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PATHOMORPHOLOGICAL AND MEAT QUALITY ALTERATIONS CONNECTED WITH WOODEN BREAST IN BROILER CHICKENS OF DIFFERENT GENOTYPES AND SLAUGHTER AGES

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This study examined pathomorphological changes and meat quality alterations associated with Wooden Breast Myopathy (WB) in total of 192 broiler chickens divided into Ross 308 (n=96) and Cobb 500 (n=96) heavy hybrids at ages 42, 60, and 70 days. WB occurrence remained consistently high (>73%) across periods, peaking on day 70 (83% for Ross, 90% for Cobb). Cobb broilers had better production results and carcass traits parameters after day 42 and day 60 of the experiment ($p \leq 0.05$). Genotype did not affect WB occurrence or severity, while slaughter age influenced severe cases WB occurrence, increasing from 11.67% on day 42 to 36.67% on day 70 ($p=0.003$). The presence of WB was associated with higher ultimate pH, lightness (L*), redness (a*), and yellowness (b*) of the muscle ($p<0.0001$), except on day 70. Physicochemical and color parameters were also influenced by slaughter age ($p<0.0001$). On day 42, drip loss ($p<0.0001$), cooking loss ($p \leq 0.05$) and shear force ($p<0.0001$) were affected by genotype. On days 60 and 70, the differences in water retention capacity were observed only between normal and severely affected breasts ($p<0.0001$). For each slaughter age severely affected WB had higher shear force compared to normal breasts ($p<0.0001$). Additionally, with the increasing slaughter age of broilers, drip loss, cooking loss, and shear force of the breast meat were increased ($p<0.0001$). The results obtained regarding the occurrence and severity of WB and its consequent meat quality alterations suggest that extended fattening is not recommended for poultry production.

Keywords: broiler chicken, wooden breast myopathy, myodegeneration, meat quality, prolonged fattening.

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INTRODUCTION

Selection of broilers for fast growth and high yield led to rapid muscle hypertrophy coupled with inadequate vascularization and an increase in white striping (WS), wooden breasts (WB), and spaghetti meat (SM) myopathies [1,2]. These muscle abnormalities affect the *pectoralis major* (*p. major*) muscle, the most valuable chicken part in the broiler industry, resulting in the downgrading of broiler breasts and enormous economic losses to the poultry industry worldwide [3]. Macroscopically, a WB is characterized by palpably hard, pale ridge-like bulges, accompanied by a clear, viscous fluid, small hemorrhages, and white striping, which may manifest individually or concurrently. The underlying histological lesions of WB are polyphasic myodegeneration characterized by degenerative and atrophic fibers, vacuolar degeneration, occasional regeneration, mononuclear cell infiltration, interstitial inflammation, and accompanying fibrosis and lipidosis [4]. Wooden breast myopathy impairs not only breast meat appearance, but also its quality. Due to visual defects and unappealing texture, consumers exhibit low acceptance of WB fillets as raw meat products. Furthermore, the compromised functional properties resulting from increased fat and connective tissue, along with the loss of functional proteins, restrict further processing and decrease the nutritional value of abnormal meat [1,5-7]. The occurrence of WB myopathy is most frequent in fast-growing, high-breast-yielding strains of broilers. The onset and severity of WB also seem to be higher in broilers that are male, fed high-energy diets, or slaughtered at older ages and heavier weights [8-12].

Despite the fact that WB myopathy is a worldwide problem, to author's knowledge, there are no available reports on the occurrence of WB in broilers in Serbia. Given that the broiler rearing system in Serbian households relies on an extended fattening period for broilers, with some birds reaching up to 10 weeks of age, the objective of this study was to compare the occurrence and severity of wooden breast myopathy in the two most common fast-growing broiler lines in Serbia, Ross 308 and Cobb 500, at 42, 60 and 70 days (d) of age. Furthermore, histological features and meat quality of normal broilers and broilers affected by WB were compared.

MATERIALS AND METHODS

The study was approved by the Ethical Committee for Animal Experimentation (Faculty of Veterinary Medicine) of the University of Belgrade, Serbia (Approval number: 10-5/2022).

Animals, housing and diets

The experiment was conducted at the registered animal breeding facility, during the period April – June 2023, Obrenovac, Ljubinić (44°32'17"N 20°03'19"E). A total of 192 one-day-old chickens of both sexes were taken from the same incubator station

(Agrovet d.o.o., Žička 8, Beograd) by authorized transport means to the farm. Half of the chicks (96) belonged to a Ross 308 hybrid; the other half were from Cobb 500 genotype. On arrival, chicks were individually weighed and 16 chicks were housed in each of 12 pens made of solid wood and a fence (stocking density 6 birds/m^2), randomly allocated to experimental groups, i.e., 2 genotypes (Ross 308 *vs*. Cobb 500) × 3 slaughtering ages (42 d, 60 d and 70 d). Each pen had a concrete floor covered with 5 cm wood shaving. The temperature in the room was 32°C from days 1 to 5, then gradually lowered to 22°C on day 21. This temperature was maintained until the end of the study. The artificial lighting program 16 hours of light, 8 hours of dark during all experimental periods was used. To enable broilers to reach their maximum production potential, water and feed were available *ad libitum*. The feeder and drinker were circular with manual filling. During the experiment, the birds were fed standard commercial diets based on corn and soybean meal (Table 1) in a mash and granulated form of the rearing phases: starter (1–21 days) and finisher (22-70 days). Health conditions and mortality were monitored throughout the experiment. Individual weights were recorded at different ages, i.e., days 0, 21, 42, 60, and 70 and weight gain was calculated for each period.

Slaughter procedure

A total of 30 birds per genotype at 42, 60 and 70 d of age (180 in total) were slaughtered in a commercial slaughterhouse, after about 8h of feed withdrawal and about 4h of water withdrawal. Immediately before slaughter, individual measurements were performed and after electric stunning, broilers were slaughtered by cutting the jugular veins. After 24h chilling, the carcasses were measured and subjected to gross examination to evaluate the occurrence and degree of WB in the *p. major* muscles. Then, the carcasses were cut into basic parts and deboned breast meat (fillets) was taken for further histology and meat quality assessment.

Macroscopic scoring

Immediately after chilling, the separated pectoral musculature was categorized based on the degree of expression of WB changes according to Tijare et al. [6] by bilateral palpation of the breast meat *(p. major*) in the craniocaudal direction. Depending on the degree of hardness and the area affected by the change, breast fillets were divided into four categories (Figure 1): grade 0 (normal) = fillets of elastic consistency, without any changes in color or consistency; grade 1 (**mild**) = normal fillets with the subtle focal changes that were harder in consistency and pale in color, mostly in the cranial part compared to rest of the muscle; grade 2 (**moderate**) = changes in the cranial part of the muscle more pronounced than in the previous category, covering the entire cranial part of *p. major* which is much harder in consistency and pale in color. The middle and caudal part of the muscle without changes; grade 3 (**severe**) = extremely hard and rigid fillets with diffuse changes, affecting the entire muscle; the presence of transparent,

mucous content on the surface of the fillet can also be noticed**.** After scoring, each fillet was individually weighed, photographed on top of a white sheet labeled with an individual case identification number and subjected to histology evaluation.

Table 1. Ingredient composition and calculated analysis of the diets

	Starter (1-21)	**Finisher (22-70)**
Ingredients (%)		
Maize	29.50	44.27
Wheat	9.50	13.80
Triticale	13.00	9.70
Soy grits	12.00	8.50
Soybean meal (CP 44%)	15.00	6.50
Soybean cake (CP 41.5%)	13.00	7.80
Sunflower meal (CP 33%)	4.00	6.30
Monocalcium phosphate	0.20	0.20
Chalk	1.10	0.60
Salt	0.45	0.45
Premix[1]	1.00	1.00
Lysine	0.50	0.22
Methionine	0.25	0.16
Adsorbent	0.50	0.50
Total	100.0	100.0
Calculated analyses (%)		
AME_n (MJ/kg)	12.62	13.37
Crude protein	22.14	17.15
Moisture	10.98	11.56
Total ash	7.65	5.90
Total lipids	6.14	3.95
Crude fiber	4.89	4.70
Calcium	1.15	1.21
Phosphorus	0.72	0.84
Lysine	1.36	0.90
Methionine+ Cysteine	0.79	0.66
Tryptophan	0.35	0.23
NFE[2]	48.20	56.74

[1] Mineral- vitamin premix provided the following per kg of diet: Vitamin A 12500 IU; Vitamin D3 2000 IU; Vitamin E 40 IU; Vitamin B1 3.76 mg; Vitamin B2 6 mg; Vitamin B6 3.76 mg; Vitamin B12 0.02 mg; Vitamin K3 3.78 mg; Choline 400 mg; Nicotinic acid 62.5 mg; Pantothenic acid 10 mg; Folic acid 1.25 mg; Zinc 50 mg; Manganese 80 mg; Iron 40 mg; Copper 8 mg; Iodine 0.8 mg; Selenium 0.15 mg; Antioxidant 125 mg; [2] Nitrogen Free Extract

Histology

For histology evaluation of WB myopathy, samples of pectoral musculature were taken from all macroscopically examined fillets. As WB is considered to be a bilateral disease and predominantly manifest in the cranial section of the fillet, samples for histological analysis were taken from the cranial part of the right *m. pectoralis major superficialis*, size 1×1×1 cm. All tissue samples were fixed in 10% buffered neutral formalin, standard processed and embedded in paraffin blocks, then cut at a 4 μm thickness for evaluation of the muscle fibers morphology, presence of myodegeneration and regeneration using hematoxylin and eosin (H&E) staining. Evaluation of the perimysial and endomysial distribution of connective tissue, specifically collagen was achieved using Masson's Trichrome staining. Immunohistochemical staining was conducted using EnVision FLEX Mini Kit based on Peroxidase/DAB method [13] with anti-CD3 antibody (monoclonal mouse anti–human F.7.2.38; DAKO, Glostrup, Denmark) and anti-CD21 antibody (monoclonal mouse anti–human Clone 1F8 ready to use; DAKO Glostrup, Denmark).

The histological scoring was conducted with a light microscope (BX51, Olympus Optical, Japan), and pictures were taken with Olympus Color View III® digital camera.

Myopathic lesions were scored using four scale system by Trocino et al. [11]: grade 0 (normal) = presented samples with typical polygonal shape and noticeable cross striation of fiber on cross-section, normal or central nuclei, without necrotic fibers or infiltration of connective tissues; grade 1 (mild) = represents the morphology of fibers as in score 0, but can be detected some fibers with hyaline cytoplasm and central nuclei or necrotic fibers, connective tissue infiltration normally distributed in endomysium and perimysium; grade 2 (moderate) = samples with diffusely organized necrotic fibers, evident thickening of interstitial connective tissue, detection of inflammatory cells, and aggregates of adipocytes; the highest score, grade 3 (severe) = samples that exhibited a significant amount of interstitial connective tissue, myofibrillar degeneration represented with hyalinized fibers that have lost cross striation, as well necrotic and fragmented fibers of various size with surrounding macrophages or heterophilic granulocytes, and great amount of adipose tissue).

Meat Quality Analysis

The L*a*b* color indexes were measured on the ventral side of the fillet (L* – lightness, a*-redness, and b*-yellowness) using a Minolta CM-2600d spectrophotometer (Konica Minolta, Ramsey, NJ, USA). For each sample, the meat color result was determined as the average of three measurements taken from parts of the fillets that were devoid of obvious color defects such as bruises, discolorations, hemorrhages, or any other conditions that might have interfered with uniform color readings. The meat pH was measured in triplicate on the cranial parts of fillets using a portable pH meter (Testo 205, Testo AG, Lenzkirch, Germany). Drip loss in meat samples from the *p. major* muscle was assessed using the "bag" drip loss method [14]. Briefly, each meat sample

was weighed and stored for 24h at 4°C in a container. After storage, the meat samples were reweighed, and the percentage of drip loss was calculated. Cooking loss was assessed by initially weighing each sample individually. Subsequently, the samples were wrapped in aluminum foil and placed in a convection air oven once the temperature reached the predetermined value of 175°C. The cooking temperature was maintained for a duration of 45 minutes. After completion of the cooking process, the samples were allowed to cool at room temperature for one hour before being subjected to analysis for cooking loss. This was expressed as the percentage difference in meat weight after cooking, with the initial weight before cooking considered as 100%. Shear force evaluation was conducted on cylinder samples taken from the center of each fillet, longitudinal to the muscle fibers (1.27 cm in diameter). A Warner-Bratzler blade, using testing machine Texture Analyser TA XP (Stable Micro System, Godalming, England), was used to shear the samples across to the muscle fibers. Ten measurements were performed on each sample to obtain mean values expressed as shear force (N).

Statistical Analysis

Data are presented as the mean ± SEM and analyzed using SPSS software package version 20.0 (SPSS Inc., Chicago, USA). Physicochemical parameters and meat quality data were evaluated using a General Linear Mixed Model (GLMM), with genotype, myopathy degree, and slaughter age as fixed effects and the pen as a random effect. For productive performance characteristics and carcass traits, the independent samples t-test was used, while the intergroup comparisons for all data were appraised by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests. Differences were considered significant at $p \leq 0.05$. In the regression models, genotype and slaughter age were assigned as independent variables. Binary logistic regression was employed to identify associations between the independent variables and the development of WB (non-WB = 1, WB = 2). Multinomial logistic regression described the effects of independent variables on WB degree (normal = 1, mild WB = 2, moderate WB = 3 and severe WB = 4). The results from the logistic regression analysis were reported as odds ratios including 95% CI and the respective p-values for each variable. The odds ratio >1 indicates an increased chance whereas <1 denotes a decreased chance of a dependent category as a result of an increase in the independent variable by one unit.

RESULTS

Macroscopic Findings

Macroscopically, *p. major* muscles affected by WB appeared pale, outbulging, and hardened. The affected areas exhibited petechiae or slightly larger hemorrhagic foci and were often covered with a clear viscous fluid, predominantly localized to the cranial end of the breast muscles. Upon examination, a significant disparity was noted

between samples categorized as having moderate and severe cases of WB compared to unaffected samples. Within both the Ross and Cobb groups, all cases classified as severe WB exhibited diffuse lesions. Conversely, samples categorized as mild to moderate WB displayed more focal lesions, primarily distributed in the cranial parts of the fillets (Figure 1).

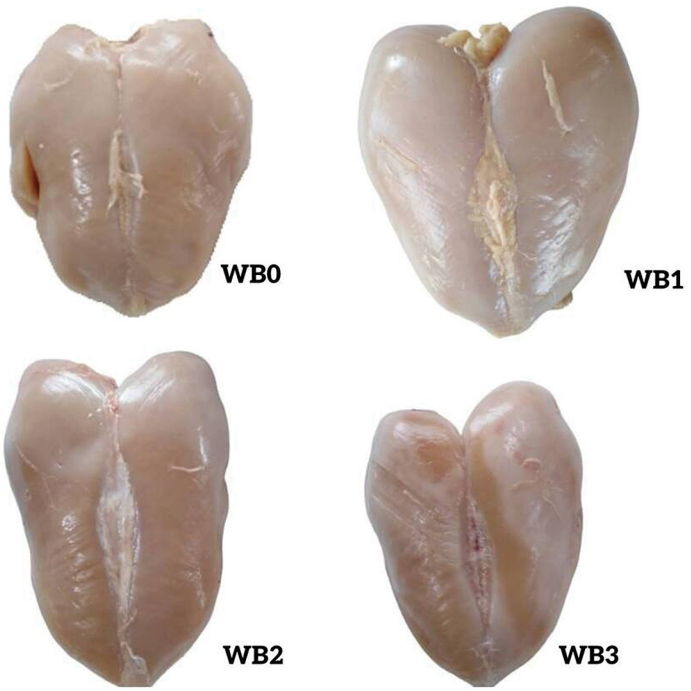


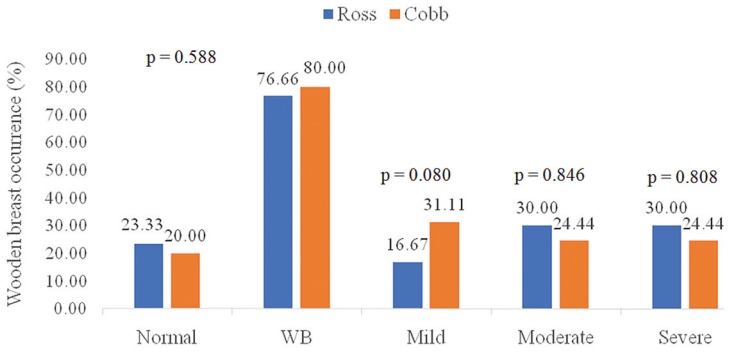
Figure 1. **WB** categorization based on macroscopic findings. Grade **WB0** (normal) – unaffected muscle of normal color and consistency; Grade **WB1** (mild) – subtle presentation of focal myodegenerative lesions in cranial area of the muscle surrounded by unaffected muscle; Grade **WB2** (moderate) – the entire cranial part of the muscle exhibits diffuse involvement characterized by hardened consistency and pale coloration**;** Grade **WB3** (severe) – the fillets exhibit extreme hardness and rigidity with diffuse changes, impacting the entire muscle (bilateral in presentation).

The occurrence and degree of wooden breast in relation to genotype and slaughter age

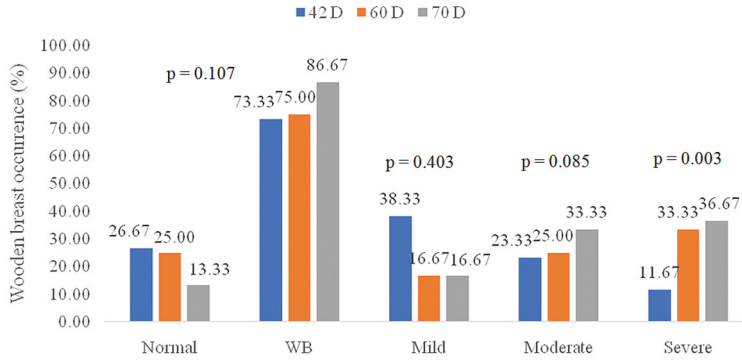
The occurrence and degree of wooden breasts in different genotypes and slaughter age are shown in Graph 1, Graph 2, Supplementary Table 1, 2 and 3. The genotype did not affect ($p>0.05$) the occurrence and degree of wooden breast (Graph 1, Suppl. Table 2). On the contrary, slaughter age affected the occurrence of wooden breasts,

whereby the highest (p=0.003) percentage of severe degree was recorded on day 70 (11.67 *vs.* 36.67%, Graph 2, Suppl. Table 3).

At day 42 of experiment, the WB occurrence was approx. 73% in both genotypes (Supplementary table 1). Both genotypes on day 70 had more than 85% affected cases of WB, without significant differences between groups (p>0.05). The occurrence of WB in Cobb broilers on day 70 was 90% from which 40% were classified as severe. In Ross broilers on day 70, 33% of *p. major* were evaluated as WB severe category. Marked discrepancy between the right and left *p. major* muscles in the presence and degree of the WB lesion was not noted.



Graph 1. Occurrence (%) of chickens showing wooden breast (total, mild, moderate, severe): effect of genotype (the reference category is normal score)



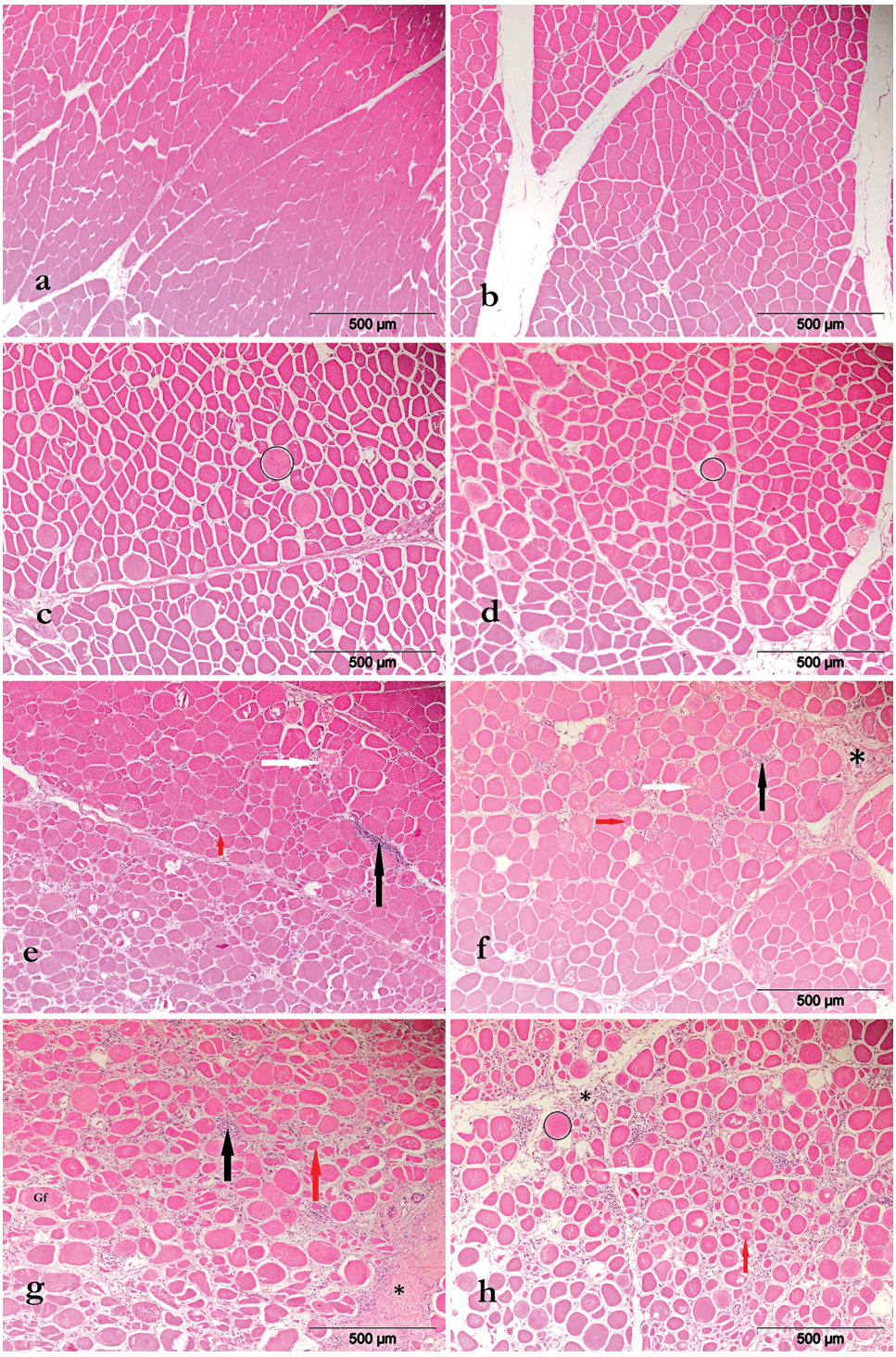
Graph 2. Occurrence (%) of chickens showing wooden breast (total, mild, moderate, severe): effect of slaughter age (the reference category is normal score)

Histologic Findings

On a histological level, *p. major* muscle macroscopically classified without myopathy, revealed a typical polygonal shape and uniform size of myofibers on cross section, with nuclei mostly peripherally located, with no presence of myopathic lesion, fibrosis or lipidosis (Figure 2 a, b). Samples classified as mild on gross appearance showed myofibers in some areas surrounded by a sparse amount of connective tissue within endomysium, scattered inflammatory cells and rare areas of adipose tissue accumulation (Figure 2 c, d). All samples categorized as moderate and severe WB in both broiler groups, as determined by macroscopic examination, were confirmed to exhibit severe myodegeneration and fibrosis with high number of hyalinized and necrotic fibers, accompanied with regeneration (Figure 2 e, f, g, h). Conversely, some of the fillets classified as unaffected based on macroscopy revealed only mild myodegeneration and infiltration without evident fibrosis. Some adipose tissue was present in all histologically evaluated cases, with evident increased amount of perivascular adipose tissue and myodegeneration in WB of moderate and severely affected samples.

Furthermore, a diffuse thickening of the perimysial network was observed, accompanied by variable amounts of loose connective tissue, granulation tissue, or collagen-rich connective tissue, indicative of fibrosis, which separated the muscle fibers (Figure 3).

In all cases of wooden breast, perivascular inflammatory cell infiltration, primarily around the veins, was consistently observed. This infiltration exhibited an irregular pattern and was predominantly composed of lymphocytes, occasionally resulting in disruption of the vascular wall. Immunohistochemical staining with the anti-CD3 antibody showed strong positivity, primarily in the cytoplasm, of perivascular cells (Figure 4). Additionally, occasional cells within the interstitium showed positivity for the anti-CD21 antibody.


a
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c
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Figure 2. *P. major*, Hematoxylin eosin staining, transverse section, **a, c, e** and **g** (Ross, day 70) **b, d, f** and **h** (Cobb, day 70). **Circle –** dystrophic muscle fiber, **black arrows –** mononuclear infiltrate, **asterisks –** collagen fibers, **white arrows –** necrotic muscle fiber, **Gc–** giant cells, **red arrows –** regenerative muscle fibers.

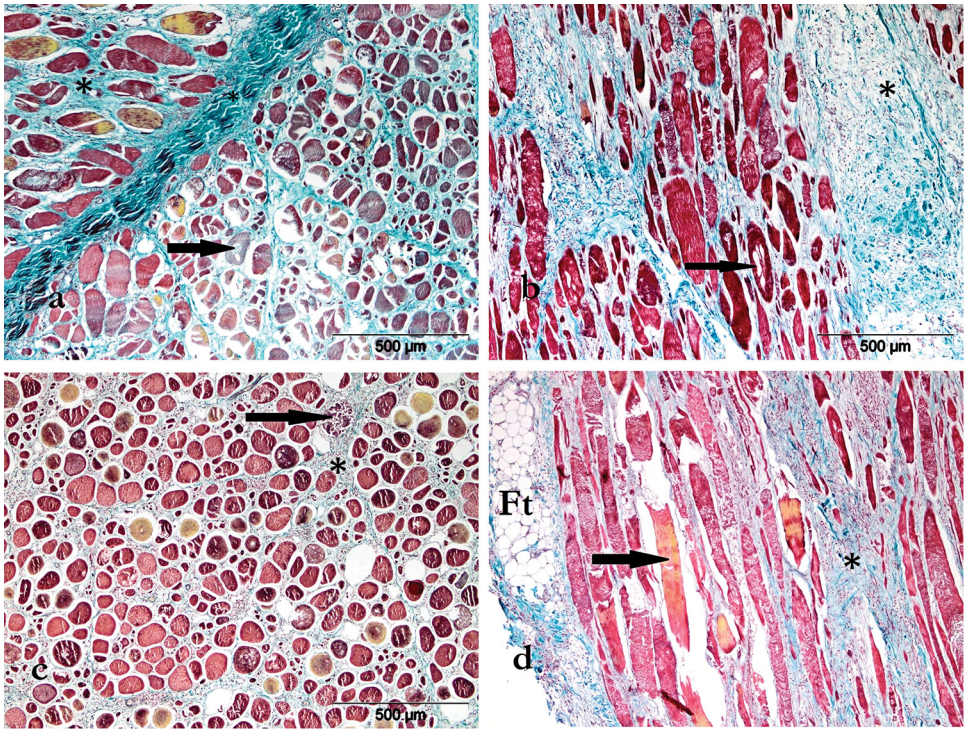

Figure 3. *P. major*, Masson trichrome staining, **a –** transverse section (Ross, day 70); **b –** longitudinal section (Ross, day 70) **c –** transverse section (Cobb, day 70); **d –** longitudinal section (Cobb, day 70). **Asterisk –** collagen fiber, **Black arrow –** necrotic muscle fiber, **Ft –** fat tissue

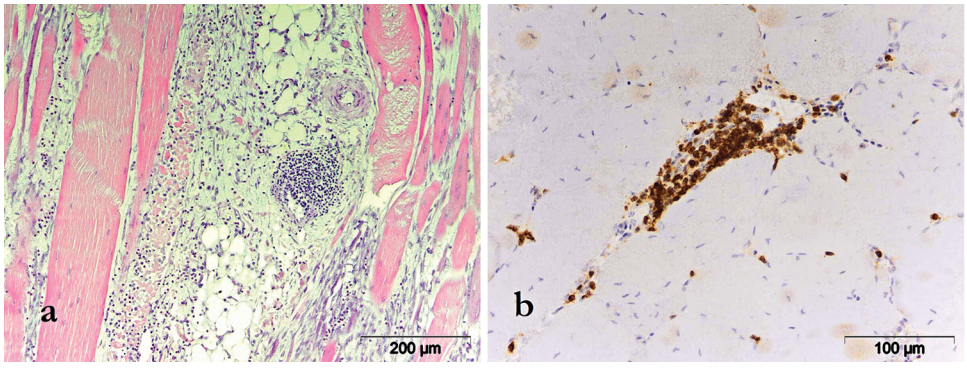

Figure 4. a - *P. major*, broiler. Accumulation of mononuclear cells within the perivenular region, exhibiting mild infiltration into the vein wall, with no discernible alterations observed in the arteria. HE. **b** – *P. major,* broiler. The perivenular mononuclear cells stain intensely with CD3 antibody, primarily within the cytoplasm mononuclear cells cytoplasm. Immunohistochemistry for CD3.

Productive performance and carcas traits of broiler chicken in relation to genotype and slaughter age

The effects of genotype and slaughter age on the productive performance of broiler chickens are shown in Table 2. Genotype affected the productive performance in broiler chickens, whereby Cobb had higher ($p<0.0001$) body weight on all sampling days, except ($p>0.05$) on day 70. In addition, Cobb had higher ($p<0.0001$) daily weight gain during the first and second periods.

Effects of genotype and slaughter age on carcass traits of broiler chickens are shown in Table 3. Most of the carcass traits differed ($p\leq0.05$) between genotypes, except ($p>0.05$) for cold carcass weight and breast weight on day 70. Cobb had higher cold carcass weight ($p<0.0001$ and $p=0.0040$, respectively), dressing percentage ($p=0.0189$ and $p=0.0007$, respectively), and breast yield ($p<0.0001$) on days 42 and 60. In contrast, Ross had higher breast yield ($p<0.0001$) on day 42 and dressing percentage ($p<0.0001$) on day 70.

Table 2. Productive performance (Mean±SE) of broiler chickens in relation to genotype and slaughter age.

Parameters	Genotype		p value
	Ross 308	Cobb 500	
Body weight (g)			
Day 1	39.10±0.27 [a]	41.90±0.26 [b]	<0.0001
Day 21	848.60±9.67 [a]	978.60±7.34 [b]	<0.0001
Day 42	2240±25.04 [a]	2462±20.90 [b]	<0.0001
Day 60	3530±69.54 [a]	3878±82.81 [b]	0.0122
Day 70	4123±117.90	4019±65.78	0.4444
Daily weight gain (g/d)			
First period (1 to 21 d)	40.48±0.47 [a]	46.84±0.36 [b]	<0.0001
Second period (22 to 42 d)	65.81±0.79 [a]	70.44±0.70 [b]	<0.0001
Third period (43 to 60 d)	79.22±2.10	86.48±3.83	0.0710
Fourth period (61 to 70 d)	70.72±3.28	74.91±5.88	0.5154

Note:[a,b] Different superscripts within the same row indicate a significant difference ($p < 0.05$).

Table 3. Carcass traits (Mean±SE) in broiler chickens in relation to genotype and slaughter age

Parameters	Genotype		p value
	Ross 308	Cobb 500	
Day 42			
Cold carcass weight (CC, g)	1651±21.42 [a]	1830±28.01 [b]	<0.0001
Dressing percentage (%)	70.25±0.25 [a]	71.14±0.27 [b]	0.0189
Breast weight (g)	638.20±9.54 [a]	674.60±13.31 [b]	0.0299
Breast yield (% CC)	38.63±0.23 [a]	36.81±0.26 [b]	<0.0001
Day 60			
Cold carcass weight (CC, g)	2624±60.75 [a]	2981±72.82 [b]	0.0004
Dressing percentage (%)	73.37±0.22 [a]	74.68±0.29 [b]	0.0007
Breast weight (g)	972.90±21.87 [a]	1174±29.69 [b]	<0.0001
Breast yield (% CC)	37.11±0.19 [a]	39.36±0.16 [b]	<0.0001
Day 70			
Cold carcass weight (CC, g)	3093±94.17	2887±61.66	0.0720
Dressing percentage (%)	74.92±0.35 [a]	71.68±0.63 [b]	<0.0001
Breast weight (g)	1205±40.96	1164±27.56	0.4004
Breast yield (% CC)	38.92±0.41 [a]	40.26±0.32 [b]	0.0133

Note: [a,b] Different superscripts within the same row indicate a significant difference ($p < 0.05$).

Meat quality of broiler chickens in relation to genotype, WB degree and slaughter age

Effects of genotype, WB degree, slaughter age, and their interactions on physicochemical parameters of the *p. major* muscle of broiler chickens are displayed in Table 4. The effect of genotype on meat color traits (L* and a* values, p=0.016 and p=0.014, respectively) was found. However, observing these parameters at separate slaughtering ages did not reveal a difference between Ross and Cobb broilers (p>0.05). The presence of WB, regardless of the degree of severity, was associated (p<0.0001) with higher ultimate pH, lightness (L*), redness (a*), and yellowness (b*)

of *p. major* muscle, except on day 70 when no differences in pH and lightness were observed between meat affected by WB and normal breasts. Further, slaughter age affected ($p<0.0001$) physicochemical and color parameters, whereby ultimate pH, redness (a*), and yellowness (b*) decreased, while lightness (L*) increased with broiler age from day 42 to 60. Additionally, the differences between days 60 and 70 were less notable and limited to meat yellowness and pH.

Effects of genotype, WB degree, slaughter age, and their interactions on water-holding capacity and textural traits of *the p. major* muscle of broiler chickens are depicted in Table 5. Genotype affected the water-holding capacity and textural traits; Cobb had lower drip loss ($p<0.0001$) on day 70, cooking loss ($p=0.020$) on day 42, and shear force ($p<0.0001$) on day 42 and 60 than Ross chickens. Cooking loss and shear force were higher in severely affected WB than in normal breasts, whereas mild and moderate WB exhibited intermediate values. In addition, slaughter age affected ($p<0.0001$) water-holding capacity and textural traits, whereby drip loss, cooking loss, and shear force increased with broiler age, with noticeable differences observed between day 42 and two later periods of the experiment.

Table 4. Physicochemical and color parameters of *pectoralis major* muscle in broiler chickens in relation to genotype, wooden breast degree and slaughter age

Item		Genotype (G)		Wooden breast (WB) degree				p value		
		Ross 308	Cobb 500	Normal	Mild	Moderate	Severe	G	WB	G×WB
pH										
	42	5.80±0.01^{acA}	5.77±0.01^{aA}	5.70±0.01^{bA}	5.79±0.01^{aA}	5.81±0.01^{acA}	5.87±0.01^{cA}			
	60	5.40±0.02^{aB}	5.40±0.01^{aB}	5.31±0.01^{bB}	5.41±0.02^{aB}	5.42±0.02^{aB}	5.47±0.02^{aB}	0.053	<0.0001	0.812
	70	5.40±0.01 ^{B}	5.38±0.01 ^{B}	5.40±0.02 ^{C}	5.38±0.02 ^{B}	5.38±0.01 ^{B}	5.41±0.01^{C}			
Age	p value	<0.0001								
Age × G	p value	0.879								
Age ×WB	p value	<0.0001								
Age × G ×WB	p value	0.034								
*L**										
	42	54.86±0.60^{aA}	55.53±0.56^{aA}	51.82±0.61^{bA}	55.99±0.45^{aA}	56.89±0.77^{aA}	56.92±0.98^{aA}			
	60	58.20±0.56^{abB}	59.98±0.71^{aB}	55.40±1.12^{bAB}	59.07±0.67^{abB}	59.63±0.79^{aAB}	61.04±0.78^{aAB}	0.016	<0.0001	0.453
	70	60.27±0.93^{B}	61.88±0.81^{B}	59.14±1.30^{B}	59.70±0.95^{B}	62.99±1.33^{B}	62.75±1.26^{B}			
Age	p value	<0.0001								
Age × G	p value	0.965								
Age ×WB	p value	0.520								
Age × G ×WB	p value	0.793								

Table 4. continued

*a**										
	42	2.21±0.14^{aA}	2.01±0.15^{aA}	1.28±0.11^{bA}	2.17±0.12^{aA}	2.59±0.21^{aA}	2.86±0.14^{a}			
	60	1.47±0.09^{acB}	1.49±0.12^{acAB}	0.77±0.09^{bAB}	1.36±0.09^{cB}	1.47±0.09^{cB}	2.07±0.10^{d}	0.014	<0.0001	0.201
	70	1.87±0.24^{acAB}	1.03±0.16^{bcB}	0.61±0.21^{bcB}	0.96±0.28^{cB}	1.56±0.27^{acB}	2.42±0.28^{a}			
Age	p value	<0.0001								
Age × G	p value	0.002								
Age ×WB	p value	0.388								
Age × G ×WB	p value	0.005								
*b**										
	42	7.33±0.23^{A}	7.19±0.33^{A}	6.57±0.26^{A}	6.97±0.31^{A}	8.04±0.52^{A}	8.22±0.28^{A}			
	60	5.31±0.21^{B}	6.09±0.29^{B}	5.16±0.33^{B}	5.46±0.31^{B}	5.50±0.34^{B}	6.43±0.36^{B}	0.626	<0.0001	0.529
	70	4.98±0.22^{aB}	4.30±0.19^{abC}	3.66±0.20^{bC}	4.13±0.20^{bC}	4.69±0.27^{abB}	6.33±0.23^{cB}			
Age	p value	<0.0001								
Age × G	p value	0.003								
Age ×WB	p value	0.119								
Age × G ×WB	p value	0.774								

Note: ^{a,b,c,d} Different lower-case superscript letters within the row indicate a significant difference between groups on the same slaughtering period ($p< 0.05$); ^{A,B,C} Different upper-case superscript letters within the column for each parameter indicate differences within the same group on different slaughtering periods ($p< 0.05$).

Table 5. Water-holding capacity and texture traits in broiler chickens in relation to genotype, wooden breast degree and slaughter age

Item		Genotypes (G)		Wooden breast degree (WB)				p value		
		Ross 308	Cobb 500	Normal	Mild	Moderate	Severe	G	WB	G×WB
Drip loss (%)										
	42	2.03±0.06^{aA}	1.96±0.09^{aA}	1.60±0.07^{bA}	2.05±0.06^{aA}	2.15±0.11^{aA}	2.41±0.06^{aA}			
	60	3.23±0.10^{aB}	3.27±0.11^{aB}	2.89±0.13^{aB}	2.93±0.11^{aB}	3.32±0.15^{abB}	3.77±0.06^{bB}	<0.0001	<0.0001	0.028
	70	3.53±0.12^{acB}	2.76±0.14^{bC}	2.40±0.18^{bC}	2.76±0.21^{bB}	3.10±0.12^{abB}	3.92±0.11^{cB}			
Age	p value			<0.0001						
Age × G	p value			<0.0001						
Age ×WB	p value			0.010						
Age × G ×WB	p value			0.081						
Cooking loss (%)										
	42	21.02±0.41^{aA}	18.43±0.31^{bA}	18.53±0.55^{bA}	19.30±0.43^{bA}	20.27±0.50^{abA}	22.38±0.91^{aA}			
	60	24.49±0.50^{aB}	24.50±0.60^{aB}	21.26±0.44^{bA}	24.47±0.60^{acB}	25.13±0.56^{acB}	26.92±0.6^{cAB}	0.020	<0.0001	0.006
	70	26.38±0.53^{aC}	25.79±0.74^{aB}	23.54±0.69^{aC}	24.26±0.59^{aB}	26.08±0.81^{aB}	29.73±0.81^{bC}			
Age	p value			<0.0001						
Age × G	p value			0.463						
Age ×WB	p value			0.143						
Age × G ×WB	p value			0.137						
Shear force (N)										
	42	24.39±0.57^{aA}	17.96±0.45^{bA}	18.03±0.49^{bA}	20.29±0.69^{bcA}	21.04±0.64^{cA}	30.00±1.19^{dAB}			
	60	26.49±0.54^{aB}	23.50±0.49^{bB}	22.69±0.65^{bB}	23.86±0.71^{abB}	25.68±0.73^{abB}	26.53±0.72^{aA}	<0.0001	<0.0001	0.444
	70	27.19±0.55^{aAB}	28.59±0.56^{acC}	24.42±1.16^{bB}	25.52±0.67^{bB}	27.89±0.64^{abcC}	30.53±0.73^{cB}			
Age	p value			<0.0001						
Age × G	p value		<0.0001							
Age ×WB	p value			0.040						
Age × G ×WB	p value			0.834						

Note: ^{a,b,c,d} Different lower-case superscript letters within the row indicate a significant difference between groups on the same slaughtering period ($p< 0.05$); ^{A,B,C} Different upper-case superscript letters within the column for each parameter indicate differences within the same group on different slaughtering periods ($p< 0.05$).

DISCUSSION

The objective of the present study was to evaluate the effect of three slaughtering ages and genotype on the rates and degree of WB, changes in breast muscle at the histological level, and meat quality in two widely used fast-growing strains of broiler chickens.

Concerning the WB, in the present study the incidence of this myopathy in both genotypes was high (between 73% on day 42 and 83-90% on day 70), as reported by Cruz et al. [15] (89.2%). Contrarily, under experimental conditions, Trocino et al. [11] reported a lower WB occurrence of approximately 24% (8.0% in females and 16.3% in males) in broilers slaughtered at 46 d of age. As for the slaughter age, continuous fattening increased the final body weight of chickens and WB rates. In fact, the occurrence of WB has been reported to increase as the birds became older and heavier [16], as observed in the present study. Moreover, Che et al. [17] reported increased live weight as a risk factor for WB occurrence due to fiber hypertrophy and reduced capillary density, ultimately leading to elevated WB rates in heavier birds. Thus, similarly to the present findings, Tijare et al. [6] found 96.1% of breasts showing WB in broilers slaughtered at 61 d of age. Indeed, the results from the combined database of 4,332 broilers pooled from 7 research experiments conducted at Texas A&M University showed that the occurrence of WB in broilers of 8 weeks of age could be as high as 86% [18]. The generally high rates of myopathies reported in the studies conducted in controlled environmental conditions, such as in our study, could be due to the ideal growing conditions [3,6] favoring the development of these muscles abnormalities. Due to the relatively small number of broilers used in the present trial, a larger-scale study is required to verify the high WB rates reported herein. Even in industrial conditions, during gross examination of 1,920 breasts from 9-week-old broilers, approximately 85% were found to display signs of WB, of which, similarly to our findings, more than 42% were severe or very severe [16]. Moreover, Xing et al. [19] reported a relatively high WB rate (61.9%) under commercial conditions in China. Regarding myopathy scores, prolonged fattening resulted in decreased rates of normal and mild scored WB and increased occurrence of moderate and severe WB in both genotypes. The prolonged fattening increases the probability of severe WB in both genotypes. The impact of extended fattening on carcass traits is usually consistent with the results of final live weight, as found in the present study. Thus, the increase in the rate and degree of myopathies during the experiment was likely related to the increased weight and yield of *p. major,* as previously reported [12,20].

Regarding genotype, Cobb had a higher body weight and weight gain during the first 42 days of the experiment. The better productive performance for Cobb than Ross broiler chickens reported in the present study corroborates previous trials [21,22] reporting Cobb broilers to exhibit superior body weight. However, these differences between genotypes disappeared during fattening at the end of the experiment (day 70). Despite the higher breast weight, Cobb chickens had lower breast yield than Ross birds

on day 42 of age. In contrast, on days 60 and 70 of the study, Cobb broiler chickens had a higher breast yield than Ross ones. Differences in the production results are likely due to genetic potential factors, as suggested by Olanrewaju et al. [23]. Nevertheless, despite the differences in the body weight and breast yield between the two genotypes, no effect of the genotype on WB was found on day 42, as previously reported by other authors [11,24], nor later during fattening. Contrarily, Hammemi et al. [22] reported a higher rate of WB myopathy in Cobb than in Ross chickens, with Ross 308 chickens having a higher rate of mild WB (22.5% *vs.*15.62%) and Cobb 500 higher occurrence of moderate (4.38% *vs*. 12.5%) and severe (1.88% *vs*. 3.12%) WB.

Although the results of the WB occurrence among the studies are inconstant, likely due to different factors such as age [16], sex [11,12], genotype [22], diet and feeding strategies [11,25], final weight [18], etc., the histological findings reveal damaged muscle fibers in over 97% of breasts examined [4,7,26]. In agreement, several macroscopically normal *p. major* muscles from the present study exhibited mild myodegeneration and infiltration without evident fibrosis at the microscopic level. Microscopic lesions reported in *p. major* muscle affected by WB from the present study corroborate previous histological findings [7,11,26]. Some studies reported initial microscopic features of WB in the *p. major* muscles could be found in broilers as early as two weeks of age [27,28]. Papah et al. [29] reported phlebitis to appear in *p. major* muscle as early as in the first week of life, before other WB microscopic lesions. Namely, these authors proposed that impaired venous drainage may lead to local accumulation of metabolic waste and ROS, triggering myofibrillar degeneration and necrosis. As in the present study, vasculitis and perivascular infiltrations of inflammatory cells (mostly CD3 T lymphocytes) have been observed in the breasts of market-age birds affected by WB [4,11,28,29]. However, it is worth noting that multiple authors [27-29] reported the presence of robust lymphocytic phlebitis in *p. major* without other characteristics lesions for WB and *vice versa*. The etiology and pathogenesis of WB and other myopathies onset are under extensive investigation. Available data suggest that genetic selection of broilers for the hypertrophic growth of the muscle fibers and inadequate vascular support lead to the impaired oxygen and nutrient supply to the muscle tissue on one side, and poor drainage and accumulation of metabolic by-products on the other [2]. This mechanism is supported by transcriptomic data showing altered expression of genes involved in carbohydrates and lipid metabolism, remodeling of the extracellular matrix, inflammation/immune response, fatty acid beta oxidation, and oxidative phosphorylation [30-32].

In agreement with the present findings, other authors [7,27,33] reported a reduction in muscle fibers in *p. major* with WB, loss of typical polygonal shape, an abnormally rounded fibers with nuclear internalization, loss of cross striations, vacuolar degeneration and necrosis of fibers coupled with fibrotic response in form of proliferation and diffuse thickening of endomysium and perimysium network, variable amount of loose connective tissue and granulation tissue. The severity of microscopic lesions increased as the degree of WB increased from normal to severe, reaching the highest

levels of myodegeneration in *p. major* affected by severe WB. Microscopic findings of myodegeneration, accompanied by regeneration, have been previously associated with the WB [27]. In agreement, in the present study, necrotic fibers detected in the myofibers at all defective degrees of WB were surrounded by regenerating fibers. Namely, the disruption of the sarcolemma due to myofiber degeneration initiates necrosis from the influx of calcium from the sarcoplasmic reticulum, triggering an immune response [34], as confirmed in the present study where infiltration of CD3-immunoreactive cells was found. Following tissue damage, activated satellite cells undergo proliferation and differentiation and engage in fusion with existing myofibers or new fibers synthesis, thus facilitating muscle regeneration [35-37]. Depending on the niche environment, these multipotent cells can follow myogenic or adipogenic pathways [35,37,38]. Considering adequate vascularization to be imperative during muscle regeneration, hypoxic conditions in WB cause transdifferentiation of satellite cells to adipocytes, which might explain, at least partially, the deposition of fat in WB-affected muscles, as demonstrated by Emami et al. [37]. Other authors also reported adipocyte infiltration in *p. major* muscle with WB [7,11]. Muscle fibrosis, the structural hallmark of the WB, found in previous and the present study, is a secondary process generated as a response to muscle necrosis resulting in the progressive overproduction and deposition of fibrillar collagens Types I and III in the perymisial and endomysial connective tissue spaces [19,34].

Alterations to the morphological structure of the muscle has been linked to changes in meat chemical composition, decreased nutritional value and impaired meat quality [1,7,39].

The higher ultimate pH of *p. major* affected by WB (regardless of the degree of WB severity), compared with the normal muscles, is in line with previous reports [5,7,16,40]. In this regard, proteomic data suggest the downregulation of carbohydrate metabolic pathways related to reduced glycolysis, gluconeogenesis, tricarboxylic acid cycle, glycogen degradation, and pyruvate fermentation to lactate to reduce glycolytic potential and elevate ultimate pH in defected muscles [16]. In addition, previous findings found that the degree of the myopathy can influence meat pH [41], as observed in the present study for day 42, with no differences between degrees of WB at later ages. As for the age, although it would be expected for older birds with greater breast weight and higher fiber diameter to have a reduced *in vivo* glycogen storage and, thus, exhibit higher ultimate pH as previously reported [42], in the present study the decline in ultimate pH of *p. major* was reported. Nevertheless, in agreement with present findings, Schneider et al. [43] reported higher pH in the breast meat of broilers slaughtered at 49 d than at 56 d, although no difference was found for day 42. Accordingly, Schneider et al. [43], Coban et al. [44], and Glamočlija et al. [45] also reported an age-related decline in pH in the *p. major* muscles (from 42 d to 63 d, 42 d to 56 d, and 42 d to 50 d, respectively) likely as the consequence of a greater degree of postmortem glycolytic metabolism in more mature muscles. As in the previous studies, genotype did not influence pH [11,24].

Regarding color, meat lightness increased in abnormal *p. major* muscles compared to the normal counterparts, as seen before [41]. However, the color did not differ between breasts affected by different degrees of WB. Contrarily, the yellowness and redness increased in breasts affected by WB, as found by other authors [5,16,41]. Corroborating previous findings, the degree of WB increased b* values [16]. These alterations in color indices are likely due to the accumulation of fat (along with liposoluble pigments from the feed) in abnormal breasts and the altered light reflection caused by edema and changed muscle tissue structure [5,41]. As for redness, increased redness in WB-affected muscles could be due to the high expression of myoglobin genes [46]. Overall, redness and yellowness of breast meat decreased with age of the birds, whereas lightness increased, regardless of the myopathy degree. It is worth noting that changes in color of *p. major* muscle were evident between birds of 42 and 60 days of age, whereas minor color differences were reported between broilers slaughtered on days 60 and 70. The literature data on age-related color changes in breast meat are inconsistent. Opposite to present findings, previous reports showed that the older chicken muscles were darker [42,47,48] and redder [47,48] than the chicken muscles of younger birds. However, corroborating present findings few authors [47-49] reported the tendency of the breast meat to show a lower yellowness and redness due to the decrease in the heme pigment content, as birds get older. As for genotype, although the broiler line was found to be a relevant factor in color indices of breasts, as previously reported by other authors [49,50] when considering birds' age, there was no difference in color between genotypes. Similarly, Hammemi et al. [22] and Gratta et al. [24] did not find a color difference between the breasts of two genotypes slaughtered at 35 and 45 days of age, respectively.

Considering fluid losses, contrary to the expectation that muscles affected by WB may increase water-holding capacity due to the higher ultimate pH, in comparison to normal breast, abnormal muscles had higher drip and cooking loss, in agreement with available literature data [7,16,51,52]. These losses could be attributed to the alteration in the structure of the *p. major*, mainly degeneration of muscle fibers, thus decreased myofibrillar proteins, accompanied by accumulation of interstitial connective tissue seen at the microscopic level [7]. Indeed, the present study found that the fluid losses were generally higher in breasts with severe WB, whereas the moderate and mild degrees of WB did not significantly increase drip loss except for day 42, nor cooking loss except for day 60, confirming that ability to retain liquids depends on the severity of lesions in the muscle tissue. The imparted ability of WB muscles to bind water influences their marinade uptake limiting further processing of abnormal meat [5]. Regarding slaughter age, drip and cooking losses increased over time and were higher in older birds, as expected due to the increased muscle damage and occurrence of myopathies. Overall, the genotype did not influence drip losses, except on day 70 when the muscles of Cobb exhibited lower drip loss than Ross chickens.

Finally, the shear force of cooked *p. major* was affected by the presence of severe WB, associated with the high levels of collagen crosslinking due to fibrosis of WB-affected

muscles [34] and higher cooking losses in such meat. The absence of the myopathy effect on breast toughness in mild and moderate affected WB breasts could be due to the cooking effect on shear values [53]. Other authors [7,54,55] reported the same tenderness in cooked normal and WB breasts, regardless of the condition degree. Indeed, corroborating the present study, some authors found WB-affected meat to exhibit a higher hardness than the normal filets [12,39]. Furthermore, the difference in shear force and cooking loss were observed between genotypes for day 42, with Ross exhibiting higher *p. major* toughness and lower ability to retain liquid compared to Cobb, likely due to a higher occurrence of severe WB in former compared to later. From this moment onward, the differences in meat cooking loss between genotypes were not evident as severe WB rates in Cobb increased during prolonged fattening, whereas the differences in hardness disappeared after day 60 of the experiment. As for slaughter age, the shear force of muscles increased with age, probably as a result of greater fiber size [56] and increased collagen cross-links in older birds [57].

CONCLUSION

As for production performances and carcass traits, Cobb 500 genotype had higher body weight and breast weight, except for day 70, and greater breast yield during all trials than Ross 308 birds. The occurrence of WB was high (73-90%) but was not significantly different between genotypes. As for birds' age, the occurrence of severe WB increases with age. The presence of WB, especially severe WB, impaired meat quality in terms of altered color, decreased ability to retain liquid, and increased shear force compared to normal chicken breasts, which can have repercussions for the processing of such meat. Moreover, gross examination proves to be effective as the primary method for detecting WB in moderate and severe cases, while mild cases may require subtler confirmation methods such as microscopic evaluation. In the present study, both macroscopy classified as severe and moderately affected WB were scored as severe at the histological level, sharing microscopic lessons including nuclear internalization, hyaline and vacuolar degeneration, infiltration and marked fibrosis. In conclusion, considering myopathy occurrence and severity, as well as meat quality alterations, extended fattening is not recommended for poultry production.

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Authors' contributions

AR conceptualization, formal analysis, investigation and methodology, and manuscript draft preparation. MBC conceptualization, project administration, supervision, validation, writing original draft, review, and editing of the draft. MG data curation, formal analysis, statistical analysis, validation, review, and draft editing. NČ is writing the original draft. VT investigation and methodology regarding texture. ML experimental part during farming animals and production results. SN conceptualization, project administration, supervision, validation, review, and draft editing. All authors read and approved the final manuscript.

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REFERENCES

1. Petracci M, Soglia F, Madruga M, Carvalho L, Ida E, Estévez M: Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Comprehensive Reviews in Food Science and Food Safety 2019, 18(2):565-583.
2. Soglia F, Petracci M, Davoli R, Zappaterra M: A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles. Poult Sci 2021, 100(6):101180.
3. Kuttappan VA, Hargis BM, Owens CM: White striping and woody breast myopathies in the modern poultry industry: a review. Poult Sci 2016, 95(11):2724-2733.
4. Sihvo HK, Immonen K, Puolanne E: Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet Pathol, 2014, 51(3):619-623.
5. Mudalal S, Lorenzi M, Soglia F, Cavani C, Petracci M: (2015). Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Anim 2015, 9(4):728-734.
6. Tijare VV, Yang FL, Kuttappan VA, Alvarado CZ, Coon CN, Owens CM: Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult Sci 2016, 95(9):2167-2173.

7. Soglia F, Mudalal S, Babini E, Di Nunzio M, Mazzoni M, Sirri F, Cavani C, and Petracci M: Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poult sci 2016, 95(3): 651-659.
8. Kuttappan VA, Brewer VB, Waldroup PW, Owens CM: Influence of growth rate on the occurrence of WS in broiler breast fillets. Poult Sci 2012, 91:2677–2685
9. Lorenzi M, Mudalal S, Cavani C, Petracci M: Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. J Appl Poult Res 2014, 23:754–758
10. Russo E, Drigo M, Longoni C, Pezzotti R, Fasoli P, Recordati C: Evaluation of white striping prevalence and predisposing factors in broilers at slaughter. Poult Sci 2015, 94:1843–1848
11. Trocino A, Piccirillo A, Birolo M, Radaelli G, Bertotto D, Filiou E, Petracci M, Xiccato G: Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poult Sci 2015, 94:2996-3004.
12. Bordignon F, Xiccato G, Boskovic Cabrol M, Trocino A: Factors affecting breast myopathies in broiler chickens and quality of defective meat: a meta-analysis. Front Physiol 2022, 13: 933235
13. Gjurovski I, Bozinovski S, Janevski A, Atanaskova PE, Konstadinova KS, Ristovski T: Immunohistochemical investigation of fipv3-70 antigen expression in the ileum of cats with effusive feline infective peritonitis, Acta Vet Belgrade 2003, 73(3):432-438
14. Honikel KO: (1998). Reference methods for the assessment of physical characteristics of meat. Meat Sci 1998, 49(4):447-457
15. Cruz RFA, Vieira SL, Kindlein L, Kipper M, Cemin HS, Rauber SM: Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels. Poult Sci 2017, 96(2): 501-510.
16. Kuttappan VA, Owens CM, Coon C, Hargis BM, Vazquez-Anon M: Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. Poult Sci 2017, 96(8): 3005-3009.
17. Che S, Wang C, Iverson M, Varga C, Barbut S, Bienzle D, Susta L: Characteristics of broiler chicken breast myopathies (spaghetti meat, woody breast, white striping) in Ontario, Canada. Poult Sci 2022, 101(4): 101747.
18. Aguirre ME, Leyva-Jimenez H, Travis R, Lee JT, Athrey G, Alvarado CZ: Evaluation of growth production factors as predictors of the incidence and severity of white striping and woody breast in broiler chickens. Poult Sci 2020, 99(7): 3723-3732.
19. Xing T, Zhao X, Zhang L, Li JL, Zhou GH, Xu XL, Gao F: Characteristics and incidence of broiler chicken wooden breast meat under commercial conditions in China. Poult Sci 2020, 99(1): 620-628.
20. Lake JA, Brannick EM, Papah MB, Lousenberg C, Velleman SG, Abasht B: Blood gas disturbances and disproportionate body weight distribution in broilers with wooden breast. Front in Physiol 2020, 11: 525514.
21. Hristakieva P, Mincheva N, Oblakova M, Lalev M, Ivanova I: Effect of genotype on production traits in broiler chickens. Slovak J Anim Sci 2014, 47(1): 19-24.
22. Hammemi C, Askri A, Létourneau-Montminy MP, Alnahhas N: Current state of breast meat quality in standard-yielding broiler strains. JAPR 2024, 33(1): 100383.
23. Olanrewaju HA, Miller WW, Maslin WR, Collier SD, Purswell JL, Branton SL: Interactive effects of light-sources, photoperiod, and strains on growth performance, carcass

characteristics, and health indices of broilers grown to heavy weights. Poult Sci 2019, 98(12): 6232-6240.

24. Gratta F, Cabrol MB, Xiccato G, Birolo M, Bordignon F, Trocino A: (2023). Effect of light restriction on productive results and behavior of broiler chickens. Poult Sci 2023, 102(12): 103084.
25. Vieira V, Marx FO, Bassi LS, Santos MC, Oba A, de Oliveira SG, Maiorka A: Effect of age and different doses of dietary vitamin E on breast meat qualitative characteristics of finishing broilers. Anim Nutr 2021, 7: 163-167
26. Radaelli G, Piccirillo A, Birolo M, Bertotto D, Gratta F, Ballarin C, Vascellari M,. Xiccato G, Trocino A: Effect of age on the occurrence of muscle fiber degeneration associated with myopathies in broiler chickens submitted to feed restriction. Poult Sci 2017, 96(2): 309-319.
27. Sihvo HK, Lindén J, Airas N, Immonen K, Valaja J, Puolanne E: Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. Vet Pathol 2017, 54(1):119-128.
28. Chen LR, Suyemoto MM, Sarsour AH, Cordova HA, Oviedo-Rondón EO, Wineland, M, Barnes J, Borst LB: Temporal characterization of wooden breast myopathy ("woody breast") severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. Avian Pathol 2019, 48(4): 319-328.
29. Papah MB, Brannick EM, Schmidt CJ, Abasht B: Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens. Avian Pathol 2017, 46(6): 623-643.
30. Papah MB, Brannick EM, Schmidt CJ, Abasht B: Gene expression profiling of the early pathogenesis of wooden breast disease in commercial broiler chickens using RNA-sequencing. PLoS One 2018, 13(12): e0207346.
31. Wang Z, Khondowe P, Brannick E, Abasht B: Spatial transcriptomics reveals alterations in perivascular macrophage lipid metabolism in the onset of Wooden Breast myopathy in broiler chickens. Scientific Reports 2024, 14(1): 3450.
32. Che S, Pham PH, Barbut S, Bienzle D, Susta L: Transcriptomic profiles of Pectoralis major muscles affected by spaghetti meat and woody breast in broiler chickens. Animals 2024, 14(2): 176.
33. Malila Y, Juthawut U, Srimarut Y, Chaiwiwattrakul P, Uengwetwanit T, Arayamethakorn S, Punyapornwithaya V, Sansamur C, Kirschke CP, Huang L, Visessanguan W: Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (Gallus gallus). AJAS 2018, 31(11): 1807.
34. Velleman SG: Pectoralis major (breast) muscle extracellular matrix fibrillar collagen modifications associated with the wooden breast fibrotic myopathy in broilers. Front Physiol 2020, 11: 523915.
35. Velleman SG, Clark DL: Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. Avian Dis 2015, 59(3): 410-418.
36. Clark DL, Velleman SG: Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poult Sci 2016, 95: 2930-2945
37. Emami NK, Cauble RN, Dhamad AE, Greene ES, Coy CS, Velleman SG, Orlowski S, Anthony N, Bedford M, Dridi, S: Hypoxia further exacerbates woody breast myopathy in broilers via alteration of satellite cell fate. Poult Sci 2021, 100(7): 101167.
38. Velleman SG: Satellite cell-mediated breast muscle growth and repair: the impact of thermal stress. *Front Physiol 2023,* 14: 1173988

39. Tasoniero G, Cullere M, Cecchinato M, Puolanne E, Dalle Zotte A: Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by white striping and wooden breast myopathies. Poult Sci 2016, 95(11): 2707-2714.
40. Baldi G, Yen CN, Daughtry MR, Bodmer J, Bowker BC, Petracci M, Gerrard DE: Exploring the factors contributing to the high ultimate pH of broiler pectoralis major muscles affected by wooden breast condition. Front Physiol 2020, 11: 528714.
41. Campo MDM, Mur L, Guerrero A, Barahona M, Resconi VC, Magalhaes DR, Lisbinski E, Boito B, Oliveira IMD, Olleta JL: Differentiating breast myopathies through color and texture analyses in broiler. Foods 2020, 9(6): 824.
42. Baéza E, Arnould C, Jlali M, Chartrin P, Gigaud V, Mercerand F, Durand C, Meteau K, Le Bihan-Duval E, Berri C: Influence of increasing slaughter age of chickens on meat quality, welfare, and technical and economic results. J Anim Sci 2012, 90(6): 2003-2013.
43. Schneider BL, Renema RA, Betti M, Carney VL, Zuidhof MJ: Effect of holding temperature, shackling, sex, and age on broiler breast meat quality. Poult Sci 2012, 91(2): 468-477.
44. Coban O, Lacin E, Aksu MI, Kara A, Sabuncuoglu N: The impact of slaughter age on performance, carcass traits, properties of cutup pieces of carcasses, and muscle development in broiler chickens. European Poult Sci 2014/Archiv für Geflügelkunde, 78.
45. Glamoclija N, Starcevic M, Janjic J, Ivanovic J, Boskovic M, Djordjevic J, Markovic R, Baltic MZ: The effect of breed line and age on measurements of pH-value as meat quality parameter in breast muscles (m. pectoralis major) of broiler chickens. Proc Food Sci 2025, 5:89-92.
46. Mutryn MF, Brannick EM, Fu W, Lee WR, Abasht B: Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genom 2015, 16: 1-19.
47. Fletcher DL: Poultry meat quality. Worlds Poult Sci J 2002, 58: 131–145.
48. Li J, Yang C, Peng H, Yin H, Wang Y, Hu Y, Yu C, Jiang X, Du H, Li Q, Liu Y: Effects of slaughter age on muscle characteristics and meat quality traits of Da-Heng meat type birds. Animals 2019, 10(1): 69.
49. Janisch S, Krischek C, Wicke M: Color values and other meat quality characteristics of breast muscles collected from 3 broiler genetic lines slaughtered at 2 ages. Poult Sci 2011, 90(8): 1774-1781.
50. Debut M, Cécile B, Elisabeth B, Nadine S, Cécile Ar, Daniel G, Jehl N: Variation of chicken technological meat quality in relation to genotype and preslaughter stress conditions. Poult Sci 2023, 82(12): 1829-1838.
51. Sun X, Koltes DA, Coon CN, Chen K, Owens CM: Instrumental compression force and meat attribute changes in woody broiler breast fillets during short-term storage. Poult Sci 2018, 97(7): 2600-2606.
52. Bowker BC, Maxwell AD, Zhuang H, Adhikari K: Marination and cooking performance of portioned broiler breast fillets with the wooden breast condition. Poult Sci 2018, 97(8): 2966-2970.
53. Soglia F, Gao J, Mazzoni M, Puolanne E, Cavani C, Petracci M, Ertbjerg P: Superficial and deep changes of histology, texture and particle size distribution in broiler wooden breast muscle during refrigerated storage. Poult Sci 2017, 96(9): 3465–347
54. Mazzoni M, Petracci M, Meluzzi A, Cavani C, Clavenzani P, Sirri F: Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult Sci 2015, 94(1):123-130.

55. Dalgaard LB, Rasmussen MK, Bertram HC, Jensen JA, Møller HS, Aaslyng MD, Hejbøl EK, Pedersen JR, Elsser-Gravesen D, Young JF: Classification of wooden breast myopathy in chicken pectoralis major by a standardised method and association with conventional quality assessments. IJFST 2018, 53(7): 1744-1752.
56. Chen XD, Ma QG, Tang MY, Ji C: Development of breast muscle and meat quality in Arbor Acres broilers, Jingxing 100 crossbred chickens and Beijing fatty chickens. Meat Sci 2007, 77(2): 220-227.
57. Coró FA, Youssef EY, Shimokomaki M: Age related changes in poultry breast meat collagen pyridinoline and texture. J Food biochem 2002, 26(6):533-541.

PATOMORFOLOŠKE KARAKTERISTIKE I PROMENE KVALITETA MESA DRVENASTIH GRUDI KOD BROJLERA RAZLIČITIH GENOTIPOVA I STAROSTI

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Ovom studijom ispitivane su patomorfološke karakteristike i promene kvaliteta mesa povezane sa miopatijom drvenastih grudi (WB) kod ukupno 192 brojlera podeljenih u dve grupe na osnovu provenijencije teških hibrida (Ross 308, n=96 i Cobb 500, n=96) tokom 42, 60 i 70 dana tova. Tokom sva tri perioda uzgoja, incidenca WB je bila konstantno visoka (>73%) dostižući maksimum 70. dana (83% Ross, 90% Cobb, pojedinačno). Cobb brojleri su imali bolje proizvodne rezultate i parametre kvaliteta trupova nakon 42. i 60. dana eksperimenta ($p \leq 0,05$). Genotip nije uticao na incidencu ili stepen izraženosti promena WB, dok je dužina tova uticala na povećanje učestalosti nalaza WB sa jako izraženim promenama od 11,67% 42. dana do 36,67% 70. dana ($p=0.003$). Nalaz WB u mesu brojlera zahvaćenih ovom miopatijom uticao je na povećanje pH vrednosti, svetloće (L*), udela crvene boje (a*) i udela žute boje (b*) u mesu ($p<0,0001$), izuzev 70. dana. Dužina tova uticala je na fizičko-hemijske i parametre boje mesa ($p<0,0001$). Značajan uticaj genotipa na sposobnost vezivanja vode ($p<0,0001$), kalo kuvanja ($p \leq 0,05$) i silu sečenja mesa ($p<0,0001$) utvrđen je 42. dana. Razlike u sposobnosti vezivanja vode zabeležene su 60. i 70. dana između normalnih i fileta WB sa jako izraženim promenama ($p<0,0001$). Tokom sva tri perioda uzgoja, uzorci pektoralne muskulature zahvaćeni sa jako izraženim promenama WB imali su veću silu sečenja u poređenju sa normalnim uzorcima ($p<0,0001$). Pored toga, sa povećanjem starosti brojlera, vrednosti sposobnosti vezivanja vode, kaloa kuvanja i sile sečenja pektoralne muskulature su bile veće ($p<0,0001$). Dobijeni rezultati u vezi sa pojavom i intenzitetom promena WB i posledičnim uticajem na kvalitet mesa ukazuju na to da se produženi tov ne preporučuje kod intenzivnog uzgoja brojlera.