Iraqi Journal of Veterinary Sciences

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Myofibril architecture in prediction and evaluation of broiler wooden breast: Histopathological and immunohistochemical study

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Article information

Article history:
Received 22 March, 2024
Accepted 03 June, 2024
Published online 24 June, 2024

Keywords:
Actin
Expression
Woody
Muscle
Immunohistochemistry

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Abstract

Wooden breast is one of the myopathies that affected broilers and caused economic loss, but the causes are still unknown. This experiment aimed to investigate the effect of a high percentage of soybean oil in inducing this abnormality. Thirty chicks were fed into basal ratio as a control group and basal ratio with soybean oil 5.20% as the treatment group for 35 days. The histopathological examination of the Pectoralis muscle of broiler rearing for 12 days was represented by abnormal myofiber architecture, edema, and increased endomysium formation at percentages <10% and occupied scor1. By 13-21 days, adipose tissue deposition with fibrosis occupies between >40% and <80%, with phlebitis and atrophied myofibers at 35 days. Fiji-ImageJ analysis was utilized as a unique quantification method to estimate myofiber diameter. The treatment group showed a significant decrease in myofiber diameter 0.187 and 0.705 at 21 and 35 days compared to the control group 0.706 and 2.276, respectively. The score/grading system investigated the viewpoints in histochemical analysis, so the fibrosis grade was moderate and severe. In contrast, the grade of carbohydrates was mild and weak in an insight myofiber sections obtained at 12 and 35 days of treatment. Immunohistochemistry staining of smooth muscle actin (α-SMA) expression was elevated from moderate to mild with progressive lesions in the treatment group. It has been found that adding a large proportion of soybean oil leads to a wood myofiber, and the use of semi-quantitative analysis and artificial intelligence aids in diagnosing myopathies.

DOI: 10.33899/ijvs.2024.148083.3547,

Introduction

The poultry industry has been rapidly developing by introducing many technologies, vaccines, and food programs to optimize muscle development and rapid poultry growth, which are the two main features of recent broilers. Nevertheless, the expansion in development rate and muscle mass was linked to alterations in the functional aspects of skeletal muscle, particularly in terms of its structure and biochemical characteristics (1). In addition, broilers bred to grow quickly and have a more significant body weight and breast production are more prone to stressors before and after slaughter, including factors such as humidity, temperature, chilling, and stunning methods (2). The occurrence of these chemical and physical alterations causes muscle abnormalities (3). such as white striping and wooden breast muscle, spaghetti meat (SM), and pale, soft, and exudative (PSE)-like condition. The pectoralis major (PM) muscles are particularly susceptible to metabolic-associated deficiencies and myopathies, which have recently raised significant concerns over myopathies (4). Wooden breast (WB) is an emerging chronic myodegeneration disease in broiler chickens, mainly characterized by hardness and firmness resulting from the replacement of the normal muscle tissue by fibrous connective tissue (5,6). Wooden breasts commonly occur in fast-growing breeders and may relate to metabolic disturbances. Other risk factors, such as environment, nutrition, and genetic selection (7,8), lead to

hypertrophic myofiber with increased distance between the affected muscle fiber and vessels. Additionally, it has been suggested that in chickens bred for meat production, the connective tissue in the muscles does not expand fast enough to match the radial expansion of the muscle fibers, resulting in injury and damage to the muscles (9). However, it is difficult to investigate the mechanisms causing wooden breast myopathy, and its etiology remains unidentified. The results of WB have been improved by experimenting with different feeding and production regimes (10).

This study is one of the few studies that aim to determine the ability of a high percentage of soybean oil to induce wooden breast, relying on histological alteration and semi-quantity statistical analysis of the affected myofiber upon the application of Artificial Intelligence (Fiji software program), and estimating the level of fibrosis and carbohydrates, as well as smooth muscle actin (α-SMA) expression upon immunohistochemistry.

Materials and methods

Ethical approve

Experiments on animals were conducted after the approval of the Scientific Ethical Committee / Faculty of Veterinary Medicine/ University of Mosul - UM.VET.2023.028.

Broiler housing

All thirteen chicks' broiler Ross-308 (11) were initially housed in two pens (170 x 170 x 175 cm/ pen) across the room on a veterinary college farm. A pen floor was concrete with bedding with wood shaving at a thickening 10 cm, and each pen from others by mesh walls with an average height of 125 cm. After being placed, the chicks were kept at 32-35°C. The housing temperature dropped by 3°C every week until it reached 21-23°C at 4 weeks of age, and it remained there until the end weeks of the experiment's fifth week. The rearing cage had the typical closed broiler house layout and was outfitted with automatic heating, ventilation, and temperature monitoring systems in addition to a 24-hour artificial lighting system (12).

Experimental regime

The recommendations for formulating the basal diet for broiler chicks were based on the National Research Council (13). Birds were administered water and feed as 2 *ad libitum* provided to a three-period feeding design from 0-12 days as a starter, while grower began from 13-21 days and finisher period 22-35 days. Birds were fed with a balanced ratio; their chemical composition is represented in table 1.

Muscle sample collection

As part of the study design, flocks were slaughtered when reaching 35 days, and pectoralis muscles were examined. Samples were taken from the ventral surface (facing the skin), according to the procedure Soglia *et al.* (14). Briefly, samples collected for histology and immunohistochemical analyses were sectioned from the superficial - cranial region of the breast muscle along myofiber, quickly kept on 10% formalin for fixation at least 72 hours for the histological sectioning procedure.

Table 1: Chemical composition of broiler component diet throughout varying periods of rearing

Nutrient name	Starter	Grower	Finisher
	Control/Treatment	Control/Treatment	Control/Treatment
Crud protein (%)	22.4/22.4	21/21	19.0000/19.000
Crude fat (%)	3.0000/5.2019	2.6848/5.1756	3.0142/5.2299
Lysine (%)	1.37/1.37	1.1800/1.1800	1.0800/1.0800
Methionine (%)	0.5659/0.5300	0.5100/0.5100	0.4800/0.4800
metabolizable energy kcal (ME/kg)	2800	3050	3100
Crud material (%)	89.8/89.9	89.8/89.9	90.06/90.23
Fiber (%)	3.6/4.0	3.1/3.6	3.0/3.6
Ashes (%)	5.9/6.0	4.7/5.1	4.5/4.6
Methionine and cysteine (%)	0.9/0.9	0.8/0.8	0.7/0.7
Threonine (%)	0.97/0.97	0.7/0.7	0.7/0.7
Tryptophan (%)	0.26/0.27	0.22/0.23	0.19/0.20
Calcium (%)	1.0/1.0	0.75/0.80	0.65/0.65
Phosphite (%)	0.48/0.48	0.42/0.42	0.36/0.36
Sodium (%)	0.16/0.17	0.16/0.17	0.18/0.18
Potassium (%)	0.93/0.97	0.85/0.89	0.76/0.80
Chloride (%)	0.24/0.24	0.21/0.23	0.23/0.23
Linoleic acid (%)	1.70/2.94	1.45/2.85	1.57/2.77
Choline (%)	1.23/1.26	1.10//1.09	971.5/961.9
Sodium and chloride potassium (%)	244.9/258.3	227.8/239.9	208.5/219.2

Histological image analysis workflow

The fixed muscle samples were trimmed to approximately 5 µm thickness and embedded in paraffin following routine tissue processing. The paraffin-embedded muscle tissues were sectioned to a thickness of 5 µm, rising on glass slides, and then dried sufficiently after clearance with xylene. Then routine (H&E) and special stains (Masson Trichrome) for fibrous connective tissue and carbohydrate staining (Periodic acid stain-PAS) (15,16) were performed on the muscle tissues. The individual lesions in muscle - tissues revealed in previous reports by Sihvo *et al.* (5) and Kawasaki *et al.* (17) were evaluated using a five-score index of severity considering the following features in the muscle (Table 2). The evaluation criteria for each lesion were the scores according to the apparent ratio of each lesion to the visual area of the tissue section. The scores of features for each histological lesion were applied according to the apparent ratio of each pathological lesion to the visual field of the tissue section (Table 3).

Table 2: Histopathological catalog lesions of wooden breast broiler

Disturbance	Histological features
Cell injury	Vacuolar degeneration, necrosis
Circulatory disorder	Perivascular cuffing, infiltration of inflammatory cells, hemorrhage, and congestion
Newly tissue formation	Thickening of endomysium tissue, Fibrosis
Abnormal myofiber	Variable in diameters, loss of polygonality and striation, splitting of myofiber, and intermittently

Table 3: Score sand ratio of wooden breast lesions

Score	Ratio of histological lesions
0	Absence lesions
1	The percentage of lesions <10%
2	The percentage of lesions>10%
3	The percentage of lesion >40% - <80%
4	The percentage of lesions <80%

Fiji-ImageJ analysis

Quantification was performed according to a standard Fiji-ImageJ (version 1.53t) measurement tool (18); the code and script are accessible as a Zenodo source [available here]. The processing of the image involvement recognition of the muscle fibers with (the PTBIOP Cellpose wrapper plugin) and the translation of the label covers to ROIs (regions of interest) with the MorphoLibJ plugin. The morphologic analysis included the muscle fiber measurement system; the miner diameter (mm) and transverse section mm 2 were saved to excel documents for statistical analysis.

Protocol of immunohistochemistry and semi-quantitative analysis

Hydrate and remove wax from the paraffin section. If necessary, apply thermal repair or digesting therapy to the tissue section's antigen by the antigen/antibody situation. For ten minutes, incubate with 3% H_2O_2 to eradicate endogenous peroxidase activity, then rinse with PBS or TBS (2-3) times; after that, the blocking buffer will be added and incubated for thirty minutes at 37°C. Shake off any extra liquid, then add the primary antibody (from a rabbit) at the recommended dilution ratio and incubate for 1 to 2 hours at 20 to 37°C or overnight at 4°C (then reheat for 30 minutes at 37°C), then rinse twice or three times with PBS or TBS, absorbent paper to dry the section has been used, then the polyperoxidase-anti- rabbit IgG and added and let it sit for 20 minutes at room temperature or 37°C and rinse with PBS or TBS for two to three minutes, then add one drop (around 50 µL). Mix thoroughly after adding 1 drop (about 50 µL) of DAB Concentrate for every 1 mL of DAB Substrate. It is necessary to control the period of coloring DAB, as the brown-yellowish or tan color is a vital indicator of the positive marker. Okoye and Nnatuanya (19) documented the semi-quantities of IHC (score and grade) according to the intense of immune stain reactivity (%) as the following: 0%=0/non, < 5-10% = 1/weak, 10-25% =2/mild, 25-50%= 3/moderate, and 50-75% =4/strong.

Statistical analysis

The statistical analysis of the data of this study was based on a t-test at values less than $P \leq 0.05$ and $P \leq 0.01$ (20).

Results

Histopathological features

The evaluation of histopathological lesions in the transverse myofiber section in broilers fed with the basal diet with a high percentage of soybean oil 5.20% during the starter rearing period shows deposition of fatty tissue, thin endomysium formation with fibrosis (Figure 1), myofiber appear homogenous round in shape and loss polygonate with variable in diameters and splitting all these lesions score at 3, edema and increase endomysium formation at percentage <10% (Figure 2).

Histologic lesions of WB myopathy in the PM of broiler at 13-21 days of age have progressed over time. The ratio of all lesions occupies between >40% and <80%, which is characterized by increased adipose tissue deposition with fibrosis splitting myofiber, severe congestion, and progress thickening and infiltration of inflammatory cells in

endomysium tissue with progress multifocal infiltration of inflammatory cells, myositis, and eosinophilic homogenous necrotic myofiber (Figure 2).

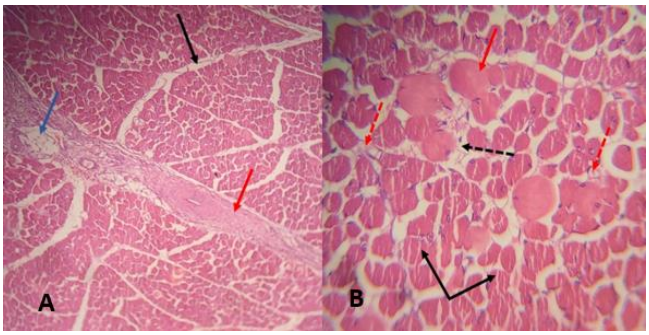

Figure 1: Image of PM in broiler fed with a basal diet with a high dose of soybean oil 5.20% during the starter period 12 days show: (A) deposition of adipose tissue (blue arrow), thine endomysium black arrow, fibrosis (red arrow), H&E, 40x (B) myofiber appear homogenous round in shape (red arrow), myofiber splitting black arrow, endomysium forming tissue (red -dot arrow) and edema, H&E, 400x.

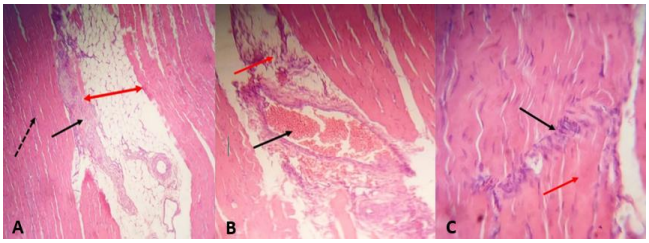

Figure 2: Image of PM in broiler fed with a basal diet with a high dose of soybean oil 5.20% during the grower period 21 days show : (A) increase adipose tissue deposition (red two-head arrow) with fibrosis (black arrow) and splitting myofiber (black dot arrow,)100X (B) sever congestion (black arrow) and progress thickening and infiltration of mononuclear inflammatory cells in endomysium tissue (red arrow),(C) with multifocal infiltration of inflammatory cells (black arrow)and eosinophilic homogenous necrotic myofiber (red arrow), 400X, H&E.

Represented microscopical lesions of wooden breast myopathies in broiler fed with a basal diet with high oil 5.20% during finisher period 22-35 days characterized by severe disrupting in myofiber architecture as loss of alignment and tortuosity of muscle fibers with atrophied and intermittently myofiber Zenker necrosis which represented by segmental myofibrillar interruption and multifocal infiltration of inflammatory cells these lesions scoring 4 with ration occupy more than 80%, concurrent progress lesions occupy between >40% and <80% represented by sever heterophilic cellularity a round myofiber (myositis) with phlebitis and atrophied myofiber with sever deposition of adipose tissue (Figure 3), also the result of microscopic examination investigated necrosis thickening of endomysium tissue and regeneration characterized by fibrosis which occupies more than 80%, furthermore there was edema and hemorrhage (Figure 4).

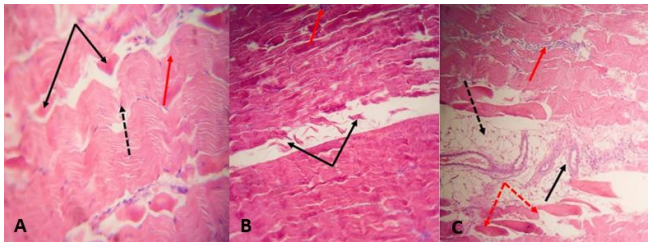

Figure 3: Image of PM in broiler fed with high dose soybean oil 5.20% during finisher period 35-day show: (A) loss of alignment of muscle fibers (black dot arrow) and tortuosity (red arrow) with atrophied and intermittently myofiber (black arrow) 400x, (B) Zeinker necrosis which represented by segmental myofibrillar interruption (black arrow) and multifocal infiltration of inflammatory cells (red arrow) 100x, (C) sever heterophilic cellularity a round myofiber (myositis)- (red arrow) with phlebitis (black arrow) and atrophied myofiber (red dot arrow) with sever deposition of adipose tissue (black dot arrow), H&E, 100x.

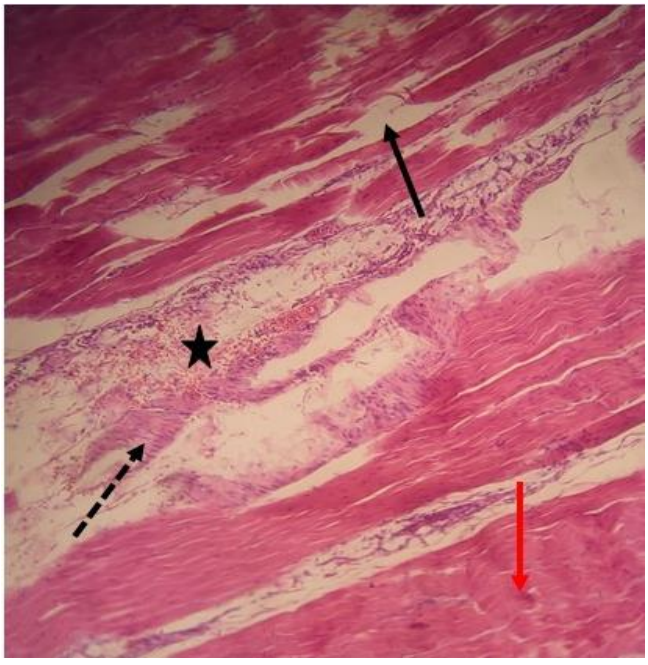

Figure 4: This image of PM in a broiler fed with high-dose soybean oil 5.20% during the finisher period of 35 days shows necrosis (red arrow), thickening of endomysium tissue, and fibrosis (black dot arrow). Furthermore, there was edema (black arrow) and hemorrhage (black star), H&E, 400X.

Semi-quantities System analysis (SSA)

Through this study, artificial intelligence software (Fiji) was applied to evaluate the area and measurements of muscle fibers, which helps apply semi-quantitative analysis and give a statistical description of pathological alteration in muscle tissue. The statistical analysis reveals significance improved less than in the woody myofiber of pectoralis muscle in broiler rearing for 12 days 0.12389 while in the control group, it reached 0.09364 while in broiler continuously treated with a high concentration of soybean oil for 21 and 35 day the result reveals decrease significantly in woody myofiber area in broiler rearing for two rearing period 0.18727 and 0.70556 respectively in contrast measurement to myofiber area in broiler of the control group which represented statistically by 0.70650 and 2.27638 (Table 4).

Table 4: Semi-quantitively analysis of myofiber area in pectoralis muscle in broiler fed different ration for variable rearing period

Days	Groups	Mean ± SD	Sig
12	Control	0.09364 ± 0.028438 **	0.005
	Treatment	0.12389 ± 0.058162	
21	Control	0.70650 ± 0.357239 **	0.0001
	Treatment	0.18727 ± 0.078509	
35	Control	2.27638 ± 0.757181**	0.0001
	Treatment	0.70556 ± 0.232897	

Histochemical analysis

The chemical composition of myofiber in myopathy, mainly wooden breast, may be affected, so in this study, the histochemical analysis of fibrous connective tissue exhibits various scores/grades in muscle fiber of breast broiler rearing for 35 days in the control group the microscopic examination revealed (+/ mild) in sectioned obtained from the control group at 12 and 35 days while in broiler fed in starter and finisher ration with a high concentration of soybean oil 5.20% revealed (++/ moderate and +++/ sever) reactivity for fibrous connective tissue, respectively. This study evaluated the histochemical analysis of carbohydrates contained in broiler breast myofiber using periodic acid-Schiff staining. The section myofibers control group analysis in the period starter and finisher 12 and 35 days revealed (+/ mild and +++/severe), respectively. Furthermore, (+/ mild and-/ negative) in sections obtained from the treatment group with 5.20% of soybean oil for 12 and 35 days, respectively (Figure 5).

Immunohistochemistry features

The immune histochemistry reactivity of α-SMA in pectoralis muscle in the broiler with wooden breast was investigated during starter and finisher period rearing, which revealed variable immune reactivity as in control group the immune staining of α-SMA expression in pectoralis broiler muscle during 12 and 35 days of rearing period was occupied as strong grades and represented by 50-75% respectively in myofiber even in the muscular layer of blood vessels as in catalog, and moderate expression represented by 25-50 % to mild expression represented by 10-25% detected in woody pectoralis myofiber in broiler rearing with high soybean in the ration for at least 12 and 35 days respectively (Figure 6).

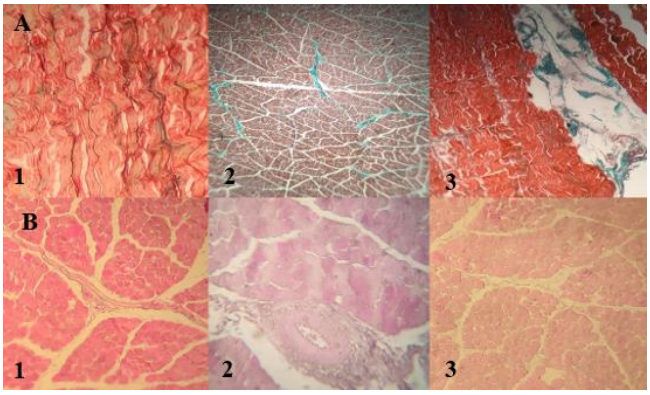

Figure 5: Catalog image of histochemical component's analysis of breast muscle in broiler revealed Masson Trichrome for fibrous connective tissue from (A-1, 100x), (A-2, 40x), and (A-3, 10x) as mild, moderate, and severe, respectively, and periodic acid-Schiff staining for carbohydrate from (B- 1,2 and 3) as severe, mild, and negative, respectively, 100x.

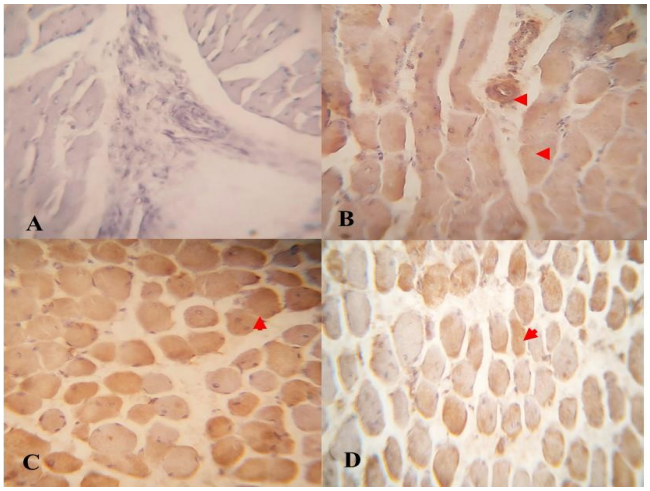

Figure 6: Immune histochemistry staining of α-SMA expression in woody pectoralis myofiber in broiler revealed (A- negative, B- strong, C- moderate, and D- mild) in broiler fed with ration with high soybean for 12 and 35 days, 400X.

Discussion

Many studies and reporters have been conducted on wooden breast, which is a major, modern, and new problem in the poultry industry, but the causative agents are still

unclear; its occurrence may be associated with the increased rate of both food intake and appetite, and consequently used newly genetic strains which characterized by rapid growth (21,22). Bailey *et al.* (23) and Meloche *et al.* (24) suggested that environmental conditions, genetics, management, and restricted nutritional programs represented WB development risk factors.

The results of a recent study investigated that high inclusion of soybean oil 5.20% to the broiler ratio during the rearing period (starter, grower, and finisher) led to induction WB at 12 days from rearing the histological features of WB characterized by cell injury, splitting of myofiber which appear homogenous and round with endomysium forming tissue the lesions progress and become more severity according to the long period of exposure to 21 and 35 days, a combined by circulatory disturbances and necrosis (25,26), Sihvo *et al.* (6) reported significance association of mononuclear vasculitis and myo-degeneration.

Soy oil is a vegetable oil considered a source of fat in broiler production; it is rich in ω-6s (Linoleic acid) and low levels of ω-3s (Linolenic acid). Poultry needs a balanced ratio of fatty acids (ω-6:3), as an imbalance may lead to oxidative stress in the muscles (27); oxidative stress also affects liver function, reducing the synthesis of proteins that bind with lipids to form a lipoprotein complex (28). Consequently, there will be an increase in levels of triglycerides and cholesterol, which may be ectopic concentrated and deposition in the extracellular matrix; a round pectoralis myofiber investigated in this study as white streak parallel to the longitudinal muscle axis, blood vessels, and cause vein stenosis or obstructive which is the pathological key for phlebitis and myositis as well as fibrosis (29-31). Unbalanced hemodynamic and circulatory disorders such as edema, congestion, and loss of venous outflow led to the accumulation of the end product of metabolic activity and the release of free radicals, which cause oxidative stress and muscle damage (32). Hypoxia is the main sequelae of circulatory disturbances and a responsible factor for WB development; it causes an alternative pathway for cellular respiration through suppression catabolism of pyruvate in the mitochondria and switches cellular aerobic respiration to anaerobic glycolysis of glucose metabolism from oxidative phosphorating and decline muscle glycogen and downregulated lipid metabolism (33,34). Our results agree with Papah *et al.* (35), who suggest that vascular disturbances cause hypoxic conditions at the start of the early rearing period (one week) and progressive development with advancing age. Our results investigated pathological lesions represented by adipose tissue deposition and fibrosis. These results are due to the genetic selection of broiler breeds to increase production, especially the breast muscle.

As a consequence of this selection, muscle fibers become hypertrophy. With a decline in blood supply, this mechanism's outcome was myocyte degradation, atrophy of myofiber, and necrosis, leading to the formation of new tissue (adiposis and fibrosis) (36). This scientific fact reinforces the results of the semi-quantitative statistical analysis that was determined in this study, which demonstrated a significant decrease in woody myofiber in pectoralis muscle in broiler rearing for 21 and 35 days.

This histopathological alteration as myodegeneration, which progressed with the rearing period combined with accumulation of fibrous connective tissue endomysia and perimysium (upon Masson Trichrome it takes blue) and also investigated decline carbohydrate content in the muscle fiber with WB, this result also reported by Maharjan *et al.* (37), the decline in the level of carbohydrates, which is expressed by the weak PAS stain, may be due to degeneration of muscle fiber and atrophy. Under hypoxia variable gene expression of affected myofiber will be occur, which can be detected by immunohistochemistry staining, and the intensity of staining can be detected through the score-grade system (38,39), the outcome of recent study that expression of α-SMA in treatment group for 12 and 35 days was variable from moderate and mild grade of immunostaining respectively in contrast to strong in control group, this results unagreement with previous studies Velleman *et al.* (40) and Liu *et al.* (41) who reported that the level of actin unchanged in WB myofiber, Zhao *et al.* (42) reported that the α-SMA cells doesn't responsible as markers for fibrogenic cell in muscle diseases while its very intense immunostaining in the smooth muscle cells lining the intramuscular layer in the wall of blood vessels, the decline in grading expression in this study may related to the histopathological alteration of myofiber which represented by hydropic degeneration and Zenker necrosis and this lesions resulted from alteration in the ultracellular organelles and may affected the gene responsible for α-SMA expression, high body weight and superior breast productivity that birds achieve over a short period of time can contribute to myopathy. However, the pathological mechanisms at the molecular level and the influence of the genetic component in the development of WB remain unclear. It should be distinguished that the analysis performed in this study is based on single muscle fibers. At the same time, the architecture and function of the whole muscle will also be affected by fiber injury and the progress of fibrosis in the later phase of the disorder.

Conclusion

Based on the data of this study, an imbalance in the diet and an increase in the proportion of vegetable oils lead to the occurrence of wooden breast broiler muscle, which is characterized by histological lesions such as vascular disorders, lipidosis, and cell adaptation with abnormal in myofiber architecture. Through this study, it becomes clear the possibility of using artificial intelligence and immunohistochemistry in addition to traditional methods of diagnosis and identifying histological and morphological lesions. The semi-quantitative analysis is one of the most

accurate and essential statistical methods that can be applied in research or diagnosis as an evaluation tool for the occurrence of myopathies. To understand the mechanism of the occurrence of rigidity of the chest muscle in broilers, there must be new studies aimed at studying changes at the cellular and genetic levels.

Acknowledgment

The researchers thank and appreciate the College of Veterinary Medicine, University of Mosul.

Conflict of interest

Researchers undertake no conflicts of interest.

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التركيب الليفي العضلي في التنبؤ وتقييم تخشب عضلة الصدر في الفروج اللحم: دراسة نسجية مرضية وكيميائية مناعية

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الخلاصة

يعد الصدر المتخشب أحد الاعتلالات العضلية التي تصيب الفروج اللاحم ويسبب خسائر اقتصادية ولكن الأسباب لا تزال غير معروفة. هدفت هذه التجربة إلى دراسة تأثير النسبة العالية من زيت فول الصويا في إحداث هذا الاضطراب. تم تغذية ثلاثين فرخا على العليقة الأساسية كمجموعة سيطرة والعليقة الأساسية وزيت فول الصويا ٥,٢٠٪ كمجموعة معاملة لمدة ٣٥ يوما. تمثلت التغييرات المرضية النسجية للعضلة الصدرية في الفروج اللاحم والذي تمت معاملته لمدة ١٢ يوما ببنية غير طبيعية لليف العضلي والوذمة وزيادة تكوين اللفافة الليفية الداخلية بنسبة أقل من ١٠٪ والتي كانت بدرجة ١، وعند١٣- ٢١ يوما كانت نسبة ترسب الأنسجة الدهنية مع التليف ما بين > ٤٠٪ و< ٨٠٪، مع التهاب الاوردة والضمور الليفي العضلي عند اليوم ٣٥. تم استخدام تحليل صورة فيجي كطريقة قياس منفردة كمية لقطر الليف العضلي، إذ لوحظ الانخفاض المعنوي في مساحة الليف العضلي والذي كان ٠,١٨٧ و ٠,٧٠٥ عند اليوم ٢١ و ٣٥ على التوالي، مقارنة مع مجموعة السيطرة ٠,٧٠٦ و ٢,٢٧٦ على التوالي. استخدم نظام الدرجة /المستوى كنقاط التفتيش لتحديد شدة المحتوي الكيميائي إذ كان مستوى التليف معتدل وشديد، في حين كان مستوى الكربوهيدرات خفيف وضعيف في داخل مقاطع الألياف العضلية التي تم الحصول عليها بعد ١٢ و ٣٥ يوما من المعاملة. كانت صبغة الكيمياء المناعية النسجية لتعبير اكتين العضلة الملساء من معتدل الى خفيف مع تقدم الآفة في مجموعة المعاملة. يستنتج بان إضافة نسبة عالية من زيت فول الصويا يؤدي إلى ألياف عضلية متخشبة وان تطبيق التحليل شبه الكمي والذكاء الاصطناعي يساعد في تشخيص الاعتلال العضلي.

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