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ANIMAL PRODUCTION

Histomorphometry of muscle fibers in breast fillets of broilers with wooden breast myopathy

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ABSTRACT. Wooden breast myopathy (WB) strongly affects the poultry industry mainly in terms of consumers rejection and economical loses, due to morphological changes in broiler muscle tissue and consequently low meat quality. The aim of this study is to evaluate the histomorphometry of muscle fibers of breast fillets of broilers with severity levels of WB myopathy. The histological evaluation considered 30 samples of the *pectoralis major* muscle and the level of WB myopathy (ten normal fillets, ten moderate fillets, and ten severe fillets). Fillets with a severe level of WB present low average fiber number, high average fiber diameter, low percentage of fibers with diameter of less than 20 μm, low percentage of fibers with diameter between 20 and 40 μm, and high percentage of fibers with diameter between 40 and 70 μm. Fiber cross-sectional area is greater in fillets affected by moderate and severe WB. Thus, fillets with a severe level of WB damage the muscle fiber structure and contribute more severely to the degenerative processes of breast meat.

Keywords: abnormalities; breast meat; fiber size; muscle morphology.

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Introduction

In recent years, the high breast yield in broilers has led to changes in their morphological structure. In broilers selected for a high breast yield, the increase in muscle fiber diameter has reduced the space available in the connective tissue, decreasing blood supply, altering muscle metabolism, and causing muscle lesions, such as myopathies (Clark & Velleman, 2017).

Currently, wooden breast (WB) myopathy has strongly affected the poultry industry mainly because of morphological changes in muscle tissue and low meat quality. In this sense, is possible to highlight total or cut condemnations, lower yield and product value due to reduction in water retention capacity, lower emulsification and gelation capacity and consumers rejection as factors responsible for economic loss in the poultry sector (Petracci et al., 2019). Microscopically, WB myopathy presents fibers of different sizes, necrosis, muscle degeneration, connective and adipose tissue infiltration, and low meat quality, such as high dripping, cooking losses, and high final pH values (Soglia et al., 2016; Kawasaki, Iwasaki, Yamada, Yoshida, & Watanabe, 2018; Assunção et al., 2020). Such changes in broiler breast fillets and meat quality may affect industrial processing and the final quality of the products. Therefore, the aim of this study is to evaluate the histomorphometry of muscle fibers of broilers with severity levels of WB myopathy.

Material and methods

All procedures performed in this study were approved by the Ethics Committee on the Use of Animals BRF/SIF 18 (protocol no. 076/18/14). Histological evaluation considered 30 samples of the *pectoralis major* muscle and levels of WB myopathy (ten normal fillets, ten moderate fillets, and ten severe fillets). Fillets were collected from male Cobb 500® chicken, raised in a dark house facility, slaughtered at 40 days of age and average weight of 3.066 kg. Slaughtering was conducted after an average fasting time of

7h and 25 min., and an average waiting time in the shed of 1h and 15 min. After weighing, the birds were stunned (voltage: 60 V, frequency: 1000 Hz, and alternating current: 120 mA), scalded (2 min. at 60°C), plucked, and eviscerated mechanically. The carcasses were submitted to a pre-chiller (12°C) and a chiller (2°C), totaling 1h and 10 min. before deboning. Assunção et al. (2020) described the visual assessment, myopathy classification, and sample collection in detail. Samples were stored at 4°C for up to 24 hours *post-mortem*, then the superficial layer (ventral side) of fillets of each WB level were cut along the muscle fibers. The cuts had approximately 0.5 cm in width, 1.5 cm thickness, and 3 cm in length (Figure 1; method adapted by Soglia et al., 2017).

Figure 1. Design of the histological analysis of the ventral portion of broiler fillets according to the severity of wooden breast myopathy.

Subsequently, the samples were immediately fixed in 10% buffered formalin solution for 24 hours at room temperature and transferred to 70% alcohol for preservation. The samples were then dehydrated in a graded alcohol series (70 to 100%), diaphanized in xylol, and impregnated in paraffin. From each sample, ten serial cross-sections (tissues) with a 5-μm thickness were obtained in duplicate using a rotary microtome (American Optical 820, New York, USA) mounted on stoned no slides (Perfecta Exacta, São Paulo, Brazil) and stained with hematoxylin and eosin (H&E), according to the method suggested by Michalany (1990). After staining, the slides were made with permount balsam and a coverslip for mounting permanent slides.

Digital photomicrographs were taken using a biological microscope (BEL Photonics Bio 2, Italy) and a Moticam digital camera (Moticam 2300, Hong Kong, China) equipped with the software Motic Images Plus 2.0 (Motic, Hong Kong, China). Twenty slides (ten tissue samples/slide) of each level of myopathy (ten normal fillets, ten moderate fillets, and ten severe fillets) were prepared, totaling 60 slides. Five photomicrographs were captured of each tissue at a 10X magnification and read by scanning technique by only one person (Joiner, Hamlin, Lien, & Bilgili, 2014), totaling 3,000 photomicrographs.

The histomorphometric analyses aiming a quantitative evaluation between levels of WB myopathy were average fiber number, average fiber diameter, and fiber cross-sectional area. Average fiber diameter was measured by counting 30 fibers/sample (Clark & Velleman, 2017) so that the smallest distance between each fiber was measured to minimize variability associated with the stage of muscle contraction of neighboring fibers (Joiner et al., 2014). The fiber cross-sectional area was estimated in 100 fibers of each sample (Petracci, Sirri, Mazzoni, & Meluzzi, 2013). In addition, the percentage of fibers with diameters < 20 μm, ≥ 20 μm and < 40 μm, ≥ 40 μm and < 70 μm, and ≥ 70 μm were determined as described by Wang, Clark, Jacobi, and Velleman (2020). Myopathic lesions (muscle degeneration and necrosis), and lipid and connective tissue accumulation were described according to Malila et al. (2018).

Statistical analysis

The data set was initially subjected to normality and homogeneity tests using the Shapiro-Wilk Normality test and, whenever necessary, transformed into a logarithmic scale, however always showing the original values. Subsequently, data were analyzed using the ANOVA option of the General Linear Model (GLM) procedure in the statistical software Minitab (version 17, Inc. State College, PA 2014). When significant, the means were compared by Tukey test ($p < 0.05$).

Results

The observations of photomicrographs performed in fillets affected by moderate and severe WB show that both had an absence of the characteristic polygonal shape of fibers, hypertrophic fibers, infiltration of endomysial connective tissue separating the fibers (fibrosis), accumulation of adipose tissue, fibers with degenerations, and necrosis (Figure 2A and B).

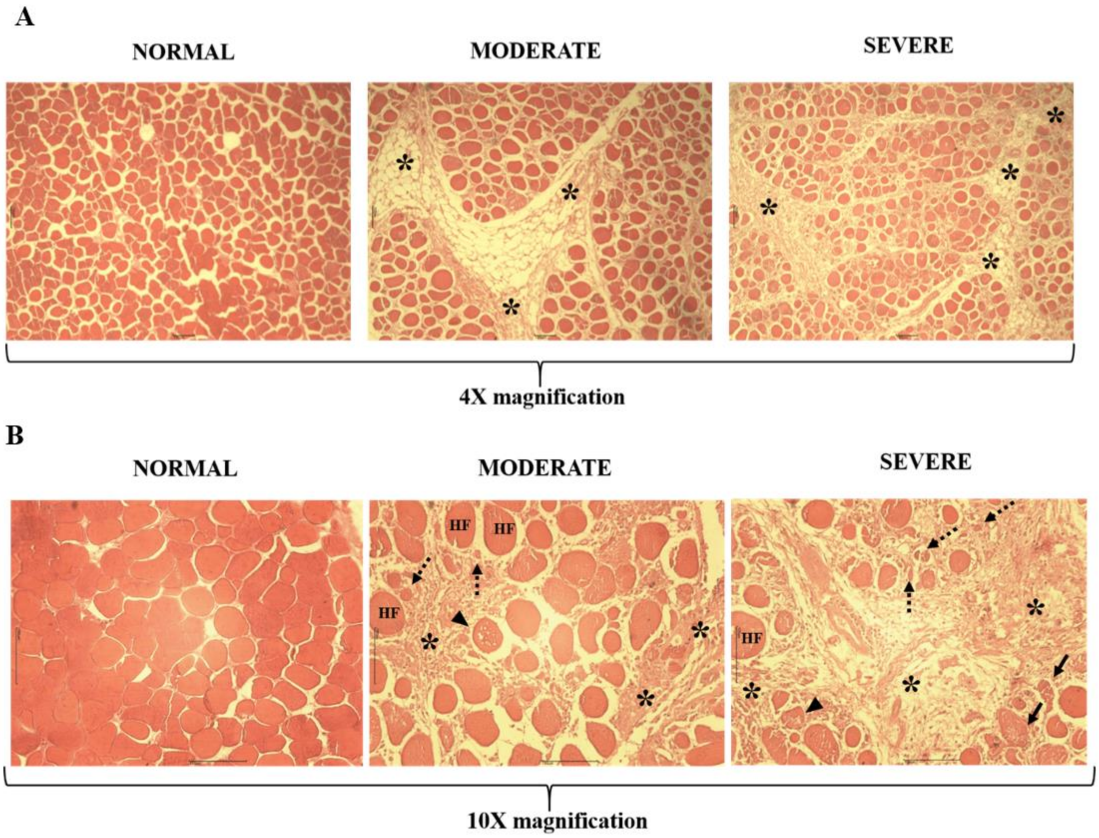

Figure 2. Photomicrography of broiler fillets based on the severity of wood breast myopathy (Normal, Moderate, and Severe).

A) Asterisks: replacement of muscle tissue for connective and adipose tissue. B) Asterisks: accumulation of endomysial connective tissue. Arrows: fibers with muscle degeneration. Dotted arrows: small-size fibers. Arrow heads: fibers with necrosis. HF: hypertrophic fibers. Hematoxylin and eosin (H&E) staining. Scale: 100 μm.

There was no difference ($P = 0.06$) for the parameter fiber diameter (FD) ⩾ 70 μm (%) among WB myopathy levels. The fiber cross-sectional area (μm^2) was greater ($P = 0.0001$) in fillets affected by moderate and severe WB. The lowest average fiber number occurred in fillets with a severe level of WB ($P = 0.015$) and the highest average fiber number was observed in normal fillets, while moderate fillets showed intermediate values. Fillets with severe WB levels showed a larger ($P = 0.001$) average fiber diameter compared to normal and moderate fillets, which did not differ from each other. Normal fillets and those with a moderate level of WB showed higher values ($P = 0.004$ and $P = 0.001$) for the parameters FD < 20 μm (%) and 20 μm ⩾ FD < 40 μm (%) compared to severe level fillets. Regarding the parameter 40 μm ⩾ FD < 70 μm (%), fillets with severe level of WB showed higher ($P = 0.004$) values compared to normal and moderate fillets, which did not differ from each other (Table 1).

Table 1. Effects of levels of severity of WB myopathy on muscle fiber histomorphometry.

Parameters	WB myopathy level[1]			MSE	P-value
	Normal (0)	Moderate (1)	Severe (2)		
Fiber CSA (μm^2)[2]	2,375.07[b]	3,359.23[a]	2,973.48[a]	112	0.0001
AFN/field[3]	77[a]	71[ab]	64[b]	1.92	0.0150
AFD/field (μm)[4]	43.08[b]	42.34[b]	49.00[a]	0.88	0.0010
FD < 20 μm (%)[5]	8.58[a]	10.65[a]	5.79[b]	0.03	0.0040
20 μm ⩾ FD < 40 μm (%)	33.04[a]	33.03[a]	22.82[b]	1.38	0.0010
40 μm ⩾ FD < 70 μm (%)	54.12[b]	52.87[b]	61.80[a]	1.28	0.0040
FD ⩾ 70 μm (%)	4.26	3.46	9.58	0.09	0.0600

MSE = Mean standard error. [a,b] Mean values of the same parameter followed by different overwritten letters differ significantly ($p < 0.05$). [1] *n* = 10/level of severity. [2] CSA = cross-sectional area. [3] AFN = average fiber number. [4] AFD = average fiber diameter. [5] FD = fiber diameter.

Discussion

Changes observed in the photomicrographs (Figure 2A and B) are consistent with the inferior results for meat quality obtained in a previous study (Assunção et al., 2020), such as higher weight loss per cooking and drip losses in increasing levels of myopathies. Similarly, Kawasaki et al. (2018) found small fibers with degenerative changes and decreased polygonal shape and fibrosis. According to the authors, these morphological changes are due to an insufficient blood supply. In our study, blood supply histological structures were not evaluated. Fillets affected by moderate and severe WB had a larger fiber cross-sectional area (μm^2). Likewise, Dalle Zotte et al. (2017) found a larger fiber cross-sectional area and a lower fiber number in fillets with WB. On the other hand, fillets with severe WB levels showed a larger average fiber diameter and a higher value for the parameter 40 μm ⩾ FD < 70 μm (%) compared to normal and moderate fillets. Large fibers with a greater cross-sectional area and a greater diameter are indexes of muscle abnormalities that may contribute to necrosis and degenerative processes due to reduced space for connective tissue, limiting vascularization, lactic acid accumulation, and inhibition of satellite cell mediated fiber regeneration (Clark & Velleman, 2017). These indexes of abnormalities are related to the process of muscle growth and development, as they may limit the circulatory system, resulting in broilers more prone to oxidative stress associated with WB myopathy (Wang et al., 2020). In addition, the presence of necrotic fibers, as reported in this study, may be a sign of a structural muscle fiber damage (Petracci et al., 2013).

Conclusion

Fillets with severe level of WB provide evidence that changes in histomorphometric measurements cause injuries to muscle fiber structure. In addition, the severe WB level contributes most severely to degenerative processes in breast meat.

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