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Histopathologic evaluation of wooden breast and white striping myopathy in different broiler genotypes using light microscopy and image analysis

Evaluación histopatológica de la miopatía de estría blanca y pechuga de madera en diferentes genotipos de pollos de engorde mediante microscopía óptica y análisis de imágenes

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ABSTRACT

Broiler myopathies cause significant economic losses in the poultry industry, adversely affecting meat quality and animal welfare. Cobb 500 and Ross 308 are widely cultivated commercial lines globally; however, Anadolu–T is a newly developed genotype with limited research on the histopathological evaluation of myopathic lesions. This study focuses on the histopathological evaluation of breast muscle myopathies in three different broiler lines (Cobb 500, Anadolu–T, and Ross 308). Additionally, histopathological lesions such as lipidosis, inflammatory cell infiltration, connective tissue formation, and degeneration were compared among genotypes using manual scoring with traditional light microscopy, as well as less commonly utilized digital image analysis software, including ImageJ and QuPath. Macroscopically, the Cobb genotype exhibited the highest WB scores (mean score: 2) ($P<0.05$), while the Anadolu–T genotype had the highest WS scores (1.11) ($P>0.05$). In the evaluation of histopathological lesions, the highest fibrosis scores were observed in the Cobb genotype (0.86), the highest mononuclear inflammatory cell infiltration scores in the Ross genotype (2.25), the highest lipidosis scores in the Anadolu–T genotype (3.22), and the highest degeneration scores in the Cobb genotype (3) ($P>0.05$). In this study, the evaluation of myopathy scores revealed significant differences in myopathy susceptibility among different genotypes. The Anadolu–T genotype was found to be less susceptible to WB myopathy severity ($P<0.05$) but more prone to WS myopathy severity ($P>0.05$).

Key words: Broiler; Anodolu-T; myopathy; image analysis; histopathology

RESUMEN

Las miopatías en pollos de engorde causan pérdidas económicas significativas en la industria avícola, afectando negativamente la calidad de la carne y el bienestar animal. Cobb 500 y Ross 308 son líneas comerciales ampliamente cultivadas a nivel mundial; sin embargo, Anadolu–T es un genotipo recientemente desarrollado con investigaciones limitadas sobre la evaluación histopatológica de las lesiones miopáticas. Este estudio se centra en la evaluación histopatológica de las miopatías en los músculos pectorales de tres líneas diferentes de pollos de engorde (Cobb 500, Anadolu–T y Ross 308). Además, las lesiones histopatológicas como la lipidosis, la infiltración de células inflamatorias, la formación de tejido conectivo y la degeneración se compararon entre los genotipos utilizando puntuaciones manuales con microscopía óptica tradicional, así como programas de análisis de imágenes digitales menos utilizados, incluidos ImageJ y QuPath. Macroscópicamente, el genotipo Cobb presentó las puntuaciones más altas de WB (puntuación media: 2) ($P<0,05$), mientras que el genotipo Anadolu–T tuvo las puntuaciones más altas de WS (1,11) ($P>0,05$). En la evaluación de las lesiones histopatológicas, las puntuaciones más altas de fibrosis se observaron en el genotipo Cobb (0,86), las puntuaciones más altas de infiltración de células inflamatorias mononucleares en el genotipo Ross (2,25), las puntuaciones más altas de lipidosis en el genotipo Anadolu–T (3,22) y las puntuaciones más altas de degeneración en el genotipo Cobb (3) ($P>0,05$). En este estudio, la evaluación de las puntuaciones de miopatías reveló diferencias significativas en la susceptibilidad a las miopatías entre los diferentes genotipos. Se encontró que el genotipo Anadolu–T era menos susceptible a la gravedad de la miopatía WB ($P<0,05$), pero más propenso a la gravedad de la miopatía WS ($P>0,05$).

Palabras Clave: Pollo de engorde; Anodolu-T; miopatía; análisis de imágenes; histopatología

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INTRODUCTION

In recent years, the global demand for poultry meat has significantly increased, prompting intensive genetic selection and feed optimization in broiler production. These advancements have resulted in faster growth rates and enhanced breast meat yield [1]. Among the most popular commercial broiler (*Gallus gallus domesticus*) genotypes known for their rapid growth and feed efficiency are Cobb 500 and Ross 308 [2, 3]. Anadolu–T is a new broiler breed, with breeding efforts initiated in 2017 and officially registered as Anadolu–T in 2020 [4].

However, it is known that these genetic selections have contributed to the development of a group of myopathies, particularly affecting the *Pectoralis major* muscle (breast muscle), leading to defects in both appearance and function [5, 6]. Among breast muscle myopathies, White Striping (WS), Wooden Breast (WB), and Spaghetti Meat (SM) are more commonly observed [7, 8, 9]. SM is characterized by the degradation and separation of muscle fibers, whereas WB is defined by increased firmness, pale coloration, and occasional petechial hemorrhaging, along with varying amounts of clear, viscous fluid on the epimysial surface [10]. WS is characterized by numerous white streaks running parallel to the muscle fibers [4, 11, 12].

Additionally, there is a type of myopathy known as Deep Pectoral Myopathy (DPM), which primarily affects the *supracoracoideus* or *pectoralis minor* muscles [13]. This condition leads to ischemic necrosis, impairing meat quality and causing histological and biochemical changes in the muscle tissue. Initially, the muscle appears dark red or purplish, but as necrosis progresses, it gradually turns to a greenish hue [5]. Although factors such as hereditary muscular dystrophy, heat stress, trauma, exertion, nutrition, and toxic myopathies have been identified as potential contributors to *P. major* muscle myopathies [10, 14] the precise etiology of these conditions remains unclear. However, since these myopathies are not caused by infectious agents, they do not pose a public health concern [9].

In most studies, pathological lesions in broiler myopathies are microscopically characterized by muscle degeneration, lipidosis, fibrosis, and inflammatory cell infiltration [10, 15, 16]. There are, however, a limited number of studies focused on the histopathological evaluation of chicken myopathies using digital image analysis software such as QuPath and ImageJ [17, 18, 19]. Traditionally, subjective scoring using light microscopy has been a common method in histopathological evaluations [15, 20, 21]. In recent years, the use of digital image analysis software has significantly increased, particularly due to its ability to provide more objective and reproducible results in the scoring of pathological lesions.

In this study, pathological lesions in the *P. major* muscle of different broiler breeds (Cobb 500, Anadolu–T, and Ross 308) were evaluated both macroscopically and microscopically. Notably, there is a lack of significant studies in the existing literature regarding the histopathological examination of myopathic lesions in the Anadolu–T genotype. This study contributes to the identification of susceptibility to myopathy across different chicken breeds, providing valuable insights for genetic selection. The variation in pathological lesions in the breast muscles between breeds is expected to be valuable for improving poultry breeding programs and enhancing poultry meat quality. This study aims to contribute to the scientific community by providing novel and significant data in this field, thereby enhancing the existing literature.

MATERIAL AND METHODS

Sample collection and preparation

The broiler muscle samples used in this study were collected under standard conditions from three different genotypes reared at the Poultry Breeding Unit of the Research and Application Center, Faculty of Veterinary Medicine, Bursa Uludag University. These genotypes included female Cobb (n=8), Anadolu–T (n=10), and Ross (n=9) broiler chickens. The samples were refrigerated (Arcelik, 475-T, Turkey) at +4°C for 14 hours and subsequently examined for muscle myopathies. As this study was conducted on muscle samples obtained post-slaughter from animals raised for food production, ethical approval was not required.

The study consisted of three main groups (Cobb 500, Ross 308, and Anadolu T), each comprising 50 female chicks. Each group was further divided into five replicates, with 10 chicks per replicate. A total of 150 one-day-old chicks were raised in the experimental unit of the faculty farm. For this experiment, the chicks were housed in group pens measuring 1 × 1 m, each equipped with plastic slatted floors. Housing density, lighting program, and other management practices in the groups were arranged in accordance with the "Regulation on Minimum Standards for the Protection of Broiler Chickens." The animals in the groups were raised under standard care and feeding conditions (*ad libitum*) for broilers over a period of 44 days (d). After the macroscopic scoring of breast fillets, 1 × 1 × 1 cm muscle tissue samples were collected from the right *P. major* muscle of each animal. Both longitudinal and transverse sections were prepared for each tissue sample [16]. The fresh muscle tissues collected were fixed in 10% neutral buffered formalin.

The tissues were then processed through graded alcohol series. After paraffin embedding and blocking, 4 µm thick sections were obtained using a microtome (Leica, RM 2125, Germany) to prepare muscle tissue slides. Hematoxylin and eosin (HE) staining (Merck Millipore, MA, USA) was performed on slides to identify histopathological changes, such as mononuclear cell infiltration and lipidosis, under a light microscope (Olympus Corporation, CX41, Japan). In addition, Masson's Trichrome (MT) staining (Bio Optica, Italy) was used to assess muscle fiber disruption, degeneration and collagen deposition [10, 15, 16].

Macroscopic scoring of pathological lesions

The presence and severity of WS and WB myopathies were assessed macroscopically on deboned right breast fillets by two evaluators. For WB, breast fillets showing no firmness or pale areas upon palpation, and having flexible consistency throughout, were classified as normal breast fillets (score 0). Fillets with mild firmness in the cranial region but otherwise flexible were classified as mildly affected (score 1). Fillets firm throughout the cranial region but flexible in the middle to caudal regions were classified as moderately affected (score 2). Fillets that were extremely firm and rigid from the cranial region to the caudal end were classified as severely affected (score 3) [22]. Breast fillets exhibiting WS were evaluated based on the presence of visible white stripes running parallel to the muscle fibers. Fillets with no visible white stripes were classified as normal (score 0). Fillets with white stripes less than 1 mm thick running parallel to the muscle fibers were classified as moderately affected (score 1). Fillets with white stripes 1-2 mm thick were considered severely affected (score 2).

Fillets where the surface was almost entirely covered by white bands thicker than 2 mm were classified as extremely affected (score 3) [23].

Scoring of histopathological lesions under light microscopy

The scoring of myopathic lesions in the *P. major* muscle of broilers was conducted under a light microscope (Olympus Corporation, CX41, Japan), taking into account the distribution and degree of structural deterioration of the lesions. This assessment allowed for a more precise determination of the severity and extent of the myopathic lesions. The scoring system ranged from 0 to 5. A score of (0) represents healthy muscle tissue without degeneration, fibrosis, or lipidosis. A score of (1) was assigned when the lesions covered less than 20% of the tissue, (2) when covering 20-40% of the tissue, (3) when covering 40-60%, (4) when covering 60-80%, and (5) when the lesions were extensive, covering greater than 80% of the tissue. The presence of inflammatory cells was classified as none (0), minimal (1), mild (2), moderate (3), or marked/severe (4/5) [21, 24].

Scoring pathological lesions using digital image analysis programs

Images from five distinct areas, where fibrosis and mononuclear inflammatory cell infiltration were most prominent in muscle tissue slides, were digitized under a light microscope (Olympus Corporation, CX41, Japan) at magnifications of 100× and 200×, respectively. After these images were uploaded to the ImageJ (v.1.53d) image analysis software, the following steps were selected sequentially: image, adjust, and color threshold. The threshold values used to calculate the fibrosis area were set as follows: hue: 0-255, saturation: 0-255, and brightness: 0-177. For the calculation of mononuclear inflammatory cell infiltration areas, the threshold values were defined as hue: 0-255, saturation: 0-255, and brightness: 0-185, and the same threshold values were consistently applied across all images. Subsequently, area calculations were performed using the analyze and measure functions [17].

Images from five distinct areas with the most intense myofibril degeneration and lipid infiltration were digitized under 100× magnification using a light microscope. These images were then uploaded to QuPath software (v.0.4.3), a cell image analysis software. To calculate lipid infiltration areas, the “wand tool” measurement tool was manually applied to delineate lipid infiltration regions. For the assessment of myofibril degeneration, the following steps were conducted sequentially: classify, pixel classification, train pixel classifier, and full (downsample = 1.00) segments were selected. Next, using the annotation module, the regions with myofibrillar degeneration were selected via the “wand tool” and classified as degeneration areas. Regions containing healthy muscle myofibrils and regions to be excluded (interstitial space) were separately classified and visualized with different colors. Subsequently, the “live prediction” mode was chosen to identify the areas of the classified measurements [18]. In both image analysis programs, the designated areas were calculated in pixels and then converted to square micrometers (μm²) for quantitative analysis.

Statistical analysis

All macroscopic measurements, including hardness for WB and white streak for WS, as well as the scores obtained from microscopic assessments, were tested for normal distribution using the Shapiro-Wilk normality test. Since the data did not follow a normal distribution, the Kruskal-Wallis test, a nonparametric method, was employed. A significance level of $P<0.05$ was considered statistically significant. All results are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS Statistics (v.20), and graphs were generated using GraphPad Prism (v.8.4.2).

RESULTS AND DISCUSSIONS

Macroscopic results

In the tactile evaluation of breast fillets, no pathological lesions were observed in the *P. major* muscle samples taken from one specimen in each group (FIG. 1A). Breast fillets exhibiting WB were characterized by distinctly firm areas, pale discoloration, an outwardly bulging appearance, and widespread petechial hemorrhages (FIG. 1B). Macroscopically, the severity of WB was moderate in Cobb and Ross genotypes, while it was mild in Anadolu–T. Cobb exhibited the highest WB scores (mean score: 2), followed by Ross (1.88) and Anadolu–T (0.78) groups, respectively ($P<0.05$) (FIG. 2A, TABLE I). The macroscopic evaluation of breast fillets for WS revealed the presence of numerous fine white striations, each less than 1 mm in width, aligned parallel to one another on the muscle surface (FIG. 1C). In all genotypes, the severity of WS was mild. The Anadolu–T genotype exhibited the highest mean WS score (1.11), followed by the Ross (0.75) and Cobb (0.57) groups, respectively, with no statistically significant differences observed among the groups ($P>0.05$) (FIG. 2A, TABLE I). WB and WS myopathies were observed simultaneously in all animals. In this study, the evaluation of myopathy scores revealed that across the three genotypes, Anadolu–T was less susceptible to the severity of WB myopathy but more prone to the severity of WS myopathy ($P<0.05$) (FIGS. 2A and 2B).

Previous studies have investigated the prevalence of WB and WS myopathies in commonly used broiler genotypes (Ross 308, Cobb 500) [25] or examined susceptibility to myopathies focusing

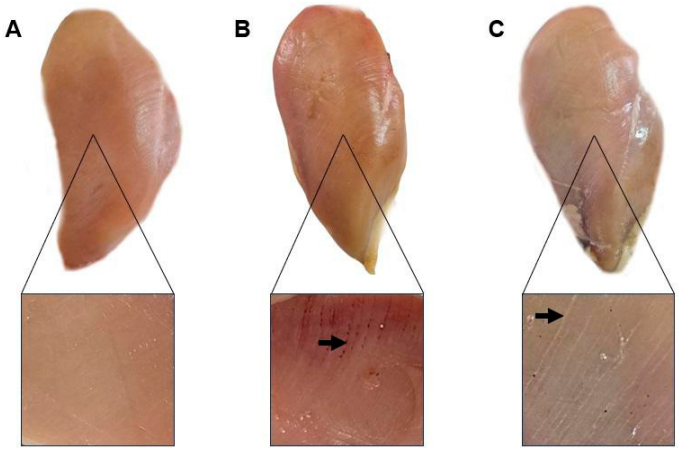

FIGURE 1. Macroscopic changes observed in the *Pectoralis major* muscle in myopathies. A) Normal appearance of the *P. major* muscle. B) WB. Pale and scattered petechial hemorrhages on the surface of the *P. major* muscle (arrow) (Cobb, score: 2. C) WS. Numerous white streaks less than 1 mm in size running parallel to myofibers in the *P. major* muscle (arrow) (Anadolu–T, score: 1), ($P<0.05$)

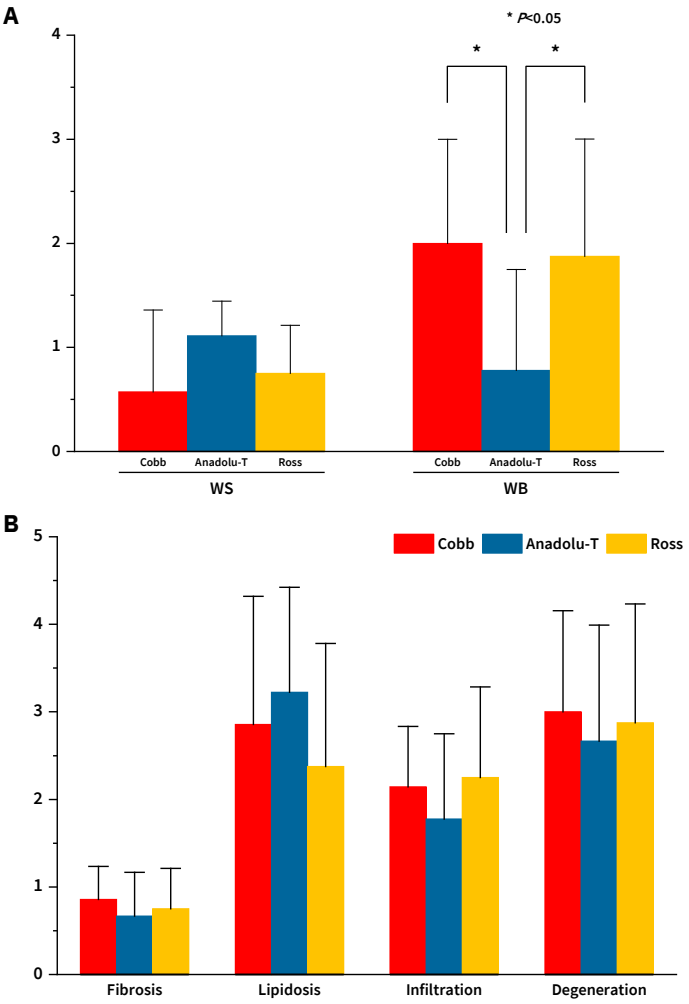

FIGURE 2. Representation of macroscopic and histopathologic findings in statistical graphs. A) Macroscopic findings score. B) Histopathologic findings score. WB: Wooden breast, WS: White striping, *P<0.05, ns: not significant. All data are presented as mean ± standard deviation (SD)

TABLE I
Macroscopic scoring of myopathies in three different genotypes

	Cobb (n=7)		Ross (n=8)		Anadolu-T (n=9)	
	Mean score (0-3)	SD	Mean score (0-3)	SD	Mean score (0-3)	SD
WB	2^{a*}	0.93	1.88^{a*}	1.03	0.78^{b}	0.92
WS	0.86	0.64	0.75	0.33	1.11	0.43

$*^{a-b}$ P<0.05. According to the Kruskal-Wallis test, a significant difference was observed for WB in the comparison between genotypes, whereas no significant difference was found for WS.

on a single genotype [16, 26, 27]. However, studies comparing WB and WS myopathy scores across different genotypes remain limited [28, 29]. In particular, the macroscopic evaluation of WB and WS in a novel genotype such as Anadolu–T has not yet been conducted. Previous literature reported that macroscopic scoring performed on d 42 in Ross 308 and Cobb 500 genotypes revealed that WB was of moderate severity (score 2) in both genotypes [28]. Similar results were obtained in this study, with WB scores being of moderate severity in both genotypes. However, this study identified a statistically significant difference in WB scores for the Anadolu–T genotype compared to other genotypes. This finding suggests that the Anadolu–T genotype may exhibit a distinct sensitivity to WB, potentially influenced by genetic or environmental factors.

White fatty striations on breast meat have been identified as a key pathognomonic feature of WS myopathy [15, 23, 30, 31]. The thickness of these striations has been correlated with the severity of WS and is characterized by greater lipid infiltration in the muscle tissue compared to other breast myopathies [12]. Another study in the literature conducted on Ross 308 and Cobb 500 genotypes reported that WS was of mild severity (score 1) on d 42 [29]. Similarly, in this study, WS scores were found to be of mild severity in both genotypes. However, no statistically significant differences were observed in intergroup comparisons regarding WS. This finding suggests that WS may have a more homogeneous distribution across genotypes. This study also observed the simultaneous occurrence of WS and WB myopathies. This finding aligns with previous literature [5, 27], supporting the notion that both myopathies may be associated with similar pathophysiological mechanisms. In particular, oxidative stress, hypoxia, and disruptions in muscle protein metabolism are thought to play significant roles in these mechanisms.

Histopathology results

In this study, the histopathological lesions in the *P. major* muscle of broiler chickens affected by myopathies were evaluated. Specifically, the presence of fibrosis in the muscle tissue was confirmed through minimal collagen detection using MT staining. In the transverse and longitudinal sections examined, myofibrils were observed as red, collagen as blue, and degenerated myofibrils as yellow-orange in the interstitial areas (FIGS. 2A and 3C). The collagen area was highlighted in red and visualized using the ImageJ analysis software (FIG. 3B). Among the genotypes analyzed, Cobb exhibited the highest observed fibrosis scores (mean score: 0.86), followed by Ross (0.75) and Anadolu–T (0.67) groups, with no statistically significant differences between them (P>0.05) (FIG. 2B, TABLE II). Mild multifocal mononuclear inflammatory cell infiltration (mononuclear infiltration) was observed around interstitial areas and fragmented myofibrils in muscle tissue (FIG. 3C). This infiltration area was highlighted in red using the ImageJ analysis software (FIG. 3D). The highest mononuclear infiltration scores were observed in the Ross genotype (mean score: 2.25), followed by Cobb (2.14) and Anadolu–T (1.78) groups (P>0.05) (FIG. 2B, TABLE II). Mild to moderate multifocal endomysial lipid infiltration areas appeared as white vacuolar deposits between muscle bundles (FIG. 4A). The lipid infiltration areas were visualized in green using the QuPath analysis software (FIG. 4B). The severity of lipidosis was moderate in the Anadolu–T and Cobb genotypes, while it was mild in the Ross genotype. Anadolu–T exhibited the highest recorded lipidosis scores (mean score: 3.22), followed by Cobb (2.86) and Ross (2.4) groups, with no statistically significant differences among them (P>0.05) (FIG. 2B, TABLE II).

In transverse sections of muscle tissue, moderately severe multifocal degenerated myofibrils with non-polygonal shapes and varying diameters were confirmed using HE and MT staining. In HE staining, the degenerated myofibrils were observed as hyper-eosinophilic amorphous structures with the loss of cross-striations

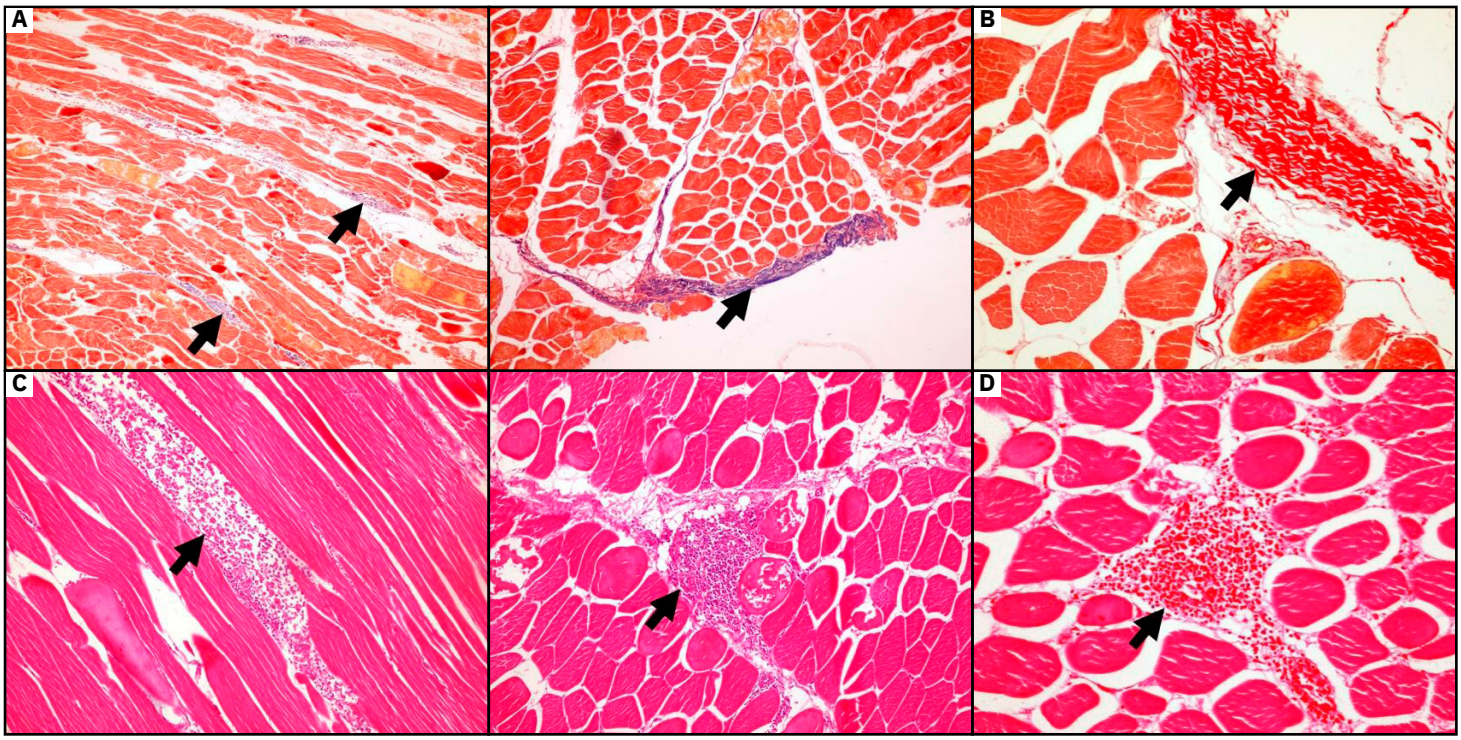

FIGURE 3. Histopathological findings and ImageJ analysis. A) Fibrosis. In longitudinal and transverse sections, the connective tissue (collagen) separating the muscle fibers in the perimysium appears blue (arrows), while myofibrils appear red (MT; 10×). B) The collagen area is displayed in red using the ImageJ analysis program (MT; 40×). C) Mononuclear inflammatory cell infiltration. Appearance of mononuclear inflammatory cell infiltrates around fragmented myofibrils showing loss of striations in longitudinal and transverse sections (arrow) (HE; 20×). D) The area of mononuclear inflammatory cell infiltration is shown in red with ImageJ analysis analysis program (arrow) (HE; 40×)

TABLE II
Histopathologic scoring of myopathic lesions in three different genotypes

	Cobb (n=7)		Ross (n=8)		Anadolu–T (n=9)	
	Mean score (0-5)	SD	Mean score (0-5)	SD	Mean score (0-5)	SD
Fibrosis	0.86	0.35	0.75	0.43	0.67	0.47
Infiltration	2.14	0.64	2.3	0.97	1.78	0.92
Lipidosis	2.86	1.36	2.4	1.32	3.22	1.13
Degeneration	3	1.07	2.88	1.27	2.67	1.25

According to Kruskal-Wallis test, there was no significant difference between genotypes in terms of histopathologic lesions $P>0.05$

(necrosis) (FIG. 3C). In MT staining, degenerated myofibrils appeared yellow-orange (FIG. 4C). Myofibrillar degeneration areas were visualized in green using the QuPath analysis software (FIG. 4D). The Cobb genotype exhibited the highest observed degeneration scores (mean score: 3), followed by Ross (2.88) and Anadolu–T (2.67) groups, with no statistically significant differences between them ($P>0.05$) (FIG. 2B, TABLE II). In this study, similar results were obtained in the scoring of histopathological lesions using both light microscopy and digital image analysis software. When comparing histopathological lesions across the three genotypes, it was noted that only lipidosis showed higher scores in the Anadolu–T genotype, while other lesions were recorded at lower scores (FIG. 2B).

Previous studies have investigated the histopathological lesions associated with WS [11, 19] and WB [9] myopathies in Ross 308 and Cobb 500 genotypes. However, research focusing on the comparison of microscopic lesions between these genotypes has been limited [8] Additionally, the histopathological evaluation of WB and WS myopathies in a new genotype such as Anadolu–T has not been previously conducted. In this study, no statistically significant differences were found in the comparison of histopathological lesions among the genotypes. However, it is suggested that the variations in histopathological lesions observed in these genotypes could provide valuable insights into the interactions between muscle development mechanisms and genetic factors.

Consistent with previous studies, this research demonstrated that WB and WS myopathies exhibit similar pathological features at both macroscopic and microscopic levels. Despite their distinct macroscopic appearances, the presence of similar histopathological characteristics in WB and WS myopathies suggests the involvement of shared mechanisms in their development [7, 12, 20, 30]. WS myopathy is typically associated with lipid accumulation (lipidosis) in the perimysial region, whereas WB myopathy is characterized by localized or diffuse hardening of the *P. major* muscle. This hardening often leads to a marked thickening of the perimysial network due to connective tissue proliferation (fibrosis) [15, 16, 32].

A study demonstrated that the presence of hardened muscle tissue in WB and WS myopathies, without fibrotic tissue, indicates that fibrosis is not the sole cause of muscle stiffness [10, 33]. The low fibrosis scores observed in this study are consistent with these findings in the literature. Additionally, it has been reported in the literature that muscle fiber degeneration and fibrosis collectively contribute to muscle stiffness [21]. In this study, the observation of

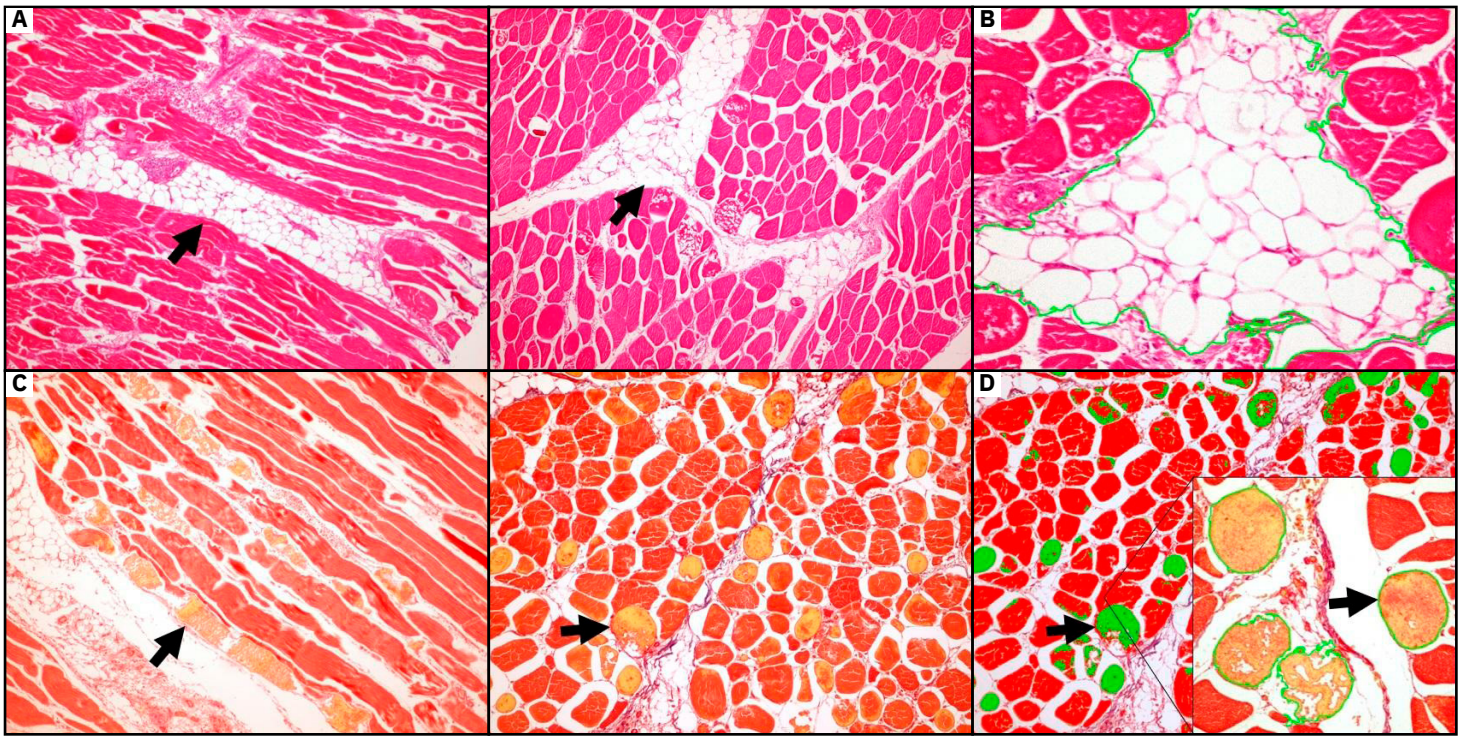

FIGURE 4. Histopathological findings and QuPath analysis. A) Lipidosis. appearance of white areas of lipid infiltration around the interstitium, muscle fibers, and muscle bundles in longitudinal and transverse sections (arrow) (HE; 10×). B) The area of lipid infiltration is shown in green using the QuPath analysis program (HE; 40×). C) Degeneration. Yellow-orange appearance of fiber separation, segmental myofibril disruption, fiber size changes, and flocular or vacuolar degeneration in longitudinal and transverse sections (arrow) (MT; 10×). D) Degeneration area highlighted in green using the QuPath analysis program. The larger image is shown at MT; 10× magnification, while the smaller image is at MT; 40× magnification (arrow)

higher rates of fibrosis and myodegeneration in the Cobb genotype compared to other genotypes suggests a strong association between this genotype and WB myopathy [16, 34]. As reported in a previous study, this study also confirmed the association between WS and lipid accumulation through macroscopic and microscopic analyses [12]. Furthermore, free lipids around venous vessels are thought to play a significant role in the initiation of reactive lesions (e.g., phlebitis, myositis, fibrosis) and the progression of myopathies. The inflammatory effects of lipid accumulations may trigger immune responses, leading to mononuclear infiltrations [35, 36]. This mechanism is considered significant in clearing necrotic and degenerative muscle tissue.

In two different studies, the lesion scores of mild WS myopathy were different from each other in the histopathologic evaluation of breast fillets in Ross genotype. In one of these studies, degeneration/necrosis score was mild, lipidosis was moderate, fibrosis and infiltration were not observed [30], while in the other study, degeneration/necrosis and infiltration scores were moderate, fibrosis and lipidosis were mild-moderate [19]. In this study, similar findings were observed in Ross genotype WS myopathy, with lipidosis being mild to moderate and degeneration of moderate severity. In another study on the Cobb genotype, myopathic lesions, fibrosis, and lipidosis scores were reported as mild to moderate in cases of moderate WS myopathy, whereas in severe WS, fibrosis was mild, and the scores for other lesions were of moderate severity [14]. In this study, similar to severe WS myopathy, lipidosis and degeneration were found to be of moderate severity. In a study on the Ross genotype, mild WB myopathy was reported to exhibit predominantly moderate myodegeneration, severe vasculitis/mononuclear infiltration, and moderate adipose tissue/lipidosis. In cases of severe WB myopathy, myodegeneration and vasculitis were severe, and adipose tissue/lipidosis was also reported to be severe [16].

In this study, similar to mild WB myopathy, degeneration was found to be of moderate severity. The variability in histopathological lesion scores identified in these studies is thought to result from multiple factors, including genetic diversity between genotypes, sex, age, type and severity of myopathy, rearing conditions, nutritional regimen, the anatomical region of the muscle tissue examined, and differences in histopathological evaluation methods used.

CONCLUSION

In this study, macroscopic and microscopic examinations of breast fillets obtained from female Cobb, Anadolu–T, and Ross genotypes revealed the presence of both White Striping (WS) and Wooden Breast (WB) myopathies in all three genotypes. WB was recorded with the highest score in the Cobb genotype, while WS was observed with the highest score in the Anadolu–T genotype. Microscopic examinations identified fibrosis, lipidosis, degeneration, and mononuclear inflammatory cell infiltration. Fibrosis was most frequently observed in the Cobb genotype, while lipidosis was most prominently detected in the Anadolu–T genotype. The use of light microscopy and digital image analysis software in the evaluation of microscopic lesions has enhanced the consistency and reliability of the results obtained in this study. This study

demonstrated significant differences in the susceptibility of different genotypes to WB and WS myopathies. Notably, the resistance of the Anadolu–T genotype to WB myopathy highlights the need for further investigation into the mechanisms underlying the susceptibility and resilience of this genotype to muscle pathologies.

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Conflict of Interest

The authors declare no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Petracci M, Mudalal S, Soglia F, Cavani C. Meat quality in fast-growing broiler chickens. Worlds Poult. Sci. J. [Internet]. 2015; 71(2):363-374. doi: https://doi.org/f7gvb7

[2] Khalid N, Ali MM, Ali Z, Amin Y, Ayaz M. Comparative productive performance of two broiler strains in open housing system. Adv. Life. Sci. [Internet]. 2021 [cited 29 Oct. 2024]; 8(2):124-127. Available in: https://goo.su/lgBqj1U

[3] Erensoy K, Sarıca M. Fast growing broiler production from genetically different pure lines in Turkey: Broiler traits: growth, feed intake, feed efficiency, livability, body defects and some heterotic effects. Trop. Anim. Health Prod. [Internet]. 2023; 55(1):61. doi: https://doi.org/g8xpst

[4] Sarıca M, Erensoy K, Özkan İ, Oğuzhan E, Çağlak S. Anadolu–T Etlik Piliç Saf Hatlarının Gelişme ve Karkas Özellikleri [Growth and carcass traits of Anadolu–T broiler pure lines]. Türk Tarım-Gıda Bilim ve Teknoloji Dergisi [Turkish JAF Sci. Tech]. [Internet]. 2021; 9(11):1980-1987. Turkish. doi: https://doi.org/g8xpsv

[5] Petracci M, Cavani C. Muscle growth and poultry meat quality issues. Nutrients [Internet]. 2012; 4(1):1-12. doi: https://doi.org/fxnsw6

[6] 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. [Internet]. 2020; 99(1):620-628. doi: https://doi.org/gmpdbc

[7] Baldi G, Soglia F, Petracci M. Current status of poultry meat abnormalities. Meat Muscle Biol. [Internet]. 2020; 4(2). doi: https://doi.org/gk9z5j

[8] 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. Compr. Rev. Food Sci. Food Saf. [Internet]. 2019; 18(2):565-583. doi: https://doi.org/gk9z6d

[9] Barbut S. Recent myopathies in broiler's breast meat fillets. Worlds Poult. Sci. J. [Internet]. 2019; 75(4):559-582. doi: https://doi.org/gk9z5c

[10] Sihvo HK, Immonen K, Puolanne E. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. [Internet]. 2014; 51(3):619-623. doi: https://doi.org/g8xpsw

[11] Kuttappan VA, Brewer VB, Apple JK, Waldroup PW, Owens CM. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. [Internet]. 2012; 91(10):2677-2685. doi: https://doi.org/f388pq

[12] Baldi G, Soglia F, Mazzoni M, Sirri F, Canonico L, Babini E, Laghi L, Cavani C, Petracci M. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal [Internet]. 2018; 12(1):164-173. doi: https://doi.org/gct65m

[13] Jordan FTW, Pattison M. Poultry Diseases 4th ed. Deep pectoral myopathy of turkeys and chickens. London (UK): Saunders; 1998. p. 398-399 p.

[14] Maxie MG, editor. Jubb, Kennedy, and Palmer's Pathology of domestic animals. 5th ed. Vol. 1. [Internet]. Philadelphia (PA, USA): Saunders; 2007. Chapter 2, Muscle and tendón. p. 185-186. doi: https://doi.org/djpz7s

[15] Kuttappan VA, Shivaprasad HL, Shaw DP, Valentine BA, Hargis BM, Clark FD, McKee SR, Owens CM. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. [Internet]. 2013; 92(2):331-338. doi: https://doi.org/f4zmh8

[16] 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. [Internet]. 2017; 54(1):119-128. doi: https://doi.org/gnpswx

[17] Dayan J, Goldman N, Waiger D, Melkman-Zehavi T, Halevy O, Uni Z. A deep learning-based automated image analysis for histological evaluation of broiler pectoral muscle. Poult. Sci. [Internet]. 2023; 102(8):102792. doi: https://doi.org/g8xpsx

[18] Li B, Lindén J, Puolanne E, Ertbjerg P. Effects of wooden breast syndrome in broiler chicken on sarcoplasmic, myofibrillar, and connective tissue proteins and their association with muscle fiber area. Foods [Internet]. 2023; 12(18):3360. doi: https://doi.org/g8xpsz

[19] Prisco F, De Biase D, Piegari G, d'Aquino I, Lama A, Comella F, Mercogliano R, Dipineto L, Papparella S, Paciello O. Pathologic characterization of white striping myopathy in broiler chickens. Poult. Sci. [Internet]. 2021; 100(7):101150. doi: https://doi.org/gmpdbb

[20] 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. [Internet]. 2022; 101(4):101747. doi: https://doi.org/g8xps2

[21] 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. [Internet]. 2017; 46(6):623-643. doi: https://doi.org/g8xps3

[22] 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. [Internet]. 2016; 95(9):2167-2173. doi: https://doi.org/f85wp7

[23] Kuttappan VA, Hargis BM, Owens CM. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. [Internet]. 2016; 95(11):2724-2733. doi: https://doi.org/f9b46c

[24] Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet. Pathol. [Internet]. 2013; 50(6):1007-1015. doi: https://doi.org/g8xps4

[25] Bordignon F, Xiccato G, Cabrol MB, Birolo M, Trocino A. Factors affecting breast myopathies in broiler chickens and quality of defective meat: A meta-analysis. Front. Physiol. [Internet] 2022; 13:933235. doi: https://doi.org/g8xps5

[26] Lake JA, Dekkers JC, Abasht B. Genetic basis and identification of candidate genes for wooden breast and white striping in commercial broiler chickens. Sci. Rep. [Internet] 2021; 11(1):6785. doi: https://doi.org/g8xps6

[27] Muñoz-Lapeira M, Font-i-Furnols M, Brun A, Jofré A, Botella M, Zomeño C. Breast myopathy co-occurrence and its impact on carcass and meat quality attributes in broiler chickens. Poult. Sci. [Internet] 2025; 104(1):104625. doi: https://doi.org/g8xps7

[28] Rajčić A, Bošković Cabrol M, Glišić M, Čobanović N, Tomović V, Laudanović M, Nešić S. Pathomorphological and meat quality alterations connected with wooden breast in broiler chickens of different genotypes and slaughter ages. Acta Vet. Sciendo [Internet] 2024; 74(2):183-209. doi: https://doi.org/g8xps8

[29] Panisson JC, Bassi LS, Barrilli LEN, Dias RC, Maiorka A, Krabbe EL, Lopes L, Oliveira SG. Energy and nutrient intake on white striping, wooden breast and carcass composition in broilers from three genetic lineages at different ages. Anim. Feed Sci. Technol. [Internet] 2022; 285:115225. doi: https://doi.org/g8xps9

[30] 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. [Internet]. 2015; 94(8):1843-1848. doi: https://doi.org/f7krr2

[31] Petracci M, Mudalal S, Bonfiglio A, Cavani C. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. [Internet]. 2013; 92(6):1670-1675. doi: https://doi.org/f4z9r7

[32] Soglia F, Mazzoni M, Petracci M. Spotlight on avian pathology: current growth-related breast meat abnormalities in broilers. Avian Pathol. [Internet]. 2019; 48(1):1-3. doi: https://doi.org/g8xptb

[33] Pavanello ACL, Mendonça FJ, Oliveira TES, Torezan GB, Di Santis GW, Soares AL. Physicochemical and histopathological parameters of broilers with dorsal cranial myopathy. Anim. Biosci. [Internet]. 2023; 36(6):953-961. doi: https://doi.org/g8xptc

[34] 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. [Internet]. 2017; 96(9):3465-3472. doi: https://doi.org/gbtrjx

[35] Maxwell MH, Robertson GW. The avian heterophil leucocyte: a review. Worlds Poult. Sci. J. [Internet]. 1998; 54(2):155-178. doi: https://doi.org/dptgvr

[36] Kozakowska M, Pietraszek-Gremplewicz K, Jozkowicz A, Dulak J. The role of oxidative stress in skeletal muscle injury and regeneration: focus on antioxidant enzymes. J. Muscle Res. Cell. Motil. [Internet]. 2015; 36:377-393. doi: https://doi.org/f8cqc9