168, and 264 post mortem; 76.7, 74.7, and 73.7% in wooden breasts at 24, 168 and 264 h post mortem.
[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

$P < 0.001$) (Table 1), and the latter was also different between WS and WB meat. Previous studies used breasts within 24 to 48 h after slaughtering and showed that WS and WB meat exhibited significantly increased meat ultimate pH (Petracci et al., 2013b; Bowker and Zhuang, 2016), especially when both meat abnormalities were simultaneously present (Tasoniero et al., 2016; Zambonelli et al., 2016; Kuttappan et al., 2017).

Some authors reported no difference in lightness (Petracci et al., 2013b; Trocino et al., 2015; Tasoniero et al., 2016) or color indices (Sánchez-Brambila et al., 2016; Zambonelli et al., 2016) because of myopathies. Others observed a higher redness (present study; Petracci et al., 2013b) and yellowness index (Mudalal et al., 2015) in affected breasts compared to normal ones, which have been associated with increased fibrotic response and reduced amount of heme pigments (Petracci et al., 2017).

Consistent with the present results, there is a common consensus that myopathies increase meat cooking losses, especially in the case of WB meat (Trocino et al., 2015) and with the simultaneous presence of WS and WB (Mudalal et al., 2015; Tasoniero et al., 2016; Zambonelli et al., 2016; Soglia et al., 2016b). A lower ability of holding and binding water has been measured in WB meat compared to normal and WS meat (Tijare et al., 2016) due to the degeneration of muscle fibers and myofibrils that usually retain the majority of water (Soglia et al., 2016b).

The degeneration process in defective muscles at a histological level also affects meat chemical composition (Kuttappan et al., 2012a; Bowker and Zhuang, 2016); myodegeneration is associated with lipidosis (especially in the case of WS) and fibrosis (especially in the case of WB) (Petracci et al., 2014; Soglia et al., 2016b; Radaelli et al., 2017). In fact, in the present trial, WB meat exhibited the highest water and the lowest protein content (75.0 and 21.4%, respectively) compared to the other breasts (73.4 and 23.9% in normal, 73.6 and 23.2% in WS, respectively) ($P < 0.001$) (Table 2). Moreover, WB meat showed higher ether extract content than normal meat (1.88 vs. 1.09%; $P < 0.001$), and WS meat exhibited intermediate values. Finally, WB meat showed the lowest ash content compared to normal and WS samples (Table 2), which may be due to cellular liquid losses and altered ion homeostasis following the occurrence of breast myopathies (Petracci et al. 2014; Zambonelli et al., 2016).

In our trial, a significant interaction was found between meat quality and storage time ($P < 0.05$); water

Table 3. Effect of myopathy occurrence and storage time on the FA composition (% total FA) of *Pectoralis major* in broiler chickens.

	Meat type[1] (M)			Storage time[2] (h) (H)			*P*-value			
	Normal	White striping	Wooden breast	24	168	264	M	H	M × H	SEM
Breasts (n)	24	24	24	24	24	24				
C14:0	0.45	0.43	0.43	0.43^{a,b}	0.42^{b}	0.45^{a}	0.41	<0.05	0.43	0.008
C16:0[3]	22.8^{a}	20.4^{b}	20.4^{b}	20.9	20.9	21.7	<0.001	0.10	<0.05	0.31
C16:1 n9	0.38^{b}	0.42^{a}	0.43^{a}	0.43^{a}	0.40^{b}	0.40^{b}	<0.001	<0.05	0.82	0.006
C16:1 n7	3.58^{a}	3.19^{b}	3.14^{b}	3.32	3.52	3.08	<0.05	0.06	0.60	0.128
C18:0	7.48^{a}	6.63^{b}	6.59^{b}	6.72^{b}	6.38^{b}	7.60^{a}	<0.001	<0.001	0.35	0.126
C18:1 n9	31.8^{a}	30.7^{b}	30.8^{b}	30.8	31.7	30.8	<0.05	0.11	0.10	0.33
C18:1 n7	1.78	1.73	1.71	1.73^{a,b}	1.69^{b}	1.80^{a}	0.13	<0.05	0.34	0.020
C18:2 n6[4]	25.6^{b}	29.9^{a}	29.7^{a}	29.0	28.8	27.4	<0.001	0.12	<0.05	0.57
C18:3 n3	2.58^{b}	3.06^{a}	3.08^{a}	2.97^{a,b}	3.00^{a}	2.75^{b}	<0.001	<0.05	0.15	0.061
C20:4 n6	0.69	0.72	0.79	0.77^{a,b}	0.61^{b}	0.83^{a}	0.34	<0.001	0.27	0.061
C20:5 n3	0.07	0.06	0.08	0.07	0.07	0.08	0.27	0.20	0.99	0.005
C22:6 n3	0.07	0.07	0.08	0.08	0.07	0.08	0.21	0.89	0.84	0.006
SFA[5]	31.3^{a}	28.0^{b}	28.0^{b}	28.6^{b}	28.2^{b}	30.4^{a}	<0.001	<0.001	<0.05	0.37
UFA[6]	68.7^{b}	72.0^{a}	72.0^{a}	71.4^{a}	71.8^{a}	69.6^{b}	<0.001	<0.001	<0.05	0.37
MUFA	38.2^{a}	36.7^{b}	36.7^{b}	36.9	37.8	36.8	<0.05	0.17	0.14	0.43
PUFA[7]	30.5^{b}	35.3^{a}	35.4^{a}	34.4	34.0	32.8	<0.001	0.26	<0.05	0.69
$\sum$ n-3	2.89^{b}	3.34^{a}	3.39^{a}	3.26	3.28	3.08	<0.001	0.14	0.23	0.078
$\sum$ n-6[8]	27.4^{b}	31.7^{a}	31.7^{a}	30.9	30.4	29.5	<0.001	0.28	<0.05	0.62

SFA, saturated FA; UFA, unsaturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA.

[1]Mean of the different storage times within each meat type.

[2]Mean of the different meat types within each storage time.

[3]Significant probability of the interaction Meat type × Storage time: C16:0, 23.3, 21.9, and 23.1% in normal breasts at 24, 168, and 264 h post mortem; 19.5, 19.9, and 21.7% in white striping breasts at 24, 168, and 264 h post mortem; 19.9, 20.8, and 20.3% in wooden breasts at 24, 168, and 264 h post mortem.

[4]Significant probability of the interaction Meat type × Storage time: C18:2 n6, 25.0, 27.0, and 24.9% in normal breasts at 24, 168, and 264 h post mortem; 31.6, 30.8, and 27.2% in white striping breasts at 24, 168, and 264 h post mortem; 30.5, 28.5, and 30.2% in wooden breasts at 24, 168 and 264 h post mortem.

[5]Significant probability of the interaction Meat type × Storage time: SFA, 31.8, 29.6, and 32.4% in normal breasts at 24, 168, and 264 h post mortem; 26.7, 27.1, and 30.2% in white striping breasts at 24, 168 and 264 h post mortem; 27.4, 27.9, and 28.5% in wooden breasts at 24, 168, and 264 h post mortem.

[6]Significant probability of the interaction Meat type × Storage time: UFA, 68.2, 70.4, and 67.6% in normal breasts at 24, 168, and 264 h post mortem; 73.3, 72.9, and 69.8% in white striping breasts at 24, 168, and 264 h post mortem; 72.6, 72.0, and 71.5% in wooden breasts at 24, 168, and 264 h post mortem.

[7]Significant probability of the interaction Meat type × Storage time: PUFA, 29.6, 31.9, and 30.0% in normal breasts at 24, 168 and 264 h post mortem; 37.2, 36.3, and 32.5% in white striping breasts at 24, 168, and 264 h post mortem; 36.4, 33.7, and 36.1% in wooden breasts at 24, 168, and 264 h post mortem.

[8]Significant probability of the interaction Meat type × Storage time: $\sum$ n6, 26.5, 28.6, and 27.0% in normal breasts at 24, 168, and 264 h post mortem; 33.4, 32.5, and 29.1% in white striping breasts at 24, 168, and 264 h post mortem; 32.7, 30.1, and 32.3% in wooden breasts at 24, 168, and 264 h post mortem.

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

content was higher in WB (76.7%) than in normal and WS meat (74.0 and 73.8%, respectively) only 24 h after slaughtering, whereas no differences among groups were found at 168 or 264 h post mortem (Table 2). Likely, protein denaturation during storage altered the water holding capacity of meat (Hughes et al., 2014; Warner, 2017), which in turn increased water losses and reduced water content of all samples, as described during chilling and frozen storage of lamb meat and pork (Coombs et al., 2018; Medić et al., 2018).

The FA profile was similar in WS and WB meat. Compared to normal breasts, WB and WS samples exhibited lower saturated FA (**SFA**) (31.3 vs. 28.0 and 28.0%, respectively) and higher unsaturated FA (**UFA**) (68.7 vs. 72.0 and 72.0%, respectively) and therefore a lower SFA/UFA ratio (0.46 vs. 0.39 and 0.39, respectively) ($P < 0.001$) (Table 3). Differences found in UFA were mainly due to variations in polyunsaturated FA (PUFA), which were higher in affected than in normal breasts (35.3% in WS and 35.4% in WB vs. 30.5% in normal, respectively; $P < 0.001$). Conversely, monounsaturated FA (**MUFA**) was lower in WS and WB meat (36.7%) than in normal meat (38.2%) ($P < 0.05$). Furthermore, both n-3 and n-6 FA proportions were higher in WB and WS than in normal samples ($P < 0.001$).

Among individual FAs, palmitoleic (C16:1 n7) and oleic (C18:1 n9) acids primarily accounted for differences in MUFAs between affected and normal meat ($P < 0.05$); among the PUFAs, linoleic (C18:2 n6) and α-linolenic (C18:3 n3) acids changed the most (Table 3).

Poultry meat has a high nutritional value because of its high protein and low lipid contents as well as its high PUFA rate. As measured in our data, linoleic acid (C18:2 n6) and α-linolenic acid (C18:3 n3) contribute to the high UFA rate (Grashorn, 2017). Indeed, FA composition of the meat is notoriously influenced by the lipid dietary intake (Liburn, 2017), but little data are available on the effect of myopathies on FA meat composition. Some authors found higher SFA rates in normal breasts than in WS and WB meat (Kuttappan et al.,

2012a; Soglia et al., 2016a), consistent with the results of the present trial, whereas others (Traffano-Schiffo et al., 2017) observed an opposite trend. Soglia et al. (2016a) did not find any significant difference in total MUFA or PUFA. Nevertheless, they found that the linoleic acid (C18:2 n6) rate was lower in normal compared to WB and WS/WB breasts, and the α-linolenic acid (C18:3 n3) rate was lower in normal breasts compared to WS/WB breasts. These last findings are consistent with the results of Kuttappan et al. (2012a) and those of the present study.

A comparison with the results of Traffano-Schiffo et al. (2017) is difficult because they measured FA composition by Differential Scanning Calorimetry on the superficial meat layers. They found that WS samples had approximately 5% SFA, 23% PUFA, and 72% MUFA, whereas normal breasts had no SFA, 76% PUFA, and 24% MUFA.

The increased PUFA content in WS and WB meat also reported by other authors for the whole muscle has been associated with sarcolemma damages, inflammatory processes, and consequent myopathy development (Kuttappan et al., 2012a; Soglia et al., 2016a). Cells involved in the inflammatory response usually contain a relatively high proportion of the n-6 arachidonic acid in their membrane phospholipids (Calder, 2012). Moreover, the degeneration and regeneration processes of muscle fibers in affected breasts (Daughtry et al., 2017) may bring about some changes in muscle fiber metabolism (Pette and Staron, 2000; Gošnak et al., 2010) and as a consequence, in meat FA composition. Normally, the *Pectoralis major* in chickens comprises mostly or exclusively fast, glycolytic IIB fibers (Chauhan and England, 2018), in which lipids are generally more saturated than in those within slow and oxidative fibers (Alasnier et al., 1996; Realini et al., 2013). However, in case of myopathies, a reduced glycolytic potential has been reported (Abasht et al., 2016). Thus, a fiber-type switching (Mutryn et al., 2015) and a shift towards oxidative metabolism could be hypothesized (Chauhan and England, 2018), but this has not been proven (Kuttappan et al., 2017). Further research is necessary to clarify the mechanism and the causes of changes in the FA profile of poultry meat affected by myodegeneration.

Effect of Storage Time on Meat Quality Traits

Under our conditions, meat pH remained stable until the end of storage (11 d). A longer time (15 d) would be necessary to record increased pH as a consequence of the accumulation of protein degradation products (Marcinkowska-Lesiak et al., 2016).

The L^* index decreased from 24 h (48.1) to the following sampling times (45.9 on average), but it significantly increased again (47.1) at 216 h ($P < 0.001$) (Table 1). The b^* index showed the highest value after 24 h and the lowest value after 168 h (8.28 vs. 6.98; $P < 0.05$), without significant differences between the other sampling times. Cooking losses during the first 72 h post mortem averaged 24.2%, decreased until 22.0% between 120 h and 168 h, and finally reached an average of 26.6% between 216 and 264 h ($P < 0.001$). The differences among storage times were different according to meat type (probability of the interaction, $P = 0.05$) (Table 1). Cooking losses of WB meat significantly changed from 29.2% at the first sampling time (24 h) to lower values between 72 h and 168 h (24.8% on average), and finally increased again at 216 h and 264 h (29.6 and 27.8%, respectively). Lastly, the highest shear force was measured at 24 h of storage time (5.08 kg/g), decreased by 72 h, and reached the lowest value by 120 h (2.83 kg/g; $P < 0.001$). From this time onwards, shear force remained stable (Table 1).

Changes in lightness, cooking losses, and shear force during storage were consistent with changes in water retention ability and myofibrils structure of the meat. The high initial meat lightness and cooking losses likely depend on the high rate of free water in meat; during storage, there is a loss of juice (drip loss), which decreases the proportion of free water. Finally, lightness and cooking losses increased again likely because the water previously bound to myofibrils was released following protein denaturation due to oxidation and microbial spoilage. Myofibrils changes also explain the reduction of meat shear force during storage, as observed in previous studies (Soglia et al., 2017; Sun et al., 2018) both in normal and WB meat. Corresponding to our results, Sun et al. (2018) also found a similar reduction in the compression force over an 8-d storage time at 4°C in all breast categories (normal, mild, and moderate/severe WB), and higher changes in drip loss in moderate/severe WB meat than in normal breasts over time.

Meat water content was higher at 24 h and significantly decreased by 168 and 264 h post mortem (74.8 vs. 73.8 and 73.4%, respectively; $P < 0.001$) (Table 2). On the contrary, crude protein content was lower at 24 and 168 h and increased by 264 h (22.2 and 22.6 vs. 23.6%, respectively; $P < 0.001$). Similar changes were observed for ash content ($P < 0.001$). Variations in ether extract content, despite being significant ($P < 0.001$), were in a narrow range. Likely, changes in water content depended on the higher drip losses of aged meat, which in turn may have increased the relative proportion of crude protein in meat after 264 h, consistently with previous results (Coombs et al., 2018; Medić et al., 2018).

With increasing storage time, chicken breasts also changed their FA profile (Table 3). The SFA rate increased (28.6 and 28.2% at 24 and 168 h vs. 30.4% at 264 h; $P < 0.001$) due to increased rates of C16:0 (20.9 to 21.7%; $P = 0.10$) and C18:0 from 24 and 168 to 264 h (6.72 and 6.38 vs. 7.60%; $P < 0.001$). The most abundant UFAs were oleic acid (C18:1 n9) and linoleic acid (C18:2 n6), and their rates did not change

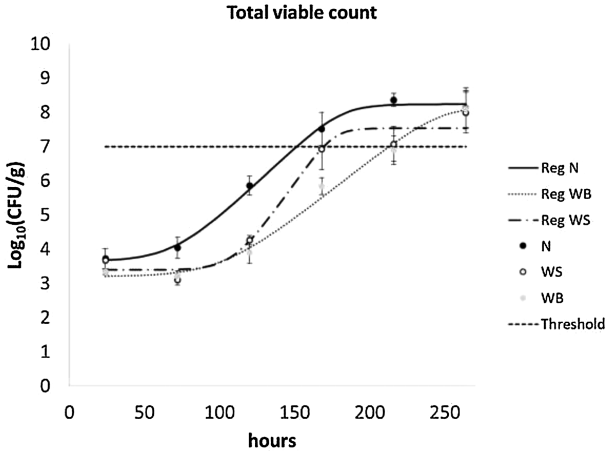

Figure 1. Total viable counts (means ± SD) and model fit in normal (N) chicken meat and in meat affected by white striping (WS) and wooden breast (WB). Threshold was set to 7 log10 CFU/g according to Rukchon et al. (2014).

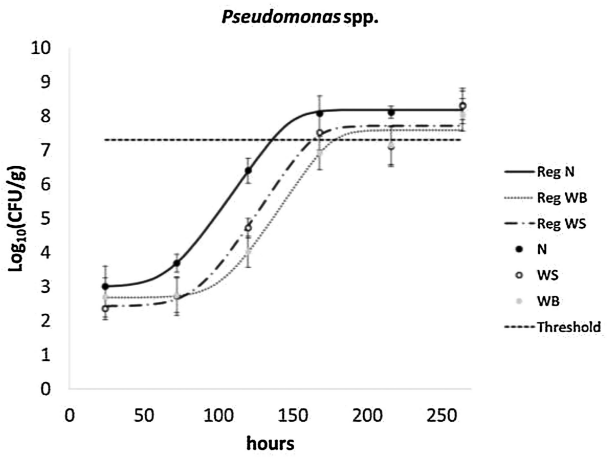

Figure 2. *Pseudomonas* spp. counts (means ± SD) and model fit in normal (N) chicken meat and in meat affected by white striping (WS) and wooden breast (WB). Threshold was set to 7 log10 CFU/g according to Raab et al. (2008).

over time. Nevertheless, small changes in other MUFAs and PUFAs accounted for a reduction in UFA rates from 24 and 168 to 264 h (71.4 and 71.8 vs. 69.6%; $P < 0.001$). Some differences were recorded across meat type (probability of the interaction meat type × storage time, $P < 0.05$). Normal breasts had lower SFA and C16:0 rates and higher UFA rates at 168 h than at 24 or 264 h; WS meat showed a lower C18:2 n6 rate and consequently, lower PUFA and n-6 rates at the end of storage (264 h). These changes were not observed in the other meat types.

The increase in SFA rate with storage time may be due to changes in MUFA and especially PUFA contents, which are expected to decrease during storage because of lipid oxidation (Wood, 2017; Medić et al., 2018).

Microbiological Shelf Life of Meat

Under optimal chilling conditions, fresh poultry meat can have 4 to 10 d of shelf life depending on the packaging solution (Patsias et al., 2008; Raab et al., 2008; Vasconcelos et al., 2014). Nevertheless, among food, poultry meat has the highest pathogenic and spoilage bacteria counts (Galarz et al., 2016) and the liable organisms for spoilage are *Pseudomonas* spp., *Brochothrix thermosphacta*, *Shewanella putrefaciens, Acinetobacter, Moraxella, Psychrobacter* spp., including *A. johnsonii* and *P. immobilis*, and *Enterobacteriaceae* spp. (Mead, 2004; Hinton, 2017; Rouger et al., 2017).

Indicators used to estimate poultry meat shelf life may differ according to storage conditions (Nychas et al., 2007; Doulgeraki et al., 2012; Rossaint et al., 2015). Among them, TVC and *Pseudomonas* spp. are the most applied descriptors when meat is stored under aerobic conditions (Bruckner et al., 2012; Hinton, 2017; Rouger et al., 2017). In the present trial, microbial growth parameters of TVC and *Pseudomonas* spp. were different between normal and affected meat during storage (Figures1 and 2). In fact, normal breasts had the highest initial TVC value and the shortest TVC lag phase in comparison to WS and WB meat (Figure 1, Table 4). Differences in lag phases indicate that WS and WB samples underwent a later microbial spoilage, and their exponential growth started after 85.2 and 77.8 h of storage time, respectively, while the normal ones started at 46.3 h. Therefore, a shelf life threshold (7 $\log_{10}$ CFU TVC/g) was achieved sooner in normal breasts (130 h) than in WS (149 h) and WB (192 h) meat. After this threshold was reached, the TVC final count was similar in normal and WB meat, but lower in WS samples (Table 4).

Pseudomonas spp. are commonly considered a specific spoilage organism for several types of animal-origin food, mainly pork, poultry, and seafood. This microbial target is directly involved in spoilage mechanisms associated with meat protein degradation and production of unpleasant flavors, off-odors, abnormal colors, and slimes (Andreani and Fasolato, 2017; Hinton, 2017). The *Pseudomonas* spp. count was initially similar in normal and WS meat compared with WB meat; during storage, normal breasts showed a shorter lag phase (42.0 vs. 60.9 and 77.4 h, respectively) (Figure 2, Table 4). The spoilage threshold proposed by Raab et al. (2008) for *Pseudomonas* spp. (7.3 $\log_{10}$ CFU/g) was achieved first in normal breasts compared to WS and WB meat (110 vs. 139 and 154 h, respectively). The estimated shelf life obtained in this work for normal and abnormal breasts is consistent with previous data for poultry meat stored under different conditions (chilling at 4°C, freeze chilling, packaging with or without modified atmosphere) (Patsias et al., 2008; Raab et al., 2008).

A larger sample size per meat type at each storage time could have been used to calculate microbial growth parameters. However, differences among meat types and storage times were confirmed by the NPC test, a robust multivariate statistical approach (Pesarin and Salmaso, 2010).

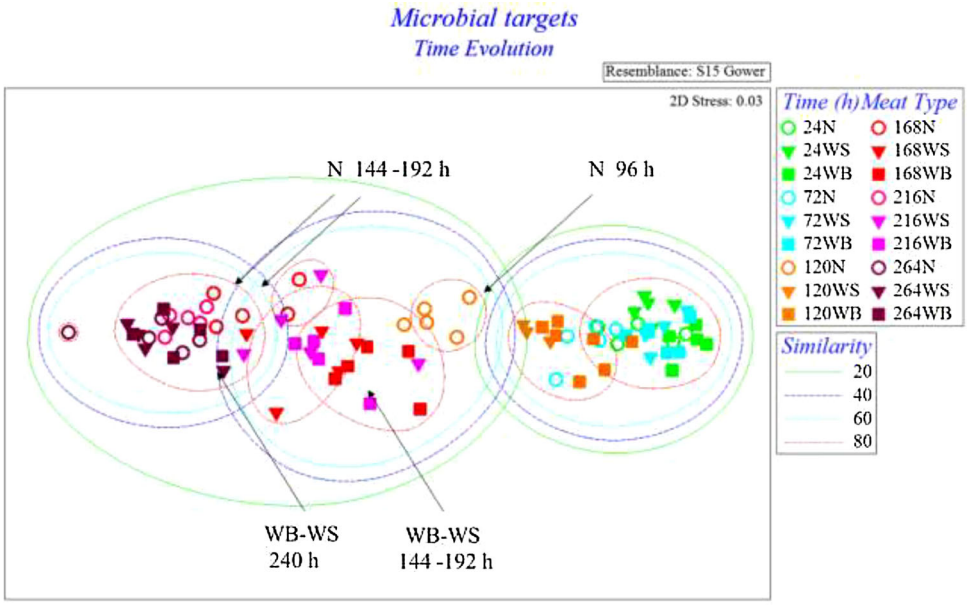

Figure 3. Non-metric multidimensional scaling plot overlapped with the similarities obtained by hierarchical cluster analysis of samples. Data are labeled according to the storage time (different colors); Circles = N, normal breasts; Triangles = WS, white striping breasts; Square = WB, wooden breasts. Some peculiar clusters were highlighted with arrows (h, h post mortem).

Table 4. Estimated growth parameters (± SE) of total viable counts and *Pseudomonas* spp. of *Pectoralis major* in normal, white striping and wooden breasts of broiler chickens.

	Normal	White striping	Wooden breast
Breasts (n)	30	30	30
Total viable counts (TVC)			
Initial value (log_{10} CFU/g)	3.70 ± 0.17	3.39 ± 0.17	3.20 ± 0.15
Lag phase (h)	46.3 ± 9.36	85.2 ± 9.44	77.8 ± 8.80
Maximum rate (log_{10} CFU/g/h)	0.040 ± 0.004	0.062 ± 0.001	0.035 ± 0.003
Final value (log_{10} CFU/g)	8.24 ± 0.13	7.53 ± 0.17	8.21 ± 0.26
Shelf life (h (d))	130 (5)	149 (6)	192 (8)
R^2	0.96	0.93	0.96
SE fit	0.39	0.54	0.37
Pseudomonas spp.			
Initial value (log_{10} CFU/g)	3.00 ± 0.30	2.43 ± 0.23	2.69 ± 0.19
Lag phase (h)	42.0 ± 7.55	60.9 ± 12.4	77.4 ± 6.9
Maximum rate (log_{10} CFU/g/h)	0.063 ± 0.008	0.067 ± 0.015	0.066 ± 0.009
Final value (log_{10} CFU/g)	8.18 ± 0.11	7.72 ± 0.19	7.59 ± 0.18
Shelf life (h (d))	110 (5)	139 (6)	154 (6)
R^2	0.97	0.94	0.94
SE fit	0.40	0.61	0.55

The global *P*-value of the full model, which tested the effects of meat type and storage time, showed an overall effect on the different microbial target counts ($P < 0.001$) (Table 5). For the single effects, meat type was significant only for *Enterobacteriaceae* spp. count, whereas storage time was significant for all targets. Nevertheless, the NPC tests among meat types stratified by storage time showed that TVC and *Pseudomonas* spp. counts significantly changed with meat type between 72 and 216 h of storage (Table 5). During this period, normal meat showed the highest microbial counts compared to breasts affected by myopathies. Moreover, NPC tests showed that *Enterobacteriaceae* spp. and LAB counts significantly changed with meat type since the first sampling, after 24 h of storage, and until 216 h. Overall, normal meat displayed the highest load, WB meat had the lowest one, and WS meat exhibited intermediate values. Nevertheless, all differences in microbial targets across meat types disappeared by 264 h of storage (i.e., 11 d). The literature reports *Pseudomonas* spp. as dominant in poultry meat stored under aerobic conditions (Vasconcelos et al., 2014; Hinton, 2017; Rouger et al., 2017), but in our trial most of the microbial targets reached 7 log_{10} CFU/g by the end of storage (Table 5).

Lastly, the non-metric multidimensional scaling plot in Figure 3, based on all microbial targets, shows two main cluster groups. The first cluster includes all samples at 24 and 72 h of storage associated with WS and WB samples at 120 h (20% similarity). The second cluster includes all other samples (20% similarity). Within the first cluster, samples at 24 h are separated from

Table 5. Effect of myopathy occurrence and storage time on microbial targets (mean ± SD) (log_{10} CFU/g) of *Pectoralis major* in broiler chickens (global and partial *P*-values are referred to the non-parametric combination test).

Storage time (h)	Meat type	TVC[1]	*Pseudomonas* spp.	H_2S producer	*Enterobacteriaceae*	LAB[2]	Global *P*-value
Tests among meat types stratified by storage time							<0.001
24	Normal	3.72 ± 0.30	3.01 ± 0.58	2.58 ± 0.14	2.45 ± 0.32	3.22 ± 0.44	
	White striping	3.67 ± 0.34	2.36 ± 0.32	2.44 ± 0.18	2.32 ± 0.30	3.63 ± 0.29	
	Wooden breast	3.34 ± 0.09	2.68 ± 0.57	2.12 ± 0.72	1.84 ± 0.29	2.85 ± 0.27	
	P-value	0.08	0.17	0.15	<0.001	<0.01	
72	Normal	4.04 ± 0.31	3.68 ± 0.25	2.90 ± 0.36	2.96 ± 0.06	3.61 ± 0.16	
	White striping	3.10 ± 0.16	2.71 ± 0.56	2.60 ± 0.42	2.19 ± 0.22	3.47 ± 0.22	
	Wooden breast	3.21 ± 0.14	2.70 ± 0.51	2.59 ± 0.35	2.17 ± 0.42	3.22 ± 0.40	
	P-value	<0.001	<0.001	0.37	<0.001	0.13	
120	Normal	5.85 ± 0.28	6.40 ± 0.13	3.69 ± 0.17	4.26 ± 0.45	4.74 ± 0.37	
	White striping	4.26 ± 0.13	4.71 ± 0.28	3.78 ± 0.46	3.44 ± 0.43	3.95 ± 0.11	
	Wooden breast	3.90 ± 0.31	4.02 ± 0.45	3.48 ± 0.47	3.01 ± 0.24	3.43 ± 0.55	
	P-value	<0.001	<0.001	0.50	<0.001	<0.001	
168	Normal	7.51 ± 0.48	8.07 ± 0.51	5.62 ± 0.70	6.32 ± 0.22	5.69 ± 0.60	
	White striping	6.92 ± 0.51	7.51 ± 0.51	5.99 ± 0.94	4.93 ± 0.55	5.11 ± 0.43	
	Wooden breast	5.83 ± 0.25	6.91 ± 0.48	5.44 ± 0.46	4.56 ± 0.66	4.84 ± 0.06	
	P-value	<0.001	<0.01	0.51	<0.001	<0.05	
216	Normal	8.10 ± 0.64	8.03 ± 0.24	6.22 ± 0.44	6.40 ± 1.02	6.52 ± 0.36	
	White striping	7.07 ± 0.51	7.11 ± 0.58	5.23 ± 0.98	5.23 ± 1.00	5.91 ± 0.19	
	Wooden breast	6.90 ± 0.48	7.01 ± 0.57	5.48 ± 0.27	4.11 ± 1.00	5.40 ± 0.56	
	P-value	<0.05	<0.01	0.06	<0.01	<0.05	
264	Normal	8.11 ± 0.59	8.31 ± 0.41	6.83 ± 0.28	7.48 ± 0.46	6.97 ± 0.80	
	White striping	7.98 ± 0.59	8.29 ± 0.51	6.82 ± 0.21	7.09 ± 0.60	7.07 ± 0.39	
	Wooden breast	8.09 ± 0.54	8.03 ± 0.47	6.77 ± 0.20	6.71 ± 0.58	6.80 ± 0.43	
	P-value	0.93	0.57	0.92	0.13	0.77	
Tests among levels of meat type and storage time factors							
Meat type	Partial *P*-value	0.15	0.08	0.75	<0.01	0.07	<0.05
Storage time	Partial *P*-value	<0.01	<0.001	<0.01	<0.001	<0.001	<0.001

[1]TVC, total viable counts; [2]Lactic acid bacteria.

samples at 120 h (80% similarity). Within the second cluster, normal breasts at 120 h are grouped with WS and WB samples analyzed at 168 h and 216 h (60% similarity), and normal breasts at 216 h are grouped with samples analyzed at 264 h (80% similarity).

The microbiological profile of poultry meat affected by myopathies has not been widely investigated. The only study available to our knowledge (Dalgaard et al., 2018) used storage conditions that were different from ours. These authors evaluated the microbiological profile of normal and moderate or severe WB breasts after 6 and 8 d of storage at 4°C in modified atmosphere packaging (70% nitrogen, 30% CO_2). Under these conditions, at the end of the storage, the TVC on the meat surface did not differ between normal and WB meat. Nevertheless, *Enterobacteriaceae* spp. counts in severe WB samples significantly increased from 6 to 8 d of storage. According to the same authors, alterations in muscle tissue, moisture content, mobile water fraction, drip loss, and pH affected the microflora growth and composition in WB samples stored in modified atmosphere packaging, favoring *Enterobacteriaceae* spp. and microflora diversification.

On average, microbial counts 24 h after slaughtering were consistent with previous data collected within 24 h after slaughtering (Raab et al., 2008; Vasconcelos et al., 2014; Rouger et al., 2017). Moreover, based on the sampling procedure, no difference in initial contamination of the different meat types was likely, which could have affected shelf life. Thus, differences in microbial counts at 24 h and shelf life evolution depended on the meat type itself. Water holding capacity, protein, and collagen quality of the meat are known to affect microbial proliferation (Maxcy, 1981). The alteration in muscle tissues of WS and WB meat modifies muscle fiber structure and environment, which affects the selection of ephemeral and specific microbial association and consequent spoilage behaviors (Nychas et al., 2007; Dalgaard et al., 2018). The growth of some foodborne bacteria (specifically *E. coli* O157:H7) is dependent on their ability to adhere to muscle fiber extracellular matrix (endomysium, perimysium, epimysium) (Chagnot et al., 2017). Different muscle fiber structure and a lower glycolytic potential is usually associated with abnormal meat (Chauhan and England, 2018) and could have reduced the availability of nutrients (glucose, acid lactic, low-molecular weight soluble compounds, nitrogen compounds) for microbial growth (Nychas et al., 2007) and increased the meat shelf life in our trial. Alternately, in the present study, meat pH did not differ among meat types and thus did not play a specific role in controlling microbial spoilage, such as in DFD meat (Nychas et al., 2007). Finally, in our study, differences in FA composition between normal and affected meat could have decreased shelf life in the former compared to the latter. In fact, antimicrobial activities of long-chain UFAs compared to SFAs are documented (Zheng et al., 2005). These FAs have inhibitory effects both on Gram (+) and Gram (−) bacteria (Lee et al., 2002), likely due to their action in bacterial cell membranes, and changes

in FA composition are being investigated as a strategy to slow down or prevent food spoilage (Lee et al., 2002; Zheng et al., 2005; Desbois and Smith, 2010).

In conclusion, both normal and abnormal meat exhibit similar evolution during storage at 4°C under aerobic conditions, but surprisingly, the microbial shelf life is shorter in normal than in abnormal meat. Further studies are necessary to elucidate the factors and the mechanisms that may modulate microbial growth and composition during storage of poultry meat affected by myopathies.

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