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MYOPATHIES IN BROILER CHICKENS: ANALYSIS OF EPIDEMIOLOGICAL FACTORS, PATHOMORPHOLOGICAL CHARACTERISTICS, AND IMPACT ON THE PHYSICAL AND CHEMICAL PROPERTIES OF POULTRY MEAT

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CONTENTS

LIST OF ABBREVIATIONS

°C – degree Celsius
µg – microgram
µL – microliter
µm – micrometer
µmol – micromole
*a** – redness
AC – alternating current
ACRBC – Athens Canadian Random Bred Control
AKP – alkaline phosphatase
ALD – *anterior latissimus dorsi*
ALP – alkaline phosphatase
ALT – alanine aminotransferase
AST – aspartate aminotransferase
ATP/ADP – adenosine triphosphate/adenosine diphosphate
*b** – yellowness
BA – biogenic amine
BW – body weight
CAD – cadaverine
CE – European Community (Communauté Européenne)
CK – creatine kinase
cm – centimeter
CO_2 – carbon dioxide
DCM – dorsal cranial myopathy
dL – deciliter
DNA – deoxyribonucleic acid
DPM – deep pectoral myopathy
EU – European Union
FA – fatty acid
FAME – fatty acid methyl esters
FTU – phytase unit
g – gram
GAA – guanidinoacetic acid
GC-HS – gas chromatography–mass spectrometry
GGT – gamma-glutamyl transferase
h – hour
H&E – hematoxylin and eosin
HDL – high-density lipoprotein
HIS – histamine

HS-SPME	–	headspace solid-phase microextraction
Hz	–	hertz
IARC	–	International Agency for Research on Cancer
ID	–	inner diameter
IU/L	–	international units per liter
kg	–	kilogram
KOH	–	potassium hydroxide
*L**	–	lightness
L	–	liter
LDH	–	lactate dehydrogenase
m. anterior latissimus dorsi	–	*musculus anterior latissimus dorsi*
m. pectoralis major	–	*musculus pectoralis major*
m. pectoralis minor	–	*musculus pectoralis minor*
m/z	–	mass-to-charge ratio
MDA	–	malondialdehyde
mg	–	milligram
min	–	minute
mL	–	milliliter
mm	–	millimeter
mmol	–	millimole
mol	–	mole
MUFA	–	monounsaturated fatty acid
No.	–	number
pCO_2	–	partial pressure of carbon dioxide
pH	–	potential of hydrogen
PHE	–	phenylethylamine
PMM	–	*pectoralis major* myopathies
pO_2	–	partial pressure of oxygen
PUFA	–	polyunsaturated fatty acid
PUT	–	putrescine
ROI	–	region of interest
ROS	–	reactive oxygen species
rpm	–	rotations per minute
s	–	second
SD	–	standard deviation
SFA	–	saturated fatty acid
sO_2	–	sulfur dioxide
SPER	–	spermine
SPRMD	–	spermidine
TBA	–	thiobarbituric acid

TBARS	–	thiobarbituric acid reactive substances
TYR	–	tyramine
TRY	–	tryptamine
U	–	unit
USA	–	United States of America
VOC	–	volatile organic compound
WB	–	wooden breast
WS	–	white striping

INTRODUCTION

Change or adaptation is fundamental for survival in an ever-changing environment that constantly puts pressure on living organisms. Charles Darwin‘s principal theory of evolution through natural selection posits that nature determines which animals should survive and reproduce. In contrast, artificial selection, or selective breeding, involves farmers and breeders who select animals with desired traits, highlighting a significant difference from natural selection [1–3]. Farm animals, including broiler chickens, have been domesticated and changed. The selection practices of broilers began in the 1940s in order to increase muscle growth and development. The selection procedures allowed to achieve remarkable production performance [4]. Current heavy-weight hybrids (Ross 308) reach their average weight of 2.4 kg in 42 days, whereas the Athens Canadian Random Bred Control (ACRBC) strain would be approximately 0.55 kg at the same age. Ross 308’s average live weight is five times greater compared to that of the 1957 ACRBC genotypes that are not selected for meat production [5]. All those modifications have been implemented to satisfy global demand for chicken meat.

Nowadays, poultry meat is the most produced and the second most widely eaten meat after pork in the world [6,7]. Furthermore, consumption of chicken is still increasing because of it relatively low cost, easy and quick preparation [8] and desirable sensory characteristics [9]. Moreover, poultry meat is recommended by nutritionists as a lean meat and a healthier option than red meat [10]. Negative image of red meat identified as “possible carcinogenic to humans” by the International Agency for Research on Cancer (IARC). This statement has also contributed to the popularity of white meat [11]. Additionally, poultry meat complies with most cultural and religious standards [9,12]. Moreover, consumers’ preferences have changed over the years. In 1960, most people, about 77%, bought whole chickens, but nowadays, that number is only about 8% [13], and more people buy boneless cut-up chicken meat. People’s lifestyle changes have contributed to this shift. Processed meat, sold as ready-to-eat chicken products, has been gaining popularity. Processing costs do not depend as much on size, therefore, larger birds are more suitable for this industry [14,15].

Intensive and exhausting production systems have resulted in undesirable consequences. The physiology of broilers responds to human interventions. The altered physiology of chickens not only leads to cardiovascular disorders, leg deformities and bone deformations but also impacts the muscular system, resulting in the formation of muscle abnormalities known as myopathies [12,16,17]. Wooden breast (WB), white striping (WS) and spaghetti meat

(Stringy-Spongy) are myopathies related to the *pectoralis major* muscle. Another widely known myopathy is deep pectoral myopathy (DPM), also titled as "green muscle disease" or Oregon disease. DPM affects the *pectoralis minor* muscle of broilers, and this myopathy has been extensively studied for decades. Additionally, dorsal cranial myopathy (DCM) affects the *anterior latissimus dorsi* (ALD) muscle and is a relatively recent myopathy for poultry specialists [17–21].

Muscle abnormalities not only affect the visual appearance of the muscles but also influence the quality of poultry meat [12,21,22]. In slaughterhouses, after meat inspection, affected muscles are withdrawn from the food chain or downgraded based on the severity of a particular myopathy. Even if such breast meat was accepted for human consumption, it could lead to economic losses due to decreased yield value or increased time spent on manual sorting. Nevertheless, if it fell into the food production chain, it could still be rejected by consumers because of undesirable quality traits [12,17,23].

The incidence of myopathies varies widely across regions. This variation may arise due to a lack of one universally recognized system for the evaluation of myopathies. The absence of a universally accepted classification leads to inconsistencies in disease characterization and severity grading across countries. Furthermore, information about the causes and consequences of broiler myopathies is still lacking. Myopathies are established only after postmortem examination. Therefore, establishing early diagnostic tools and non-invasive biomarkers of myopathies that utilize non-destructive approaches is a significant challenge for the poultry industry. Moreover, damage and pathologies of muscles, internal organs and systemic metabolic disturbances are reflected in changes in the blood serum or plasma profiles. Therefore, integrating blood biochemistry into research not only enhances early detection but also contributes to understanding the nature of broilers' myopathies. It is essential to broaden this knowledge to prevent and resolve these muscle pathologies effectively.

Myopathies cause challenges for veterinarians, scientists, farmers, poultry producers and consumers. These conditions result in poor texture, appearance, also it alters nutritional composition of the meat. Consumers tend to reject affected muscles and it leads to financial losses to the poultry industry. Furthermore, consumers become more aware of animal welfare and the quality of the meat that they purchase. Visual alterations of the meat affected by myopathies often indicate underlying health issues cause by intensive farming practices. As poultry demand is projected to grow significantly in the next decade, researching these conditions is necessary because it could offer the solutions to ensuring animal welfare standards and improving poultry meat production efficiency without incurring meat quality losses. Epidemiological

data could help evaluate how widely myopathies are distributed. This information is essential for poultry producers to identify the spread and take action on myopathy control and prevention. Generally, more information and its wider dispersion globally about broiler health and myopathies could help improve consumer confidence in poultry products.

The data presented in this dissertation combine epidemiological studies with veterinary and food science fields.

The aim of the study

The study aimed to analyze the incidence, main etiological factors and pathomorphological characteristics of myopathies in broiler chickens and to evaluate their impact on fast-growing chickens' health and poultry meat quality.

Objectives of the study

1. To determine the incidence of myopathies in broiler chicken flocks and to analyze the risk factors potentially influencing the etiopathogenesis of broiler myopathies;
2. to investigate the impact of *pectoralis major* myopathies on the physicochemical parameters of chicken breast meat;
3. to analyze the changes in the serum biochemical parameters of broilers affected by wooden breast myopathy;
4. to analyze the histopathological features of *pectoralis major* and *anterior latissimus dorsi* muscles of broilers affected by myopathies;
5. to evaluate the histomorphometry results of *pectoralis major* and *anterior latissimus dorsi* muscles of broilers affected by myopathies.

Scientific novelty

This is the first comprehensive investigation of myopathies in Ross 308 broiler chickens in which these muscle pathologies are analyzed in a complex manner. In this research, we investigated the prevalence of myopathies in Lithuania and their possible causes and assessed the physicochemical parameters and histopathological characteristics of the affected muscles. We investigated not only *pectoralis major* myopathies (PMM) but also included dorsal cranial myopathy (DCM) and deep pectoral myopathy (DPM). The inclusion of DCM in this research is particularly novel, because during the review of the scientific literature, we observed that this myopathy has been overlooked due to large interest in PMM. Notably, the incidence of DCM has only been documented in Brazil. This highlights the limited research on this myopathy in other regions.

According to the current knowledge in this dissertation for the first time volatile organic compounds (VOCs) and biogenic amines (BAs) were analyzed in the *pectoralis major* muscle of broilers affected by myopathies. This study demonstrated novel insights into PMM by identifying the significant increase in hexanal, a lipid oxidation marker, in muscle affected severely by PMM. This finding justifies that oxidative stress is important in the etiopathogenesis of PMM. Nevertheless, aldehyde hexanal appears to make the most significant contribution to flavor quality. Therefore, this study confirm that myopathies not only affect the physical features of poultry meat but also alter its sensory and chemical characteristics that may influence consumer acceptance.

From a pathomorphological point of view, we visually evaluated the histopathologic changes of PMM and DCM. Pathologies such as degeneration/necrosis, accumulation of fibrous connective and adipose tissue and inflammation of muscle and blood vessels were scored. We found that the means of all those pathologies of the *pectoralis major* muscle increased in severe PMM compared to those in normal muscle without myopathies, but the means of adipose tissue accumulation and inflammation of the blood vessels were not significantly increased in the DCM group.

Additionally, for the first time, by using image analysis techniques during histomorphometric analysis, the percentage of not only fibrous connective and muscle tissue but also adipose tissue was measured in muscles affected by PMM and DCM. Furthermore, for the first time, the diameter of myofibers was measured in muscles affected by DCM. The diameter of myofibers tends to decrease in PMM, whereas in DCM, the diameter of myofibers is typically increased in affected muscles. Overall, these pathological changes of the muscles lead to reduction of meat quality, especially due to decreased muscle and increased fibroadipose tissue in severe cases of PMM and pronounced fibrosis in DCM.

Practical significance

This research combines information for veterinarians, scientists, poultry producers and consumers. It gives valuable insights into the prevalence and distribution of myopathies in Lithuania. Understanding the incidence and the main risk factors will broaden the knowledge about myopathies and may suggest corrective actions in poultry management that are necessary to minimize the incidence of poultry muscle abnormalities and improve broilers' health.

Furthermore, biomarkers are measurable indicators that allow to identify disorders and are a useful tool in research. Blood analysis and detection of biomarkers such as creatine kinase (CK) helps to indicate muscle damage before slaughter and reduce the production costs associated with downgraded

meat and manual sorting efforts during postmortem examination. It is an indicator of muscle tissue damage and could also be a potential diagnostic tool in research for flock health monitoring before slaughter.

Additionally, myopathies are established at slaughterhouses during postmortem examination. Research data could be used to more accurately diagnose and categorize myopathies. Histopathological and histomorphometric analyses are essential methods that allow to accurately and more precisely investigate the myopathies in broilers. Quantitative data on the tissue composition and quality changes in muscle allows for more objective assessments of myopathy severity and its influence on meat quality. Research enables a more reliable grading system for broiler meat, which is essential for ensuring consistent product quality.

Overall, this research offers practical tools and insights that can directly be used for improving economic efficiency and sustainability in poultry production. Furthermore, consumer education may reduce rejection rate and minimize financial losses for the poultry sector. The findings of this dissertation give information for future studies and could be as a foundational dataset for further investigations into broiler myopathies in Lithuania.

Structure of the dissertation:

The dissertation consists of a list of abbreviations, introduction, literature review, materials and methods, results, discussion, conclusions, recommendations, summary in Lithuanian, references, list of publications, list of scientific conference abstracts, curriculum vitae (CV), and acknowledgements.

The dissertation comprises 28 tables and 52 figures, and its total length is 131 pages.

1. LITERATURE REVIEW

1.1. Myopathies in modern broiler chickens

In the 1920s, chicken meat was largely seen just as a secondary product of the commercial egg industry. The “Chicken of Tomorrow” was introduced in the 1940s, and the origin of commercial meat-type chickens started to change. The vision of broilers changed into developing a heavy meat-type chicken [24].

In 1960, 10 billion tons of poultry meat were produced. Nowadays, 120 billion tons are produced annually, predicted to reach 180 billion tons in 2050 [25]. Poultry meat popularity and consumption keep increasing [6,7].

Heavy-weight broilers are characterized by their rapid growth rate, high feed efficiency and high breast meat yield [6,7,17]. Over the past decades, the age of slaughter has been reduced, while the average slaughter weight has increased significantly. Today, the typical slaughter age of modern broilers ranges between 5 and 7 weeks, depending on the production method and broiler hybrid, while in the 1950s, broilers were slaughtered at 12–16 weeks of age [26,27]. Additionally, the slaughter weight of 2.5 kg can nowadays be reached in 38 days, compared to 63 days in the 1960s [28,29]. In Europe, approximately 6 billion broiler chickens are raised annually, yielding about 13.3 million tons of poultry meat. The birds are bred for their rapid muscle development [30].

However, many years of effort by researchers and poultry specialists have led to both favorable and unfavorable outcomes. Although the breast muscle weight of Ross 308 broilers is significantly greater than that of broilers from 1975 [5], high-efficiency production systems in broiler farming have caused various health issues and also profoundly impacted the muscular system of broilers. Myopathies have alarmed and raised significant concerns within the poultry industry [12,16,17]. In recent years, new myopathies of pectoral muscles have emerged, including DPM, WS, WB and spaghetti meat myopathy, which leads to the separation of muscle fiber bundles due to defects in intramuscular connective tissue [31]. Additionally, dorsal cranial myopathy (DCM), a lesion of the ALD muscle, also contributes to carcass downgrading [19]. All these myopathies are a huge problem in the poultry sector, presenting substantial challenges and economic losses.

1.2. Incidence, etiopathogenesis and pathomorphological characteristics of deep pectoral myopathy

DPM is also known as "green muscle disease", Oregon disease or compartment syndrome [32,33]. This muscle pathology was first described by Dickinson et al. (1968) in adult turkeys in USA as a "degenerative myopathy" [20]. DPM is more common in meat-type chickens that were selected with greater breast muscle [34–36]. DPM develops in the deep pectoral muscles (*musculus pectoralis minor*) [37]. According to the literature, the condition appears to be more common in males than in females [37,38]. Harper et al. (1983) found that viral or bacterial infection is not associated with this muscle pathology. In addition to that, it has been established that antioxidants such as selenium, methionine and vitamin E as well as changes in diet type cannot reduce the incidence of DPM [32,33,39,40].

The etiopathogenesis of this myopathy is closely related to the anatomy of the *pectoralis minor* muscle. The muscle is located between the sternum and inelastic fascia and is unable to fully expand during physiological activities such as wing flapping. The pressure exerted to the *pectoralis minor* during winging makes the muscle increase in size [33]. Furthermore, Martindale et al. (1979) have indicated that during wing exertion, the tenders can increase in weight by up to 20 percent due to enhanced blood circulation to the muscle [41]. Therefore, due to limited space for extension, the muscle becomes compressed, leading to blood circulation disorders and occlusion of blood vessels. This results in hypoxic conditions and causes ischemic necrosis [35]. In meat-type heavy birds, the pressure is maintained long enough to lead to irreversible changes—necrosis of the *pectoralis minor* muscle. It has been observed that in lighter birds, this pressure rapidly returns to normal levels, preventing the occurrence of necrosis [36].

Gross lesions of DPM are divided into stages according to its severity and can be either unilateral or bilateral. Early lesions show edema and hemorrhages, and the muscle is very red due to ruptured vessels in deep pectoral muscles. Later, the affected *pectoralis minor* becomes green and shrunken and then pale green (old stage) [36]. The green color is the result of the gradual breakdown of hemoglobin and myoglobin in the damaged muscle [42].

Histologically, the affected muscle shows necrosis, Zenker's degeneration and atrophy of myofibers. Increased acidophilia and edema are observed. Additionally, an inflammatory infiltrate predominantly composed of macrophages, along with some heterophil leukocytes, is observed. Connective tissue proliferation is also noted. In most advanced lesions, normal muscle tissue is replaced by fibrous connective and adipose tissue [17,43,44].

However, the muscle pathology does not impair the general health of birds and does not have an effect public health, but it is aesthetically undesirable [33,42]. Nevertheless, this muscle abnormality is also associated with pododermatitis [43]. However, DPM is established only after postmortem examination in the slaughterhouse and goes undetected until processing. After the removal of the fillet, the rest of the carcass is still usable and fit for human consumption [33,42]. DPM affects a valuable part of the chicken carcass and therefore leads to economic losses [45]. It is believed that wing flapping is a major cause of this myopathy. Generally, knowledge about the etiology and the main possible causes allows slaughterhouse management to control its incidence in broiler flocks. For example, management practices to help minimize wing flapping are trying to avoid excessive activity that may cause unnecessary winging, minimizing excessive noise levels and using a sunrise/sunset feature to control light brightness and intensity [37,42].

The incidence of DPM is not that high, possibly due to the previously mentioned successful management practices and control strategies. Barbut et al. (2024) found that nowadays, DPM is observed rarely, as breeders have effectively selected against birds that exhibit these conditions [46]. In Italy, the average incidence of DPM is 0.84%. However, the range of total DPM incidence varies highly, from 0% to 16.7% [45]. In Bulgaria, the incidence of DPM is 0.51% [44]. In Poland, the incidence of DPM ranges from 0.02% up to 1.9% in flocks of five- to seven-week-old broilers [47]. According to Lien et al. (2011), spontaneous DPM cases vary from 3% to 17% and are more commonly established in broilers with greater growth rates [38].

1.3. Incidence, etiopathogenesis and pathomorphological characteristics of dorsal cranial myopathy

DCM affects *musculus anterior latissimus dorsi* (*m. anterior latissimus dorsi*) of broilers. The etiology of this myopathy is still unknown [19,48]. According to the literature, this muscle pathology affects fast-growing, modern meat-type healthy male broilers of high-yielding strains that are in good body condition with the greatest slaughter weights and no other apparent diseases. Additionally, DCM is more common in older broilers [19]. Furthermore, according to Zimermann et al. (2012), vaccines (Marek's disease and fowl pox) are commonly administered in this anatomic region to one-day-old chicks, and the possibility that this could initiate the pathologic process cannot be eliminated [19]. It is also believed that an important factor for DCM development is large pectoral muscles. ALD muscles are antagonists of pectoral muscles and are, from a physical and mechanical perspective, poorly balanced [19]. Additionally, the ALD muscle has only type I myofibers

that are more sensitive to oxygen deficiency [49,50]. Therefore, when the wings move over the very large pectoral muscles, blood flow is intermittently interrupted, and the focal lesion of this myopathy could be caused by ischemia followed by reperfusion [19,49].

DCM is localized and affects only the ALD muscle. Macroscopically, DCM is characterized by yellowing and swelling of the skin area covering the ALD muscle. Subcutaneous edema, hemorrhagic areas, increased density, pallor, thickening and adhesion to adjacent muscles is observed. Moreover, other muscles, such as *rhomboideus superficialis* and *scapulohumeralis caudalis*, present superficial hemorrhages [19].

Histologically, DCM is characterized by chronic myopathic polyphasic lesions, which include degenerative, necrotic or regenerating myofibers, with fibrosis and adipose tissue accumulation. Furthermore, an inflammatory response is present, which is predominantly mononuclear, with lymphohistiocytic, macrophage and heterophils infiltration [19,51]. Zimmerman et al. (2012) suggests that lymphohistiocytic infiltration is observed at minimal or mild levels, indicating that the inflammatory process is a secondary event in the pathogenesis to primary muscle degeneration and necrosis [19]. Proliferation of satellite cells, sarcolemma nuclei and nuclear rowing on basophilic myofibers suggest the presence of regenerative myofibers. Variations in the sizes of myofibers with cytoplasmic fragmentation, floccular necrosis and regeneration are present. Additionally, vascular granulation tissue and fibrin are present at the borders of the affected muscle, and in some sections, variable amounts of lymphocytes are present [19,51].

In the adjacent muscle groups such as *rhomboideus superficialis, scapulohumeralis caudalis and triceps brachii*, the features include myofiber hyalinization, necrosis and superficial fibrosis, more prominent than in other muscles such as the *supracoracoideus, pectoralis thoracicus* and *sartorius*. These latter muscles exhibit hyalinization and necrosis of a few fibers, without fibrosis. Interestingly, histologic examination of apparently normal ALD muscle also reveals moderate microscopic lesions. These include some myofiber hyalinization, segmental necrosis and lymphocytic infiltration, with mild fibrosis. Additionally, histologic changes of the skin covering the ALD muscle reveal focal and perivascular lymphocyte infiltration of the dermis and subcutaneous tissue [19].

In Brazil, the incidence rate of this myopathy ranges up to 7.35% [19,51,52]. Additionally, in 2009, this muscular lesion was found to be the main cause of downgrading in winter and the second cause in the entire year [53]. DCM is detected almost exclusively in the processing lines at the slaughterhouses by trained personnel. This myopathy of the ALD muscle impairs the visual quality of carcasses [19].

Carcasses affected by DCM undergo trimming, involving the removal of the skin (often from the neck to the cloaca), of the affected and adjacent muscles, as well as of the humerus and part of the thoracic muscles. Carcasses with extensive lesions that compromise their overall visual quality are condemned as a whole, resulting in economic losses [52].

1.4. The incidence and main risk factors of *pectoralis major* myopathies: wooden breast and white striping

The incidence of WB and WS myopathies has been reported in several countries, and the number is higher than what the chicken industry would admit. The values are extremely high, up to 95% [12,54]. As shown in Table 1.4.1, the prevalence of WB and WS myopathies shows high variation across different regions and countries. Nevertheless, information about the incidence of myopathies is still missing in many countries all around the world.

In Italy, the incidence of PMM has been investigated extensively by several researchers. According to Lorenzi et al. (2014), the overall incidence of WS in both medium and heavy birds is 43.0%, with 36.8% being moderate and 6.2% severe. Additionally, it has been observed that heavy flocks exhibit significantly higher percentages of both moderate and severe WS compared with those in medium flocks of broilers. Moreover, the effect of genotype has also been noted—high-breast-yield hybrids exhibit a higher incidence of both moderate and severe WS compared with that in standard-breast-yield birds [55]. Similarly, Gratta et al. (2017) observed that the incidence of WS depends on genotype, with an average rate of 75.5% at slaughter. Moreover, the occurrence of breast fillets severely affected by WS was higher in Ross (25.9%) than in Cobb broilers (7.41%). Additionally, it was reported that 5.1% of commercial broilers were affected by WB, with no variation due to feeding system or genotype [56]. Russo et al. (2015) also found a high prevalence of WS at 78.4%, more severe in heavier broilers. However, the research result showed that genetics did not affect the prevalence of WS. Moderate WS prevalence was 56.9% in medium-weight broilers and 56.8% in heavy-weight broilers, and severe WS prevalence was 13.3% in medium-weight broilers and 25.7% in heavy-weight broilers [57]. Petracci et al. (2013) reported a total incidence of moderate and severe WS at 12.0%, with 8.9% for moderate and 3.1% for severe cases. Additionally, breast yield hybrids exhibited a higher overall incidence of WS compared to standard breast yield birds [58].

In France, the incidence of WS is 50.7% [59]. This result is in line with previously reported findings in Italy [55–57]. Additionally, it has been

observed that the presence of WS was specifically associated with increased yield of the *pectoralis major* muscle but not overall growth of the body [59].

In Finland, WB incidence similar to that of WS in other Europe countries was reported, overall about 65% [60].

In Turkey, generally, the proportion of 36–39-days-old broilers affected by WS moderately and severely is more than 70% (51.32% moderately and 20.56% severely). Additionally, the incidence of WS has been compared among different age groups of broilers, and the highest incidence of WS was in the oldest group of broilers. Incidence increased substantially with increasing age [61].

In North America, the incidence of WS and WB myopathies is very high. According to Che et al. (2022), in Canada, WS prevalence is 96.0% (93.8% mild and 2.2% moderate). Total WB incidence is also very high at 82.4%. Additionally, according to their research results, mild WS appears to be the "new normal" for breast fillets. Nevertheless, it should be noted that in their study, severe WS was not observed [62]. According to Kuttappan et al. (2013), in the USA, overall WS prevalence reaches 83.34% (71.53% for moderate and 11.81% for severe) [63], while Tijare et al. (2016) reported that WS and WB incidence separately in the USA reached 96.1% [64].

In South America, the prevalence of WS and WB myopathies is not as high as in North America. In Brazilian slaughtering plants, the prevalence of WS and WB myopathies in chickens slaughtered at 6–7 weeks of age is 28.3% and 11.2%, respectively. Additionally, 17.5% of breast fillets are affected by both WB and WS myopathies. Higher incidence of myopathies has also been observed in breasts from older birds [54]. In Argentina, the prevalence of WB is 50.29%, with 4.00% affected severely and 46.29% affected moderately [65].

In Thailand, the prevalence of breast fillets affected by both WB and WS myopathies at various levels of severity from 6–7-weeks-old broilers is 7–8%. Overall, the prevalence of WS is 97.8% (55.7% mild, 39.0% moderate and 3.3% severe). Furthermore, the incidence of WB is 6.6% [66], different from the results in China. It has been established that the incidence of WB in China is approximately 61.9%, with 31.3% scored as mild, 23.2% scored as moderate and 7.6% scored as severe [67].

According to the findings presented in Table 1.4.1 that detail the incidence of WB and WS, North America exhibits the highest incidence rates of WB and WS myopathies globally. Additionally, WS is the most common myopathy, with the highest incidence rates observed in Thailand, reaching up to 97.8% [66]. The high prevalence underscores the widespread impact of these conditions to the poultry industry.

Despite the lack of information about WB and WS incidence in smaller countries, Table 1.4.1 reflects a global challenge regarding the incidence of PMM in poultry production. Both WS and WB myopathies, whether occurring separately or in combination, are common worldwide in regions where fast-growing, heavy-weight broilers are raised [12,54]. However, there are some reasons why the incidence varies significantly across different countries. One primary reason is the subjective classification criteria for these myopathies, which could influence the ability to consistently classify myopathies, leading to variations in prevalence rates. Additionally, differences in the age, sex, and the average weights of broilers at slaughter contribute to these fluctuations [62]. All these factors necessitate caution when making direct comparisons between the studies.

Table 1.4.1. *The incidence of wooden breast (WB) and white striping (WS) myopathies in different countries*

Continents and their countries	Type of myopathy	Incidence rate	References
Europe (Turkey included)			
Italy, France, Turkey	WS	12–78.4%	[55,56,57,58,59,61]
Italy, Finland	WB	5.1–65.0%	[56,60]
North America			
Canada, USA	WS	83.34–96.1%	[62,63,64]
	WB	82.4–96.1%	[62,64]
South America			
Brazil	WS	28.3%	[54]
Argentina, Brazil	WB	11.2–50.29%	[54,65]
Brazil	WB+WS	17.5%	[54]
Asia			
Thailand	WS	97.8%	[66]
Thailand, China	WB	6.6–61.9%	[66,67]
Thailand	WS+WB	8%	[66]

WB, wooden breast; WS, white striping; WS+WB, WS together with WB.

1.5. Underlying causes of *pectoralis major* myopathies: wooden breast and white striping

The etiopathogenesis of chicken breast myopathies remains unclear, but several possible factors have been identified. The development of PMM is complex and multifactorial and has several possible biological pathways and response mechanisms. It is influenced by demographics, health, husbandry

and even external environmental factors [18,62]. Myopathies are particularly associated with intensive and exhausting animal production systems [58,68–71]. Studies have shown that the heaviest male broilers with high growth rate, feed efficiency and high breast meat yield are more susceptible to PMM [68,69,72,73]. It has also been indicated that WB and WS do not occur spontaneously. The cellular disruption of the breast muscle starts at 2 weeks of age and develops as a widespread fibrotic injury [74]. Moreover, according to Santos et al. (2023), WS is exhibited before WB myopathy. Additionally, WB condition does not occur as an isolated myopathy, but rather always concomitantly with WS, suggesting that WB may be an aggravated state of WS [75]. Similarly, Shivo et al. (2014) have found that broilers affected by WB are likely to show gross lesions of WS [76].

According to the literature, important roles in WB pathogenesis are played by intracellular calcium dysregulation, possible fiber-type switching, hypoxia and oxidative stress. Furthermore, differentially expressed genes associated with these causes are observed, but the major causative gene has not been revealed [77,78]. According to the literature, non-genetic factors have a significantly greater influence compared to that of genetic factors, as the heritability of WS and WB myopathies is low to moderate, ranging from 0.185 to 0.338 and from 0.024 to 0.097, respectively [79,80].

Histological analysis of myopathies has revealed that WB and WS have a lot of similarities. Both myopathies are characterized by hyperplasia and hypertrophy of myofibers. Hyperplasia occurs during embryonic development, while hypertrophy occurs post-hatch. Therefore, the selection of chickens is based on hypertrophy. Increased size of myofibers and altered muscle physiology determine reduced blood supply and altered breast muscle metabolism. The muscle fiber enlargement is mediated through the fusion of an adult myoblast stem cell (called satellite cells) into the existing myofibers that adds nuclei to the existing muscle fibers, leading to myonuclear deoxyribonucleic acid (DNA) accretion and increased protein synthesis [81,82]. The satellite cells are located between the basement membrane and sarcolemma of skeletal myofibers and play a crucial role in post-hatch skeletal muscle growth. Once hatching occurs, the number of myofibers is already fixed, and growth occurs primarily through hypertrophic growth. Therefore, this enlargement of these pre-existing myofibers is facilitated by satellite cells [81,83–85]. Additionally, Sihvo et al. (2018) have observed a significant decrease in the numbers of blood vessels, accompanied by an increase in myofiber area per vessel in chickens affected by focal WB [86]. These findings suggest that capillaries and blood supply may play a direct or possibly indirect role in the progression of WB myopathy. Enlarged myofibers and reduced blood supply lead to hypoxia [18].

Additionally, based on evaluations of the histological features in WB-affected muscles, it has been hypothesized that hypoxic conditions may develop as early as the first week in broilers, with the severity of the resulting lesions progressively increasing as the chickens grow [74]. According to Radaelli et al. (2017), muscle fiber degeneration associated with WS and WB occurs after 2 weeks of growth and increases within 28 days, and has been detected in almost all birds with histology within 46 days of age [87]. Furthermore, WB and WS primarily affect the skin-facing cranial portion of the *pectoralis major* muscle, and in the later phase, involve the entire muscular tissue. Moreover, higher myofiber density has been observed in the cranial part of *pectoralis major* muscle. Therefore, the cranial portion of the muscle is affected primarily and more severely [88–91]. Enlarged myofibers and insufficient vascularization cause metabolic stress due to the increased diffusion distances for oxygen, nutrients, and waste products. This makes it challenging to supply adequate oxygen, remove metabolic waste, and can lead to muscle damage [12].

The development of hypoxic conditions due to inadequate vascularization initiates a cascade of events, including phlebitis and oxidative stress. Generally, hypoxia and oxidative stress are multifactorial contributors in the pathogenesis of myopathies. This sequence activates various response mechanisms, such as changes in energy metabolism, inflammation, degeneration and regeneration, all of which are closely related to the progression of these myopathic disorders [18].

Nevertheless, skeletal muscle tissue tries to combat hypoxia by increasing blood flow, partly through the production of nitric oxide. While nitric oxide helps improve oxygen delivery, it can also lead to the generation of more reactive oxygen species (ROS), which are byproducts of normal cellular metabolism. This creates a pro-oxidative environment, which can also cause oxidative stress and promote inflammation and eventually muscle tissue damage [92].

It has been observed that the initial ultrastructural changes in the early phase of WB in 22-day-old broilers primarily affect the mitochondria and the sarcoplasmic reticulum [86].

Bordini et al. (2024) found that the increased demand for protein synthesis that is necessary to support the hypertrophic growth of pectoral muscle may lead to the accumulation of misfolded or dysfunctional protein such as collagen type IV [93]. This rise in protein synthesis may overburden the sarcoplasmic reticulum, which manages protein folding. The downregulation of genes in the eIF2 signaling pathway and reduced expression in the ubiquitin proteasome pathway suggests a buildup of misfolded proteins, impairing sarcoplasmic reticulum function and inducing sarcoplasmic reticulum stress [18,94]. The

strain on the sarcoplasmic reticulum may result in cellular stress, exacerbating muscle damage and contributing to the progression of myopathies such as WB and WS [93].

It is hypothesized that after this process, phlebitis, oxidative stress and altered calcium homeostasis take place, and response mechanisms such as energetic metabolism, inflammation, degeneration and regeneration are activated [46].

Multiple lines of evidence support this hypothesis of sarcoplasmic reticulum stress, primarily caused by the accumulation of misfolded proteins (along with factors such as altered calcium homeostasis and fatty acid accumulation) [18]. The sarcoplasmic reticulum is the main calcium store compartment of the cell and is very sensitive to intracellular redox changes. Calcium controls muscle excitation and contraction, gene transcription, cell growth and proliferation [95–97]. Therefore, damage to the sarcoplasmic reticulum, which surrounds muscle fibers, leads to increased calcium influx. This activates calcium-dependent proteases. Those proteases initiate muscle necrosis [17,88]. Additionally, long-term cytoplasmic calcium overload could cause a "vicious cycle" where mitochondrial calcium overload leads to mitochondrial dysfunction. This dysfunction increases ROS production and oxidative stress, exacerbating cell damage and causing mitochondria-mediated cell death [98].

When ROS levels are increased, mitochondrial damage levels are increased in various membranes of the cell, leading to the alterations of normal physiological functions in WB-affected muscle [99]. Mitochondrial membranes are composed of lipids and proteins and are vulnerable to ROS attack. Overproduction of ROS causes oxidative stress and cell damage by affecting membrane structure, protein function and lipid metabolism, and disturbs the function and homeostasis of skeletal muscle [90,100]. In the electron transport chain of the inner mitochondrial membrane, complexes I and III are the main contributors to mitochondrial ROS production [101]. According to a study by Zhang et al. (2023), the activities of complexes I and III were decreased significantly in a WB-affected group [95]. Inhibition of the mitochondrial electron transport chain induces ROS overproduction, and these ROS can cause oxidative stress, potentially leading to cellular damage. Therefore, mitochondrial dysfunction might be an important reason for muscle damage of WB-affected broilers [95,102]. Moreover, structure changes in the mitochondria, such as the collapse of cristae and dissolution of the mitochondria matrix, are a simultaneous consequence that have been observed in WB-affected groups [95].

Furthermore, the *pectoralis major* muscle of chickens consists of IIB myofibers [103]. A myofiber type switching from II type (fast-twitch) to

I type (slow-twitch) has been documented in myopathic muscles of broilers. This switch is believed to be a response to the rapid growth of the *pectoralis major* muscle that occurs over a short period of time. The transition to slow-twitch myofibers contributes to the metabolic dysregulations and carbohydrate and lipid metabolism alterations due to myopathic conditions. This switch may be the muscles' attempt to adapt to the rapid growth, but it also results in reduced energy efficiency and worsened myopathy symptoms [17,88,104].

There are a lot of studies that analyze a wide range of biological processes of broilers affected by PMM. However, the current research suggests that both hypoxia and oxidative stress share a common origin, leading to disrupted growth and development within muscle tissue [12,18].

1.6. Pathomorphological characteristics of *pectoralis major* myopathies: wooden breast and white striping

WB myopathy is a degenerative pathology that affects broilers' *pectoralis major* muscle [12,60,76]. WS is another myopathy of broiler chickens. WS affects not only the *pectoralis major* muscle but also could be detected in thighs (*iliotibialis* muscle), tenders (*pectoralis minor* muscle) and drumsticks (*gastrocnemius* muscle) [12,105]. Furthermore, WS could be detected alone or often coexist with WB myopathy [60]. In addition, both myopathies have similarities to other muscle conditions like hereditary muscular dystrophy, nutritional myopathy, toxic myopathies and marbling [70].

The names to WS and WB myopathies are given due to macroscopic lesions of the breast muscle [76,105]. The white striations that run parallel to the myofibers are a characteristic feature of WS myopathy [105]. White stripes usually appear in the cranial part of the *pectoralis major* and may or may not extend along the muscle to the caudal region [106]. WB is also named according to its gross lesions. Pale expansive areas of substantial hardness of bulged, hard and rigid muscles appear along with clear viscous fluid and hemorrhages on the *musculus pectoralis major* (*m. pectoralis major*) surface [76].

Based on visual examination of myopathies, both of them are graded into categories depending on severity [64,79,86,105,107,108].

WS is mainly graded by the brightness and thickness of white striations. According to Kuttappan et al. (2012) [108], normal fillets lack white lines, while moderately affected breasts exhibit white lines less than 1 mm thick, parallel to the myofibers. Severely affected fillets display parallel white lines thicker than 1 mm that are very visible on the muscle surface. Nevertheless, Bailey et al. (2015) [79] expanded this gradation by adding the mild category. Mild, focal white stripes on a part of the fillet; moderate, extensive white

stripes across the muscle surface; and severe, very thick white stripes with extensive coverage over the breast surface.

According to the macroscopical examination, palpation and visual evaluation, Tijare et al. (2016) graded WB myopathy into stages as follows: 0 (normal) = fully flexible fillets, 1 (mild) = fillets that were hard primarily in the cranial region but flexible elsewhere; 2 (moderate) = hardness throughout but flexible in the mid to caudal region; 3 (severe) = fillets that were extremely hard and rigid from cranial to caudal [64]. Other authors simplify grading [86,107]. Oliveira et al. (2021) separated WB into moderate and severely affected muscle, according to the presence of hardness only in the cranial or caudal region and hardness over the entire length of the breast fillet, respectively [107]. Sihvo et al. (2018) graded into unaffected with normal consistency, focal WB myopathy with localized hardening or diffuse WB myopathy with the complete muscle area being hardened [86].

Despite their macroscopical differences, histologically, WB and WS have a lot of similar features. Both myopathies have been characterized with degeneration and necrosis, fibrosis, adipose tissue accumulation and regenerative changes of the muscle [70,105].

Microscopically, WB and WS are characterized by the presence of degenerative, necrotic and atrophic myofibers, vacuolar degeneration and myofiber lysis. The affected muscle exhibits fragmented, hypereosinophilic amorphous myofibers, with loss of cross striation and internalization of nuclei. There is also mild mineralization and occasional regeneration, hyalinization, mononuclear cell infiltration, primarily of macrophages and heterophils, with occasional lymphocytes, indicating inflammatory, degenerative and necrotic processes. Additionally, the accumulation of adipose tissue and fibrosis are visible. The affected areas show diffuse thickening of the interstitium with varying amounts of loose connective tissue, granulation tissue or collagen-rich connective tissue (fibrosis) separating the myofibers. Moderate to severe edema and accumulation of loose connective tissue with moderate numbers of lymphocytes and macrophages are also evident in the perimysium surrounding the muscle bundles [76,88,105]. Additional features in WB-affected muscle include perivasculitis, typically occurring around veins and characterized by irregular T lymphocyte infiltration that sometimes disrupts the vascular wall. The epimysium covering the *pectoralis major* muscle is slightly to moderately thickened by loose connective tissue, containing abundant amorphous extracellular material, variable amounts of collagen fibers, mixed inflammatory cell infiltrate and small vascular profiles with plump endothelium [76].

1.7. Physicochemical properties of *pectoralis major* myopathies: wooden breast and white striping

Poultry meat is a low-fat meat with a high unsaturation degree of fatty acids (FAs) and low sodium and cholesterol levels. It is considered a "functional food" because of bioactive substances such as linoleic acid, vitamins and antioxidants, and balanced n-6 to n-3 polyunsaturated fatty acids (PUFAs) ratio [109,110].

Nowadays, the increasing incidence of emerging breast meat abnormalities, such as WS and WB, is leading to higher rates of downgrading, posing significant sustainability challenges for the poultry industry. Additionally, there is a decline in the nutritional, sensory and technological quality of the meat, which may negatively influence current consumer attitudes towards poultry products [9,108].

Myopathies impact the visual appearance of the breast meat, potentially leading to consumer reluctance towards affected breast fillets [108]. According to the literature, WS and WB both affect the color of the breast fillet. That kind of breast meat may exhibit higher redness (a^*), yellowness (b^*) and lightness (L^*). A higher L^* value means that the affected meat has greater luminosity or is whiter than normal [22,58,65,107].

Overall, according to Table 1.7.1, WB has more significant impact on meat color compared to that of WS. Generally, WB increases b^*, L^* and a^* of the poultry meat [22,111,112]. In contrast, the WS effect on meat color is varied, sometimes increasing these parameters [58] but often having no effect at all [58,59,89,113,114]. These findings highlight that WB has more pronounced influence and more consistent effect on the color of poultry meat. These color changes may be explained due to histologically observed alterations in the muscle tissue, such as polyphasic muscle fiber degeneration and fibrosis [76].

In addition to that, meat affected by WS and WB separately or together exhibits a higher pH [22,59,88,89,107,111,113,115]. A more pronounced effect on pH has been established in muscles affected severely by WS or WB [58,107,111] or when WS occurs together with WB [88,113,115]. According to the literature, the highest pH values observed in samples affected by WB could be linked to glycogen storage, breast muscle weight and the correlation between these two variables. This relationship causes larger breast muscles to exhibit reduced glycolytic potential, leading to a higher final pH [116]. It has also been noted that the higher final pH is also likely attributable to the energy status and alterations in muscle metabolic pathways. In chicken fillets affected by severe WB, the amount of lactic acid produced during rigor mortis is insufficient to significantly lower the pH [115,117].

Table 1.7.1. *Effect of white striping (WS) and wooden breast (WB), occurring individually or combined on physical parameters of the pectoralis major muscle*

Technological parameters	WS	WB	WS+WB	References
*L** (lightness)	↑ / –	↑ / –	↓ / –	[22,65,107,59,58,89,111,112,113,114, 115]
*a** (redness)	↑ / –	↑ / –	↑ / –	
*b** (yellowness)	↑ / –	↑ / –	↑ / –	
pH	↑ / –	↑ / –	↑ / –	[22,59,58,65,88,89,107,111,112,113, 114,115]
Shear force	↓ / –	–	↑	[22,65,59,58,107,111,114,115]
Cooking loss	↑ / –	↑ / –	↑ / –	[22,59,58,88,107,111,113,114,115,1 21]
Drip loss	↑ / ↓ / –	↑	–	[59,58,65,88,111,113,115]
Hardness	–	↑	↑	[65,88]

*L**, lightness; *a**, redness; *b**, yellowness; WB, wooden breast; WS, white striping; ↑, increased compared to normal *m. pectoralis major*; ↓, decreased compared to normal *m. pectoralis major*; –, no effect.

Furthermore, fillets affected by WS have a lower Allo–Kramer shear force [58], while shear force tends to increase when poultry breasts are affected by both WS and WB myopathies [114,115]. Shear force reflects meat tenderness, while hardness shows how firm or soft meat feels during palpation. Hardness of breast fillets is used for determination of WB in slaughterhouses. According to the literature, severe WB or the combination of WS and WB leads to increased hardness of pectoral muscles of broilers [65,88]. It is believed that this muscle change could be due to extensive fibrosis resulting from the accumulation of cross-linked collagen fibers in affected breast fillets. However, it is hypothesized that fibrosis is not the only one factor behind the hardness of the WB meat and the lesion is not restricted to the *pectoralis major* muscles only [17,118]. Furthermore, Soglia et al. (2017) reported that collagen may play a major role in the increased firmness associated to WB myopathy [119]. Moreover, another study found the proteoglycan decorin connection to WB. Decorin is an extracellular small leucine-rich repeat proteoglycan, and its core protein binds to fibrillar collagens regulating collagen crosslink formation. Therefore, it has been suggested that excesses production of decorin in broilers could lead to increased hardness of *pectoralis major* potentially due to enhanced collagen crosslinking [120].

Most studies indicate that *m. pectoralis major*, especially affected severely by WS and WB, displays the greatest cooking loss and drip loss [58,59,65,88,107,111,113,121]. Some authors attribute it to the increased

release of water due to extensive loss of cell membrane integrity. Water can escape, resulting in higher drip loss as well as cooking loss, leading to the presence of a thin layer of fluid viscous material on the surface of the muscle [76]. Furthermore, in breast muscle severely affected by WB, increased drip loss may be due to replacement of myofibrillar proteins with connective tissue proteins (collagen) that form a scar tissue. This process results in reduced membrane integrity and altered muscle structure. All these pathological changes allow water to escape more easily, contributing to higher drip and cook loss [46,64,88,105,113].

Moisture, protein, lipid and ash content also provides information about the quality of the meat. According to the literature, moisture content is increased in chicken breast fillets affected by WS in combination with WB [88,115, 122]. Although WS alone often does not affect moisture content (especially in moderate forms) [69,89,122,123], severe cases can lead to increased moisture content [121]. Furthermore, WB alone or in combination with WS has more significant impact on increasing moisture retention than WS does in chicken breast meat, especially in severe cases [65,88,107,111,112,122]. It is hypothesized that the higher moisture content in WB samples might be due to the possible occurrence of moderate to severe edema as a consequence of an inflammatory process [76].

The combination of WS with WB myopathies could significantly reduce protein content in chicken breast muscle [88,115,122]. However, WS has a larger impact on protein level in poultry meat. According to the literature, WS alone consistently reduces protein levels, especially in severe forms [69,121,123]. According to Kuttappan et al. (2012), higher degrees of WS have lower amounts of protein content and greater amounts of fat content, resulting in higher net calorie content in the meat [69]. Nevertheless, WB shows variable effects, sometimes reducing protein content [65,88,111,112,122] and sometimes having no effect at all, even in severe WB forms [107]. However, in most cases, protein content is significantly lower in all abnormal fillets, regardless of whether they are affected by WS, WB, or both [122]. According to the literature, degeneration of muscle fibers due to myopathies may lead to a decrease in protein content. Moreover, the replacement of degenerated muscle fibers by adipose tissues may increase the fat content [88]. In addition to that, fillets with severe WS result in reduced purchase intent because of looking fattier and having a marbled appearance [108]. Furthermore, intramuscular fat content is generally higher and tends to increase in fillets severely affected by WS [121,123]. Lipid content is influenced regardless on severity of WS, WB and WS together with WB. Severe WS tends to increase lipid content [69], and severely affected cases have thicker and more bright white stripes on the muscle surface. WB whether alone or together with WS also tends to

increase lipid content in breast fillets [65,88,111,112,115,122]. According to the literature, the reduction of protein content may lead to an increase in fat deposition because of more space for the adipocytes to expand. Nevertheless, the degeneration during myopathies could be associated with differentiation of muscle stem cells to adipocytes, resulting in hyperplasia of adipocytes. Additionally, the increased amount of lipids in affected muscle could be due to increased lipogenesis in the liver or increased uptake of circulating fat due to hyperplasia of adipocytes [69,105].

Pluripotent stem cells of the muscle could also differentiate into fibroblasts, which in turn lead to fibrosis and therefore collagen content being increased in broiler muscles affected by WB and WS myopathies [63,69,115,122,123]. However, it should be noted that an increase in collagen in breasts with WS is not observed in all of the reports [9,66]. For example, Baldi et al. (2018) [89] and Soglia et al. (2016) [122] reported no effect of the WS condition on collagen content. WB alone and in combination with WS has variable effects, with some cases showing no effect at all [122] and others with collagen content increased [88]. These increased alterations of fat and collagen in WS- and WB-affected cases could be related to increased connective and adipose tissue in affected breast fillets [122].

Nevertheless, while in most cases WS myopathy does not affect ash content [65,69,89,107], in breast fillets affected severely by WB [65,88], WS together with WB [88,115] and even some cases when meat is severely affected by WS [121], ash content tends to decrease.

In conclusion, pectoral muscles of broilers affected by WS, WB or both exhibit higher moisture, lipid, intramuscular fat and collagen contents, while protein and ash contents are reduced compared to those in normal fillets. WB has a more detrimental effect on the functional quality of meat breasts compared to that of WS. However, the combined presence of WS and WB has an even more severe impact on the nutritional and chemical properties of *pectoralis major* muscle [17].

Myopathies also have influence on the amounts of FAs in the *pectoralis major* muscle. PUFAs are essential for cell membrane structure. Phospholipids are major components of the plasma membrane and have a higher PUFA ratio compared to triglycerides, making them more susceptible to oxidation [124]. It is known that oxidative stress can induce lipid peroxidation particularly in PUFAs of the cell membranes. Not much studies have done to ground this theory, but Table 1.7.2 shows that the amount of PUFAs is decreased in affected breast fillets. This finding supports the occurrence of lipid peroxidation in affected breast fillets [63,125]. Nevertheless, the results about the amount of saturated fatty acids (SFAs) suggest that the impact of WS, WB or both myopathies on SFAs could vary depending on severity of

those conditions [63,69,107,122,125]. Moreover, monounsaturated fatty acids (MUFAs) are more resistant to oxidation, but still undergo peroxidation. Elevated MUFA levels in breast muscle affected severely by WS and WB or both could be a compensatory response to oxidative stress [63,69,125].

Table 1.7.2. *Effect of white striping (WS) and wooden breast (WB), occurring individually or combined, on fatty acids (FA) of breast fillets*

Fatty acids	WS	WB	WS+WB	References
SFA	↓	↑ / ↓ / –	↓ / –	[63,69,107,122,125]
MUFA	↑ / –	↑ / –	↑ / –	
PUFA	↓ / –	↓ / –	↓ / –	

FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; WB, wooden breast; WS, white striping; ↑, increased compared to normal *m. pectoralis major*; ↓, decreased compared to normal *m. pectoralis major*; –, no effect.

Malondialdehyde (MDA) is a substance that is generated during secondary lipid oxidation and is used as an oxidation marker [126]. Sometimes MDA content in meat is determined using thiobarbituric acid reactive substances (TBARS) [127]. The presence of oxidized lipids in the diet of humans and animals lead to an increase in TBARS in plasma and tissues [126,128,129]. According to some researchers, as seen in Table 1.7.3, WS, WB or both myopathies do not have an effect to TBARS content [59,107,122]. However, according to Soglia et al. (2016), a group affected severely by WB exhibited higher TBARS values compared to that in normal muscle [122]. This result may indicate higher levels of lipid oxidation, which lead to rancidity and off-flavors, compromising meat quality. However, according to the literature, MDA content determination using TBARS is simple and reproducible, but MDA content is often overestimated, so it should be used cautiously when evaluating results [130].

Table 1.7.3. *Effect of white striping (WS) and wooden breast (WB), occurring individually or combined, on thiobarbituric acid reactive substances (TBARS)*

Parameter	WS	WB	WS+WB	References
TBARS (MDA/kg of meat)	–	↑ / –	–	[59,107,122]

TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; WB, wooden breast; WS, white striping; ↑, increased compared to normal *m. pectoralis major*; ↓, decreased compared to normal *m. pectoralis major*; –, no effect.

Oxidative stress could induce lipid peroxidation. This process leads to formation of aldehydes, ketones and other secondary products also known

as volatile organic compounds (VOCs). Secondary lipid peroxidation products are not only MDA, but also other aldehydes such as hexanal and 4-hydroxynonenal [131–134]. VOCs influence the sensory characteristics of the poultry meat. FAs and amino acids are essential precursors of VOCs in meat. Lipids are the sources of VOCs. Changes in FA composition could affect meat flavor and therefore that might decrease consumer acceptability [135].

Furthermore, according to Luo et al. (2022), the main VOC components in chicken breast muscle are hexanal, heptanal, octanal, nonanal, octadecanal, benzaldehyde, (E,E)-2,4-nonadienal, hexadecanal, and 1-octen-3-ol [135].

Oxidative stress not only affects lipid stability but also influences metabolic pathways of the chicken. One of the key attributes contributing poultry meat popularity is high protein content. High protein content provide suitable conditions for biogenic amines (BAs) formation. BA's are low-molecular-weight compounds that are generally produced by decarboxylation of amino acids by microorganisms, reductive amination, and transamination of aldehydes and ketones, and result of activity of body tissues [136].

Pathological process of the cell leads to breakdown of proteins and lipids. This could result in release of free amino acids, because normal metabolic pathways are disrupted, and these pathological changes could further promoting BAs formation [136]. Furthermore, pH and chemical composition may influence BAs presence in the food. In raw meat, the presence of BAs depends on factors such as: meat origin, storage conditions, specific microbiota, and meat shelf-life [137]. Alessandroni et al. (2022) highlighted that chicken meat is more susceptible to BA accumulations due to specific protein composition and softer texture in comparison with pork and beef meats [138]. Broiler chickens meat affected by myopathies have exhibited collagen contents and hardness is increased in severe WB cases [65,88]. Extensive studies of WB myopathy also have shown that fillets affected by severe typically receive negative consumer feedback. Common complaints include descriptors such as higher springiness, hardness, denseness, cohesiveness, crunchiness, fracturability, fibrousness and chewiness compared to normal fillets. These unfavorable sensory characteristics significantly impact marketability of the affected meat [139].

However, BA content in meat account for less than 10% of scientific papers [136]. Furthermore, specific relationships between WB and WS myopathies and BA formation are very limited in the scientific literature, therefore, it is hard to do comparisons between studies. The connection between WB and WS myopathies and BAs formation as well as VOCs remains still an unexplored field in scientific literature.

1.8. Blood biochemical parameters of broilers affected by myopathies

Rhabdomyolysis refers to the breakdown of the skeletal muscle tissue [76,140]. Various enzymes, muscle cell content, myoglobin, sarcoplasmic proteins, electrolytes, and various organic acids leak into the plasma or serum and may be indicative of injury to the muscles or other organs [140–143]. Damage occurring in the muscle, because of myopathies could be reflected in biochemical profiles of blood [63].

However, not much research has been done to analyze blood parameters of broilers affected by myopathies. Kuttappan et al. (2013) found that creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) enzymes were elevated in the serum of broilers severely affected by WS myopathy. Elevated serum levels of enzymes reflect the muscle damage and degenerative changes due to severe WS. However, Kuttappan et al. (2013) observed unchanged gamma-glutamyl transferase (GGT) and decreased AKP (alkaline phosphatase) levels in the serum of WS-affected birds [63]. Additionally, according to the literature, CK and LDH values increased in the blood serum together with the severity of WB and WS scores [144]. Furthermore, according to Kong et al. (2021), CK could be a predictive blood marker for the prediction of WB in 42-day-old broilers [142]. Additionally, Xing et al. (2021) found that AST, AKP and GGT were elevated in the serum of WB-affected broilers, and this result suggests that liver injury occurs in broilers affected by WB myopathy [145]. Additionally, in another study of Xing et al. (2021), LDH as well as CK were elevated in WB-affected broilers' blood serum [146].

Nevertheless, broilers affected by WB myopathy may also exhibit secondary pathophysiologic perturbations in blood circulation and in other organ systems. Insufficient cardiopulmonary capacity to accommodate sustained rapid growth, resulting in pulmonary hypertension syndrome, or ascites [147]. Additionally, WB is associated with blood gas disturbances characterized primarily by increased venous potassium ions and pCO_2 and decreased pH, sO_2 and pO_2. The accumulation of carbon dioxide and acidification of venous blood occurs when the metabolic demands of the tissue exceed the capacity of the respiratory or circulatory system [148].

Moreover, Sesterhenn et al. (2017) and Amaral et al. (2017) analyzed blood biochemical parameters of broilers affected by DCM [51,53]. According to research results of Amaral et al. (2017) [53] only AST and CK enzymes activities were higher in the DCM group than in the control group. In addition, Sesterhenn et al. (2017) found that blood serum levels for ALT, AST, CK and LDH were significantly greater in broilers affected by DCM [51].

The authors also analyzed histological lesions of liver and found diffuse hepatocyte vacuolization, cholangitis, cholestasis, and bile duct hyperplasia, moderate bile duct proliferation [48,53]. Moreover, researchers suggest that not only muscle damage, but also hepatic damage is linked to DCM [53].

Additionally, blood analysis was performed of DPM affected broilers, and the results showed that turkeys with plasma CK levels above 1,000 units/mL could be considered susceptible to DPM [35].

Structural and biochemical alterations in muscle, evidenced by elevated plasma levels of the intracellular enzyme CK, have been noted in broiler chickens bred for high production performances. Specifically, the increased release of CK into bloodstream is likely a direct result of muscle damage but also may be closely related to the protein turnover associated with the rapid growth rate of meat-type chickens [63,142,144,149].

Additionally, studies examining the blood parameters of broilers affected by myopathies showed no systemic infections are associated with pectoral muscles as well as ALD myopathies [53,63].

1.9. The strategies how to control *pectoralis major* myopathies: wooden breast and white striping

Long-term genetic selection will help reduce the incidence of myopathies. However, in the shorter term, exploring management strategies to capture the non-genetic effects is critical [80]. Non-genetic factors may have more instant and greater influence on the incidence of myopathies [46]. Additionally, Che et al. (2022) indicate that the occurrence of breast myopathies could be reduced by growing broilers to less than 2.46 kg, rather than encouraging excessive weight gain [62]. Furthermore, Meloche et al. (2018) found that restricting feed intake by 95%, 90% and 85% significantly reduced the incidence of WB and WS myopathies. However, this reduction came at the cost of negatively impacting overall performance and yield of broilers [150].

Satellite cells are essential for muscle growth and development [151]. Therefore, proper incubation conditions, including optimal temperature, oxygen and CO_2 levels are crucial for embryo development and the number of satellite cells [152].

Nevertheless, ensuring early access to feed, good ventilation, proper lighting conditions, management of built-up litter heat, and flock thinning are important to maintaining optimal environmental conditions though the whole chicken life. These measures align with the breeder recommendations is pivotal to reduce the risk of myopathies formation [151,152].

Furthermore, nutrition plays a vital role in mitigating WB and WS myopathies. Nutrients support and improve blood flow by reducing oxidative

stress and inflammation [46,153,154]. Feeding broilers all-plant-based diets that contain guanidinoacetic acid (GAA) could reduce the incidence of myopathies [155,156]. The inclusion of GAA in the diets of broilers on all-plant-based feeds helps to ensure sufficient creatine production, which is an alternative energy source, also it improves vascularization, vasodilatation and oxygenation [151,155–157,158]. It has also been found that "super dosing" phytase at a level of 2000 FTU significantly reduces the incidence and severity of WB in broilers. High level of phytase acts as an antioxidant, modulating genes associated with oxygen homeostasis and thereby reducing oxidative stress [159].

Moreover, broilers fed with 85% of the recommended lysine level exhibited lower incidence of WS. Lysine is essential for protein synthesis and muscle development in poultry. [153]. Additionally, the authors found that WB and WS could be reduced with increasing L-Arginine above traditional recommendations. L-Arginine is involved in the synthesis of nitric oxide, which is a vasodilator and is essential to produce muscle creatinine [160,161].

Furthermore, vitamin E and selenium are known as antioxidants. However, dietary vitamin E level had little effect on the reduced incidence of WS, while dietary selenium did not affect the occurrence of WB [60,162,163].

Nevertheless, it has been hypothesized that, supplementing broiler diets with vitamin C may reduce the over-activation of the ascorbate biosynthesis pathway, potentially preventing glycogen depletion in muscles affected by WB. This could help to reduce oxidative stress by maintaining redox balance. Overall, vitamin C could mitigate muscle damage of broilers affected by WB myopathy [164–166].

Overall, gastrointestinal tract is important organ of the chicken that has a complex microbiota. Microorganisms are natural inhabitants of the gut that play a critical role in the host immune system, physiology of gastrointestinal tract, health, and productivity. Microbiota could be affected by genetics, the age of the chickens, diet, litter management, and the use of antibiotics [102,167,168]. Nowadays, it is hypothesized about the gut microbiota's influence on WB formation. Therefore, further studies in this research area are crucial. According to the literature, WB incidence could be reduced by characterizing and regulating the gut microbiota at an early age in broilers [169].

Generally, management of environmental factors and nutrition are two main categories that should be under supervision to reduce the incidence of myopathies. The whole poultry production chain (from farm to fork) is important and demands scientists and poultry management attention to reduce the incidence of myopathies and improve bird health as well as poultry meat quality [12].

2. MATERIALS AND METHODS

The research was conducted at the Pathology Centre of Department of Veterinary Pathobiology and at the Animal Reproduction Laboratory, Faculty of Veterinary Medicine as well as at the Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, from 2020 to 2024. All samples for research were collected from the two main broiler's slaughterhouses in Lithuania.

2.1. Ethical statement

The study did not require consent or ethical approval in accordance to European Directive 2010/63/EU, as no live animals were used for experimental purposes and no animals were specifically killed for the purposes of the research. All of the samples (muscle tissue, blood) were obtained postmortem from animals sacrificed for non-scientific purposes and killed as part of commercial food routine. Permission to collect the samples for research was granted by the slaughterhouse management before the research commenced. The broilers were slaughtered according to standard industrial practices under the supervision of the official veterinary authority. All procedures involving animals were carried out in strict compliance with European slaughter regulations (CE No. 1099/2009 of 24 September 2009) for the protection of animals at the time of killing (Ref. Official Journal of the European Union L 303/1).

2.2. Design of the study

In this dissertation, myopathies in fast-growing Ross 308 male and female 40–43-day-old broilers weighing around 2.4–2.6 kg were analyzed. Figure 2.2.1 illustrates the sequence of how the research was conducted. As outlined in Table 2.2.1, the research was performed in stages, each focusing on different aspects of chicken broiler myopathies: incidence and risk factors of myopathies, microbiological analysis of subcutaneous material of myopathies affected area, physicochemical analysis of the chicken breast meat, blood serum biochemical analysis, and histopathological analysis of the muscles affected by myopathies.

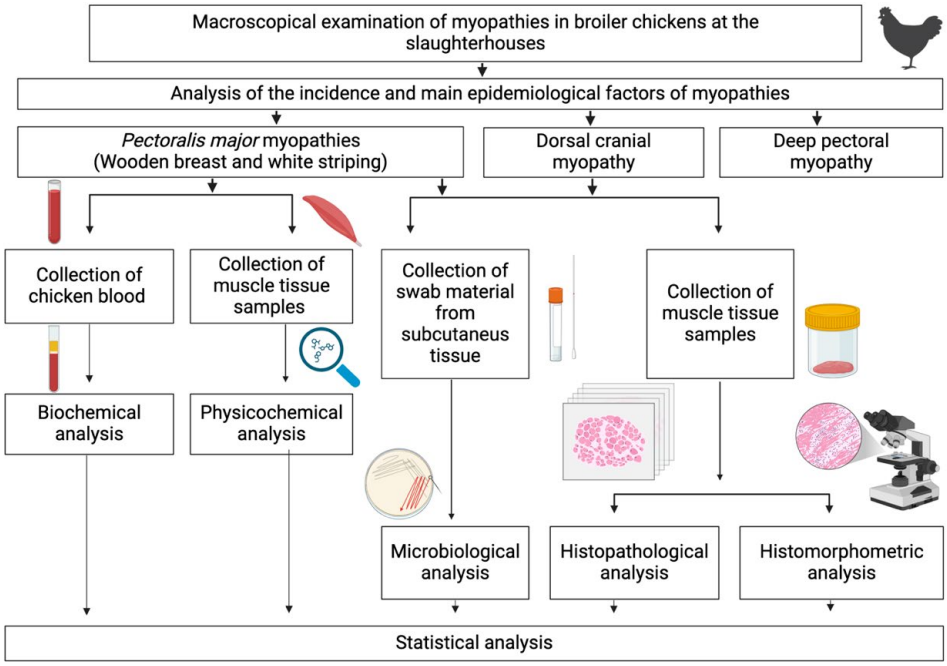

Figure 2.2.1. *Methodological design of the research on broiler myopathies (author's illustration). Created in https://BioRender.com*

Table 2.2.1. *Summary of studies on myopathies in Ross 308 broilers*

Study No.	Description of analysis	Number of broilers (n)	Analyzed myopathies	Sample type of chicken
1.	The incidence and risk factors analysis	19,500	DPM	*Pectoralis minor* muscle
		124,200	DCM	ALD muscle
		54,000	PMM (WB+WS)	*Pectoralis major* muscle
	Microbiological analysis	12	PMM (WB+WS)	Swab material from the subcutis of *pectoralis major* muscle area
		12	DCM	Swab material from the subcutis of ALD muscle area
2.	Physicochemical analysis	18	PMM (WB+WS)	*Pectoralis major* muscle
3.	Biochemical analysis	66	WB	Blood
4.	Histopathological analysis	60	PMM (WB+WS)	*Pectoralis major* muscle
		50	DCM	ALD muscle
5.	Histomorphometric analysis	60	PMM (WB+WS)	*Pectoralis major* muscle
		50	DCM	ALD muscle

ALD, *anterior latissimus dorsi*; DCM, dorsal cranial myopathy; DPM, deep pectoral myopathy; PMM, *pectoralis major* myopathies (wooden breast and white striping included); WB, wooden breast.

Chicken broiler myopathies such as DPM, DCM, WB and WS were investigated. WB and WS were grouped together under the term of *pectoralis major* myopathies (PMM) due to their frequent co-occurrence and similar pathological features. For biochemical blood analysis, only broilers affected by WB were included. DPM was analyzed only in epidemiological studies. It was not investigated further due to its relatively low incidence, already known etiology and prevention factors.

2.3. The incidence and risk factors of broiler myopathies in Lithuania

In the first study the incidence and risk factors of myopathies were analyzed (Publication 1). Male and female Ross 308 broiler chickens were slaughtered between 39 and 42 days of age according to standard industrial practices. After slaughtering broiler muscles were analyzed in order to do gross examination, to evaluate broiler myopathies incidence in Lithuania and to establish main risk factors. *M. pectoralis major, m. anterior latissimus dorsi* and *musculus pectoralis minor* (*m. pectoralis minor*) were investigated from 3 processing lines and PMM, DCM and DPM were detected.

Additionally, microbiological analysis of subcutis of myopathies affected area were done to investigate microbial culture.

The incidence of broiler myopathies in the slaughterhouses

Overall, 54,000 broilers were examined during gross examination of *pectoralis major* muscle. Two main categories of PMM were determined: a control group—*pectoralis major* without myopathies, where no pale areas or white striations were visible on the breast fillet, and *pectoralis major* affected by myopathies—pale muscle in the cranial area of the breast fillet or throughout the breast fillet, also white striations could be visible.

Furthermore, DCM was investigated. Overall, 124,200 broilers were examined for this myopathy. DCM gross lesions of the skin covering ALD muscle were classified into the groups. First, ALD without DCM (normal)—no visible discoloration of the skin area covering the ALD muscle; second, ALD affected by DCM (moderate)—slightly yellowish or a little bit of green discoloration of the skin area covering ALD muscle, and third, ALD affected by DCM (severe)—yellow or green discoloration of the skin area covering ALD muscle.

Lastly, *m. pectoralis minor* was investigated. A total of 19,500 broilers were examined in this part of the study. DPM lesions were also categorized. The first stage (fresh lesion)— muscle is very red and hemorrhagic, hemorrhages also appear on the fibrous sheath and obvious suffusion of serous fluid could be seen; the second stage (a couple days old)—muscle is pale pink to plum

color; the third stage (old)—muscle is "putty like" in consistency, green, which in some parts is turned white and grey. DPM was classified into stages according to Bilgili and Hess (2008) scoring system [170]. *M. pectoralis minor* without visible lesions of DPM was considered normal.

The risk factors of broiler myopathies

The second part of the research was to establish parameters, that could have highest influence on the presence of PMM, DCM and DPM. All following data were collected of each examined flock in the following order: incidence of myopathies in each flock (methodology described above), average broiler live body weight (BW) at slaughter, age at slaughter, treatment of flocks and season at the time of slaughter. Average broiler live BW at slaughter, age at slaughter, treatment of flocks and season at the time of slaughter were considered as a potential risk factors in etiopathogenesis of myopathies.

In order to easier present and handle the collected data, the incidence of myopathies in flocks and all investigated traits were divided into classes (please see the results section for specific details).

Microbiological analysis of the subcutis of myopathies affected area

Microbiological analysis was as the third part of the same epidemiological study. Total 24 microbiological samples were collected exceptionally from broilers raised without antibiotics. Firstly, broilers were grossly examined for PMM and grouped accordingly. The control group (without PMM) (n = 6) had no pale areas, no hardness and no thick liquid or white striations on the *pectoralis major* muscle surface, and broilers affected by PMM (PMM group) (n = 6) had hardness and pale muscle and white stripes visible on the *pectoralis major* muscle surface. Secondly, for DCM: in the control group (without DCM) (n = 6), no visible discoloration of the skin area covering the ALD muscle, and broilers affected by DCM (DCM group) (n = 6), slightly yellowish or a little bit green, or yellow, or green discoloration of the skin area covering ALD muscle.

Samples for microbiological analysis were collected aseptically using sterile cotton swabs from subcutis of the myopathies affected and unaffected areas. Then the samples were placed into Amies transport medium and taken to the laboratory within 12 hours. In the laboratory, the samples were cultured aerobically on selective and differential media. Microorganisms were identified according to biochemical and antigenic characteristics.

2.4. Physicochemical analysis of broiler *pectoralis major* muscle

In the second study physical and chemical parameters of the *pectoralis major* muscle were analyzed (Publication 2). Physical analysis is crucial for evaluation of the quality of the poultry meat. In this study only PMM were included, because it significantly affects *m. pectoralis major*, the most valuable part of the chicken.

Collection of *pectoralis major* muscle samples

Pectoralis major muscle samples were collected from 41-day-old Ross 308 broilers (average live weight 2.5 kg), raised without antibiotics in a traditional intensive system. The samples of muscles, without bone and skin, were collected from broilers with varying severity degrees of PMM. The severity of PMM was determined visually and by palpation of the *pectoralis major* muscle. The classification was as follows: normal (n = 6): no pale areas, no hardness, and no thick liquid or white lines of breast fillet; mild (n = 6): hardness and pale muscle only in the cranial part of the breast fillet, light-yellow viscous liquid on the breast fillet, and up to 1 mm thick white lines are visible; severe (n = 6): hardness, pale areas throughout the breast fillet, a lot of light-yellow, thick, and viscous liquid on the breast fillet, and white lines are clearly visible, thicker than 1 mm. The classification criteria for WS myopathy were based according to Kuttappan et al. (2012) [108].

After classification, the samples were individually weighed, packed in identical freezer bags of the same batch, and labelled with numbered stickers. The breast meat samples were transported to a laboratory under refrigeration conditions (4 °C) and then frozen at –20 °C until further analysis.

Physical and chemical analyses were performed on all collected *pectoralis major* muscle samples.

2.4.1. Physical analysis of *pectoralis major* muscle

The technological chicken breast meat's properties were analyzed following the method described by Rozanski et al. (2017) [171] and AOAC (2019) [172].

pH of *pectoralis major* muscle was measured using a pH meter (model Inolab 3, Hanna Instruments, Italy) calibrated to pH 4.0 and 7.0.

The whole *pectoralis major* muscle was used to perform the quality evaluation. The determination of water-holding capacity, drip loss, cooking loss, and shear force was performed according to Klupsaite et al. (2020) [173].

Filter paper press method was used to determine water-holding capacity. The sample (2 g) was placed on a filter paper (Whatman filter paper 41/

ashless), compressed between two plexiglass sheets, and received a pressure exerted by a weight of 1 kg for 10 min.

Drip loss was measured as the weight loss of a standardized (40–50 g, approximately 30 × 60 × 25 mm) *pectoralis major* muscle sample suspended in an airtight container over 24 h at 4 °C.

Cooking loss was determined by calculating the weight difference of the samples (in a plastic container) before and after cooking in a water bath (internal temperature of 70 °C for 30 min).

After determination of cooking loss three cylindrical samples with a diameter of 1.27 cm were removed from each sample and shear force was evaluated. TA-XT Plus texture analyzer (TA.XT plus Texture Analyzer, Stable Microsystems Ltd., Surrey, UK) coupled to a Warner–Bratzler device was used to measure the shear force.

The dry matter content of muscles was determined after oven drying the samples at 105 °C for 24 h.

Meat color (lightness (L^*), redness (a^*), and yellowness (b^*)) was measured after a 30–40 min blooming period and remeasured later in the day, using a Minolta Chroma Meter colorimeter (CR-400, Minolta Camera, Osaka, Japan) with a closed cone. The device was set on the L^*, a^*, and b^* color systems. The chromameter was calibrated with a white tile ($Y = 92.8, x = 0.3160, y = 0.3323$) using Illuminant D-65, with 2° standard observer and an 8 mm aperture size. The color of *pectoralis major* was measured in the middle part of the muscle. It was cut in two parts, and the cut place was measured.

2.4.2. Chemical analysis of *pectoralis major* muscle

Pectoralis major muscle was minced before the chemical analysis.

Analysis of fatty acids (FAs) content in *pectoralis major* muscle

Lipid extraction for FAs analysis was performed using chloroform/methanol (2:1 *v/v*) following the method described by Pérez-Palacios et al. (2012) [174]. Further analysis was performed following the procedures outlined in Klupsaite et al. (2020) [173]. A GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped together with GCMS-QP2010 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) were used to analyze composition of the FAs. A calibration curve was used to measure the concentration of the fatty acid methyl esters (FAME), and the results were expressed as a percentage of the total concentration of the FAME in the sample.

Evaluation of malondialdehyde (MDA) in *pectoralis major* muscle

MDA was analyzed according to method described by Mendes et al. (2009) [175] with some modifications outlined in Klupsaite et al. [173]. In the sample, MDA was derived with TBA solution. Varian ProStar HPLC system (Varian Corp., Palo Alto, California, USA) was used for chromatographic analysis.

Analysis of volatile organic compounds (VOCs) profile in *pectoralis major* muscle

The VOCs of the chicken meat samples were analyzed using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). A solid phase microextraction (SPME) device with Stableflex (TM) fiber coated with 50 μm DVB-PDMS-Carboxen™ layer (Supelco, USA) was used to prepare the samples. For headspace extrac-tion, 4 g of the homogenized sample were transferred to the 20 mL extraction vial. It was sealed with polytetrafluoroethylene septa and thermostated at 60 °C for 30 min. The fiber was exposed to the headspace of the vial for 30 min. Desorption time was 2 min. Prepared samples were analyzed with GCMS-QP2010 (Shimadzu, Japan) gas chromatograph and a mass spectrometer. A Stabilwax-Da capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness) was used for the analysis. The mass spectrometer operated at full scan mode (35–500 m/z). The following method conditions were used for analysis: column flow rate (helium gas, 99.999% purity) 0.65 mL/min, injector temperature 250 °C, ion source temperature 220 °C, interface temperature of 280 °C. The temperature gradient was programmed from start at 40 °C (3 min hold) and rise to 250 °C (5 °C/min) (5 min hold). The VOCs were identified according to the mass spectra libraries (NIST11, NIST11S, FFNSC2).

Analysis of biogenic amines (BAs) in *pectoralis major* muscle

The assessment of BAs was conducted following the method of Ben-Gigirey et al. (1999) [176], with some modifications. Tryptamine (TRY), phenylethylamine (PHE), cadaverine (CAD), putrescine (PUT), histamine (HIS), tyramine (TYR), spermine (SPER), and spermidine (SPRMD) were analyzed. The standard BA solutions were prepared by dissolving known amounts of each BAs (including internal standard – 1.7-diamino-heptane) in 20 mL of deionized water. The extraction of BAs in samples (5 g) was done by using 0.4 mol/L perchloric acid. The derivatization of sample extracts and standards was performed using a dansyl chloride solution in acetonitrile (10 mg/mL) as a reagent. The Varian ProStar HPLC system (Varian Corp., Palo Alto, California, USA) was made up of the following: two ProStar 210

pumps, a ProStar 410 autosampler, a ProStar 325 UV/VIS Detector, and Galaxy software (Agilent, Santa Clara, California, USA) for data processing. For the separation of amines, a Discovery® HS C18 column (150 × 4.6 mm, 5 µm; SupelcoTM Analytical, Bellefonte, Pennsylvania, USA) was used. The eluents were ammonium acetate (A) and acetonitrile (B) and the elution program consisted of a gradient system with a 0.8 mL/min flow rate. The detection wavelength was set to 254 nm, the oven temperature was 40 °C and samples were injected in 20 µl aliquots. The target compounds were identified based on their retention times in comparison to their corresponding standards. The results were expressed in milligrams per kilogram (mg/kg) of sample.

2.5. Blood serum analysis of broilers affected by wooden breast myopathy

In the third study blood samples were collected from broilers and then analyzed (Publication 3). Biochemical blood analysis helps to monitor the overall health and metabolic status of broilers, providing insights into their physiological state and identifying any deviations from normal health that may indicate underlying issues.

Collection of broiler blood samples

The broilers were examined visually on a moving shackle line, and 100 of them were selected randomly and tagged. After electrical stunning (150 mA, 400 Hz, 15–17 s, AC), 10 mL of blood was sampled directly during exsanguination via the carotid arteries and jugular veins into test tubes (treated with gel to help to separate the clot) (Venoject, Terumo Europe N. V., Leuven, Belgium) from each individual tagged bird. After death in the processing plant, all tagged birds were examined for WB. After the post-mortem examination of the *pectoralis major* muscle, representative blood samples (n = 66) were chosen and separated into two groups. The first group comprised those without WB (control group) (n = 33): no pale areas, no hardness, and no thick liquid of the breast fillet. The second group comprised those with WB myopathy (WB group) (n = 33): hardness and pale muscle only in the cranial part of the breast fillet or throughout the breast fillet and a light yellow, viscous liquid on the breast surface.

During examination of WB, no macroscopic pathologies were found in other organs in both groups.

Biochemical analysis of broilers blood serum

Samples of chicken blood were delivered to the laboratory in 2 h. The blood was centrifugated for 5 min at 3000 rotations per minute (rpm). Then,

1 mL of blood serum from each sample was separated and frozen at −20 °C for biochemical analysis. Biochemical blood tests were performed on all collected serum samples with an automated computerized biochemistry analyzer, the SELECTRA Junior (Netherlands, 2006), using Spinreact (Spain) reagents.

The serum levels of urea, AST, ALT, alkaline phosphatase (ALP), iron, creatinine, calcium, magnesium, phosphorus, potassium, sodium, albumin, gamma-glutamyl transferase (GGT), high-density lipoprotein (HDL) cholesterol, triglycerides, total protein, and CK were determined. Additionally, the level of serum globulin was determined by subtracting the level of albumin from the total protein level.

2.6. Histopathological and histomorphometric analyses of broiler muscles affected by myopathies

Histopathological and histomorphometric analyses were performed on the muscle tissue of broilers affected by PMM and DCM. These analyses help to identify the microscopical changes in *pectoralis major* and ALD muscles affected by myopathies.

Collection of *pectoralis major* and *anterior latissimus dorsi* muscle tissue samples

At the slaughterhouse the carcasses of broilers were examined visually on a moving shackle line. A total 110 broiler carcasses were selected: 60 for PMM and 50 for DCM analysis. During necropsy, it was confirmed that all selected broilers exhibited no gross lesions in any internal organs.

60 samples of *pectoralis major* muscle were collected from broilers for PMM analysis. Three groups were separated according to severity of PMM. First, control group, without PMM (normal (n = 20)): no pale areas, no hardness, and no thick liquid or white lines of breast fillet; second group, with PMM (mild (n = 20)): hardness and pale muscle only in the cranial part of the breast fillet, and up to 1 mm thick white lines are visible; third group, also with PMM (severe (n = 20)): hardness and pale color throughout the breast fillet, light-yellow viscous liquid on the breast fillet, and white lines clearly visible, thicker than 1 mm. The classification criteria for WS myopathy were based according to Kuttappan et al. (2012) [108].

Then, 50 ALD muscle samples were collected from broilers for DCM analysis. Two groups were separated. First, the control group, without DCM (n = 25) – no visible discoloration of the skin area covering the ALD muscle; second group, with DCM (n = 25) – slightly yellowish or a little bit green, or yellow, or green discoloration of the skin area covering ALD muscle.

All histological samples were collected from skinless muscles, immediately after death of broilers. Samples of *m. pectoralis major* were collected from the ventro-cranial part, approximately 1 cm deep from the ventral surface originally facing the skin. Each sample of *pectoralis major* muscle was about 1 cm in length, width, and thickness. Samples of ALD muscle were smaller than *m. pectoralis major* and was about 1 cm in length, with a width and thickness of the samples were approximately 0.4 cm. All collected samples were immersed in 10% buffered formalin solution (Sigma Aldrich, USA).

Histological preparation of *pectoralis major* and *anterior latissimus dorsi* muscle tissue slides

After fixing each collected sample was trimmed into two sections: longitudinal and cross directions. Those two cuts were placed into cassettes. Cross-section cuts were duplicated. The cassettes containing the histopathological material were processed using a tissue processor (Thermo Scientific Shandon Pathcenter, Fisher Scientific, USA). Thereafter, the material was embedded in histological paraffin using an embedding system (Tes 99 Parafin Embedding Center, Medite, Germany). Histological sections, 4 μm thick, were prepared using a microtome (Leica RM2235, Leica Biosystems, Germany). For histopathological analysis the prepared slides were stained with hematoxylin and eosin (H&E) using an automatic staining machine (Tissue-Tek DRS™, Sakura, Japan).

Additionally, the duplicates were stained with Masson's trichrome for histomorphometric analysis. It was performed following the protocol provided in the staining kit's information sheet.

All histopathological samples were analyzed using an Olympus BX63 microscope and an Olympus DP72 digital camera system (U-TV1X-2, Olympus, Japan), both equipped with Olympus cellSens Dimension 1.14 software (Olympus, Japan). Histopathological analysis was performed on all collected *pectoralis major* and ALD muscle samples.

2.6.1. Histopathological analysis of *pectoralis major* and *anterior latissimus dorsi* muscles

Histopathological analysis was done on all collected samples. Slides were analyzed directly through the microscope, evaluating longitudinal and cross-sections of the muscle. Degeneration/necrosis, accumulation of fibrous connective and adipose tissue, inflammation of the muscle and blood vessels were analyzed and scored. All pathologies were quantified by visually scoring the muscle tissue slides under bright field microscopy. The histopathological score was based on a scale ranging from 0 to 4 for degeneration/necrosis

(Table 2.6.1.1). Inflammation of the muscle, fibrous connective tissue infiltration and adipose tissue infiltration were also graded according to severity, as described in Table 2.6.1.2, ranging from 0 to 3. Inflammation of the blood vessels was evaluated according to Sihvo et al. (2017) [60]. Table 2.6.1.3 represents the evaluation criteria of inflammation of the blood vessels of muscles, and the maximum score was 2 points. Each section (cross and longitudinal) of the sample was scored independently, and in cases where one section received a higher score, the highest value was recorded as the final score. Under identical conditions, the magnification during each sample examination was 40×, while inflammation of the blood vessels was analyzed with 100× magnification.

Table 2.6.1.1. *Evaluation criteria for assessing the degeneration/necrosis of the pectoralis major and anterior latissimus dorsi (ALD) muscles*

Pathology of the affected muscle	**0 points**	**1 point**	**2 points**	**3 points**	**4 points**
Degeneration/ necrosis	Absent	< 20% of skeletal muscle is damaged	20–30% of skeletal muscle is damaged	30–50% of skeletal muscle is damaged	> 50% of skeletal muscle is damaged

Table 2.6.1.2. *Evaluation criteria for assessing the inflammation of the muscle, accumulation of fibrous connective tissue and adipose tissue of the pectoralis major and anterior latissimus dorsi (ALD) muscles*

Pathology of the affected muscle	**0 points**	**1 point**	**2 points**	**3 points**
Inflammation of the muscle	Absent	Inflammatory cells are present just where damaged myofibers are. Interstitial inflammation is absent	Inflammatory cells infiltrated damaged myofibers, also interstitial inflammation was present	Inflammatory cells infiltrated damaged myofibers, severe interstitial inflammation
Accumulation of fibrous connecti-ve tissue	Absent	< 20% of skeletal muscle is replaced	20–50% of skeletal muscle is replaced	> 50% of skeletal muscle is replaced
Accumulation of adipose tissue	Absent	< 20% of skeletal muscle is replaced	20–50% of skeletal muscle is replaced	> 50% of skeletal muscle is replaced

Table 2.6.1.3. *Evaluation criteria for assessing the inflammation of the blood vessels of the pectoralis major and anterior latissimus dorsi (ALD) muscles*

Pathology of the affected muscle	**0 points**	1 point	2 points
Inflammation of the blood vessels	Normal vessel walls without surrounding cell infiltration	Perivenular accumulation of mononuclear leukocytes (inflammation of the blood vessel)	Lymphocytes with intramural infiltration sometimes obliterate the vascular wall (vasculitis)

2.6.2. Histomorphometric analysis of *pectoralis major* and *anterior latissimus dorsi* muscles

Histomorphometric analysis of the muscles was done using Olympus cellSens Dimension 1.14 software. All collected samples of *m. pectoralis major* (n = 60) and ALD (n = 50) were analyzed. Masson's trichrome-stained cross-sectional areas of each sample were analyzed to calculate the average diameter of myofibers (μm), cross-sectional area of the myofibers (μm^2) and to determine the percentage of muscle, fibrous connective, and adipose tissues.

Firstly, all the measurements were calculated on digital images of Masson trichrome sections. The measurements of the average diameter of myofibers were conducted under 40× magnification lens in one field of view. The arbitrary line function was used to draw a line across the each selected myofiber. 100 myofibers per field were measured to ensure statistical accuracy. The myofiber diameters were recorded, and the mean of the diameter and cross-sectional area of myofibers (μm^2) were calculated of each analyzed sample.

To quantitatively analyze the composition of fibrous connective and muscle tissue (expressed as a partial volume, %) of the collected samples, high-resolution images of *pectoralis major* and *anterior latissimus dorsi* muscles were captured using microscope at 40× magnification (Figure 2.6.2.1). Manual HSV thresholding was applied to mark specifically muscle and fibrous connective tissue. Threshold values for hue, saturation, and brightness (intensity) were manually adjusted to highlight the regions of interest (ROI). Muscle tissue was marked in blue and fibrous connective tissue was marked in green (Figure 2.6.2.2). Count and Measure tool was used to quantity the area of selected structures. The percentage of adipose tissue in the samples was then calculated. Manual thresholding was applied to select areas of adipose tissue areas (ROIs). Each ROI was analyzed for area in μm^2.

The percentage of adipose tissue in each specimen was determined using the formula:

$$\text{Percentage of adipose tissue (\%)} = \frac{\text{sum of areas of adipocytes } (\mu m^2)}{\text{area of the analyzed region of speciment } (\mu m^2)} * 100$$

Anatomically, ALD muscle is significantly smaller than the *pectoralis major*, resulting in smaller sample sizes. However, the total investigated area per specimen was standardized across all samples within each myopathy. Finally, the total percentages for each specimen were adjusted to total 100%, ensuring that any unstained areas were accounted and eliminated from the final calculations.

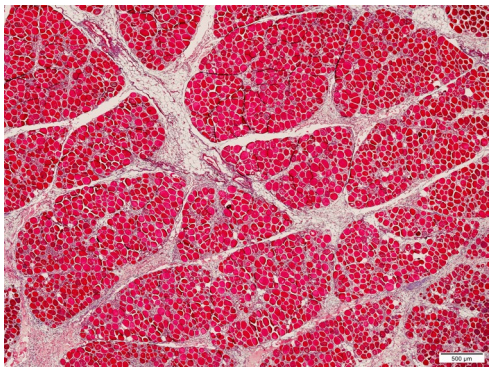

Figure 2.6.2.1. *Pectoralis major muscle stained with Masson Trichrome (nine cross-sectional images, 40×).*

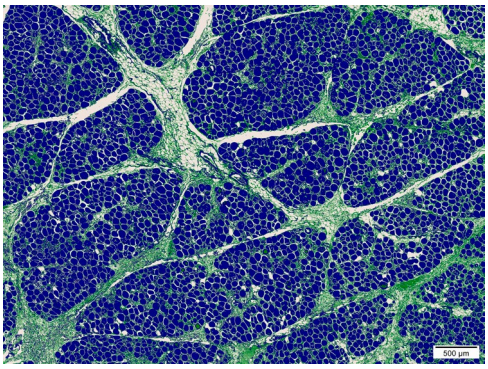

Figure 2.6.2.2. *Pectoralis major muscle processed using cellSens Dimension 1.14 software. Muscle tissue appears in blue and fibrous connective tissue is shown in green. (nine cross-sectional images, 40×).*

2.7. Statistical analysis

The data from all research were collected in a database (Microsoft Excel 2021). Histomorphometric measurements were obtained using cellSens Dimension software 1.14, and the mean values were calculated. These measurements, along with other data, were compiled in database (Microsoft Excel 2021). Once the data was compilated, it was transferred to SPSS 27.0 (SPSS Inc., Chicago, IL, USA) program software for statistical analysis. Before proceeding the analysis, all measurements were tested for a normal distribution. The descriptive statistics were calculated of investigated traits.

The data were analyzed using one-way ANOVA, and a post hoc Tukey test was performed. This statistical method was used to compare means of three independent groups to determine which groups means are significantly different from each other while controlling multiple comparisons. Additionally, in cases where comparisons between two groups were necessary, a two-tailed Student's t-test was used. This test determines whether there is a statistically significant difference between means of two independent samples.

To explore relationships between investigated traits, both Pearson's and Spearman's correlation coefficients was calculated. Pearson's correlation measures the linear relationship between two continuous variables, while Spearman's in a non-parametric test used for assessing monotonic relationships between variables.

A p value of less than 0.05 was considered statistically significant throughout all tests.

3. RESULTS

3.1. The incidence and risk factors of broiler myopathies in Lithuania

The incidence of broiler myopathies in Lithuania

Prior to recent studies, data on the prevalence of broiler's myopathies in Lithuania was unavailable. 18 flocks of broilers (n = 54,000) were evaluated for PMM. According to our research results, the total incidence of broilers affected by PMM is 18.19%. Figure 3.1.1 (A) shows *pectoralis major* muscle without myopathic changes, and Figure 3.1.1 (B) demonstrates the gross lesions of PMM.

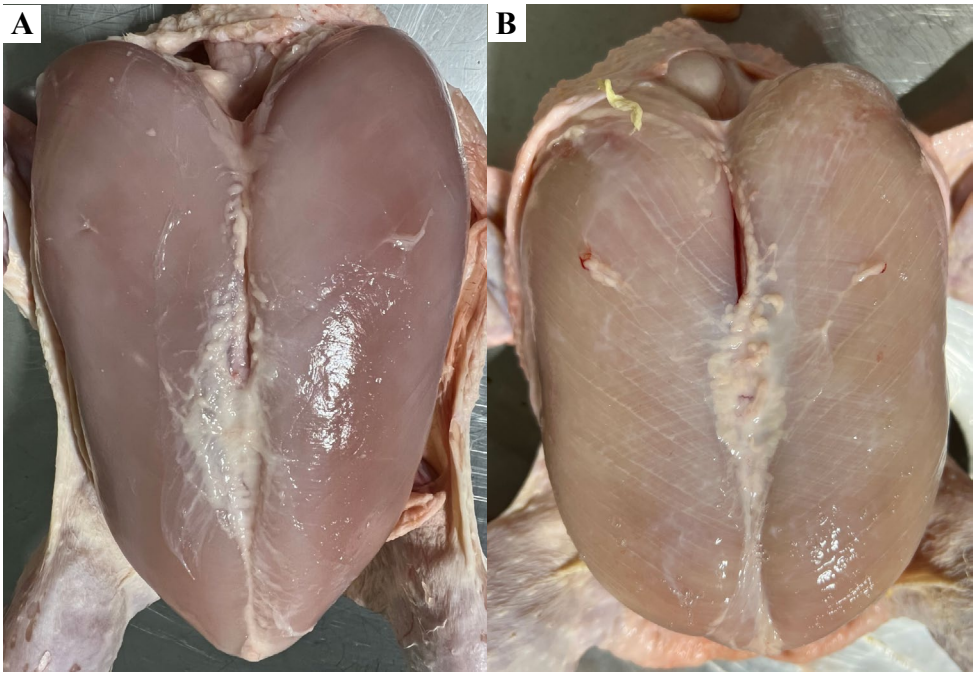

Figure 3.1.1. *Macroscopical classification of myopathies in broilers pectoralis major muscle.* ***(A)*** *Pectoralis major muscle without myopathies (without pale areas or white striations).* ***(B)*** *Pectoralis major muscle affected by myopathies (pale muscle and thick white striations throughout the breast fillet). Source: the authors.*

Furthermore, 18 flocks of broilers (n = 124,200) were evaluated for DCM. 94.84% of evaluated broilers' skin did not exhibit gross lesions of DCM (Figure 3.1.2 (A)). The total incidence of DCM in Lithuania was 5.16%, with 4.49% of broilers being moderately affected (Figure 3.1.2 (C)) and 0.67%

severely affected (Figure 3.1.2 (E)). The macroscopical characterization of DCM is presented in the Figure 3.1.2.

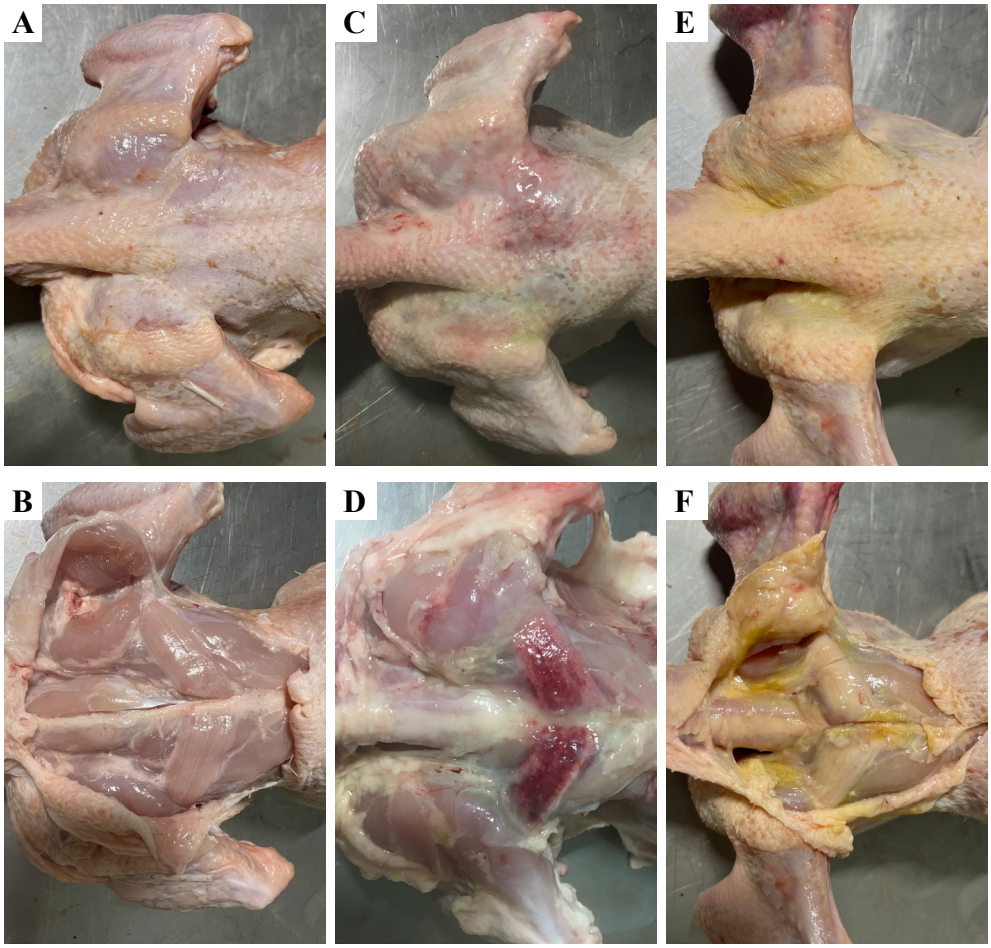

Figure 3.1.2. *Macroscopical classification of dorsal cranial myopathy (DCM) in broilers anterior latissimus dorsi (ALD) muscle.* ***(A)*** *ALD muscle without DCM (normal) (no visible discoloration of the skin area covering anterior latissimus dorsi muscle).* ***(B)*** *ALD muscle without DCM (normal) (muscle without edema, pallor and hemorrhages).* ***(C)*** *DCM (moderate) (a little bit green discoloration of the skin area covering ALD muscle).* ***(D)*** *DCM (moderate) (edematous muscle with hemorrhages).* ***(E)*** *DCM (severe) (yellow discoloration of the skin area covering ALD muscle).* ***(F)*** *DCM (severe) (muscle pale, without hemorrhages, edematous exudate on muscle surface). Source: the authors.*

Nevertheless, 13 flocks of broilers (n = 19,500) were evaluated for DPM. The incidence of DPM was the lowest among all evaluated myopathies and it was 0.27%. 99.73% of broilers did not exhibit gross lesions of DPM (Figure 3.1.3 (A)), while 0.144% exhibit fresh lesions of DPM (Figure 3.1.3 (B)). Additionally, other chickens *m. pectoralis minor* showed couple days old (Figure 3.1.3 (C)) and old lesions of DPM. (Figure 3.1.3 (D)), 0.113% and 0.01%, respectively. Macroscopical characterization of DPM is presented in the Figure 3.1.3.

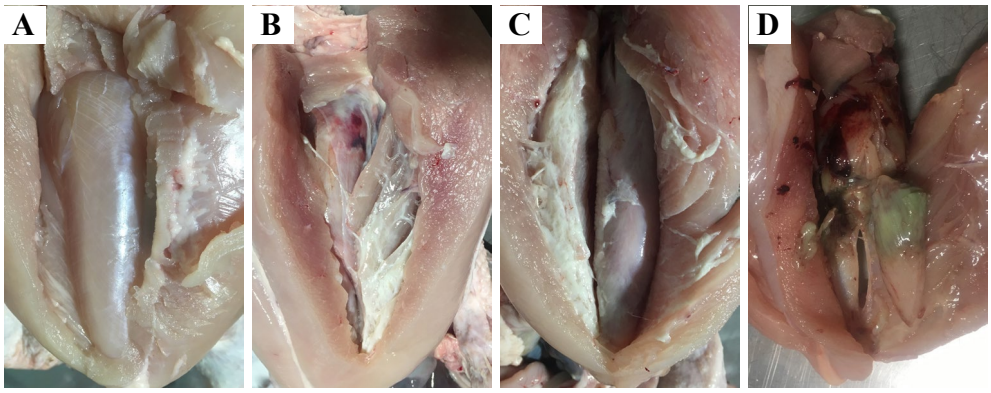

Figure 3.1.3. *Macroscopical classification of deep pectoral myopathy (DPM) in broilers pectoralis minor muscle.* ***(A)*** *Pectoralis minor muscle without DPM (muscle without hemorrhages and discoloration).* ***(B)*** *DPM (fresh lesion) (muscle hemorrhagic, hemorrhages also appear on the fibrous sheath and suffusion of serous fluid).* ***(C)*** *DPM (a couple days old) (muscle is pale pink to plum color).* ***(D)*** *DPM (old) (muscle is 'putty like' consistency, green, which in some parts is turned white and grey). Source: the authors.*

The risk factors of broiler myopathies

All variables were categorized into classes for statistical evaluation of the risk factors of PMM, DCM and DPM. The percentage of incidence of PMM in flocks was classified as follows: 1 (3–9.9%); 2 (10–24.9%); 3 (25–32.9%). The percentage of incidence of DCM in flocks: 1 (0.5–4.9%); 2 (5–7.9%); 3 (8–13%). The percentage of incidence of DPM in flocks: 1 (0.07–0.2%); 2 (0.21–0.3%); 3 (0.31–0.6%). Average broiler live BW at slaughter was grouped: 0 (2.1–2.4 kg); 1 (2.41–2.6 kg); 2 (2.61–2.9 kg). Age at slaughter was categorized: 0 (39–40 days); 1 (41–42 days). Additional factors such as treatment was classified: 0 (no treatment); 1 (antibiotics or antibiotics and coccidiostats), and seasons grouped into 0 (cold weather season); 1 (hot weather season).

Results have shown that the percentage of PMM in flocks was positively related with average live BW at slaughter (r = 0.898) and slaughter age of broilers (r = 0.693) ($p < 0.001$). Furthermore, the percentage of PMM in flocks was negatively related with treatment of broilers ($r_s = –0.535$, $p < 0.05$).

Additionally, the percentage of DCM in flocks showed positive correlation with average live BW at slaughter (r = 0.537, $p < 0.05$). However, there were no statistically significant associations between the percentage of incidence of DCM in flocks and slaughter age (r = 0.281) or treatment ($r_s = –0.170$) ($p > 0.05$). Additionally, we analyzed the seasons impact on incidence of DCM myopathies and results showed that percentage of DCM in flocks was positively associated with seasons ($r_s = 0.658$, $p < 0.01$).

However, there were no statistically significant associations between the percentage of incidence of DPM in broiler flocks and average live BW at slaughter (r = 0.015) or age at slaughter (r = 0.239) or treatment ($r_s = –0.202$) ($p > 0.05$).

Microbiological analysis of the subcutis of myopathies affected area

Microbiological analysis revealed that *Hafnia alvei* was isolated from all (100%) of the samples of subcutis that covers muscle affected by PMM. In contrast, *Enterococcus viikkiensis* was consistently isolated (100%) from control group of samples, without PMM. In broilers with DCM, *Enterococcus viikkiensis* and *Escherichia coli* were isolated from 33.33% of the samples, while *Hafnia alvei* was found in 16.67% of the cases. Notably, in 16.67% of the samples of broilers with DCM showed no bacterial presence. Furthermore, in broilers without DCM, *Escherichia coli* was isolated from all collected samples of subcutis (100%). Isolated bacteria are presented in Figure 3.1.4.

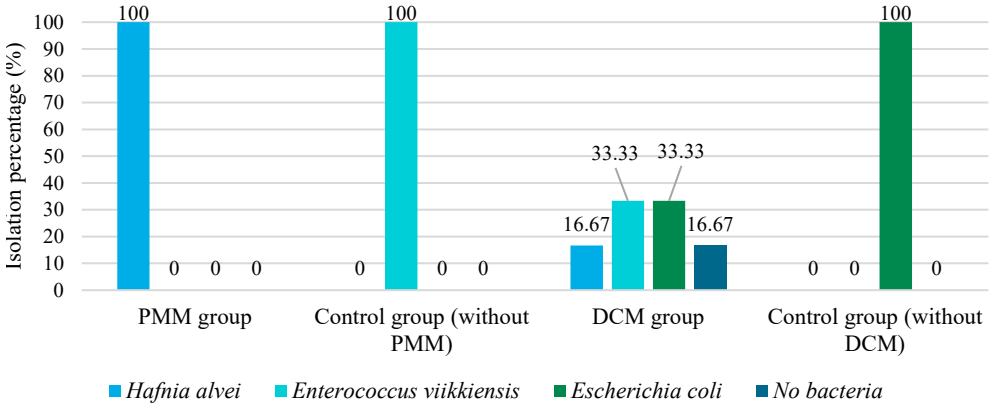

Figure 3.1.4. *Bacteria isolation from the subcutis covering pectoralis major and anterior latissimus dorsi (ALD) muscles affected (PMM and DCM groups) and not affected by myopathies (control groups)*

3.2. Physicochemical analysis of broiler *pectoralis major* muscle

3.2.1. Physical analysis of *pectoralis major* muscle affected by myopathies

Table 3.2.1.1 presents data on dry matter, drip loss, pH, water-holding capacity, cooking loss, and shear force. Samples with severe myopathies contain 6.14% less dry matter than normal muscle samples ($p < 0.01$) and 5.28% less than samples with mild myopathies ($p < 0.05$). Drip loss in severely affected muscles was 55.09% higher compared to normal samples ($p < 0.01$). Cooking loss in severely affected muscles was 68.40% higher than in muscles without myopathies ($p < 0.001$) and 38.43% higher than in muscles with mild myopathies ($p < 0.001$). However, severity of myopathies did not significantly affect shear force, pH, or water-holding capacity ($p > 0.05$).

Table 3.2.1.1. *pH, dry matter content, drip loss, water holding capacity, cooking loss and shear force of pectoralis major muscle of broilers affected by myopathies (mean ± SD)*

Variables	Severity of myopathies		
	Normal [a] (n = 6)	**Mild [b] (n = 6)**	**Severe [c] (n = 6)**
pH	6.05 ± 0.11	6.04 ± 0.16	6.03 ± 0.11
Dry matter content, %	26.38 ± 0.74**[c]	26.14 ± 0.56*[c]	24.76 ± 0.88**[a, *b]
Drip loss, %	2.85 ± 0.82**[c]	3.63 ± 0.59	4.42 ± 0.44**[a]
Water holding capacity, %	65.21 ± 1.67	65.66 ± 1.59	64.45 ± 1.99
Cooking loss, %	12.47 ± 1.29***[c]	15.17 ± 2.95***[c]	21.00 ± 1.57***[a,b]
Shear force, kg/cm^2	1.64 ± 0.32	1.48 ± 0.34	1.49 ± 0.50

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Table 3.2.1.2 indicates that the b^* of muscle samples affected by severe myopathies was 20.09% higher than that of normal samples ($p < 0.05$). Furthermore, muscle samples affected by mild myopathies showed a 27.76% increase in b^* compared to normal samples ($p < 0.01$). However, the severity of myopathies did not significantly impact the lightness or redness of chicken breast meat ($p > 0.05$).

Table 3.2.1.2. *Color parameters of pectoralis major muscle of broilers affected by myopathies (mean ± SD)*

Variables	Severity of myopathies		
	Normal [a] (n = 6)	**Mild [b] (n = 6)**	**Severe [c] (n = 6)**
Lightness (*L**), NBS	51.22 ± 5.75	52.97 ± 3.19	52.45 ± 2.77
Redness (*a**), NBS	11.61 ± 1.13	10.05 ± 1.26	10.81 ± 1.69
Yellowness (*b**), NBS	8.61 ± 1.75**[b, *c]	11.00 ± 0.55**[a]	10.34 ± 0.77*[a]

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

3.2.2. Chemical analysis of *pectoralis major* muscle affected by myopathies

Analysis of fatty acids

Table 3.2.2.1 shows that the overall amount of MUFAs was 5.09% higher in muscles affected by severe myopathies compared to normal muscles ($p < 0.05$). Similarly, the amount of MUFAs was 5.45% higher in muscles affected by mild myopathies compared to normal samples ($p < 0.05$). Regarding MUFAs, the amount of oleic acid was 4.73% higher in severely affected muscles compared to normal muscles ($p < 0.05$). Additionally, palmitoleic acid levels were 30.48% higher in muscles affected by mild myopathies compared to normal muscles ($p < 0.05$).

Furthermore, among the SFAs investigated, myristic acid levels were 6.5 fold higher in samples with severe myopathies compared to those without myopathies ($p < 0.01$). Controversially, stearic acid levels were 17.81% lower in muscles affected by severe myopathies and 16.04% lower in muscles with mild myopathies, compared to normal muscles ($p < 0.05$ for both).

The content of other FAs were not significantly altered ($p > 0.05$) in muscles affected by myopathies compared to normal samples of *pectoralis major* muscle.

Table 3.2.2.1. *Fatty acid composition (percentage of total fatty acids) of pectoralis major muscle of broilers affected by myopathies (mean ± SD)*

Fatty acids	Nomenclature	Severity of myopathies		
		Normal [a] (n = 6)	**Mild [b] (n = 6)**	**Severe [c] (n = 6)**
Myristic acid	C14:0	0.04 ± 0.01**[c]	0.20 ± 0.14	0.26 ± 0.13**[a]
Palmitic acid	C16:0	19.91 ± 1.02	19.82 ± 0.56	20.15 ± 1.28
Stearic acid	C18:0	9.04 ± 0.76*[b, *c]	7.59 ± 0.98*[a]	7.43 ± 1.12*[a]
SFA		28.99 ± 1.65	27.61 ± 1.35	27.84 ± 1.84

Table 3.2.2.1 cont.

Fatty acids	Nomenclature	Severity of myopathies		
		Normal [a] (n = 6)	Mild [b] (n = 6)	Severe [c] (n = 6)
Palmitoleic acid	C16:1	2.46 ± 0.40*[b]	3.21 ± 0.49*[a]	2.82 ± 0.48
Oleic acid	C18:1	30.84 ± 0.67*[c]	32.05 ± 0.77	32.30 ± 1.04*[a]
Gondoic acid	C20:1 ω9	0.45 ± 0.12	0.34 ± 0.06	0.36 ± 0.03
MUFA		33.76 ± 0.92*[b, *c]	35.60 ± 1.14*[a]	35.48 ± 1.27*[a]
Linoleic acid	C18:2 ω6	34.00 ± 1.50	34.09 ± 1.36	33.94 ± 1.60
γ-Linolenic	C18:2 ω6	0.04 ± 0.02	0.05 ± 0.03	0.07 ± 0.03
α-Linolenic acid	C18:3 α ω3	2.50 ± 0.53	2.01 ± 0.21	2.03 ± 0.23
Eicosadienoic acid	C20:2 ω6	0.35 ± 0.08	0.31 ± 0.06	0.29 ± 0.11
Arachidonic acid	C20:4 ω6	0.36 ± 0.11	0.32 ± 0.10	0.35 ± 0.12
PUFA		37.26 ± 1.79	36.79 ± 1.48	36.68 ± 1.54
Omega 6		34.76 ± 1.54	34.78 ± 1.37	34.64 ± 1.55
Omega 3		2.50 ± 0.53	2.01 ± 0.21	2.03 ± 0.23

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Analysis of volatile organic compounds

In our research, a total of 22 individual substances were identified during the analysis of VOCs. Among these, the compounds identified included aldehydes (11), alcohols (8), esters (2) and furans (1). The amounts of VOCs in *pectoralis major* affected by PMM are presented in Table 3.2.2.2. Our research results showed that hexanal levels were 33.76% higher in muscles affected by severe myopathies ($p < 0.01$) and 26.21% higher in muscles affected by mild myopathies ($p < 0.05$) compared to normal muscles without myopathies.

The concentration of allyl 2-ethylbutyrate was 4.3-fold higher in muscles affected by severe myopathies and 4.2-fold higher in muscles affected by mild myopathies compared to normal muscles without myopathies ($p < 0.01$ for both). In comparison to normal samples of *pectoralis major*, the amounts of 2-octenal, 2-octen-1-ol, and 2-decenal were 9.6-fold ($p < 0.01$), 2.2-fold ($p < 0.05$), and 7.9-fold ($p < 0.01$) higher, respectively, in muscles severely affected by myopathies. Additionally, the amount of 2-decenal was approximately 3.8-fold higher in muscles with severe myopathies compared to those with mild myopathies ($p < 0.05$).

Controversially, the concentration of 2-ethyl-1-hexanol and benzaldehyde were significantly lower, by 77.12% and 68.64% ($p < 0.05$ for both),

respectively, in muscles severely affected by myopathies compared to normal samples of muscles. The levels of other substances were not significantly altered ($p > 0.05$) in muscles affected by myopathies compared to normal samples of *pectoralis major* muscle.

Table 3.2.2.2. *Amounts of volatile organic compounds (area percentage according to the identified compounds) in pectoralis major muscle of broilers affected by myopathies (mean ± SD)*

Volatile organic compounds	Severity of myopathies		
	Normal [a] (n = 6)	Mild [b] (n = 6)	Severe [c] (n = 6)
Aldehydes			
Hexanal	27.58 ± 5.26*[b, **c]	34.81 ± 2.69*[a]	36.89 ± 2.95**[a]
Heptanal	0.67 ± 0.88	1.09 ± 1.05	0.99 ± 0.74
Octanal	5.38 ± 2.87	5.85 ± 0.80	5.16 ± 0.59
Nonanal	13.62 ± 4.07	13.43 ± 2.63	13.59 ± 1.84
2-Octenal	0.15 ± 0.38**[c]	0.69 ± 0.81	1.45 ± 0.11**[a]
Decanal	nd	0.36 ± 0.87	0.90 ± 1.03
Benzaldehyde	19.93 ± 12.64*[c]	11.67 ± 5.52	6.25 ± 2.93*[a]
2-Decenal	0.13 ± 0.33**[c]	0.27 ± 0.66*[c]	1.03 ± 0.24**[a, *b]
2,4-Dodecadienal	nd	nd	0.25 ± 0.39
2-Dodecenal	0.10 ± 0.25	nd*[c]	0.45 ± 0.38*[b]
Alcohols			
1-Pentanol	2.97 ± 1.11	3.07 ± 0.44	3.33 ± 0.31
1-Hexanol	0.27 ± 0.42	0.46 ± 0.50	1.03 ± 0.60
1-Octen-3-ol	9.51 ± 7.44	14.18 ± 1.18	14.98 ± 1.35
1-Heptanol	3.26 ± 1.00	2.90 ± 0.33	3.19 ± 0.32
2-Ethyl-1-hexanol	2.36 ± 1.70*[c]	1.32 ± 0.51	0.54 ± 0.34*[a]
1-Octanol	6.70 ± 2.74	5.02 ± 0.76	4.87 ± 0.35
2-Octen-1-ol	1.09 ± 0.87*[c]	1.70 ± 0.63	2.41 ± 0.47*[a]
Benzyl-alcohol	5.19 ± 3.54	2.81 ± 1.60	1.78 ± 1.21
Esters			
n-Caproic acid vinyl ester	0.77 ± 1.89	nd	0.40 ± 0.98
Allyyl-2-ethylbutyrate	0.09 ± 0.13**[b, **c]	0.38 ± 0.20**[a]	0.39 ± 0.08**[a]
Furans			
2-Pentylfuran	0.12 ± 0.29	nd	nd
Others			
1-Tetradecyne	0.12 ± 0.29	nd	0.13 ± 0.33

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***). nd, not detected.

Analysis of malondialdehyde

In addition to VOCs, MDA content was also measured. According to the concentration of MDA (μmol/kg), as seen in Table 3.2.2.3, severe myopathies expressed 61.39% higher concentrations than the control samples of muscles. However, the results showed that differences between the means of MDA levels were not statistically significant ($p > 0.05$).

Table 3.2.2.3. *Amount of malondialdehyde (μmol/kg) in pectoralis major muscle affected by myopathies (mean ± SD)*

Malondialdehyde	Severity of myopathies		
	Normal [a] (n = 6)	Mild [b] (n = 6)	Severe [c] (n = 6)
	2.02 ± 1.14	2.09 ± 1.66	3.26 ± 1.00

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Analysis of biogenic amines

According to our research, as shown in Table 3.2.2.4, the amount of TRY was 56.92% lower in severely affected *pectoralis major* fillets compared to normal, without myopathies ($p < 0.01$). Additionally, the amount of SPER was 5.85% lower in severely by myopathies affected *pectoralis major* fillets compared to normal fillets ($p < 0.05$). The amount of TYR was 72.63% higher in mildly affected samples of muscle compared to normal muscle samples ($p < 0.05$). However, the amount of other BAs was not statistically different compared to normal samples of muscle ($p > 0.05$).

Table 3.2.2.4. *Amounts of biogenic amines (mg/kg) of broilers pectoralis major affected by myopathies (mean ± SD)*

Biogenic amines	Severity of myopathies		
	Normal [a] (n = 6)	Mild [b] (n = 6)	Severe [c] (n = 6)
Tryptamine (TRY)	2.60 ± 0.97^{**c}	1.86 ± 0.62	1.12 ± 0.17^{a**}
Phenylethylamine (PHE)	36.64 ± 7.04	36.75 ± 8.24	31.26 ± 3.64
Putrescine (PUT)	2.16 ± 2.41	2.81 ± 3.32	4.09 ± 2.51
Cadaverine (CAD)	nd	nd	nd
Histamine (HIS)	nd	nd	nd
Tyramine (TYR)	5.81 ± 0.93^{*b}	10.03 ± 3.79^{*a}	9.47 ± 2.26
Spermidine (SPRMD)	29.06 ± 3.73	24.51 ± 6.31^{*c}	32.71 ± 5.28^{*b}
Spermine (SPER)	106.92 ± 5.16^{*c}	103.44 ± 4.80	100.66 ± 1.35^{*a}

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***). nd, not detected.

3.3. Biochemical analysis of broiler blood serum

As presented in Table 3.3.1, the biochemical analysis revealed significantly elevated activities of CK and ALT in the blood serum of broilers affected by WB. The levels of ALT and CK were elevated by 32.42% and 33.69%, respectively ($p < 0.05$). However, AST, ALP, and GGT did not differ significantly between broilers affected and not affected by WB myopathy ($p > 0.05$).

Table 3.3.1. *Levels of blood serum enzymes (U/L) of broilers without myopathy (control group) and those affected by wooden breast myopathy (WB group) (mean ± SD)*

Enzymes	Control group (n = 33)	WB group (n = 33)	p value
Alanine aminotransferase (ALT)	25.45 ± 12.95	33.70 ± 13.02	0.012
Aspartate aminotransferase (AST)	620.92 ± 138.02	622.09 ± 100.04	0.969
Creatine kinase (CK)	63725.21 ± 29942.14	85196.42 ± 40900.91	0.018
Alkaline phosphatase (ALP)	6679.06 ± 3367.07	8636.70 ± 5270.50	0.077
Gamma-glutamyl transferase (GGT)	27.06 ± 5.90	25.15 ± 6.01	0.197

Table 3.3.2 shows that potassium levels were 27.36% higher in the blood serum of broilers affected by WB myopathy compared to that of broilers without myopathy ($p \leq 0.01$). However, the iron, calcium, magnesium, phosphorus, and sodium concentrations in the serum did not differ significantly between broilers affected and not affected by WB myopathy ($p > 0.05$)

Table 3.3.2. *Levels of blood serum minerals of broilers without myopathy (control group) and those affected by wooden breast myopathy (WB group) (mean ± SD)*

Minerals	Control group (n = 33)	WB group (n = 33)	p value
Iron, µg/dL	87.64 ± 10.60	84.01 ± 14.92	0.260
Calcium, mmol/L	2.31 ± 0.34	2.31 ± 0.26	0.987
Magnesium, mmol/L	1.10 ± 0.10	1.14 ± 0.11	0.123
Phosphorus, mmol/L	2.69 ± 0.24	2.75 ± 0.26	0.345
Potassium, mmol/L	37.68 ± 17.37	47.99 ± 14.08	0.010
Sodium, mmol/L	144.70 ± 10.71	145.64 ± 10.24	0.717

As shown in Table 3.3.3, other parameters of broiler blood did not differ significantly between broilers affected and not affected by WB myopathy ($p > 0.05$).

Table 3.3.3. *Levels of blood serum proteins, high-density lipoprotein (HDL) cholesterol, triglycerides and creatinine of broilers without myopathy (control group) and those affected by wooden breast myopathy (WB group) (mean ± SD)*

Parameters	Control group (n = 33)	WB group (n = 33)	p value
Urea, mmol/L	1.35 ± 0.21	1.36 ± 0.18	0.755
Albumin, g/L	16.75 ± 1.33	17.08 ± 1.29	0.311
Globulin, g/L	16.10 ± 2.69	16.05 ± 2.19	0.936
Total protein, g/L	32.84 ± 3.67	33.12 ± 2.96	0.732
High-density lipoprotein (HDL) cholesterol, mmol/L	1.70 ± 0.29	1.72 ± 0.26	0.745
Triglycerides, mg/dL	66.52 ± 19.16	68.45 ± 28.69	0.748
Creatinine, µmol/L	31.12 ± 2.27	31.06 ± 2.61	0.920

3.4. Histopathological analysis of broiler muscles affected by myopathies

Pectoralis major muscle affected by myopathies

Samples of *pectoralis major* muscle of broilers were evaluated histologically. Figure 3.4.1 represents a *pectoralis major* muscle without myopathies. In *m. pectoralis major* without PMM most of the fibers are polygonal shape and the nuclei is located peripherally (Figure 3.4.4). Additionally, a few small groups of adipose tissue as well as accumulation of loose connective tissue were established in *m. pectoralis major* samples of Ross 308 broilers that macroscopically were evaluated as a control group. In addition to that, in some samples of control group degeneration and necrosis of myofibers were present (Figure 3.4.4). Inflammatory cells were infiltrated damage myofibers, also interstitial inflammation and inflammation of blood vessels in some cases were present.

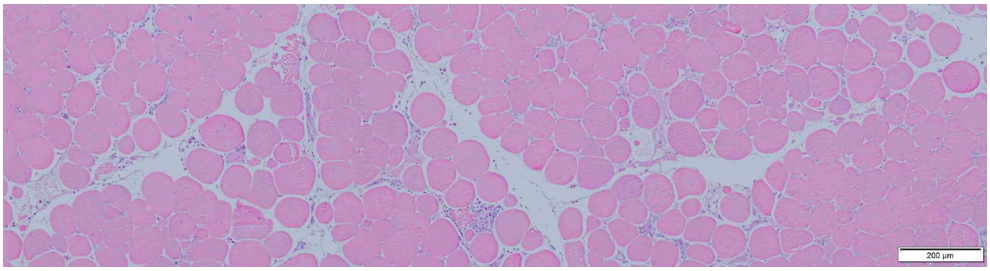

Figure 3.4.1. *Pectoralis major muscle without myopathies. Cross-section, H&E, 100× (three merged images)*

Figure 3.4.2 represents samples that were mildly affected by PMM. Expanded diameter of myofibers, fragmentation, vacuolization and hypereosinophilia showed degeneration and necrosis of myofibers (Figure 3.4.5; Figure 3.4.6). Damaged myofibers appeared infiltrated by mononuclear inflammatory cells, mainly macrophages and heterophils (Figure 3.4.7; Figure 3.4.8). Some myofibers undergoing phagocytosis. Interstitial lesions showed accumulation of fibrous connective tissue and in some cases fibrosis were present (Figure 3.4.12). Lymphocytes, macrophages and scattered heterophils in adipose and fibrous connective tissue were present (Figure 3.4.10; Figure 3.4.12). Fibrous connective tissue in some areas is associated with adipocytes that expanded the endomysium (Figure 3.4.13). Adipose tissue around blood vessels was present (Figure 3.4.11). Additionally, inflammation of blood vessels was also present. More pronounced perivenular accumulation of mononuclear leukocytes showed perivasculitis (Figure 3.4.14), usually around veins. Furthermore, sometimes lymphocytes obliterate the vascular wall, therefore, vasculitis was also present (Figure 3.4.15).

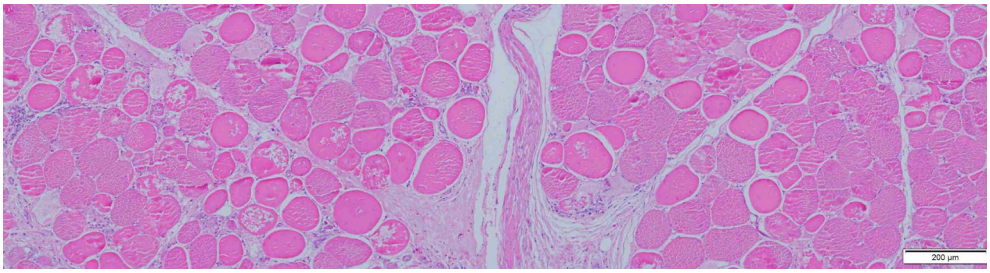

Figure 3.4.2. *Pectoralis major muscle mildly affected by myopathies. Cross-section, H&E, 100× (three merged images)*

Almost all myofibers of samples severely affected by PMM (as shown in Figure 3.4.3) undergo necrosis and degeneration (Figure 3.4.5; Figure

3.4.6). Atrophic myofibers were also present. Some of damaged myofibers are replaced by fibroadipose tissue. Fibrous connective tissue associated with adipocytes that expanded the endomysium was very pronounced. Inflammatory cells infiltration, such as macrophages and lymphocytes in fibroadipose tissue were present and showed interstitial inflammation (Figure 3.4.10; Figure 3.4.12). Additionally, vasculitis and perivasculitis were present (Figure 3.4.14; Figure 3.4.15).

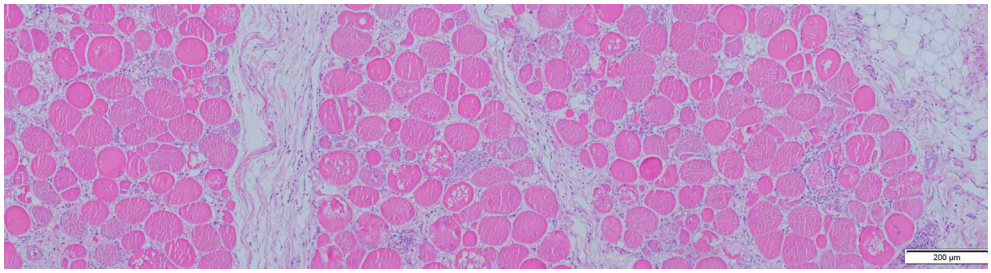

Figure 3.4.3. *Pectoralis major muscle severely affected by myopathies. Cross-section, H&E, 100× (three merged images).*

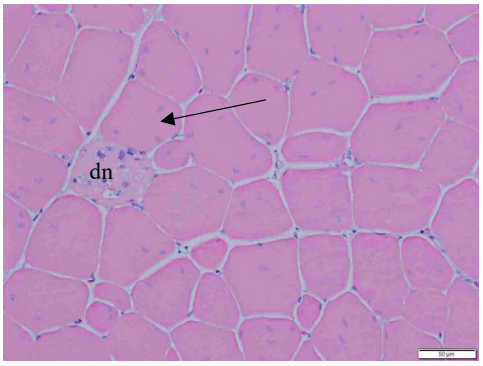

Figure 3.4.4. *Normal polygonal-shaped myofibers (arrow). Degenerative necrotic myofibers (dn) with loss of striation. Cross-section, H&E, 200×.*

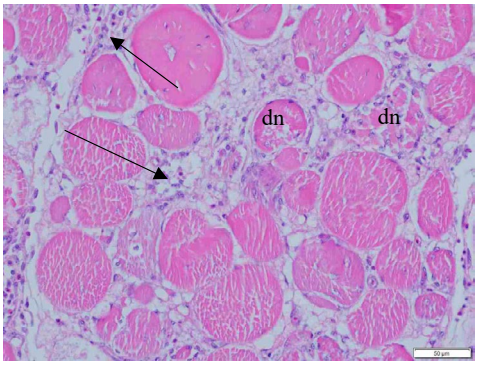

Figure 3.4.5. *Degenerative necrotic myofibers (dn) with loss of striation. Inflammatory cells infiltrate endomysium (arrows). Cross-section, H&E, 200×.*

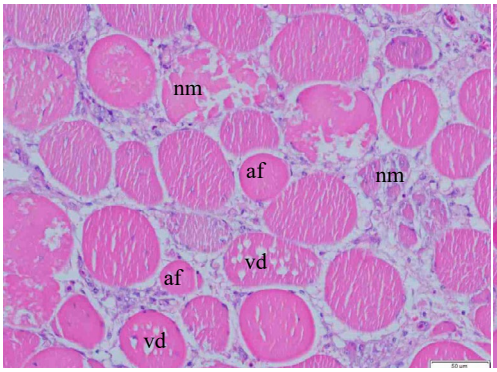

Figure 3.4.6. *Atrophic myofibers (af). Vacuolar degeneration of myofibers (vd). Necrotic myofibers (nm) with fragmented sarcoplasm, loss of striations. Cross-section, H&E, 200×.*

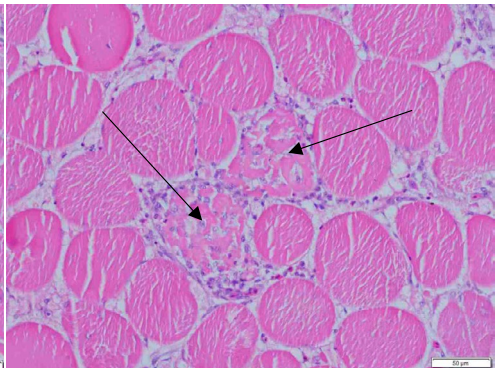

Figure 3.4.7. *Necrotic myofibers with fragmented sarcoplasm infiltrated by mononuclear inflammatory cells (arrows). Cross-section, H&E, 200×.*

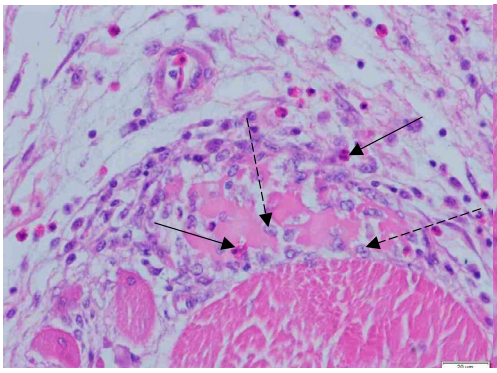

Figure 3.4.8. *Heterophils (arrows), and macrophages (lined arrows) infiltrate necrotic myofiber. Cross-section, H&E, 400×.*

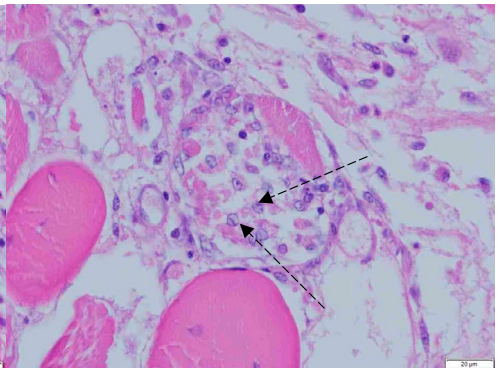

Figure 3.4.9. *Macrophages (lined arrows) infiltrate necrotic myofiber. Cross-section, H&E, 400×.*

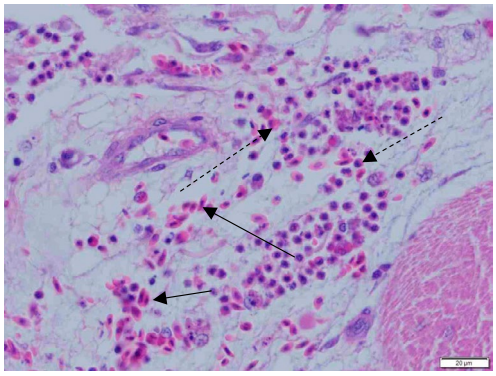

Figure 3.4.10. *Hemorrhages in the endomysium (arrows) and heterophils infiltration (lined arrows). Cross-section, H&E, 400×.*

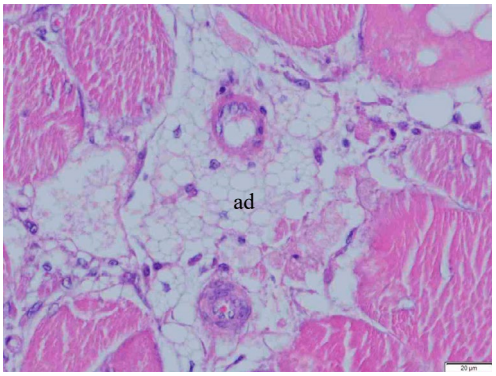

Figure 3.4.11. *Accumulation of adipose tissue (ad) around blood vessels. Cross-section, H&E, 400×.*

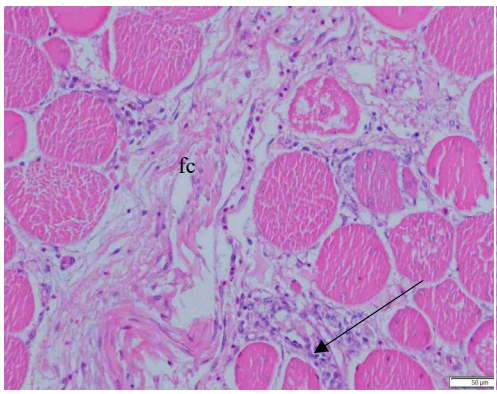

Figure 3.4.12. *Prominent expansion of fibrous connective tissue (fc) and inflammatory cells in the interstitial spaces (arrow). Cross-section, H&E, 200×.*

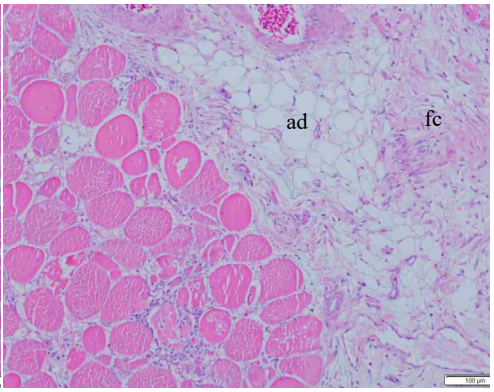

Figure 3.4.13. *Accumulation of adipose tissue (ad) and fibrous connective tissue (fc). Cross-section, H&E, 200×.*

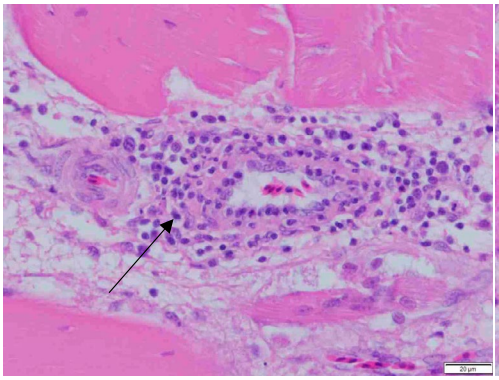

Figure 3.4.14. *Perivenular accumulation of lymphocytes (arrow) (perivasculitis).*
Cross-section, H&E, 400×.

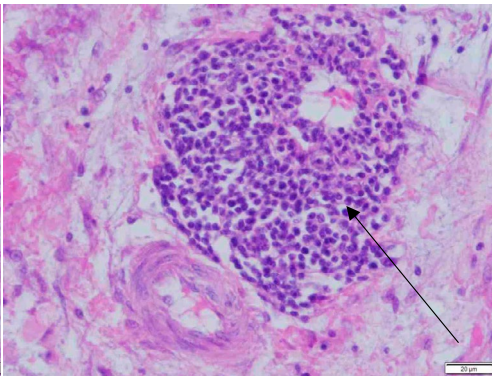

Figure 3.4.15*. Lymphocytes (arrow) with intramural infiltration sometimes obliterate the vascular wall (vasculitis).*
Cross-section, H&E, 400×.

Furthermore, as seen in Table 3.4.1, the analysis of the histopathological score revealed that as the degree of PMM increased, there was a significant increase in the degeneration/necrosis ($p < 0.001$). Furthermore, fibrous connective tissue accumulation, inflammation and inflammation of the blood vessels score were significantly increased in *m. pectoralis major* affected by severe and mild compared to that of without myopathies, normal ($p < 0.05$). Additionally, the score of adipose tissue accumulation was significantly increased in severe cases compared to that of *m. pectoralis major* affected by mild and without myopathies ($p < 0.001$). However, results of adipose tissue infiltration did not differ significantly between normal and mild cases ($p > 0.05$).

Table 3.4.1. *Histopathological scores of degeneration/necrosis, accumulation of fibrous connective tissue and adipose tissue, inflammation of the muscle, and inflammation of the blood vessels according to severity of PMM (pectoralis major myopathies) (mean ± SD)*

Pathologies of *pectoralis major* muscle	**Severity of myopathies**		
	Normal [a] (n = 20)	**Mild [b] (n = 20)**	**Severe [c] (n = 20)**
Degeneration/necrosis (on a scale from 0 to 4 points)	$1.75 \pm 0.64^{***b,c}$	$2.90 \pm 0.64^{***a,c}$	$4.00 \pm 0.00^{***a,b}$
Accumulation of fibrous connective tissue (on a scale from 0 to 3 points)	$0.35 \pm 0.59^{***b,c}$	$1.30 \pm 1.13^{***a,c}$	$2.80 \pm 0.52^{***a,b}$
Accumulation of adipose tissue (on a scale from 0 to 3 points)	$1.60 \pm 0.68^{***c}$	$2.05 \pm 0.89^{***c}$	$2.85 \pm 0.37^{***a,b}$
Inflammation of the muscle (on a scale from 0 to 3 points)	$1.25 \pm 0.64^{***b,c}$	$2.05 \pm 0.76^{***a,c}$	$2.90 \pm 0.45^{***a,b}$
Inflammation of the blood vessels (on a scale from 0 to 2 points)	$0.40 \pm 0.68^{*b,\ ***c}$	$0.95 \pm 0.83^{*a,\ ***c}$	$1.80 \pm 0.62^{***a,b}$

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

As seen in Figures 3.4.16, 3.4.17 and 3.4.18, the severity of all analyzed pathologies progressively increased from normal to severely affected muscle samples. Figure 3.4.16 shows that in normal samples of muscle the most cases of degeneration/necrosis were scored by 1–2 points. In mildly affected muscle the majority of samples scored by 2–3 points. In severely affected group all of the samples displayed degeneration and necrosis and were scored with the maximum of 4 points.

Figure 3.4.16. *Score distribution of degeneration/necrosis in pectoralis major muscle categorized as normal, mildly affected and severely affected by myopathies*

According to Figure 3.4.17, the accumulation of fibrous connective and adipose tissue was uncommon and scores by 1–2 points in normal group. In mildly affected muscle, adipose tissue accumulation were prominent, majority of samples scored by 2–3 points. Additionally, the accumulation of fibrous connective tissue and inflammation of the muscle were more evident in mildly affected samples, spread across 1–3 points. In severely affected muscle samples, fibrosis and adipose tissue infiltration were extensive, with most samples scored with 3 points. Furthermore, inflammation of the muscle was widespread, with almost all samples showing severe inflammatory changes, 3 points.

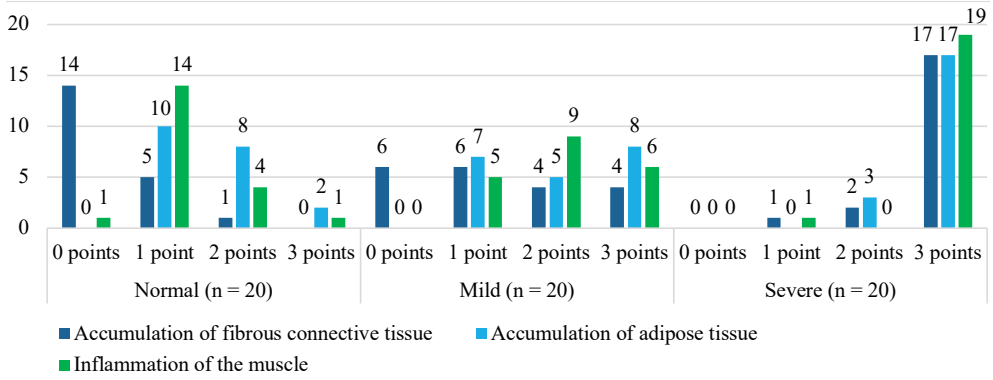

Figure 3.4.17. *Score distribution of fibrous connective tissue and adipose tissue accumulation, and inflammation of the muscle in normal pectoralis major and mildly and severely affected by myopathies*

Figure 3.4.18 shows that in the normal group, the inflammation of blood vessels was rare, but as severity increased the prevalence of vasculitis also rise. Perivasculitis was a common feature in the mild group. Additionally, vasculitis dominated in severely affected muscles.

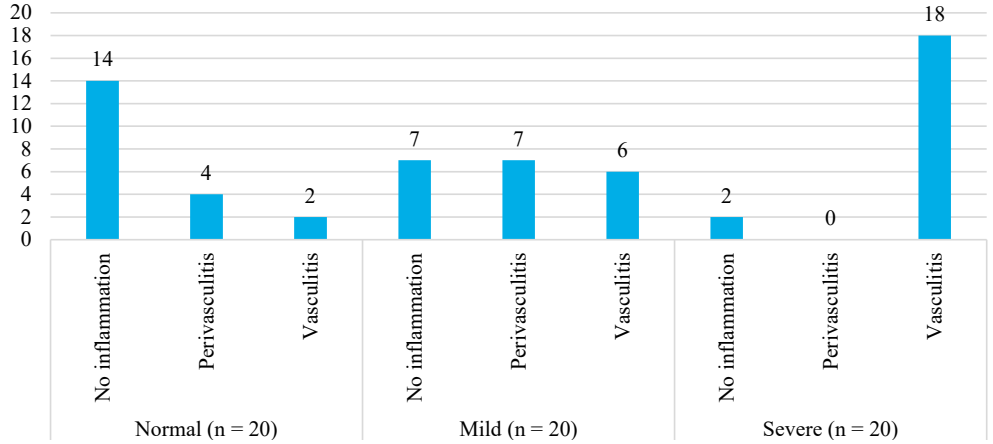

Figure 3.4.18. *Score distribution of inflammation of the blood vessels in pectoralis major muscle categorized as normal, mildly and severely affected by myopathies*

According to Table 3.4.2, Spearman's correlation test showed a significant positive correlation between severity of PMM and degeneration/necrosis of the muscle (r_s = 0.890), accumulation of fibrous connective tissue (r_s = 0.782), and inflammation of the muscle (r_s = 0.748) ($p < 0.001$).

Additionally, significant positive correlation was established between severity of PMM and accumulation of adipose tissue ($r_s = 0.617$, $p < 0.001$) and inflammation of the blood vessels ($r_s = 0.637$, $p < 0.001$).

Also, positive correlation was established between accumulation of adipose tissue and inflammation of the muscle ($r_s = 0.316$, $p < 0.05$), and other variables (degeneration/necrosis, accumulation of fibrous connective and adipose tissue, inflammation of the muscle, and inflammation of the blood vessels) were positively correlated ($p < 0.001$).

Table 3.4.2. *Spearman's correlation coefficients between different variables of pectoralis major muscle samples*

Variables	Severity of PMM	Degeneration/ necrosis	Accumulation of fibrous connective tissue	Accumulation of adipose tissue	Inflammation of the muscle	Inflammation of the blood vessels
Severity of PMM	-					
Degeneration/ necrosis	0.890***	-				
Accumulation of fibrous connective tissue	0.782***	0.809***	-			
Accumulation of adipose tissue	0.617***	0.556***	0.599***	-		
Inflammation of the muscle	0.748***	0.729***	0.711***	0.316*	-	
Inflammation of the blood vessels	0.637***	0.654***	0.573***	0.488***	0.673***	-

Correlation is significant. $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. PMM – *pectoralis major* myopathies.

Anterior latissimus dorsi muscle affected by dorsal cranial myopathy

ALD muscle of broilers was evaluated histologically. ALD muscle myofibers without DCM (as shown in Figure 3.4.19) exhibited a typical polygonal shape of the myofibers and peripherally-located nuclei. However, even in macroscopically normal ALD muscle samples, microscopic lesions were observed. Smaller and larger groups of adipose tissue infiltrated ALD muscle. Furthermore, in samples without DCM degeneration and necrosis of myofibers were evident (Figure 3.4.21), along with regenerating myofibers. Moreover, in those samples without DCM, inflammatory cells were infiltrated damage myofibers and interstitial inflammation were present (Figure 3.4.22).

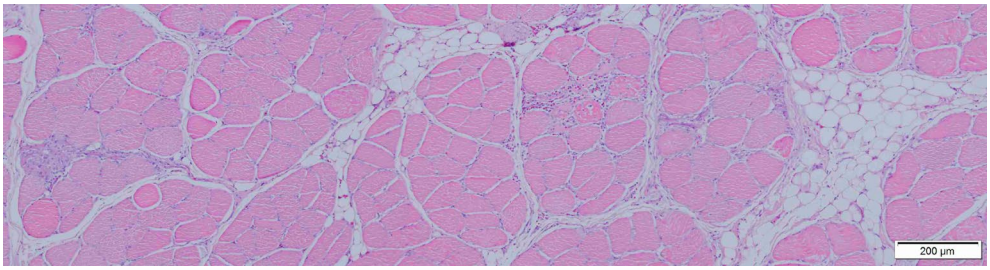

Figure 3.4.19. *Anterior latissimus dorsi (ALD) muscle without dorsal cranial myopathy (DCM). Cross-section, H&E, 100× (three merged images)*

Histological examination of ALD affected by DCM showed very pronounced accumulation of fibrous connective tissue, fibrosis was present, as seen in Figure 3.4.20. The connective tissue accumulation was heterogeneous, with regions of both immature and edematous fibrous connective tissue and areas of mature collagenized connective tissue. At the surface of the affected muscle granulation tissue, fibrin and hemorrhages are present, also variable number of lymphocytes and macrophages (Figure 3.4.24). Furthermore, ALD lesions displayed. These degenerated, necrotic and atrophic myofibers (Figure 3.4.23) were often surrounded by inflammatory cells, indicating inflammatory response (Figure 3.4.21; Figure 3.4.22). The presence of interstitial inflammation was also present. Moreover, muscle tissue was decreased microscopically in affected muscle, hypercontracted, hypereosinophilic myofibers were observed within the lesions. In addition, vasculitis and perivasculitis were also present (Figure 3.4.25; Figure 3.4.26)

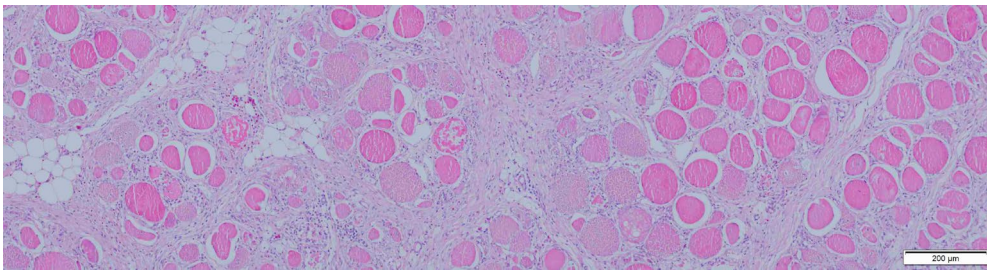

Figure 3.4.20. *Anterior latissimus dorsi (ALD) muscle affected by dorsal cranial myopathy (DCM). Cross-section, H&E, 100× (three merged images)*

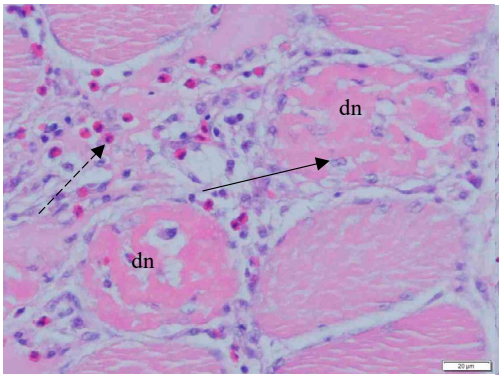

Figure 3.4.21. *Degenerative necrotic myofibers (dn) with loss of striation. Infiltrated by macrophages (arrow) and heterophils (lined arrow). Cross-section, H&E, 400×.*

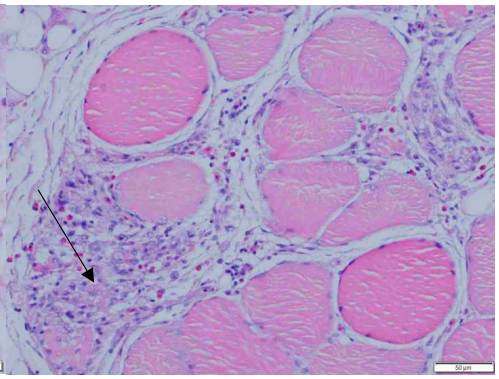

Figure 3.4.22. *Accumulation of mononuclear inflammatory cells in the epimysium (arrow). Cross-section, H&E, 200×.*

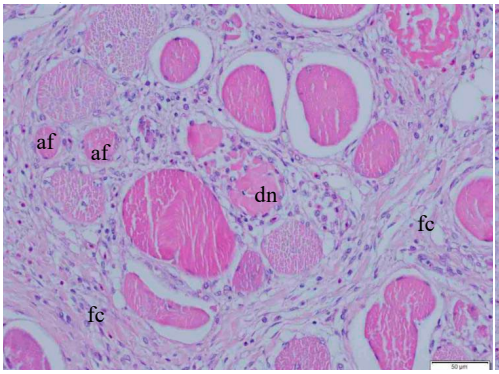

Figure 3.4.23. *Degenerative necrotic myofibers (dn) infiltrated by inflammatory cells. Atrophic myofibers (af) surrounded by inflammatory cells. Prominent expansion of fibrous connective tissue (fc). Cross-section, H&E, 200×.*

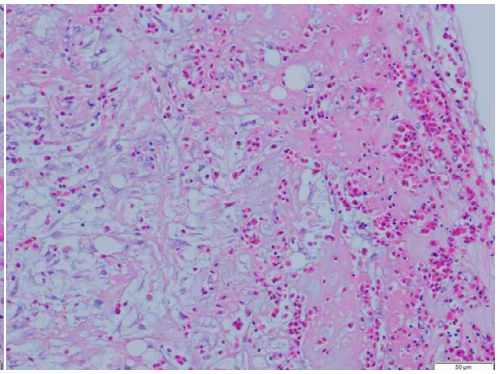

Figure 3.4.24. *Granulation tissue, fibrin, hemorrhages, variable amount of lymphocytes and macrophages at the surface of the affected muscle. Cross-section, H&E, 200×.*

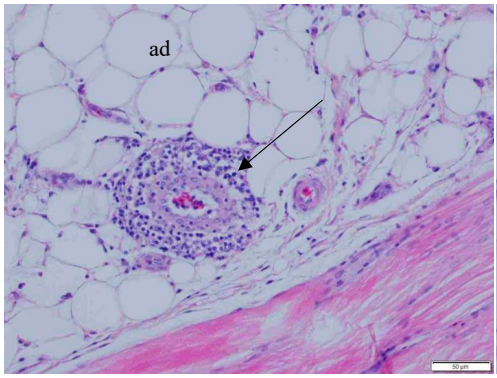

Figure 3.4.25. *Perivenular accumulation of lymphocytes (perivasculitis) (arrow) and accumulation of adipose tissue (ad). Cross-section, H&E, 200×.*

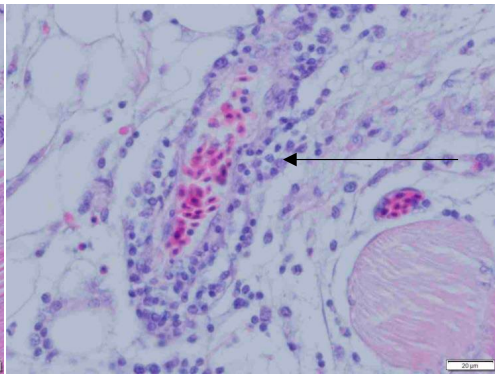

Figure 3.4.26. *Lymphocytes (arrow) with intramural infiltration obliterate the vascular wall (vasculitis). Cross-section, H&E, 400×.*

According to our research results, as seen in Table 3.4.3, the analysis of histopathological score revealed that degeneration/necrosis, accumulation of fibrous connective tissue and inflammation of the muscle were significantly increased in DCM group compared to control group ($p < 0.001$). However, adipose tissue content and inflammation of the blood vessels did not differ statistically significantly between those two groups ($p > 0.05$).

Table 3.4.3. *The histopathological scores of degeneration/necrosis, accumulation of fibrous connective tissue and adipose tissue, inflammation of the muscle and inflammation of the blood vessels according to status of dorsal cranial myopathy (DCM) (mean ± SD)*

Pathologies of *anterior latissimus dorsi* muscle	**Status of dorsal cranial myopathy**		**p value**
	Control group (n = 25)	**DCM group (n = 25)**	
Degeneration/necrosis (on a scale from 0 to 4 points)	1.48 ± 0.59	3.92 ± 0.28	< 0.001
Accumulation of fibrous connective tissue (on a scale from 0 to 3 points)	0.04 ± 0.20	2.68 ± 0.69	< 0.001
Accumulation of adipose tissue (on a scale from 0 to 3 points)	1.96 ± 0.79	2.12 ± 0.60	0.424
Inflammation of the muscle (on a scale from 0 to 3 points)	1.84 ± 0.85	2.96 ± 0.20	< 0.001
Inflammation of the blood vessels (on a scale from 0 to 2 points)	0.72 ± 0.74	0.96 ± 0.74	0.255

As seen in Figure 3.4.27, degeneration and necrosis were a common feature that affected all of the samples of the control group, but the severity of it was evaluated with most of changes scoring 1 or 2 points, indicating low to moderate ALD muscle damage. However, in the DCM group degeneration and necrosis were present and scored 4 points in the majority of DCM samples (23 out of 25), indicating severe muscle damage.

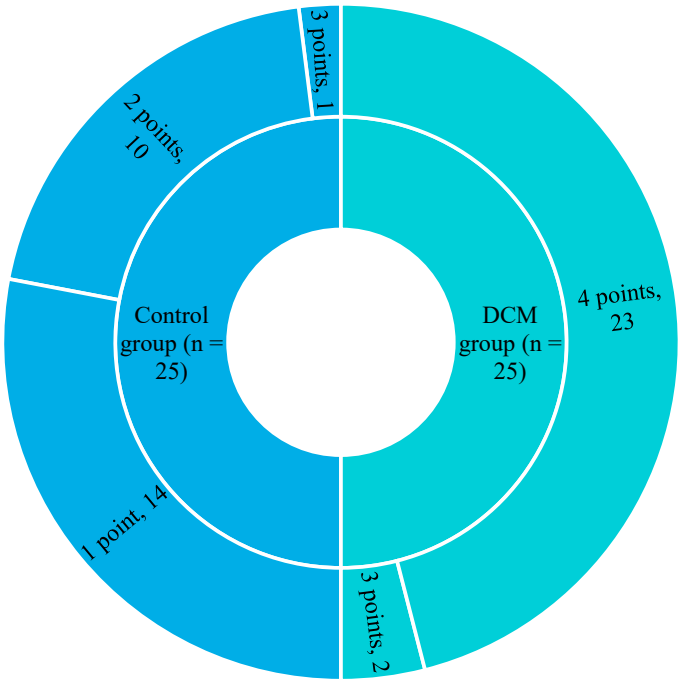

Figure 3.4.27. *Scores distribution of degeneration/necrosis in control group (without DCM) and DCM group samples*

As seen in Figure 3.4.28, in control group the most common pathologies were adipose tissue infiltration and inflammation of the muscle with most of changes scoring 1 or 2 points, indicating low to moderate ALD muscle damage, while fibrosis was absent in most of the cases (24 out of 25). In contrast to that, all pathologies in the DCM group were evaluated by higher scores and showed significant tissue damage. 3-point category was dominant in aspects, such as fibrosis and inflammation of the muscle. However, the histological analysis showed that adipose tissue accumulation was mostly evaluated by 2–3 points in control and DCM group and this pathological feature is common in both groups. Additionally, inflammation of the blood vessels was evaluated very commonly with 1 point, so perivasculitis was a common pathology in control and DCM groups.

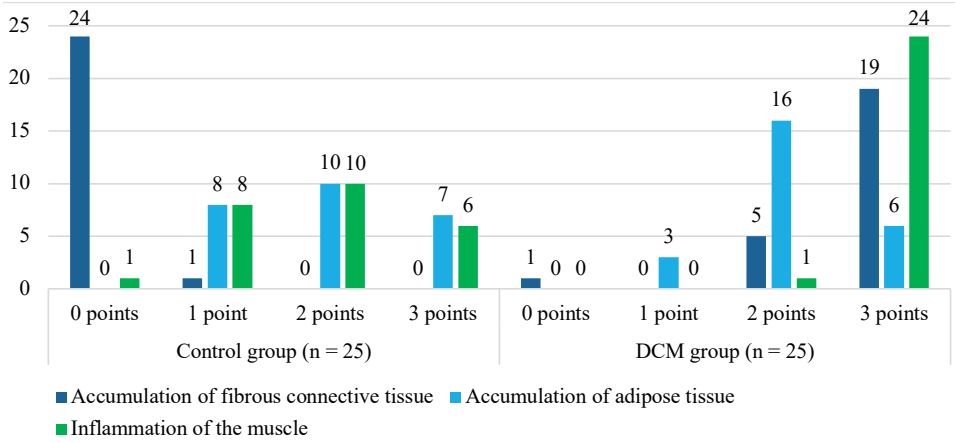

Figure 3.4.28. *Distribution of scores for accumulation of fibrous connective and adipose tissue, and inflammation of the muscle in control group (without DCM) and DCM group*

As seen in Figure 3.4.29, perivasculitis was a common pathological feature in both control group (10 out of 25) and DCM group (12 out of 25). Vasculitis was also present in both groups, but it was less common than perivasculitis, with 4 samples in the control group and 6 in the group showing DCM.

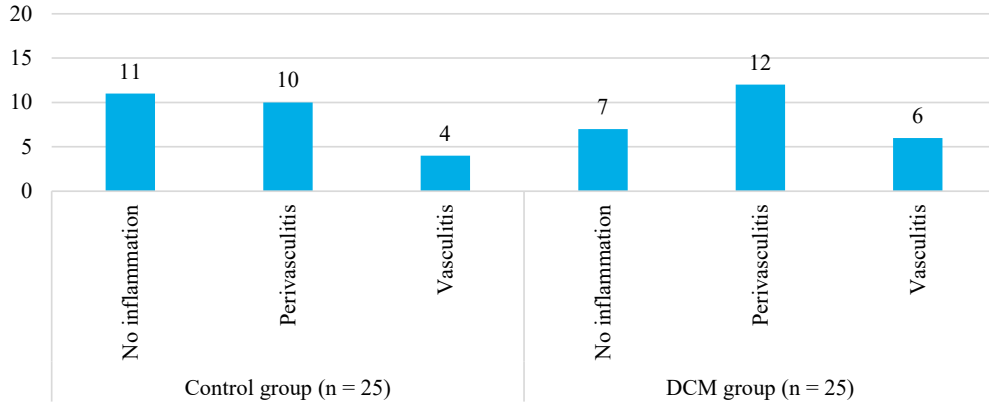

Figure 3.4.29. *Distribution of inflammation of the blood vessels by score in control group (without DCM) and DCM group*

According to Table 3.4.4, Spearman's correlation test showed a significant positive correlation between the status of DCM and degeneration/necrosis of muscle tissue ($r_s = 0.924$, $p < 0.001$), accumulation of fibrous connective tissue ($r_s = 0.917$, $p < 0.001$) and inflammation of the muscle ($r_s = 0.725$, $p < 0.001$).

Additionally, positive correlation was determined between degeneration/ necrosis and accumulation of fibrous connective tissue ($r_s = 0.882$, $p < 0.001$), and inflammation of the muscle ($r_s = 0.821$, $p < 0.001$). Additionally, a positive correlation was determined between accumulation of fibrous connective tissue and inflammation of the muscle ($r_s = 0.756$, $p < 0.001$). A positive correlation was established between inflammation of the muscle and inflammation of the blood vessels ($r_s = 0.391$, $p < 0.01$).

However, not statistically significant correlation was established between the status of DCM and accumulation of adipose tissue ($r_s = 0.111$), and inflammation of the blood vessels ($r_s = 0.167$) ($p > 0.05$). No significant association between other valuables was observed ($p > 0.05$).

Table 3.4.4. *Spearman's correlation coefficients between different variables of the samples of anterior latissimus dorsi (ALD) muscle*

Variables	**Status of dorsal cranial myopathy**	**Degeneration/ necrosis**	**Accumulation of fibrous connective tissue**	**Accumulation of adipose tissue**	**Inflammation of the muscle**	**Inflammation of the blood vessels**
Status of dorsal cranial myopathy	-					
Degeneration/ necrosis	0.924***	-				
Accumulation of fibrous connective tissue	0.917***	0.882***	-			
Accumulation of adipose tissue	0.111	0.239	0.036	-		
Inflammation of the muscle	0.725***	0.821***	0.756***	0.085	-	
Inflammation of the blood vessels	0.167	0.262	0.193	–0.025	0.391**	-

Correlation is significant. $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

3.5. Histomorphometric analysis of broiler muscles affected by myopathies

***Pectoralis major* muscle affected by myopathies**

Broiler breasts affected by severe myopathies had 15.2% smaller diameter of myofibers compared to that of mildly affected ($p < 0.001$) and 9.9% smaller compared to that of normal fillets ($p < 0.05$), as seen in Figure 3.5.1. Furthermore, the largest average myofibers diameter was established in mild myopathies affected *m. pectoralis major* (Figure 3.5.1); however, there was no statistically significant difference compared that result to normal not affected muscles ($p > 0.05$). Additionally, the mean of cross-sectional area of myofibers was smaller in severely by PMM affected muscles compared to mildly affected *pectoralis major* muscle ($p < 0.001$). There was no statistically significant difference in the cross-sectional area of myofibers between normal and mildly affected muscles, as well as normal and severely affected muscles ($p > 0.05$) (Table 3.5.1).

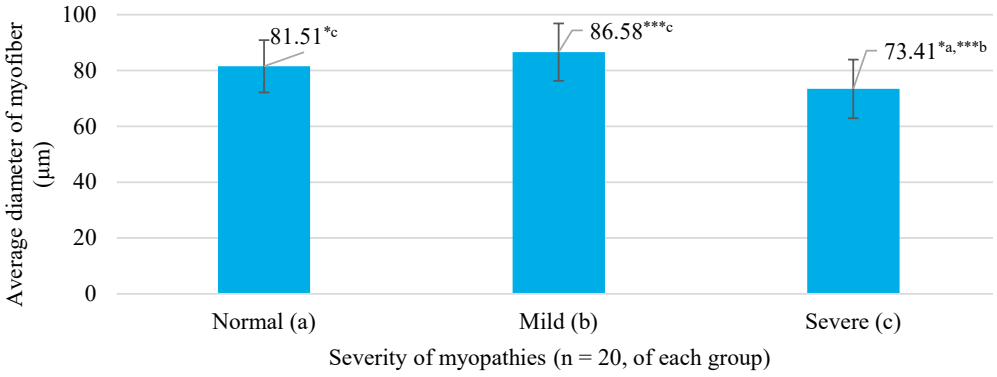

Figure 3.5.1. *Means and standard deviations of the average myofiber diameter (µm) according to severity of pectoralis major myopathies (PMM)*

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Table 3.5.1. *Cross-sectional area of broilers the pectoralis major myofibers affected by different severity of myopathies (mean ± SD)*

Cross-sectional area of the myofibers (µm²)	**Severity of myopathies**		
	Normal [a] (n = 20)	**Mild [b] (n = 20)**	**Severe [c] (n = 20)**
	5281.25 ± 1224.16	5962.81 ± 1433.56***c	4312.69 ± 1242.62***b

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Table 3.5.2 shows that in *pectoralis major* affected by severe myopathies muscle tissue was decreased by 10.5% compared to mildly affected and by 12.8% compared to normal fillets, without myopathies ($p < 0.001$). Moreover, the muscle samples that were collected from broilers muscle affected by severe myopathies have had 68.6% higher fibrous connective tissue compared to mildly affected muscle ($p < 0.01$) and 96.9% higher compared to normal muscle, without myopathies ($p < 0.001$). Adipose tissue was also 1.7-fold higher in severely affected muscle compared to mildly affected ($p < 0.05$) and 2.3-fold higher compared to that of samples collected from normal, without myopathies muscle ($p < 0.001$). However, according to our research results, if we compare the amounts of fibrous connective tissue, adipose tissue and muscle tissue in mildly affected and normal cases do not differ statistically significantly ($p > 0.05$).

Table 3.5.2. *Amounts of muscle tissue, fibrous connective tissue and adipose tissue (percentage of the evaluated area) in pectoralis major muscle of broilers affected by different severity of myopathies (mean ± SD)*

Tissues of the *pectoralis major* muscle	**Severity of myopathies**		
	Normal [a] (n = 20)	**Mild [b] (n = 20)**	**Severe [c] (n = 20)**
Muscle tissue	$89.06 \pm 3.75^{***c}$	$86.82 \pm 8.11^{***c}$	$77.68 \pm 8.43^{***a, b}$
Fibrous connective tissue	$8.64 \pm 4.30^{***c}$	$10.09 \pm 7.08^{**c}$	$17.01 \pm 7.09^{***a, **b}$
Adipose tissue	$2.30 \pm 1.91^{***c}$	$3.09 \pm 1.99^{*c}$	$5.31 \pm 3.31^{***a, *b}$

Superscripts [a, b, c] assigned to each group of myopathies severity indicate comparison group when statistically significant mean differences were found ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

Figure 3.5.2 illustrates a normal *pectoralis major* muscle, while Figure 3.5.3 and Figure 3.5.4 show *pectoralis major* muscle mildly and severely affected by myopathies. These images represent the reduction of muscle tissue, decreased diameter of myofibers and the increase of fibrous connective tissue and adipose tissue in severely PMM affected muscle compared to mildly affected and normal *pectoralis major* muscle. Images provide a comparative visualization of tissue composition changes in *pectoralis major* muscle.

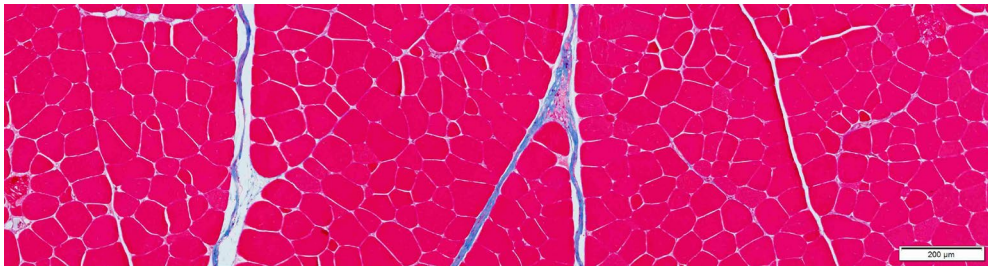

Figure 3.5.2. *Pectoralis major muscle without myopathies. Cross-section, Masson Trichrome, 100× (three merged images)*

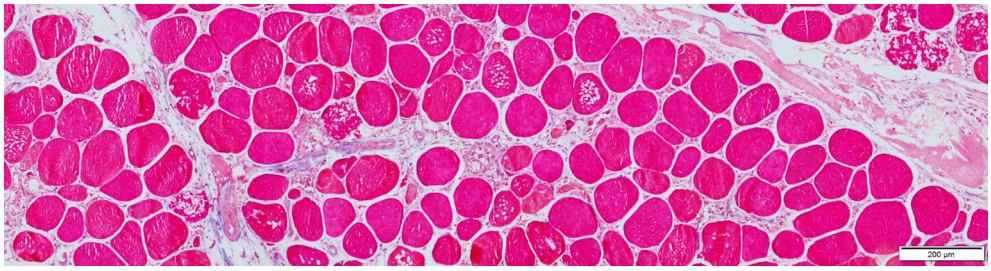

Figure 3.5.3. *Pectoralis major muscle mildly affected by myopathies. Cross-section, Masson Trichrome, 100× (three merged images)*

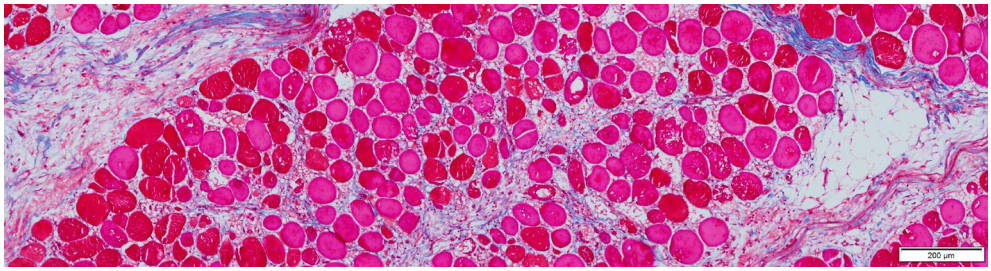

Figure 3.5.4. *Pectoralis major muscle severely affected by myopathies. Cross-section, Masson Trichrome, 100× (three merged images)*

***Anterior latissimus dorsi* muscle affected by dorsal cranial myopathy**

According to Figure 3.5.5, samples of broilers ALD affected by DCM had 10.47% increased average diameter of myofiber compared to control group of samples which were normal, not affected by DCM ($p < 0.01$). Additionally, mean of cross-sectional area of myofibers was larger in DCM group compared to control group of samples ($p < 0.01$) (Table 3.5.3).

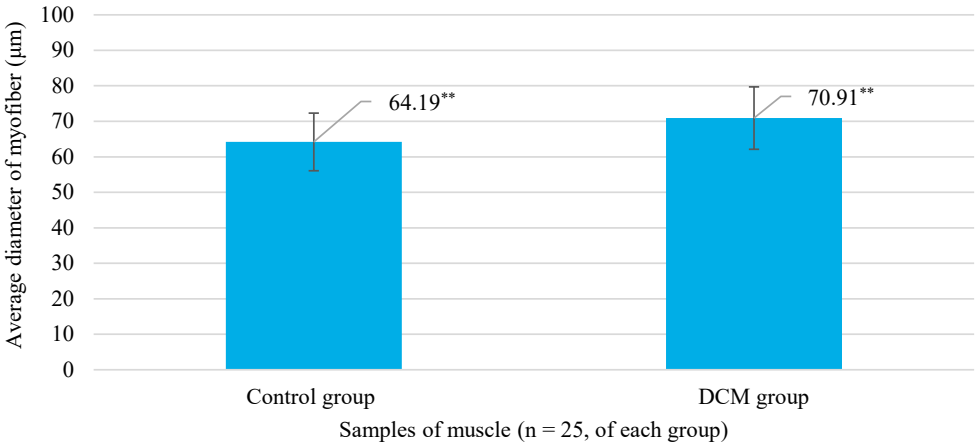

Figure 3.5.5. *Means and standard deviations of the average myofiber diameter (µm) of control group (without DCM) and affected by dorsal cranial myopathy (DCM)*

Table 3.5.3. *Cross-sectional area of broilers anterior latissimus dorsi (ALD) myofibers of control group of broilers and affected by dorsal cranial myopathy (DCM group) (mean ± SD)*

Cross-sectional area of the myofibers (µm²)	**Status of dorsal cranial myopathy**		**p value**
	Control group (n = 25)	**DCM group (n = 25)**	
	3284.56 ± 850.16	4004.98 ± 982.93	0.008

Table 3.5.4 shows that in ALD muscle affected by DCM, muscle tissue was decreased by 27.53% compared to normal samples of muscle ($p < 0.001$). Moreover, the muscle samples that were collected from broilers muscle affected by DCM have had 2.1-fold higher fibrous connective tissue compared to control group of samples ($p < 0.001$). However, according to histomorphometry research results, the amounts of adipose tissue in affected and normal cases of control group did not differ statistically significantly ($p > 0.05$).

Table 3.5.4. *Muscle tissue, fibrous connective tissue and adipose tissue (percentage of the evaluated area) amounts in anterior latissimus dorsi (ALD) muscle of control group of broilers and affected by dorsal cranial myopathy (DCM group) (mean ± SD)*

Tissues of the *m. anterior latissimus dorsi*	**Status of dorsal cranial myopathy**		**p value**
	Control group (n = 25)	**DCM group (n = 25)**	
Muscle tissue	70.97 ± 5.91	51.43 ± 10.80	< 0.001
Fibrous connective tissue	15.87 ± 4.81	33.20 ± 8.90	< 0.001
Adipose tissue	13.16 ± 4.62	15.37 ± 5.69	0.139

Figure 3.5.6 illustrates a normal ALD muscle, without DCM, while Figure 3.5.7 shows ALD muscle affected by DCM. These images represent the reduction of muscle tissue and the increase of fibrous connective tissue, and the enlargement of myofiber diameter in DCM affected muscle, providing a comparative visualization of tissue composition changes in ALD muscle.

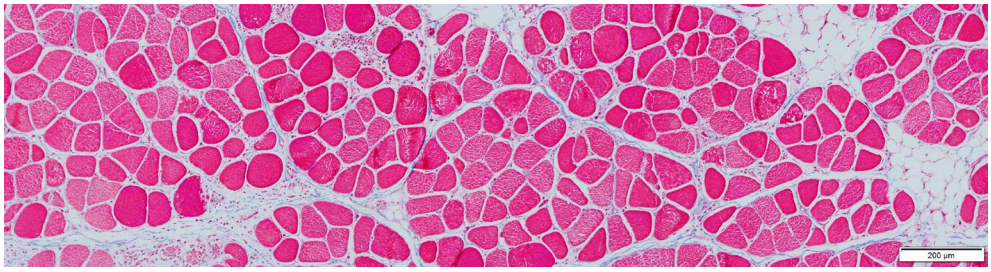

Figure 3.5.6. *Anterior latissimus dorsi (ALD) muscle without dorsal cranial myopathy (DCM). Cross-section, Masson Trichrome, 100× (three merged images)*

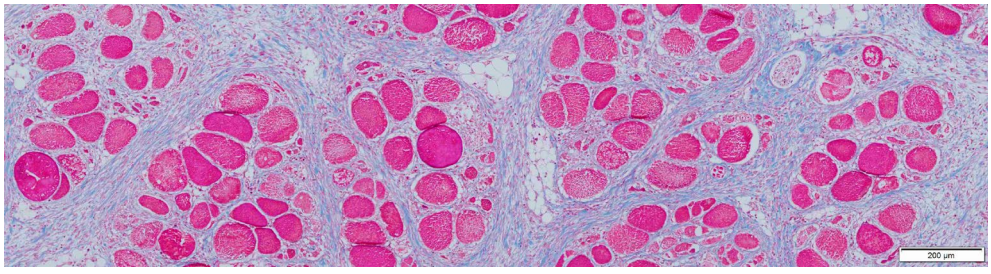

Figure 3.5.7. *Anterior latissimus dorsi (ALD) muscle affected by dorsal cranial myopathy (DCM). Cross-section, Masson Trichrome, 100× (three merged images)*

4. DISSCUSION

4.1. The incidence and risk factors of broiler myopathies in Lithuania

Myopathies are established all around the world, especially in regions where fast-growing, heavy weight broilers are used [12,54]. In Europe, including Turkey, the incidence of WS and WB separately varies significantly from 5.1% to 78.4% depending on the country and specific conditions. Notably, WS tends to be more prevalent than WB, with WS incidence starting at 12%, while WB starts at 5.1% [55–61]. It is important to notice that comprehensive statistics on the incidence of these myopathies are still lacking in many Europe countries, including some of the larger ones, likewise Spain, Norway, Sweden, Poland. In the North America the incidence rate of myopathies is alarming high. It is ranging from to 82.4% to 96.1% [62–64]. Conversely, in the South America the incidence rates are comparatively lower from 11.2% to 50.29% [54,65]. Interestingly, generally, in countries of South America, WB is more commonly established than WS. Asia also reports high incidence rates of WB and WS myopathies, ranging from 6.6% to 97.8%. The highest rate of WS among published results in Asia is 97.8% (Thailand) [66], meaning that almost every fillet is affected by myopathy, similar to the situation in North America. Overall, the highest rates of WB and WS are reported in Canada, USA and Thailand, highlighting these areas as critical hotspots for broiler myopathies. In contrast to these global incidence rates of WB and WS myopathies, according to our study results the percentage of broiler myopathies were not that as high as in other countries. According to our research results, 18.19% of broilers were affected by PMM. This lower incidence may be attributed to several factors. Possible explanations could include differences in the broiler strains commonly used in different regions or overall production intensity, environmental conditions and management practices. Nevertheless, this variation may also be attributed to differences in the macroscopic examination of myopathies and the absence of a globally accepted classification.

DCM is not as prevalent as PMM. Our study revealed that the incidence of DCM in Lithuania is 5.16%. The incidence rate of this myopathy is up to 7.35% in Brazil [19,51,52]. Our research results relate with these studies. However, it is hard to do comparisons, because the management strategies and environmental factors are different between Europe and South America continents. Additionally, in the scientific literature, there is no information about the incidence rate of DCM in other countries of the world.

Furthermore, our research revealed that the incidence of DPM in Lithuania was also at very low rates and was 0.27%. These findings are consistent

with other studies, which reported that prevalence of DPM varies from 0.02% to 17% in different continents, including Europe and North America [38,44,45,47]. The DPM is much less common compared to the incidence rates of WB and WS myopathies.

According to the literature as well as Lithuania slaughterhouse management insights, the knowledge of etiopathogenesis allows for more effective prevention strategies on poultry farms of DPM, therefore, the incidence rates are significantly lower. Among the main risk factors that have influence in DPM formation is wing flapping. Therefore, management practices trying to reduce bird activity [37,42]. Furthermore, it was established that much less incidence of DPM is in lighter birds, therefore chickens are growing not exceeding 3.6 kg weight [36,46]. However, our study results show that there were no statistically significant associations between the percentage of DPM and slaughter age or broiler BW at slaughter. This result could be explained that broilers in our study were lighter and younger. In our study broilers BW did not exceed 2.9 kg.

DCM is another pathology closely linked to the anatomical structure of broiler muscles, particularly the size of the *pectoralis major* and overall BW of broilers [19]. Our study indicates that heavier broilers exhibit greater susceptibility to DCM. This finding is in agreement with other studies, which have also observed that the heaviest male broilers with large pectoral muscles are more prone to developing DCM [19]. Moreover, our study revealed that the incidence of DCM in flocks was negatively related with seasons. This finding is in agreement with Prado et al. (2021), who state that the environment temperature could have an impact on the etiopathogenesis of DCM [177].

Furthermore, we have analyzed the potential effects on treatment, including antibiotics and coccidiostats, on the incidence rate of DCM in broiler flocks. However, the administration of antibiotics and coccidiostats appears to have no significant effect on the etiopathogenesis of DCM. Additionally, according to our study, the slaughter age did not have significant impact on the etiopathogenesis of DCM.

WS and WB myopathies nowadays get the most attention from research and poultry specialists among all myopathies of broilers. Despite the unknown etiology, researchers have found that the heaviest, male broiler chickens with the highest growth rate, feed efficiency and high breast meat yield are more prone to having pectoral muscle myopathies [68,72]. Our study results are in agreement with previous studies [12,17,57,69,178], the percentage of PMM was statistically significantly associated with heaviest and oldest chicken flocks. Furthermore, we have found the that incidence of PMM were negatively related with the treatment of broilers. In our study broilers were treated by antibiotics and coccidiostats and we hypothesized that altered balance of the

gut microbiota may be associated to development of PMM. This indication is in agreement with Zhang et al. (2021) research results. It was established that the caecum of WB-affected birds was decreased abundance of beneficial bacteria including *B. pullicacorum* and *L. hamster*, and a decline in microbial diversity [169].

The lowest incidence scores of DPM have shown that through effective management practices, poultry specialists have learned to coordinate the control of DPM and minimize its incidence. However, myopathies such as WB, WS and DCM are still prevalent in broiler chickens, with incidence rates varying significantly across different regions. Key risk factors include rapid growth, heavy body weight and large pectoral muscle mass, often associated with modern broiler genetics and intensive farming practices.

However, the etiology of WB, WS and DPM is more complex and systemic than that of DPM, making it more challenging to determine.

Bacteria, toxins, viruses, protozoa, and parasites are potential biological hazards. Bacteria consist about 90% of all foodborne illness [179,180]. Microbiological analysis of broilers subcutaneous tissue of myopathies affected area revealed that *Enterococcus viikkinesis, Escherichia coli* and *Hafnia alvei* were isolated from all groups of broilers with and without PMM and DCM. *Enterococcus viikkinesis* was isolated from all control samples without PMM, while *Hafnia alvei* was isolated from all *pectoralis major* muscle subcutaneous tissue affected by PMM. From all control group of subcutaneous tissue of ALD muscle was isolated *Escherichia coli,* while from DCM group *Enterococcus viikkinesis, Escherichia coli* and *Hafnia alvei* were isolated.

According to the literature, the most common microorganisms associated with contaminated chicken are *Campylobacter* and *Salmonella* [181]. *Enterococci* commonly inhabit the gastrointestinal tract of humans and animals. They are also frequently found in various environmental and animal sources [182,183]. Similarly, *Hafnia alvei* is also a normal component of the human and animal intestinal microbiota [183]. However, information about bacteria *Hafnia alvei* in veterinary medicine literature is quite limited, therefore, it is hard to do a conclusion about this result [183]. Furthermore, another investigated bacteria was *Escherichia coli* is also a normal inhabitant of the chicken's intestinal microbiota. However, it can be present in chicks due to of vertical transmission from the parent stock or as a result of contamination from the hatchery, transport or farm environment [184]. All established bacteria could be due to the content of intestines, because intestines were removed and carcasses of broilers could contaminate with feces of broilers. However, the limitations of this study were that we did not establish the pathogenicity profiles of *E. coli*. Our research result suggests

that subcutaneous tissue of broilers of affected area is complex microbiological environment. According to our research findings carcasses of broilers were free of any significant contamination of pathogenic microorganisms. Furthermore, all established bacteria belong to non-pathogenic environmental microbiota and could be as a normal part of digestive tract [182,183].

4.2. Physical analysis of broiler *pectoralis major* muscle

Alterations of myopathies on quality and consistency of breast fillets are reflected during physical and chemical analysis of chicken breast fillets.

According to our physical analysis results, chicken *m. pectoralis major* affected by severe PMM showed higher cooking loss and drip loss compared to those of samples collected from broilers without PMM. According to the literature, cooking loss is normally used to measure the loss of liquids as a result of protein denaturation and decomposition of cell membranes during cooking [121], while drip loss is the manifestation of the leakage of myofibers and loss of water, iron, and proteins during the transition of muscle to meat [185]. Factors which could lead to escaping of water from muscle tissue could result in higher drip loss as well as cooking loss. From raw poultry meat water could escape during storage and result in higher drip loss, while cooking loss refers to water escaping from the meat as it is cooked. Furthermore, the meat ability to hold water is influenced by quality of the muscle proteins. These proteins could be affected by oxidative stress. During stress the amount of free radicals elevates and it also triggers oxidative stress. Eventually oxidative stress damage protein cell membranes, therefore water escapes not only when animal is alive, but also from meat during storage, cooking or processing [186].

Furthermore, according to the literature drip loss may increase due to replacement of myofibrillar proteins with fibrous connective tissue proteins (collagen) forming a scar tissue in breast muscle severely affected by WB. This process results in reduced membrane integrity and altered muscle structure, which allows water to escape more easily, contributing to higher drip and cooking loss as well [46,64,88,105,113]. Moreover, the increased cooking loss and drip loss is attributed to the visual appearance of myopathies. Breast fillet affected by WB covers thin layer of fluid viscous material, this also could increase cooking as well drip loss in poultry meat [76]. This study's results corroborate those of a previous study by Wang et al. [187], it was found that cooking loss and drip loss were greater in breast meat affected by severe myopathies compared to those of breast meat without myopathies (normal). However, this finding differs from other authors' reports in which WB together with WS only significantly affect cooking loss, but not drip

loss [88,113,115]. We hypothesized that these differences could be due to the bird's age, weight, cooking procedure, chilling method and gross lesions classification during muscle examination for myopathies.

Moreover, according to this study, the dry matter content was significantly lower in *pectoralis major* samples affected by severe PMM. Myopathies alter muscles' histological structure, leading to muscle myofiber necrosis, degeneration, edema (fluid accumulation), inflammation and fibrosis [12,76]. Due to degeneration and necrosis, myopathic muscles have higher proportion of degraded myofibers. Degeneration and necrosis of myofibers lead to reduction of muscle tissue and accretion of extracellular water [188,189]. It lowers dry matter content compared to that normal muscle tissue without PMM.

Furthermore, macroscopically WB is characterized by pale areas, translucent or citrine-colored fluid on the *pectoralis major* muscle due to degeneration, fibrosis and intramuscular fat accumulation [76]. According to the study results, yellowness was significantly higher in samples affected by mild or severe myopathies compared to that in normal samples without PMM. Therefore, pathomorphological lesions of muscle affect *m. pectoralis major* color of broilers and leads to its color change to more yellowness. This finding is in agreement with Tasoniero et al. (2016), according to its research results WS together with WB, significantly increased the b^* value, yellow color of the muscle [114].

4.3. Chemical analysis of broiler *pectoralis major* muscle

FAs plays a crucial role in the technological aspects of meat quality [190]. Age, gender, genotype, temperature and feeding could influence FA profile of poultry meat [191]. Overall, our research findings show that not only those factors, but also myopathies could alter FA profile of chicken breast meat. According to our research results, *pectoralis major* samples affected by PMM had higher levels of MUFAs, including oleic and palmitoleic acids. This is in agreement with Liu et al. (2022) studies [125]. Furthermore, myristic acid also was higher in breast muscles severely affected by PMM; however, the amount of stearic acid in severely affected muscle was lower compared to that in normal samples. Moreover, phospholipids have high PUFA content, making them more susceptible to peroxidation. However, MUFAs are more resistant, but still undergo that process [63,69,124,125]. According to the literature, oxidative stress is one of the factors which could induce lipid peroxidation in fillets affected by PMM [63,125]. Additionally, according to the literature, oxidative stress is related to those chicken breast myopathies. Therefore, we believe that oxidative stress is important in the etiology of PMM. However, it

is still lack of studies that analyze FA profiles of broiler muscles affected by myopathies. Generally, lipids are an essential component of live organisms. They form cell membranes, hormones, and also are used as a source of ATP energy [192]. Additionally, our research highlights that there are specific changes in the FA profile of Ross 308 broilers that require additional analysis.

In addition to examining fatty acids, we have included the profile of VOCs in our study of broilers meat affected by PMM. According to the literature, VOCs are generated during peroxidation of lipids, particularly those containing double carbon bounds [192]. Hypoxia, inadequate vascularization of broilers muscle tissue leads to accumulation of ROS [86,193,194]. This triggers oxidative stress in broiler chickens. Lipid peroxidation occurs and wide range of VOCs are formed, including alcohols, ketones, aldehydes, alkanes [63,125,192]. Therefore, as fatty acids undergo peroxidation, these compounds are key indicators of oxidative stress [192]. According to our research results, among all evaluated VOC groups the largest established VOC group in broiler meat affected by PMM is group of aldehydes. Furthermore, among all established aldehydes, hexanal showed the highest amount in the studied samples. Additionally, this aldehyde was also significantly more abundant in muscles affected by PMM compared to normal samples of muscle.

Furthermore, the aldehydes, including hexanal, not only show the presence of oxidative stress, but also affect flavor and aroma of the meat and produce undesirable flavors [195]. This suggests that the meat from broilers affected by PMM were more susceptible to the production of unpleasant odors compared to normal *m. pectoralis major* samples, without myopathies. PMM could impact the sensory and technological qualities of the meat [195]. This finding is in agreement with Luo et al. (2022) who stated that hexanal appears to make the most significant contribution to flavor quality of broilers [135].

BAs are organic bases which participate in normal metabolic processes in living tissues, they are also considered an index of food spoilage, index for foods, meat products stability and quality [196,197]. The presence of BA's in raw meat could be influenced by some factors, such as meat origin, storage conditions, specific microbiota, and meat shelf-life [137]. Amount of free amino acids, microorganisms that contain amino acid decarboxylase, pH, temperature and atmosphere conditions, fat content and protein composition of the meat could influence the presence of BAs in the meat [198].

Our research of BAs showed that tryptamine and spermine contents were lower in muscle affected by PMM compared to that of samples without PMM. During research analysis pH, temperature, atmosphere conditions keep consistent for all samples of muscles. Additionally, according to our research results pH was not statistically significantly different between the groups. Therefore, these factors BAs formation in myopathic muscles could

not be influenced by environmental factors or pH. However, it is known that one of the possible formations of BA is the decarboxylation of amino acids by microorganisms [136,198]. According to the literature, the levels of proteins are reduced in WB- and WS-affected *m. pectoralis major* samples [88,115,122]. Amino acids are not only the precursors of BAs, but also the energy supply for microbial growth [198]. Therefore, if protein content in the meat is decreased, the amount of BA decreases as well, because the activity of bacteria is reduced. Additionally, fast-growing broiler chickens suffer from inadequate vascularization and therefore increased amount of ROS being formed [18]. We hypothesized that oxidative process could inhibit enzymes and damage bacteria that are responsible for BA formation. Furthermore, according to researchers, lipid and intramuscular fat content in WB- and WS-affected meat are increased [88,115,121–123]. High fat content reduces water activity, thereby inhibiting the growth of microorganisms and the production of free amino acids [198]. Therefore, all those processes could reduce the amount of BA's in the chicken meat. Nevertheless, this result could be due to lack of microorganisms, such as *Lactobacilli*, *Pseudomonads*, *Enterobacteriaceae*, and *Enterococci* that could utilize amino acids to produce biogenic amines due to their decarboxylase activity [199]. The microbial analysis of the caecum of WB-affected birds also showed a decline in microbial diversity. Increased abundance of *S. bovis* and *B. plebeius* and decreased abundance of beneficial bacteria, including *B. pullicaecorum* and *L. hamster*, was found [169].

4.4. Biochemical analysis of broiler blood serum

Necrosis and degeneration are evident in muscle tissue affected by myopathies. This pathologic process, known as rhabdomyolysis, results in release of enzymes, muscle cell content, myoglobin, sarcoplasmic proteins, electrolytes, and various organic acids into the plasma or serum, serving as indicators of injury to the muscles or other organs [76,140–143]. As a result of myopathies muscle tissue undergoes necrosis and degeneration, leading to the leakage of these substances into the bloodstream, providing evidence of muscle damage. Therefore, such damage associated with broiler myopathies is reflected in alterations of blood plasma or serum [63]. Other authors, such as Sihvo et al. (2014) and Soglia et al. (2018), have analyzed *pectoralis major* affected by WB and reported that this condition is characterized by the presence of degenerative, necrotic, and atrophic myofibers. The affected muscle displays fragmented, hypereosinophilic amorphous myofibers, loss of striation, and infiltration of inflammatory cells, indicating inflammatory and necrotic processes. Additionally, hyalinization, lipidosis and extensive fibrillar collagen deposition (fibrosis) are observed [76,88]. It is important

to note that our histopathological study of muscles affected by WB and WS revealed similar findings. During damage of the muscle potassium, myoglobin, phosphate, organic acids, and various enzymes, leak into the plasma or serum [141,143]. One of the main findings of our study that, the level of CK was significantly higher in the blood serum of broilers affected by WB compared to that in the serum of broilers without WB. CK is a muscle-specific enzyme that is used to diagnose muscle injury [200]. During rhabdomyolysis, CK is released into blood serum or plasma as a result of muscle damage. This leakage leads to significant elevation of CK in blood serum or plasma, which is detectable through biochemical analysis. Therefore, the findings of this study justify that rhabdomyolysis occurs in WB myopathy. This finding indicates that CK could be as a biomarker for WB. Nevertheless, it proves that selection of groups according to gross lesions of WB (palpation and visual examination) was appropriate. Furthermore, several authors have published similar results. Kawasaki et al. (2018) [189] found significantly higher CK values in a group of 20-day-old WB-affected birds (42,360 IU/L) than in those unaffected by WB (10,164 IU/L). Kawasaki et al. also found that CK values increased in unaffected birds within the broilers age, suggesting that muscular degenerative lesions progressively develop as the birds age [189]. Meloche et al. (2018) [144] also found that blood plasma CK as well as LDH increase significantly with WB and WS scores. However, according to their study results they suggested that LDH may be a more valuable predictor for myopathy. However, according to Kong et al. (2021) [142], CK may serve as a potential blood marker for predicting breast muscle defects in 42-day-old broilers and could be useful in genetic selection for broiler breeding.

Moreover, it is crucial to recognize that the release of enzymes into bloodstream is not solely indicative of the muscle damage, but also could result from other organs injury, such as liver. The liver is multifunctional organ essential for synthesis, metabolism, excretion and detoxication. It plays a key role in digestion and regulates the production, storage, and release of carbohydrates, lipids, and proteins. Aminotransferases such as ALT and AST are highly concentrated in the liver and could reflect the liver damage through biochemical analyses of chicken blood serum [201–203]. In this study, ALT was significantly higher in the blood serum of broilers affected by WB, although AST was not statistically different in the blood serum of broilers affected by WB compared to that in the serum of broilers without WB. According to the literature, both enzymes can be elevated in liver injury, but ALT is primarily found in the liver, making it more essential indicator of liver damage. In contrast, AST is present in other tissues such as heart, muscles, and kidneys, so its elevation could indicate those tissue damage as well [202,204]. According to this research results, it may be suggested that

liver failure also occurs in fast-growing broilers affected by WB myopathy. Furthermore, according to Kong et al. (2021) results the serum AST of the mild group of broilers affected by WS and WB was significantly higher than the normal group at 28 days but not at 42 days, they hypothesized that this could be due to myocardial injury and functional compensation due to rapid weight gain with a high metabolic rate and more oxygen demand at 28 days of age [58,142].

Additionally, significantly higher potassium levels were found in the blood serum of broilers affected by WB. Livingston et al. (2019) found a level of potassium in 42-day-old broilers without WB of 4.75 mmol/L, while, in those affected by mild, moderate, or severe WB, it was significantly higher—6.25 mmol/L, 5.76 mmol/L and 5.87 mmol/L, respectively [205]. Lake et al. (2020) [148] also found significantly different potassium levels: 5.04 mmol/L in broilers affected by WB and 4.86 mmol/L in unaffected broilers. Livingston et al. (2019) hypothesized that different broiler blood physiology of those affected and unaffected by WB indicate a muscle cell voltage gradient disruption [205]. In addition, high blood potassium level is also linked to intermittent muscle cell tetanus [206].

According to Lake et al. (2020), chickens with WB exhibit not only higher levels of potassium but also higher pCO_2 and a lower pH, sO_2, and pO_2. High pCO_2 and low pH indicate problems with gas exchange in the lungs, suggesting that broilers with WB have a higher metabolic rate that is not properly balanced. This imbalance could be due to issues like poor blood flow back to the heart or breathing difficulties [148]. Livingston et al. (2019) in both of the studies also found similar results. This suggests that hypoxia may be associated with chicken WS and WB myopathies [205,207]. However, our analysis focused solely on the biochemical blood profile, which limited to the analysis of mineral levels and did not account for other potential indicators of altered oxygen metabolism. Therefore, further blood analysis of chickens is needed to draw more accurate conclusions.

4.5. Histopathological analysis of broiler muscles affected by myopathies

Pectoralis major muscle affected by myopathies

Degeneration and necrosis of myofibers, accumulation of fibrous connective and adipose tissue, inflammation of the muscle and blood vessels were determined in broilers *pectoralis major* muscle mildly and severely affected by PMM. Mononuclear inflammatory cells, macrophages and heterophils infiltrated not only degenerated myofibers and necrotic muscle tissue but also was observed in the perimysium and endomysium of the muscles. Blood

vessels, veins mainly, are surrounded or infiltrated by lymphocytes. Sihvo et al. (2014, 2017) [60,76], Soglia et al. (2016) [88], Mazzoni et al. (2015) [208] research results are in agreement with ours. All of them described similar features of PMM. Sihvo et al. in 2014 [76] and 2017 [60] found that muscle affected by PMM exhibited degeneration accompanied by rounded myofibers (reduced in number), polyphasic degeneration with regeneration as well as a variable amount of interstitial fibrous connective tissue accumulation (fibrosis) was present. Kuttapan et al. (2013) [105] found that degenerative or necrotic lesions, fibrosis, and adipose tissue accumulation is increased as the degree of WS increased. Similarly, in Soglia et al. (2016) [88] studies, muscle affected by WB and WS showed myofiber degeneration and fibrosis. Samples from WB/WS fillets exhibited profound degenerative myopathic lesions together with replacement of chronically damaged muscle with adipocytes and fibrous connective tissue.

Prisco et al. (2021) [209] evaluated *m. pectoralis major* affected by WS, and found that endomysium and perivascular inflammatory infiltrate consist of macrophages and cluster of differentiation CD8 positive T lymphocytes. Additionally, severe myofiber atrophy, necrosis, fibrosis and muscle tissue replacement by adipose tissue were observed. Furthermore, myopathic features such as centralization of nuclei, myofiber splitting, and endomysium and perimysium edema were present in WS-affected muscle of Ross 308 at 55 days old. Kuttapan et al. (2013) [105] found similar results when analyzing WS-affected muscles. Floccular/vacuolar degeneration, lysis, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells) of myofibers, and interstitial inflammation along with fibrosis. There were multiple rounded hypereosinophilic myofibers with loss of cross striation and internalization of nuclei. The interstitium showed multifocal edema with infiltration by lymphocytes and macrophages. Nevertheless, Mazzoni et al. (2015) found that diffuse thickening of the perimysium network with variable amounts of loose connective tissue, granulation tissue, or collagen-rich fibrous connective tissue separating the myofibers. Myofibers were characterized by multifocal degeneration and necrosis and infiltration of CD3-immunoreactive cells (T lymphocytes) within and around the degenerative myofibers and blood vessels [208].

According to our research, affected muscles by PMM exhibited perivasculitis or vasculitis, lymphocytes were present around or inside the veins. Additionally, adipose tissue accumulation around vessels were established. Lymphocytes show that chronic inflammation is associated with PMM. Hypoxia is established as a main contributor of this pathologic feature, it plays significant role in activating endothelial cells to increase their adhesiveness for leucocytes, allowing immune cells to cross the endothelium–

cell barrier [104]. It is still hypothesized that *pectoralis major* is prone to those changes. Impaired venous drainage from phlebitis and edema may alter physiological and structural functions of the muscle tissue and trigger the development of inflammatory processes and muscle damage [18,210].

Additionally, Prisco et al. (2021) [209] and Kuttappan et al. (2013) [105] found that the severity of the histologic lesions was positively correlated with the macroscopic degree of WS myopathy. In addition to that, the results from Soglia et al. (2016) [88] showed that more severe histological lesions were determined in fillets affected by both WB/WS myopathies compared to the normal group. These findings are similar to our research results that the more severe the PMM determined, the greater and the more extended pathologic processes were established. Likewise, degeneration/necrosis, accumulation of fibrous connective and adipose tissue, and inflammation of the muscle and blood vessels.

***Anterior latissimus dorsi* muscle affected by dorsal cranial myopathy**

DCM shows similar pathomorphological changes compared to PMM. Macroscopical lesions of DCM are very similar to WB: pale muscle, increased hardness, hemorrhages and viscous fluid on the muscle surface [19,76]. It was hypothesized that macroscopically examination of DCM is not sufficient to detect this myopathy [19].

Histopathological analysis revealed that, at the surface of DCM affected samples granulation tissue, fibrin and hemorrhages and variable amount of inflammatory cells (lymphocytes and macrophages) were present. Histological analysis showed degeneration, necrosis and regeneration of myofibers. Degenerative, necrotic myofibers were infiltrated by macrophages mainly and regenerating myofibers were present. Additionally, interstitial inflammation was present, lymphocytes, heterophils and macrophages infiltrated endomysium and perimysium. It was indicated that inflammatory process is secondary process to primary muscle degeneration and necrosis [19,211]. Similar results of histopathologic evaluation of DCM were found by Zimermann et al. (2012) [19], Sesterhenn et al. (2017) [51] and Pavanello et al. (2023) [212]. Zimmerman et al. (2012) [19] hypothesized that polyphasic pattern may be associated with unbalanced anatomy of broiler chickens. Additionally, Zimmerman et al. (2012) [19] suggest that microscopic lesions result from repeated strong insults (vaccines, unbalanced muscular anatomy) to ALD muscle, because regeneration of muscle cells is no longer possible and extensive areas of fibrosis are established. Additionally, according to the literature, repeated injury can lead to the development of late polyphasic lesions, either due to the persistence of the initial causes or because the lesions self-perpetuate over the time [212].

Furthermore, in samples of control group degeneration/necrosis, accumulation of fibrous connective and adipose tissue, inflammation of the muscle and blood vessels were present. These results corroborate with other studies. Zimmerman et al. (2012) [19] found that during histological examination of ALD samples that seemed macroscopically normal, microscopic lesions including muscle myofiber hyalinization, segmental necrosis, and lymphocytic infiltration, with mild fibrosis were present. Moreover, Pavanello et al. (2023) [212] announced that degeneration/ necrosis, fibroplasia and inflammation are found in normal ALD muscles, without DCM lesions. However, differently to our research, its results have shown that fibrosis was absent in all of the samples without DCM.

Moreover, our research results showed that degeneration, necrosis, accumulation of fibrous connective tissue and inflammation of the muscle increased with the presence of DCM. Sesterhenn et al. (2017) [51] established that the microscopical lesions such as degeneration, necrosis, fibrosis and regeneration were more intense in fragments of carcasses affected by DCM. Additionally, these findings are similar to Pavanello et al. (2023) [212], according to their study DCM samples were found to have the highest microscopic scores of degeneration, necrosis, inflammation, and fibroplasia or fibrosis.

4.6. Histomorphometric analysis of broiler muscles affected by myopathies

Pectoralis major muscle affected by myopathies

Our research results showed that in *m. pectoralis major* affected severely by PMM the myofiber diameter was the smallest among all evaluated cases. Moreover, the largest diameter of myofibers was in mildly affected muscle samples compared to the samples of severely affected muscle. This result could be because breast muscle affected by severe PMM consist of more necrotic and atrophic myofibers, while in mildly affected samples high number of degenerative myofibers were present. This result is in agreement with Kawasaki et al. (2018), the mean diameter of myofibers of samples of 55-day-old WB-affected birds was smaller than that of unaffected birds [189]. Similarly to Mazzoni et al. (2015) [208] research results, samples that were collected from moderate degeneration affected muscle had the highest mean cross-sectional area of the myofibers in respect to mild and severe groups (4,956 vs. 4,688 and 4,430 μm^2). According to our research, if mildly affected compared to severe PMM the cross-sectional area of the myofibers was 5,962.81 vs. 4,312.69 μm^2, respectively. Li et al. (2023) announced that the

proportional total myofiber area decreased and the proportional connective tissue and necrosis area increased as WB stage is getting worse [213].

According to the literature, broiler chicken myopathies develop because of rapid muscle growth. Extensive muscle growth and limited blood supply due to reduced capillary density result in hypoxia and poor transportation of waste products [86,88,94]. Oxygen is essential for adenosine triphosphate production, when oxygen is depleted, oxidative phosphorylation stops as well as adenosine triphosphate declines. Because of the deficiency of ATP sodium-potassium ionic pumps fails, therefore sodium, calcium ions, and water flows into the cell cytosol, potassium and magnesium ions are lost from the cytosol. The cytosol expands, mitochondria swells, it leads to acute cell swelling and degeneration. The cell has an expanded and rounded profile with pale eosinophilic or vacuolated cytoplasm. The myofiber diameter of that muscle cells are enlarged. However, if the injury is severe or persistent, cell swells, lipid peroxidation occurs, lysosomal membrane permeability increases, cathepsins releases it leads to necrosis and reduced diameter of myofibers [214]. Therefore, theory of oxidative stress could be the cause of altered muscle morphology and may explain those size of myofiber changes in mildly and severely affected PMM.

Nevertheless, our histomorphometry results shown that the amount of fibrous connective and adipose tissue were increased in severe PMM compared it to normal and mild PMM. This result is in the agreement with previous histopathological findings of Sihvo et al. (2014) [76]. Shivo et al. (2014) found a reduction in myofiber number due to degeneration of myofibers and replacement of the necrotic myofibers with connective tissue in WB [76]. Our research results have shown that *m. pectoralis major* affected by severe PMM had less muscle tissue compared it to mildly affected and normal muscle. Additionally, Li et al. (2023) announced that the proportional total myofiber area markedly decreased in severe WB, while the proportional connective tissue and necrosis areas increased when WB severity is getting worse. In addition, the remaining muscle myofiber cross-sections change from angular to round as interstitial connective tissue separates them [213]. According to the literature fibroadipogenic progenitors are interstitial muscle-resident mesenchymal stem cells that are able to differentiate into both fibroblasts and adipocytes [215]. PMM have similarities to other muscle conditions like Duchenne muscular dystrophy, hereditary muscular dystrophy, nutritional myopathy, toxic myopathies, and marbling [70]. Therefore, due to histological similarities of animals and humans myopathies it is hypothesized that this cell type plays a similar role in development of WB and WS in broilers [215].

Anterior latissimus dorsi muscle affected by dorsal cranial myopathy

Histomorphometry of DCM results showed that the diameter of myofiber is increased in DCM affected muscle. Zimmerman et al. (2012) [19] announced that size variations of myofibers were present in DCM samples. We hypothesized that this could be due to different pathological changes in myofibers. In response to myofiber degeneration and increased loss of myofibers due to DCM the remaining myofibers may increase in size. Compensatory hypertrophy occurs to compensate the loss of the muscle tissue and to maintain muscle strength and function.

Additionally, according to our research results muscle affected by DCM had less muscle tissue and much more fibrous connective tissue compared it to normal muscle, without DCM. This result is in agreement with Sesterhenn et al. (2017) [51], according to its research results the morphometric analysis showed that broilers with DCM had less muscle tissue (34.63% ± 2.67%) when compared to those with normal muscles (47.28% ± 4.78%). Additionally, the partial volume of connective tissue was increased in those samples with DCM (29.98% ± 2.79%) compared to normal samples of muscles (15.61% ± 5.13%). Furthermore, according to Sesterhenn et al. (2017) carcass weight has strong influence on partial volume of muscle and connective tissue [51]. Nevertheless, our research histomorphometric analysis showed that adipose tissue infiltration was not statistically different between the groups. This is in agreement with Pavanello et al. (2023) [212], according to its research samples from normal samples of ALD muscle without macroscopical lesions of DCM also showed infiltration of adipose tissue.

CONCLUSIONS

1. The incidence of PMM in Ross 308 broilers in Lithuania was 18.19%, whereas DCM and DPM had incidences of 5.16% and 0.27%, respectively. The analysis of risk factors revealed that the incidence of PMM was associated with broiler live BW, age at slaughter ($p < 0.001$) and antimicrobial treatment of broilers ($p < 0.05$). DCM was associated with broiler live BW at slaughter ($p < 0.05$) and seasons ($p < 0.01$). Among all the investigated factors, broilers' BW is the main cause of myopathies. The identified bacteria from the subcutis of the affected area of DCM and PMM belonged to the non-pathogenic endogenous microbiota.
2. Myopathies had an impact on the physical and chemical properties of the meat. Chicken breast meat severely affected by PMM showed higher yellowness ($p < 0.05$), cooking loss ($p < 0.001$) and drip loss ($p < 0.01$). Furthermore, meat severely affected by PMM had lower dry matter content ($p < 0.01$). Also, PMM influenced the fatty acid and volatile organic compound profiles of chicken breast muscle. Aldehyde hexanal, which is one of the main lipid peroxidation products, was significantly more abundant in the *pectoralis major* muscle affected by myopathies ($p < 0.05$). Furthermore, the amounts of biogenic amines, specifically TRY ($p < 0.01$) and SPER ($p < 0.05$), were significantly lower in muscle severely affected by PMM.
3. Elevated serum levels of CK and potassium indicated that skeletal muscle cells were damaged in the *pectoralis major* muscle affected by WB myopathy. Increased ALT level suggested an association between WB myopathy and liver damage.
4. Degeneration or necrosis, fibrous connective tissue accumulation and inflammation of the muscle were significantly increased in the ALD and *pectoralis major* muscles affected by myopathies when compared to those in control groups ($p < 0.001$). Lymphocytic phlebitis was a common pathological feature in muscle severely affected by PMM. In PMM, adipose tissue accumulation was significantly increased in severely affected muscles compared to that in normal muscles ($p < 0.001$), while no significant difference was found between the DCM-affected and control group ($p > 0.05$).
5. In *pectoralis major* muscle severely affected by myopathies, extensive myofiber necrosis was observed, the diameter of myofibers was decreased by 9.9% and the amount of muscle tissue was decreased by

12.8% when compared to those in muscle without myopathies. At the same time, necrotic muscle fibers were replaced by fibrous connective and adipose tissue, and the amount of fibrous connective and adipose tissue increased by 96.9% and 2.3-fold, respectively.

Hypertrophic and degenerative changes developed in ALD muscle affected by DCM. The diameter of myofibers was increased by 10.47%, but the amount of muscle tissue was reduced by 27.53% compared to those in control group. Due to intensive fibrosis in the ALD muscle affected by DCM the amount of fibrous connective tissue was 2.1-fold higher. However, the amount of adipose tissue between control and DCM-affected groups did not differ statistically significantly.

RECOMMENDATIONS

1. Based on the results of pathomorphological examination, gross evaluation and histopathological analysis, a suitable classification system of myopathies should be developed. This system would ensure convenient and easily applicable affected meat sorting in slaughterhouses, aiming to minimize economic losses.
2. Creatine kinase (CK) can be used as a biological marker in research of myopathies. This enzyme could serve as an additional test to identify myopathies in live broilers prior to post-mortem examination in the slaughterhouses.
3. Further studies on metabolism, liver health and gut microbiota of broilers could provide valuable insights into the etiology of myopathies.

SANTRAUKA

Problemos aktualumas ir svarba

Gyviems organizmams tenka keistis ir prisitaikyti, norint išgyventi nuolat besikeičiančioje aplinkoje – tai neišvengiama būtinybė. Pagrindinė Charles'o Darwin'o evoliucijos teorija teigia, kad pagrindinis evoliucijos veiksnys yra gamtinė atranka. Veikiant gamtinei atrankai, išlieka geriausiai prisitaikę prie nepalankių aplinkos sąlygų individai, kurie yra pranašesni už kitus. Priešingai nei gamtinė atranka, dirbtinė atranka, arba selektyvus veisimas, yra ūkiškai vertingiausių gyvūnų atranka ir išsaugojimas veislei. Ūkininkai ir veisėjai, siekdami tobulos veislės, atrenka vertingiausių savybių turinčius gyvūnus. Dirbtinė atranka yra žmogaus valdomas procesas – tai pagrindinis skirtumas [1,2,3]. Ūkiniai gyvūnai, viščiukai broileriai, buvo prijaukinti ir pakeisti. Atranka, siekiant išveisti viščiuką broilerį, prasidėjo penktajame praėjusio amžiaus dešimtmetyje. Buvo norima pagreitinti raumenų augimą ir paspartinti jų vystymąsi. Visos intervencijos leido pasiekti puikių gamybos rezultatų [4]. Dabartiniai didelio svorio hibridai broileriai (*Ross 308*) vidutiniškai užauga per 42 dienas ir sveria 2,4 kg, o the *Athens Canadian Random Bred Control* (toliau – ACRBC) vištų genetinė linija auga lėčiau ir tokio pat amžiaus svertų maždaug 0,55 kg. *Ross 308* broilerių vidutinis gyvasis svoris yra penkis kartus didesnis, palyginti su 1957 m. ACRBC vištų genotipais [5]. Dirbtinė atranka buvo pasitelkta siekiant patenkinti vis didėjančią vištienos paklausą.

Dabartiniais laikais paukštiena intensyviausiai produkuojama ir pagal vartojimą pasaulyje yra antroje vietoje po kiaulienos [6,7]. Vištienos vartojimas vis auga – tam įtakos turi maža kaina, lengvas ir greitas paruošimas [8] bei pageidaujamos juslinės savybės [9]. Paukštieną rekomenduoja ir dietologai, nes tai liesa mėsa, labiau tinkama dietinei mitybai nei raudona mėsa [10]. Tarptautinė vėžio tyrimų agentūra nustatė, kad raudona mėsa „gali turėti kancerogeninį poveikį žmonėms". Tai taip pat lėmė didėjantį vištienos populiarumą [11]. Paukštienos vartojimas išaugo ir dėl kultūrinių bei religinių įsitikinimų [9,12]. Pasikeitė ir vartotojų pageidavimai: apie 77 proc. vartotojų 1960 m. dažniausiai pirko visą viščiuką broilerį, o šiais laikais šis skaičius siekia tik 8 proc. [13]. Šiandien vis daugiau žmonių perka pjaustytą vištieną be kaulų. Tokia mėsa įgauna vis didesnį populiarumą, keičiasi žmonių gyvenimo būdas. Šiuolaikinėje pramonėje vištienos gamybos kaštai nebepriklauso nuo broilerio dydžio, todėl šiai pramonei labiau tinka didesni paukščiai [14,15]

Intensyvi viščiukų broilerių genetinė selekcija lėmė fiziologijos sutrikimus. Pakitusi viščiukų fiziologija sukėlė net tik širdies ir kraujagyslių sistemos sutrikimus, kojų ir kaulų deformacijas, bet ir paveikė jų raumeninį audinį. Vystosi skeleto-raumenų patologijos, miopatijos [12,16,17]. Išskiria-

mos kelios didžiojo krūtinės raumens (lot. *m. pectoralis major*) miopatijos: „medinė krūtinėlė“ (angl. *wooden breast*) (WB), baltosios juostelės (angl. *white striping*) (WS) ir tarpraumeninio jungiamojo audinio defektas (angl. *stringy-spongy*). Kita plačiai žinoma miopatija yra giliųjų krūtinės raumenų miopatija (DPM), dar vadinama „žaliųjų raumenų liga“ arba Oregono liga, kuri pažeidžia broilerių mažąjį krūtinės raumenį (lot. *m. pectoralis minor*). Ši miopatija jau tiriama dešimtmečius. Dorsokranialinė miopatija (DCM) pažeidžia nugaros priekinį plačiausiąjį raumenį (lot. *m. anterior latissimus dorsi*) (ALD). Ši miopatija paukštininkystės specialistų pradėta analizuoti santykinai neseniai [17,18,19,20,21]. Miopatijos ne tik keičia raumenų išvaizdą, bet ir daro neigiamą poveikį paukštienos kokybei [12,21,22]. Skerdyklose pažeisti raumenys, priklausomai nuo miopatijos sunkumo, yra pašalinami iš maisto grandinės arba priskiriami žemesnės vertės produkcijai. Patiriami ekonominiai nuostoliai ne tik dėl sumažėjusios tokios mėsos vertės, bet ir dėl ilgesnio rankinio rūšiavimo laiko. Nepaisant to, jei tokia mėsa patenka į maisto gamybos grandinę, ji vis tiek gali būti nepaklausi dėl nepageidaujamų kokybės savybių [12,17,23].

Miopatijų dažnis skirtinguose regionuose labai skiriasi. Nėra vienos visuotinai pripažintos miopatijų vertinimo sistemos. Miopatijų sunkumas klasifikuojamas skirtingai, todėl dėl visuotinai pripažintos klasifikacijos nebuvimo įvairiose šalyse išryškėja miopatijų pasireiškimo skirtumai. Taip pat vis dar trūksta informacijos apie broilerių miopatijų priežastis ir pasekmes. Miopatijos nustatomos tik atlikus poskerdiminę ekspertizę, todėl ankstyvos diagnostikos priemonės ir neinvaziniai miopatijų biožymenys, naudojant neardomuosius metodus, yra didelis iššūkis paukštininkystės pramonei. Kraujo tyrimai leidžia diagnozuoti ligas, vidaus organų patologijas, nustatyti medžiagų apykaitos sutrikimus ir įvertinti viso organizmo būklę. Atliekant mokslinius tyrimus ir analizuojant ligas, kraujo biocheminių rodiklių analizė gali būti naudojama kaip pirminis, neinvazyvus įrankis miopatijoms diagnozuoti, suprasti jų kilmę ir vystymosi mechanizmus.

Miopatijos yra kompleksinė problema, todėl būtina plėtoti mokslinius tyrimus ir žinias, siekiant veiksmingai stabdyti šių raumenų patologijų vystymąsi.

Prognozuojama, kad paukštienos paklausa labai išaugs per ateinantį dešimtmetį, o miopatijos yra opi problema veterinarijos gydytojams, mokslininkams, ūkininkams, paukštienos gamintojams ir vartotojams. Dėl miopatijų prastėja mėsos tekstūra, išvaizda, keičiasi ir maistinė mėsos sudėtis. Vartotojai nesirenka tokios produkcijos, todėl paukštienos pramonė patiria finansinių nuostolių. Vartotojai vis atidžiau analizuoja ir vertina gyvūnų gerovę ir perkamos mėsos kokybę. Vizualiai matomi pakitimai broilerių raumenyse dažnai rodo paukščių sveikatos problemas, kurias sukelia intensyvus ūkininkavimas.

Moksliniai tyrimai yra būtini, nes jie gali pasiūlyti sprendimus, kurie leistų pagerinti paukštienos gamybos efektyvumą, užtikrinti mėsos kokybę ir gyvūnų gerovės reikalavimus. Epidemiologiniai duomenys padeda įvertinti, kaip plačiai paplitusios miopatijos. Informacija apie miopatijų pasireiškimą yra itin svarbi naminių paukščių augintojams, nes ja remiantis priimami sprendimai ir atliekami kontrolės bei prevencijos veiksmai. Informacija apie broilerių sveikatą ir miopatijas bei platesnė jos sklaida visame pasaulyje skatina vartotojų pasitikėjimą paukštienos produkcijos kokybe.

Šioje disertacijoje pateikta informacija ir įvairiapusių tyrimų duomenys susieja epidemiologijos, veterinarijos ir maisto mokslo sritis.

Mokslinis naujumas

Tai pirmasis išsamus genetinės linijos *Ross 308* viščiukų broilerių miopatijų tyrimas, kuriame šios raumenų patologijos analizuojamos kompleksiškai. Tiriamas paplitimo mastas Lietuvoje ir galimos miopatijų priežastys, paukštienos fizikinės ir cheminės savybės, raumenų miopatijos analizuojamos mikroskopiškai vertinant histologinius pokyčius. Ištirtos ne tik didžiojo krūtinės raumens (lot. *m. pectoralis major*) miopatijos (PMM), bet ir nugaros priekinio plačiausiojo raumens (lot. *m. anterior latissimus dorsi*) miopatija (DCM) bei giliųjų krūtinės raumenų miopatija (DPM). Mokslinėje literatūroje DCM nėra plačiai aprašyta ir ištirta. DCM pasireiškimas buvo užfiksuotas tik Brazilijoje. Šios miopatijos įtraukimas į disertacijos tyrimus yra naujas.

Disertacijoje pirmą kartą aprašomi rezultatai, gauti ištyrus lakiuosius organinius junginius ir biogeninius aminus raumenyse su PMM. Remiantis šio tyrimo rezultatais gautos naujos įžvalgos apie PMM, nustatytas reikšmingas aldehido heksanalio, lipidų oksidacijos žymens padidėjimas stipriai PMM paveiktuose raumenyse. Gautas rezultatas patvirtina, kad oksidacinis stresas yra svarbus PMM patogenezėje. Taip pat aldehidas heksanalis turi poveikį vištienos juslinėms savybėms. Tyrimas patvirtina, kad PMM daro įtaką ne tik paukštienos fizikinėms, bet ir cheminėms savybėms, o tai gali turėti poveikį vartotojų nuomonei apie produktą.

Patomorfologinio tyrimo metu didžiajame krūtinės (lot. *m. pectoralis major*) ir ALD raumenyse buvo balais vertinamos tokios patologijos kaip degeneracija/nekrozė, skaidulinio jungiamojo ir riebalinio audinio kaupimasis, raumenų ir kraujagyslių uždegimas. Nustatėme, kad visų šių patologijų balų vidurkis buvo didesnis raumenyse su stipriai išreikšta PMM. Vertinant ALD raumenis nustatyta, kad raumenyse degeneracijos/nekrozės, skaidulinio jungiamojo audinio kaupimosi ir raumenų uždegimo balų vidurkiai buvo didesni raumenyse su DCM, tačiau riebalinio audinio kaupimosi ir kraujagyslių uždegimo balų vidurkiai tarp dviejų tirtų grupių statistiškai reikšmingai nesiskyrė.

Atliekant histomorfometrinę analizę naudojant vaizdo analizės metodus, raumenyse paveiktuose PMM ir DCM pirmą kartą buvo apskaičiuota ne tik skaidulinio jungiamojo ir raumeninio, bet ir riebalinio audinio procentinė išraiška. Taip pat pirmą kartą buvo išmatuotas raumens skaidulų skersmuo DCM paveiktuose raumenyse. Nustatyta, kad raumenyse su DCM raumens skaidulų skersmuo buvo statistiškai reikšmingai didesnis. Vertinant didžiojo krūtinės raumens (lot. *m. pectoralis major*) skaidulų skersmenį jis buvo mažesnis stipriai PMM pažeistuose raumenyse. Visi šie patologiniai raumenų pokyčiai lemia vištienos kokybės prastėjimą, ypač dėl padidėjusio skaidulinio jungiamojo ir riebalinio audinio sunkiais PMM ir ryškios fibrozės DCM atvejais.

Darbo tikslas ir uždaviniai

Darbo tikslas: ištirti viščiukų broilerių miopatijų pasireiškimą pulkuose, išanalizuoti pagrindinius etiologinius veiksnius ir patomorfologinius pokyčius bei įvertinti miopatijų įtaką greitai augančių broilerių sveikatingumui ir paukštienos kokybei.

Darbo uždaviniai:

1. Nustatyti miopatijų pasireiškimą viščiukų broilerių pulkuose ir išanalizuoti rizikos veiksnius, galinčius turėti įtakos broilerių miopatijų etiologijai ir patogenezei.
2. Ištirti miopatijų įtaką viščiukų broilerių didžiojo krūtinės raumens (lot. *m. pectoralis major*) fizikinėms ir cheminėms savybėms.
3. Išanalizuoti broilerių, kuriems nustatyta „medinės krūtinėlės“ (angl. *wooden breast*) miopatija, kraujo serumo biocheminių parametrų pokyčius.
4. Išanalizuoti broilerių didžiojo krūtinės (lot. *m. pectoralis major*) ir nugaros priekinio plačiausiojo (lot. *m. anterior latissimus dorsi*) raumenų miopatijų histologinius pokyčius.
5. Įvertinti broilerių didžiojo krūtinės (lot. *m. pectoralis major*) ir nugaros priekinio plačiausiojo (lot. *m. anterior latissimus dorsi*) raumenų miopatijų histomorfometrijos rezultatus.

Tyrimo metodika

Mėginiai tyrimams imti skerdyklose iš sparčiai augančių *Ross 308* genetinės linijos viščiukų broilerių patinų ir patelių, 40–43 dienų amžiaus, sveriančių apie 2,4–2,6 kg. Tirtos viščiukų broilerių miopatijos, tokios kaip giliųjų krūtinės raumenų miopatija (DPM), nugaros priekinio plačiausiojo raumens

miopatija (DCM), „medinės krūtinėlės“ (angl. *wooden breast*) ir baltųjų juostelių (angl. *white striping*) miopatijos. Dėl dažno pasireiškimo ir nustatytų panašių patologinių pokyčių pastarosios miopatijos buvo nagrinėtos kaip viena grupė ir vadinamos santrumpa (PMM).

Tyrimai buvo atlikti etapais, kurių kiekvienas orientuotas į skirtingus broilerių miopatijų aspektus. Analizuoti rizikos veiksniai ir vertintas miopatijų pasireiškimas Lietuvoje (DPM = 19 500; DCM = 124 200; PMM = 54 000), atlikta poodinio audinio, dengiančio miopatijų pažeistą vietą, mikrobiologinė analizė (n = 24), didžiojo krūtinės raumens (lot. *m. pectoralis major*) fiziniai ir cheminiai tyrimai (n = 18), kraujo serumo biocheminė analizė (n = 66) ir raumenų histopatologiniai tyrimai (n = 110).

Skerdykloje atliktas makroskopinis broilerių raumenų vertinimas. Vertintas didysis krūtinės raumuo (lot. *m. pectoralis major*) ir išskirtos dvi grupės: kontrolinė grupė – be miopatijų, krūtinės raumenyje nėra blyškių plotų ar baltų juostelių, ir grupė su miopatijomis – raumuo blyškus tik kranialinėje krūtinėlės dalyje arba blyški visa krūtinėlė, gali būti matomos baltos juostelės. Kita tirta miopatija buvo DCM. Pagal odos, dengiančios ALD raumenį, makroskopinius požymius DCM buvo suskirstyta į grupes: pirmoji grupė (raumuo be miopatijos) – nematoma jokių odos spalvos pokyčių; antroji (raumuo su vidutinio sunkumo miopatija) – oda virš raumens yra gelsva arba šiek tiek žalia; trečioji (raumuo su stipraus pažeidimo miopatija) – geltonos arba žalios spalvos oda raumens srityje. Taip pat buvo vertintas mažasis krūtinės raumuo (lot. *m. pectoralis minor*). Pirmoji grupė – raumuo be matomų DPM požymių; antroji grupė – šviežias pažeidimas: raumuo raudonos spalvos, matomos kraujosruvos, serozinis skystis; trečioji grupė – keleto dienų pažeidimas: raumens spalva vyrauja nuo šviesiai rausvos iki violetinės; ketvirtoji grupė – senas pažeidimas: raumuo minkštas, tarsi molio konsistencijos, žalsvos spalvos, kai kuriose vietose balkšvas ar pilkšvas. DPM buvo susiskirstyta pagal stadijas remiantis Bilgilli‘o ir Hess‘o (2008) vertinimo sistema [170].

Atlikus makroskopinį raumenų tyrimą ir apskaičiavus miopatijų dažnį buvo įvertinti rizikos veiksniai, kurie gali turėti įtakos tirtų miopatijų pasireiškimui. Analizuoti tokie rizikos veiksniai kaip broilerių svoris ir amžius skerdimo metu, gydymui naudoti vaistiniai preparatai bei metų laikas, kai buvo skersti broileriai. Siekiant atlikti statistinę duomenų analizę, visi tirti veiksniai bei miopatijų paplitimo dažnis buvo susiskirstyti į grupes.

Mikrobiologinė analizė buvo dar vienas tyrimo etapas. Broileriai buvo įvertinti dėl PMM ir suskirstyti į grupes. Visi šio tyrimo broileriai buvo auginami be antibiotikų. Išskirtos dvi kategorijos: kontrolinė grupė – be miopatijų, krūtinės raumenyje nėra blyškių plotų ar baltų juostelių, ir grupė su miopatijomis – raumuo blyškus tik kranialinėje krūtinėlės dalyje arba blyški visa krūtinėlė, matomos baltos juostelės. Kita tirta miopatija buvo DCM. Iš-

skirtos taip pat dvi grupės – vertinta odos spalva, dengianti pažeistą ALD raumenį: kontrolinė grupė – odos spalvos pakitimų nėra; DCM grupė – ALD raumenį dengianti oda gelsva, žalsva, geltona arba žalia. Mėginiai mikrobiologiniam tyrimui imami aseptiškai. Išpreparavus odą steriliu skalpeliu sterilus tamponėlis buvo vilgomas į raumenų poodinį audinį. Mėginiai surinkti į mikrobiologines transportines terpes „Amies“ ir per 12 valandų pristatyti į laboratoriją. Laboratorijoje mėginiai pasėti į selektyvias ir diferencines maitinamąsias terpes. Mikroorganizmai identifikuoti remiantis jų biocheminėmis ir antigeninėmis savybėmis.

Antrojo tyrimo metu buvo analizuojamos fizikinės ir cheminės didžiojo krūtinės raumens (lot. *m. pectoralis major*) savybės. Raumenų mėginiai (be kaulo ir odos) buvo paimti iš broilerių su skirtingu PMM pažeidimo laipsniu. PMM sunkumo laipsnis buvo nustatytas vizualiai ir palpuojant didįjį krūtinės raumenį (lot. *m. pectoralis major*). Klasifikacija, įvertinus raumenį, buvo tokia: raumuo be miopatijų – krūtinės raumenyje nėra blyškių plotų, tiršto skysčio ar baltų juostelių, raumens konsistencija standi, nekieta; vidutiniškai pažeistas raumuo – kranialinėje dalyje raumuo kietesnis ir blyškesnis, raumenį dengia šviesiai gelsvas klampus skystis ir matomos iki 1 mm storio baltos juostelės; sunkiai pažeistas raumuo – raumuo kietas, blyškus, gausus kiekis tiršto klampaus skysčio ir aiškiai matomos storesnės nei 1 mm baltos juostelės. Baltųjų juostelių (angl. *white striping*) miopatijos klasifikacijos kriterijai buvo paremti Kuttappan‘o ir kt. (2012) metodika [108]. Visi mėginiai buvo individualiai pasverti, supakuoti į vienodus šaldymo maišelius ir paženklinti, transportuoti į laboratoriją 4 °C temperatūros sąlygomis, o vėliau užšaldyti –20 °C temperatūroje.

Technologinės fizikinės broilerių mėsos savybės tirtos remiantis Rozanski’o ir kt. (2017) bei AOAC (2019) metodikomis [171,172], pH buvo nustatytas naudojant pH matuoklį (*Inolab 3*, “Hanna Instruments”, Italija), sukalibruotas pagal pH 4,0 ir 7,0 buferinius tirpalus. Vandens rišlumas, vandeningumas, virimo nuostoliai ir kietumas buvo nustatyti pagal Klupšaitės ir kt. (2020) metodikas [173]. Vandens rišlumas buvo nustatytas paėmus 2 g mėginį, dedant jį ant *Whatman 41/ashless* filtrinio popieriaus ir spaudžiant tarp dviejų organinio stiklo plokštelių, viršuje uždėjus 10-čiai min. 1 kg svorį. Vandeningumas buvo vertinamas pagal mėginio (40–50 g, maždaug 30 × 60 × 25 mm) pokytį po 24 val. laikymo polietileno (0,45 μm) maišelyje su sandarinimo juostele 4 °C temperatūroje. Virimo nuostoliai buvo apskaičiuoti įvertinus mėginio svorį prieš ir po virimo vandens vonioje (vidinė temperatūra 70 °C, kaitinimo trukmė – 30 min.). Kietumas buvo nustatytas po terminio apdorojimo iš kiekvieno mėginio išpjaunant tris cilindro formos mėginius (skersmuo 1,27 cm). Mėginiai buvo vertinami naudojant *TA.XT Plus* analizatorių (Stable Microsystems Ltd., Surrey, JK) su *Warner Bratzler* įtaisu.

Sausųjų medžiagų kiekis buvo nustatytas mėginius džiovinant termostatinėje džiovinimo krosnelėje 105 °C 24 val. Mėsos spalvingumas (šviesumas (L^*), raudonumas (a^*), geltonumas (b^*)) buvo matuojamas po 30–40 min ir pakartotinai tą pačią dieną vėliau Minolta Chroma Meter kolorimetru (CR-400, „Minolta Camera“, Osaka, Japonija). Prietaisas nustatytas L^*, a^*, b^* spalvų sistemai, kalibruotas su balta plokštele (Y = 92,8, x = 0,3160, y = 0,3323) naudojant D-65 šviesos šaltinį, 2° *standard observer* ir 8 mm matavimo diafragmą. Spalva buvo matuojama didžiojo krūtinės raumens (lot. *m. pectoralis major*) vidurinėje dalyje, pjūvio vietoje po perpjovimo.

Mėginiai prieš cheminę analizę buvo susmulkinti (sumalti). Riebalų rūgščių analizei skirtas lipidų ekstrahavimas buvo atliktas naudojant chloroformo/metanolio (2:1, tūrio santykiu) mišinį remiantis Pérez-Palacios‘o ir kt. (2012) aprašyta metodika [174] su tam tikromis modifikacijomis [173]. Riebalų rūgščių sudėties analizei buvo naudotas dujų chromatografas *GC-2010 Plus* (Shimadzu Corporation, Kiotas, Japonija), sujungtas su masių spektrometru *GCMS-QP2010* (Shimadzu Corporation, Kiotas, Japonija). Riebalų rūgščių koncentracijai nustatyti buvo taikyta kalibracinė kreivė, o rezultatai išreikšti procentais nuo riebalų rūgščių bendros koncentracijos mėginyje.

Lakieji organiniai junginiai buvo analizuojami taikant kietosios fazės mikroekstrakciją iš dujų fazės (HS-SPME), sujungtą su dujų chromatografija ir masių spektrometrija (GC-MS). Mėginių paruošimui naudotas kietosios fazės mikroekstrakcijos (SPME) įtaisas su Stableflex™ pluoštu, padengtu 50 µm DVB-PDMS-Carboxen™ sluoksniu (Supelco, JAV). 4 g homogenizuoto mėginio buvo perkelta į 20 ml talpos ekstrakcijos buteliuką, kuris buvo sandariai uždarytas su politetrafluoretilenu ir 30 min. patalpinta į 60 °C temperatūros termostatą. Desorbcijos trukmė – 2 min. Paruošti mėginiai buvo analizuojami naudojant GCMS-QP2010 (Shimadzu, Japonija) dujų chromatografą su masių spektrometru. Analizei naudota Stabilwax-Da kapiliarinė kolona (30 m x 0,25 mm vidinis skersmuo × 0,25 µm plėvelės storis). Masių spektrometras veikė pilno jonų skenavimo režimu (35–500 m/z). Analizės sąlygos: kolonėlės dujų srauto debitas – 0,65 ml/min (naudotas helis, 99,999 proc. grynumo), injektoriaus temperatūra – 250 °C, jonų šaltinio temperatūra – 220 °C, sąsajos temperatūra – 280 °C. Temperatūros gradientas: pradinė temperatūra 40 °C (laikoma 3 min.), temperatūros kilimo greitis – 5 °C/min, iki 250 °C (laikoma 5 min). Lakieji organiniai junginiai buvo identifikuoti naudojant masių spektrų bibliotekas (NIST11, FFNSC2).

Biogeninių aminų analizė atlikta remiantis Ben-Gigirey‘io ir kt. (1999) metodika (176) su tam tikromis modifikacijomis. Analizuoti šie biogeniniai aminai: triptaminas (TRY), feniletilaminas (PHE), kadaverinas (CAD), putrescinas (PUT), histaminas (HIS), tiraminas (TYR), sperminas (SPER) ir spermidinas (SPRMD). Standartiniai biogeninių aminų tirpalai buvo ruošia-

mi ištirpinant žinomą kiekvieno biogeninio amino kiekį (įskaitant vidinį standartą – 1,7-diaminoheptaną) 20 ml dejonizuoto vandens. Biogeninių aminų ekstrakcijai iš mėginių (5 g) naudota 0,4 mol/l perchloro rūgštis. Mėginių ir standartų derivatizacija atlikta naudojant dansilchlorido tirpalą, paruoštą ištirpinus atitinkamą kiekį dansilchlorido acetonitrile (10 mg/ml). Analizei naudota *Varian ProStar HPLC* sistema (Varian Corp., Palo Alto, Kalifornija, JAV), kurią sudarė dvi *ProStar 210* pompos, *ProStar 410* automatinė mėginių įvedimo sistema, *ProStar 325 UV/VIS* detektorius ir *Galaxy* programinė įranga (Agilent, Santa Clara, Kalifornija, JAV) duomenų apdorojimui. Biogeninių aminų atskyrimui naudota *Discovery® HS C18* kolonėlė (150 × 4,6 mm, 5 µm; Supelco™ Analytical, Bellefonte, Pensilvanija, JAV). Mobili fazė A – amonio acetatas, B – acetonitrilas, o eluavimas atliktas gradientiniu režimu, esant 0,8 ml/min srautui. Detekcijos bangos ilgis – 254 nm, kolonėlės temperatūra – 40 °C, o mėginio tūris injekcijai – 20 µl. Tiriamieji junginiai buvo identifikuoti pagal jų sulaikymo laiką (*retention time*) ir palyginti su atitinkamais standartais. Rezultatai išreikšti miligramais kilogramui mėginio (mg/kg).

Malondialdehido (MDA) kiekis buvo nustatytas pagal Mendes'o ir kt. (2009) aprašytą metodiką [175] su tam tikromis modifikacijomis (173). Mėginio derivatizacija atlikta su 40 mM tiobarbitūrinės rūgšties tirpalu. Chromatografinei analizei atlikti naudota ta pati *Varian ProStar HPLC* sistema (Varian Corp., Palo Alto, Kalifornija, JAV) kaip ir biogeninių aminų analizei, tik MDA identifikavimui naudotas fluorescencinis detektorius *ProStar 363* ir *Phenomenex Gemini C18* (5 µm, 250 × 4.6 mm) kolonėlė.

Trečiojo tyrimo metu buvo tirtas viščiukų broilerių kraujo serumas. Pakabintų ant skerdimo linijos broilerių didysis krūtinės raumuo (lot. *m. pectoralis major*) buvo apžiūrėtas vizualiai, 100 broilerių buvo atrinkti atsitiktinai ir pažymėti. Po apsvaiginimo (150 mA, 400 Hz, 15–17 s, kintamoji srovė) 10 ml kraujo nuleidimo metu buvo paimta į mėgintuvėlius (Venoject, Terumo Europe N. V., Belgija). Visiems pažymėtiems paukščiams buvo atlikta poskerdiminė ekspertizė, vertinamas didysis krūtinės raumuo (lot. *m. pectoralis major*), nustatoma „medinės krūtinėlės" (angl. *wooden breast*) miopatija. Atliekant poskerdiminę ekspertizę patologijų kituose organuose nenustatyta. Atrinkti reprezentatyvūs kraujo mėginiai (n = 66) ir suskirstyti į dvi grupes. Pirmąją grupę sudarė mėginiai, imti iš broilerių be miopatijos (kontrolinė grupė): raumuo standus, nekietas, nėra blyškių plotų, nėra klampaus, tiršto skysčio. Antrąją grupę sudarė kraujo mėginiai, imti iš broilerių, kurie turi „medinės krūtinėlės" (angl. *wooden breast*) miopatiją (WB grupė): raumuo blyškus kranialinėje dalyje arba visas raumuo kietas ir blyškus, ant raumens paviršiaus šviesiai geltonas, klampus skystis. Paimti viščiukų kraujo mėginiai pristatyti į laboratoriją per 2 val. Kraujas buvo centrifuguojamas 5 minutes

3000 apsisukimų per minutę greičiu. Iš kiekvieno mėginio buvo atskirta 1 ml kraujo serumo ir užšaldyta -20 ° C temperatūroje biocheminei analizei. Visų surinktų serumo mėginių biocheminiai kraujo tyrimai buvo atlikti automatizuotu kompiuteriniu biocheminiu analizatoriumi *SELECTRA Junior* (Nyderlandai, 2006), naudojant *Spinreact* (Ispanija) reagentus. Serume tirta šlapalo (urea), geležies, kreatinino, kalcio, magnio, fosforo, kalio, natrio, albumino, didelio tankio lipoproteinų cholesterolio, trigliceridų ir bendrojo baltymo koncentracija bei asparagininės aminotransferazės (AST), alaninės aminotransferazės (ALT), šarminės fosfatazės, gama-glutamilo transferazės ir kreatinkinazės (CK) aktyvumas. Globulino koncentracija kraujo serume buvo nustatyta iš bendros baltymų koncentracijos atėmus albumino koncentraciją.

Ketvirtojo tyrimo metu buvo atlikti histopatologiniai ir histomorfometriniai tyrimai. Broilerių skerdenos, kabančios ant skerdimo linijos, buvo apžiūrimos ir vertinamos. Iš viso buvo atrinkta 110 broilerių skerdenų: 60 PMM ir 50 DCM analizei. Poskerdiminės ekspertizės metu patologijų kituose organuose nenustatyta. Makroskopiškai įvertinus raumenis, PMM analizei iš broilerių paimta 60 didžiojo krūtinės raumens (lot. *m. pectoralis major*) mėginių, kurie buvo pagal PMM sunkumą suskirstyti į tris grupes. Pirma, kontrolinė grupė: krūtinės raumenyje nėra blyškių plotų, baltų juostelių ar tiršto skysčio, raumens konsistencija standi, nekieta; vidutiniškai pažeistas raumuo – raumuo kietesnis ir blyškesnis kranialinėje dalyje, šviesiai gelsvas klampus skystis ir matomos iki 1 mm storio baltos juostelės; sunkiai pažeistas raumuo – raumuo kietas, blyškus, gausus kiekis tiršto klampaus skysčio ir aiškiai matomos storesnės nei 1 mm baltos juostelės. Baltųjų juostelių (angl. *white striping*) miopatijos klasifikacijai pasiremta Kuttappan‘o ir kt. (2012) metodika [108].

Taip pat buvo paimta 50 ALD raumenų mėginių DCM analizei. Vertinta odos spalva dengianti pažeistą ALD raumenį. Buvo išskirtos dvi grupės: pirma, kontrolinė grupė – odos, dengiančios ALD raumenį, spalvos pakitimų nėra; antra, DCM grupė – virš ALD raumens esanti oda gelsva, žalsva, geltona arba žalia.

Visi histologiniai mėginiai buvo išpjauti vienkartiniais skalpeliais iš raumenų be odos, iškart po skerdimo. Didžiojo krūtinės raumens (lot. *m. pectoralis major*) mėginiai buvo paimti iš ventro-kranialinės dalies, maždaug 1 cm gylyje nuo raumens paviršiaus. Kiekvienas didžiojo krūtinės raumens (lot. *m. pectoralis major*) mėginys buvo apie 1 cm dydžio. ALD raumenų mėginiai buvo mažesni, apie 1 cm ilgio, o mėginių plotis ir storis buvo maždaug 0,4 cm. Visi surinkti mėginiai buvo fiksuoti 10 proc. buferiniame formalino tirpale (Sigma Aldrich, JAV).

Po fiksavimo mėginiai buvo supjaustyti, daromi kiekvieno mėginio išilginis ir skersinis pjūviai. Mėginiai, atpjauti dviem pjūviais, buvo sudėti į

plastikines kasetes. Kasetės su histopatologine medžiaga buvo dedamos audinių įmirkymo procesorių *„Shadon Pathcentre“* (Thermo Scientific Shandon Pathcenter, Fisher Scientific, JAV). Po to medžiaga buvo įlieta į histologinį parafiną, naudojant audinių įliejimo įrangą *„Tes 99“* (Tes 99 Parafin Embedding Center, Medite, Vokietija). 4 µm storio histologiniai pjūviai buvo paruošti naudojant rankinį rotacinį mikrotomą (Leica RM2235, Leica Biosystems, Vokietija). Visi pjūviai buvo dubliuoti. Histopatologinei analizei paruošti mėginiai buvo nudažyti hematoksilinu ir eozinu (H&E) naudojant automatinį dažymo aparatą „*Sakura Tissue-Tek DRS*™„ (Tissue-Tek DRS™, Sakura, Japonija). Dubliuoti mėginiai buvo rankiniu būdu nudažyti *Masson‘s Trichrome* dažais siekiant atlikti histomorfometrinę analizę ir išryškinti skaidulinį jungiamąjį audinį. Procedūra buvo atlikta vadovaujantis dažymo rinkinio informaciniame lapelyje pateikta informacija. Visi histopatologiniai mėginiai buvo analizuojami naudojant Olympus BX63 mikroskopą ir Olympus DP72 skaitmeninių fotoaparatų sistemą (U-TV1X-2, Olympus, Japonija) su įdiegta *Olympus cellSens Dimension 1.14* programine įranga (Olympus, Japonija). Histopatologiškai įvertinti visi surinkti didžiojo krūtinės (lot. *m. pectoralis major*) ir ALD raumenų mėginiai. Histologinis preparatų vertinimas buvo atliktas mikroskopu, vertinant skersinius ir išilginius raumenų pjūvius. Buvo analizuotos šios patologijos: degeneracija ir nekrozė, raumenų uždegimas, skaidulinio jungiamojo ir riebalinio audinio susikaupimas ir kraujagyslių uždegimas. Visos patologijos buvo vizualiai įvertintos balais. Histopatologinis balas buvo pagrįstas patologijos stiprumu ir išplitimu raumenyje. Degeneracijos ir nekrozės skalė buvo nuo 0 iki 4 (1 lentelė). Raumenų uždegimo, skaidulinio jungiamojo ir riebalinio audinio kaupimasis buvo klasifikuojami pagal sunkumą nuo 0 iki 3 (2 lentelė). Kraujagyslių uždegimas buvo įvertintas pagal Sihvo ir kt. (2017) naudotas metodikas (60). Maksimalus įvertinimas buvo 2 balai. 3 lentelėje pateikti kraujagyslių uždegimo vertinimo kriterijai. Kiekvienas mėginio pjūvis (išilginis ir skersinis) buvo vertinamas atskirai. Tais atvejais, kai vienas iš pjūvių gaudavo didesnį balą, ši vertė buvo užfiksuota kaip galutinis balas.

1 lentelė. *Kriterijai didžiojo krūtinės (lot. m. pectoralis major) ir nugaros priekinio plačiausiojo (lot. m. anterior latissimus dorsi) raumenų degeneracijos ir nekrozės įvertinimui*

Patologija	**0 balų**	**1 balas**	**2 balai**	**3 balai**	**4 balai**
Degeneracija/ nekrozė	Nenustatyta	< 20 proc. raumens pažeista	20–30 proc. raumens pažeista	30–50 proc. raumens pažeista	> 50 proc. raumens pažeista

2 lentelė. *Kriterijai didžiojo krūtinės (lot. m. pectoralis major) ir nugaros priekinio plačiausiojo (lot. m. anterior latissimus dorsi) raumenų uždegimo, jungiamojo ir riebalinio audinio susikaupimo įvertinimui*

Patologija	**0 balų**	**1 balas**	**2 balai**	**3 balai**
Raumens uždegimas	Nenustatyta	Uždegiminės ląstelės stebimos tik pažeistose raumens skaidulose. Intersticinio audinio uždegimo nėra	Uždegiminės ląstelės pažeistose raumens skaidulose. Intersticinis audinio uždegimas	Uždegiminės ląstelės pažeistose raumens skaidulose. Stiprus intersticinio audinio uždegimas
Jungiamojo audinio kaupimasis	Nenustatyta	< 20 proc. raumens pažeista	20–50 proc. raumens pažeista	> 50 proc. raumens pažeista
Riebalinio audinio kaupimasis	Nenustatyta	< 20 proc. raumens pažeista	20–50 proc. raumens pažeista	> 50 proc. raumens pažeista

3 lentelė. *Kriterijai didžiojo krūtinės (lot. m. pectoralis major) ir nugaros priekinio plačiausiojo (m. anterior latissimus dorsi) raumenų kraujagyslių uždegimo įvertinimui*

Patologija	**0 balų**	**1 balas**	**2 balai**
Kraujagyslių uždegimas	Nenustatyta	Mononuklеariniai limfocitai susitelkę aplink kraujagysles (perivaskulitas)	Mononuklеariniai limfocitai infiltruoja kraujagyslės sienelę (vaskulitas)

Histomorfometrinė raumenų analizė buvo atlikta naudojant *Olympus cellSens Dimension 1.14* programinę įrangą. Buvo analizuoti visi mėginiai, surinkti iš didžiojo krūtinės ir nugaros priekinio plačiausiojo raumens. Apskaičiuotas kiekvieno *Masson's trichrome* dažais nudažyto mėginio vidutinis raumens skaidulų skersmuo (μm), skaidulų skerspjūvio plotas (μm^2), taip pat nustatyta raumeninio, skaidulinio jungiamojo bei riebalinio audinio procentinė išraiška raumenyje. Histomorfometrinės raumenų analizės metu analizuotas tik mėginių skerspjūvis.

Visi matavimai buvo atlikti naudojant mėginių nuotraukas, skaitmeninius vaizdus. Nuotraukos buvo daromos mikroskopu, atsitiktinai pasirinktą mėginio vietą padidinus 40 kartų. Vidutinis raumens skaidulų skersmuo buvo apskaičiuotas viename lauke, išmatuojant atsitiktinai pasirinktų 100 raumens skaidulų (griaučių miocitų). Apskaičiuotas kiekvieno analizuoto mėginio raumens skaidulų skersmens vidurkis ir bendras skaidulų skerspjūvio plotas. Siekiant kiekybiškai įvertinti surinktų mėginių raumeninio ir skaidulinio jungiamojo audinio procentinę dalį raumenų mėginiuose, buvo užfiksuoti didelės raiškos skaitmeniniai vaizdai. *Manual HSV thresholding* funkcija buvo

panaudota siekiant atskirti raumeninį ir skaidulinį jungiamąjį audinį. Atitinkamos atspalvio (*hue*), sodrumo (*saturation*) ir šviesumo (*brightness*) reikšmės buvo koreguojamos, kad būtų paryškintos norimos apskaičiuoti sritys (ROI). Raumeninis audinys buvo pažymėtas mėlynai, o skaidulinis jungiamasis audinys – žaliai. *Count and Measure* funkcija buvo naudojama pasirinktų struktūrų plotui apskaičiuoti. Taip pat buvo apskaičiuota riebalinio audinio procentinė dalis mėginiuose. Riebaliniam audiniui apskaičiuoti taip pat taikytas rankinis slenksčiavimas, pažymint ir apskaičiuojant riebalinio audinio procentinę dalį.

Riebalinio audinio procentinė dalis kiekviename mėginyje buvo nustatyta pagal formulę:

$$\text{Riebalinio audinio kiekis (\%)} = \frac{\text{riebalinio audinio plotas } (\mu m^2)}{\text{analizuoto matymo lauko plotas } (\mu m^2)} * 100$$

Siekiant eliminuoti nenudažytus plotus iš galutinių skaičiavimų, bendras kiekvieno mėginio raumeninio, skaidulinio jungiamojo ir riebalinio audinio procentas buvo pakoreguotas iki 100 proc.

Statistinė analizė

Visų tyrimų duomenys buvo surinkti į duomenų bazę, sukurtą naudojant *Microsoft Excel 2021* programą. Statistinė analizė buvo atlikta naudojant SPSS 27.0 programinę įrangą (SPSS Inc., Chicago, IL, JAV). Rezultatai pateikti naudojant vidurkius ir standartinius nuokrypius. Vidurkių palyginimui tarp dviejų nepriklausomų grupių buvo taikytas *Stjudento t* kriterijus nepriklausomoms imtims. Tarp trijų nepriklausomų grupių vidurkių palyginimui naudota vienfaktorinė dispersinė analizė (ANOVA), statistiškai reikšmingiems skirtumams identifikuoti taikytas *post hoc Tukey* testas. Kintamųjų koreliacijos įvertintos naudojant Pirsono ir Spirmeno ranginės koreliacijos koeficientus. Reikšmės buvo laikomos statistiškai reikšmingomis, kai gautoji p reikšmė buvo mažesnė nei 0,05.

Rezultatai

Vertinant genetinės linijos *Ross 308* broilerius, auginamus Lietuvos paukštynuose, PMM pasireiškimas siekė 18,19 proc. Nugaros priekinio plačiausiojo raumens (lot. *m. anterior latissimus dorsi*) miopatija buvo nustatyta 5,16 proc. broilerių: vidutinio sunkumo forma – 4,49 proc. broilerių, o 0,67 proc. broilerių turi stipraus pažeidimo miopatiją. DPM yra rečiausiai pasitaikanti miopatija, jos dažnis Lietuvoje siekė tik 0,27 proc.: iš jų 0,144 proc. atvejų buvo nustatyti švieži pažeidimai, 0,113 proc. – kelių dienų senumo, seni DPM pažeidimai sudarė tik 0,01 proc.

Nustatyta, kad PMM pasireiškimo procentas pulkuose buvo teigiamai susijęs su vidutiniu paukščių svoriu skerdimo metu (r = 0,898) ir skerdimo amžiumi (r = 0,693) (p < 0,001). Taip pat PMM procentas pulkuose buvo neigiamai susijęs su broilerių gydymu (r_s = –0,535, p < 0,05). DCM pasireiškimo procentas pulkuose buvo teigiamai susijęs su svoriu skerdimo metu (r = 0,537, p < 0,05). Statistiškai reikšmingo ryšio tarp DCM dažnio pulkuose ir skerdimo amžiaus (r = 0,281) ir gydymo (r_s = –0,170) (p > 0,05) nustatyta nebuvo. Išanalizavus sezonų įtaką DCM dažniui, rezultatai parodė, kad DCM procentas pulkuose buvo reikšmingai susijęs su sezoniškumu (r_s = 0,658, p < 0,01). Tačiau statistiškai reikšmingų sąsajų tarp DPM pasireiškimo broilerių pulkuose ir svorio skerdimo metu (r = 0,015), skerdimo amžiaus (r = 0,239) ir gydymo (r_s = -0,202) (p > 0,05) nebuvo nustatyta.

Mikrobiologinės analizės rezultatai parodė, kad *Hafnia alvei* buvo išskirta iš visų mėginių (100 proc.), imtų iš poodinio audinio, dengiančio PMM. *Enterococcus viikkiensis*, buvo aptikta visuose (100 proc.) poodinio audinio, dengiančio raumenį be PMM, mėginiuose. Mėginiuose iš poodinio audinio, dengiančio raumenį su DCM, buvo nustatyta įvairesnė mikroorganizmų sudėtis. *Enterococcus viikkiensis* ir *Escherichia coli* buvo išskirta iš 33,33 proc. mėginių, o *Hafnia alvei* – iš 16,67 proc. atvejų. Dar 16,67 proc. atvejų bakterijų nebuvo aptikta. Broilerių, kuriems nebuvo nustatyta DCM, poodinio audinio mėginiuose visais atvejais (100 proc.) buvo išskirta *Escherichia coli*.

Fizikinių savybių analizės metu buvo nustatyta, kad mėginiai su stipraus pažeidimo laipsnio PMM turėjo 6,14 proc. mažiau sausųjų medžiagų nei raumenų mėginiai be PMM (p < 0,01) ir 5,28 proc. mažiau nei mėginiai, imti iš vidutinio pažeidimo laipsnio PMM (p < 0,05). Vandeningumas mėginiuose su stipraus laipsnio PMM buvo 55,09 proc. didesnis, palyginti su kontrolinės grupės mėginiais be miopatijų (p < 0,01). Virimo nuostoliai, esant stipraus pažeidimo laipsnio PMM, buvo 68,40 proc. didesni nei raumenyse be PMM (p < 0,001) ir 38,43 proc. didesni nei raumenyse su vidutinio pažeidimo laipsnio PMM (p < 0,001). Nustatyta, kad pH, kietumo ir vandens rišlumo rezultatai tarp mėginių grupių su skirtingu miopatijos pažeidimo laipsniu statistiškai reikšmingai nesiskyrė (p > 0,05). Tyrimo rezultatai parodė, kad raumenų mėginių su stipraus pažeidimo laipsnio PMM, *b** reikšmė buvo 20,09 proc. didesnė, palyginti su kontrolinės grupės mėginiais be miopatijų (p < 0,05). O raumenų mėginiuose su vidutinio laipsnio PMM *b** reikšmė buvo 27,76 proc. didesnė, palyginti su kontrolinės grupės mėginiais (p < 0,01). Nustatyta, kad gautos *L** ir *a** reikšmės tarp mėginių grupių su skirtingu miopatijos pažeidimo laipsniu statistiškai reikšmingai nesiskyrė (p > 0,05). Mononesočiųjų riebalų rūgščių (MUFA) kiekis buvo 5,09 proc. didesnis nei raumenyse be PMM (p < 0,05). Taip pat MUFA kiekis buvo 5,45 proc. didesnis nei mėginiuose be PMM (p < 0,05). Oleino rūgšties kiekis mėginiuose su sunkaus pažeidimo

laipsnio PMM buvo 4,73 proc. didesnis nei mėginiuose be PMM ($p \leq 0,05$). Palmitoleino rūgšties kiekis raumenyse su vidutinio pažeidimo laipsnio PMM buvo 30,48 proc. didesnis nei mėginiuose be PMM ($p < 0,05$).

Iš tirtų sočiųjų riebalų rūgščių (SFA), miristino rūgšties kiekis mėginiuose su sunkaus pažeidimo laipsnio PMM buvo 6,5 karto didesnis nei mėginiuose be PMM ($p \leq 0,01$). Stearino rūgšties kiekis buvo 17,81 proc. mažesnis raumenyse su sunkaus pažeidimo laipsnio PMM ir 16,04 proc. mažesnis raumenyse su vidutinio pažeidimo laipsnio PMM nei mėginiuose be PMM ($p < 0,05$). Kitų riebalų rūgščių kiekis raumenyse tarp mėginių su PMM pažeidimo laipsniu ir be PMM statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Tyrimo metu buvo identifikuotos 22 individualios lakiosios organinės medžiagos. Nustatyti aldehidai, alkoholiai, esteriai bei furanai. Tyrimo rezultatai atskleidė, kad heksanalio koncentracija buvo 33,76 proc. didesnė raumenyse su sunkaus pažeidimo laipsnio PMM ($p < 0,01$), ir 26,21 proc. didesnė raumenyse su vidutinio pažeidimo laipsnio PMM ($p < 0,05$), lyginant su mėginiais iš raumenų be miopatijos. Alil-2-etilbutirato koncentracija, lyginant su kontroliniais mėginiais, buvo 4,3 karto didesnė raumenyse su sunkaus pažeidimo laipsnio PMM ir 4,2 karto didesnė su vidutinio pažeidimo laipsnio PMM ($p < 0,01$). Lyginant su kontroliniais mėginiais šių junginių kiekiai buvo taip pat žymiai padidėję raumenyse su sunkaus pažeidimo laipsnio PMM: 2-oktenalio – 9,6 karto ($p < 0,01$), 2-okten-1-olio – 2,2 karto ($p < 0,05$), o 2-decenalio – 7,9 karto ($p < 0,01$). Pastarojo junginio kiekis taip pat buvo apie 3,8 karto didesnis raumenyse su sunkaus pažeidimo laipsnio PMM, lyginant su vidutinio pažeidimo laipsnio PMM ($p < 0,05$). 2-etil-1-heksanolio ir benzaldehido koncentracijos, lyginant su kontroliniais mėginiais, buvo reikšmingai sumažėjusios su sunkaus pažeidimo laipsnio PMM – atitinkamai 77,12 proc. ir 68,64 proc. ($p < 0,05$). Kitų tirtų lakiųjų organinių junginių koncentracijų skirtumai tarp miopatijos paveiktų ir sveikų raumenų nebuvo statistiškai reikšmingi ($p > 0,05$). Rezultatai taip pat parodė, kad MDA kiekiai tarp grupių statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Remiantis mūsų tyrimu nustatyta, kad biogeninio amino TRY kiekis su sunkaus pažeidimo laipsnio PMM buvo 56,92 proc. mažesnis, lyginant su normaliais, miopatijų nepažeistais mėginiais ($p < 0,01$). SPER kiekis su sunkaus pažeidimo laipsnio PMM buvo 5,85 proc. mažesnis nei kontroliniuose mėginiuose ($p < 0,05$). TYR kiekis buvo 72,63 proc. didesnis su vidutinio pažeidimo laipsnio PMM, lyginant su kontroliniais raumenų mėginiais ($p < 0,05$). Kitų biogeninių aminų kiekiai tarp grupių statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Biocheminio kraujo tyrimo metu nustatytas reikšmingas CK ir ALT aktyvumo padidėjimas serume broilerių, kuriems nustatyta „medinės krūtinėlės“ (angl. *wooden breast*) miopatija. ALT aktyvumas padidėjo 32,42 proc.,

o CK – 33,69 proc. ($p < 0,05$). Taip pat tokiems broileriams kraujo serume nustatyta didesnė 27,36 proc. kalio koncentracija ($p \leq 0,01$). Kitų kraujo serumo parametrų skirtumai nebuvo statistiškai reikšmingi tarp broilerių, turinčių miopatiją ir be jos ($p > 0,05$).

Broilerių didžiojo krūtinės raumens (lot. *m. pectoralis major*) mėginių histopatologinio tyrimo metu nustatyta, kad toks raumuo be PMM turėjo nedidelį kiekį susikaupusio riebalinio audinio ir skaidulinio jungiamojo audinio. Nustatyta, kad mėginiuose be PMM taip pat stebima raumenų skaidulų degeneracija ir nekrozė bei nestiprus intersticinis uždegimas, kartais aplink venas stebimas perivaskulitas. Raumenyse su vidutinio pažeidimo laipsnio PMM buvo nustatyta raumenų skaidulų degeneracija ir nekrozė. Nekrozuotos skaidulos infiltruotos mononuklearinių uždegiminių ląstelių, daugiausia makrofagų ir heterofilų. Retais atvejais stebima fibrozė, riebalinio audinio susikaupimas. Visame jungiamajame audinyje buvo limfocitų, makrofagų ir heterofilų. Stebimas ne tik perivaskulitas, bet ir vaskulitas.

Mėginiuose, kurie imti iš raumenų su sunkaus pažeidimo laipsnio PMM, beveik visos raumenų skaidulos nekrozuotos ir degeneruotos. Yra ir atrofuotų raumeninių skaidulų. Nekrozuojant raumenų skaiduloms jos pakeičiamos skaiduliniu jungiamuoju-riebaliniu audiniu. Stebima stipri fibrozė. Gausu uždegiminių ląstelių, makrofagų ir limfocitų, ryškus vaskulitas.

Spirmeno koreliacijos koeficientas parodė reikšmingą teigiamą ryšį tarp PMM pažeidimo laipsnio stiprumo ir degeneracijos/nekrozės ($r_s = 0,890$), skaidulinio jungiamojo audinio susikaupimo ($r_s = 0,782$) riebalinio audinio kaupimosi ($r_s = 0,617$), raumens uždegimo ($r_s = 0,748$) ir kraujagyslių uždegimo ($r_s = 0,637$) ($p < 0,001$).

Broilerių ALD raumens histopatologinės analizės metu buvo nustatyta, kad net ir be makroskopiškai matomos DCM, ALD raumenų mėginiuose buvo ženklus riebalinio audinio susikaupimas. Taip pat tokiuose mėginiuose buvo matoma raumens skaidulų degeneracija ir nekrozė. Stebima uždegiminių ląstelių infiltracija nekrozuotose raumens skaidulose ir aplinkiniame jungiamajame audinyje. Makroskopiškai nustatytuose DCM mėginiuose buvo daug skaidulinio jungiamojo audinio, ryški fibrozė. Raumenų skaidulų degeneracija, atrofija, makrofagai infiltruoja nekrozuotas skaidulas. Taip pat ryškus intersticinio audinio uždegimas, vaskulitas ir perivaskulitas. Raumenų su DCM paviršiuje yra fibrino ir kraujosruvų, taip pat matomos uždegiminės ląstelės, limfocitai ir makrofagai.

Spirmeno koreliacijos koeficientas parodė reikšmingą teigiamą ryšį tarp DCM ir degeneracijos/nekrozės ($r_s = 0,924$), skaidulinio jungiamojo audinio kaupimosi ($r_s = 0,917$), raumenų uždegimo ($r_s = 0,725$) ($p < 0,001$). Tačiau statistiškai reikšmingo ryšio tarp DCM ir riebalinio audinio kaupimosi ($r_s = 0,111$), kraujagyslių uždegimo ($r_s = 0,167$) nebuvo nustatyta ($p > 0,05$).

Histomorfometrinės analizės rezultatai parodė, kad raumenyje su sunkaus pažeidimo laipsnio PMM skaidulos buvo 15,2 proc. mažesnės, lyginant su vidutinio pažeidimo laipsnio PMM ($p < 0,001$), ir 9,9 proc. mažesnės nei raumenyje be miopatijų ($p < 0,05$). Didžiausias raumenų skaidulų skersmuo buvo nustatytas raumenyje su vidutinio pažeidimo laipsnio PMM. Tačiau lyginant raumens skaidulų skersmenį su vidutinio pažeidimo laipsnio PMM ir be miopatijų, statistiškai reikšmingo skirtumo nebuvo ($p > 0,05$). Gauti rezultatai parodė, kad raumenų mėginiuose su sunkaus pažeidimo laipsnio PMM raumeninio audinio kiekis buvo 10,5 proc. mažesnis, lyginant su vidutinio pažeidimo PMM ir 12,8 proc. mažesnis, lyginant su kontroliniais mėginiais ($p < 0,001$). Raumenų mėginiuose su sunkaus pažeidimo PMM skaidulinio jungiamojo audinio kiekis buvo 68,6 proc. didesnis nei mėginiuose su vidutinio pažeidimo PMM ($p < 0,01$) ir net 96,9 proc. didesnis, lyginant su miopatijų nepažeistais mėginiais ($p < 0,001$). Riebalinio audinio kiekis su sunkaus pažeidimo PMM buvo 1,7 karto didesnis, lyginant su vidutinio pažeidimo PMM ($p < 0,05$) ir 2,3 karto didesnis, lyginant su kontroliniais mėginiais ($p < 0,001$). Tačiau, remiantis mūsų tyrimo rezultatais, raumeninio, skaidulinio jungiamojo ir riebalinio audinio kiekiai raumenų mėginiuose su vidutinio pažeidimo laipsnio PMM, lyginant juos su kontroliniais mėginiais, statistiškai reikšmingai nesiskyrė ($p > 0,05$). Broilerių raumenų mėginiuose su DCM vidutinis raumenų skaidulų skersmuo buvo 10,47 proc. didesnis, lyginant su kontrolinės grupės mėginiais ($p < 0,01$). Vertinant raumens sudėtį, nustatyta, kad ALD raumenyse su DCM raumeninio audinio kiekis buvo 27,53 proc. mažesnis, lyginant su raumenų mėginiais be DCM ($p < 0,001$). Raumenų mėginiuose, imtuose iš broilerių su DCM, skaidulinio jungiamojo audinio kiekis buvo 2,1 karto didesnis, lyginant su kontrolinės grupės mėginiais ($p < 0,001$). Riebalinio audinio kiekis tarp grupių statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Išvados

1. Tyrimo metu Lietuvoje *Ross 308* genetinės linijos broileriams buvo nustatytas PMM pasireiškimas – 18,19 proc., DCM – 5,16 proc., o DPM – 0,27 proc. Įvertinus rizikos veiksnius nustatyta, kad PMM pasireiškimas buvo susijęs su broilerių kūno svoriu, amžiumi ($p < 0,001$) ir antimikrobiniu gydymu ($p < 0,05$). DCM pasireiškimas buvo susijęs su broilerių svoriu ($p < 0,05$) ir sezoniškumu ($p < 0,01$). Tarp analizuotų rizikos veiksnių svarbiausias miopatijų etiologijoje yra broilerių kūno svoris. Nustatyta, kad visos išskirtos bakterijos iš poodinio audinio, dengiančio raumenis su PMM ir DCM, priklauso natūraliai organizmo mikrobiotai.

2. Miopatijos turėjo įtakos mėsos fizikinėms ir cheminėms savybėms. Vištienos krūtinėlė su stipriai išreikšta PMM buvo geltonesnės spalvos ($p < 0,05$), pasižymėjo didesniais virimo nuostoliais ($p < 0,001$) ir vandeningumu ($p < 0,01$). Taip pat stipriai miopatijų paveiktoje krūtinėlėje buvo mažesnis sausųjų medžiagų kiekis ($p < 0,01$). PMM turėjo įtakos riebalų rūgščių ir lakiųjų organinių junginių sudėčiai vištienos krūtinėlės mėsoje. Aldehido heksanalio, kuris yra vienas iš pagrindinių lipidų peroksidacijos produktų, koncentracija buvo žymiai didesnė miopatijų pažeistame raumenyje ($p < 0,05$). Nustatyta, kad raumenyje su stipraus pažeidimo laipsnio PMM triptamino ($p < 0,01$) ir spermino ($p < 0,05$) kiekis buvo mažesnis nei mėginiuose be miopatijų.
3. Viščiukų broilerių, kuriems diagnozuota „medinės krūtinėlės“ (angl. *wooden breast*) miopatija, kraujo serume nustatytas padidėjęs CK aktyvumas bei kalio koncentracija rodo didžiojo krūtinės raumens (lot. *m. pectoralis major*) miocitų pažaidą. ALT aktyvumo padidėjimas siejamas su kepenų pažeidimu.
4. Raumenyse su PMM ir DCM degeneraciniai, nekroziniai pokyčiai, skaidulinio jungiamojo audinio kiekio padidėjimas ir uždegimo išplitimas buvo reikšmingai didesni lyginant su kontrolinėmis grupėmis be miopatijų ($p < 0,001$). Raumenyje su stipriai išreikšta PMM dažnai nustatytas limfocitinis flebitas. Raumenyje su stipriai išreikšta PMM riebalinio audinio kiekis buvo didesnis palyginti su kontroline grupe be miopatijų ($p < 0,001$). Tarp DCM ir kontrolinės grupių riebalinio audinio kiekis raumenyse statistiškai reikšmingai nesiskyrė ($p > 0,05$).
5. Raumenyje su stipria PMM, nustatyta išplitusi griaučių miocitų nekrozė, jų skersmuo buvo 9,9 proc., o raumeninio audinio kiekis buvo 12,8 proc. mažesnis, nei raumenyje be PMM. Taip pat, raumenyje su stipria PMM, skaidulinio jungiamojo audinio kiekis buvo net 96,9 proc., o riebalinio audinio kiekis net 2,3 karto didesnis, raumeninį audinį keitė skaidulinis jungiamasis ir riebalinis audiniai.
   ALD raumenyje (lot. *m. anterior latissimus dorsi*) su DCM vystėsi hipertrofiniai ir degeneraciniai pokyčiai, todėl griaučių miocitų skersmuo buvo 10,47 proc. didesnis, bet bendras raumeninio audinio kiekis buvo 27,53 proc. mažesnis palyginus su kontroline grupe. Dėl intensyvios fibrozės raumenyje su DCM skaidulinio jungiamojo audinio kiekis buvo net 2,1 karto didesnis, tuo tarpu riebalinio audinio kiekis tarp kontrolinės ir DCM grupių statistiškai reikšmingai nesiskyrė.

Praktinės rekomendacijos

1. Remiantis makroskopinės ir mikroskopinės analizės rezultatais, turėtų būti sukurta tinkama miopatijų klasifikavimo sistema. Klasifikacija prisidėtų prie ekonominių nuostolių mažinimo, užtikrintų patogų ir lengvai skerdyklose pritaikomą mėsos rūšiavimą.
2. Kreatinkinazė gali būti naudojama kaip miopatijų biologinis žymuo. Šio fermento tyrimai gali būti taikomi kaip papildomas įrankis miopatijų diagnostikoje dar gyviems broileriams prieš skerdimą.
3. Tolimesni viščiukų broilerių medžiagų apykaitos, kepenų funkcinės būklės ir žarnyno mikrobiotos tyrimai papildytų jau esamus tyrimus ir pateiktų vertingų įžvalgų apie broilerių miopatijų etiologiją ir patogenezę.

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2. **Lebednikaitė, E.**, Klupšaitė, D., Bartkienė, E., Klementavičiūtė, J., Mockus, E., Anskienė, L., Balčiauskienė, Žana, & Pockevičius, A. (2023). Fatty Acid Profile, Volatile Organic Compound, and Physical Parameter Changes in Chicken Breast Meat Affected by Wooden Breast and White Striping Myopathies. Animals, 13(19), 1-11. https://doi.org/10.3390/ani13193136 [S1] [M.kr.: A003] [Citav. rodiklis: 2.7, bendr. cit. rod.: 2.145, kvartilis: Q1 (2023. InCites JCR SCIE)]
3. **Lebednikaitė, E.**, Sutkevičienė, N., Vilkonienė, T., Balčiauskienė, Žana, Kučinskas, K., Anskienė, L., & Pockevičius, A. (2024). Serum Biochemical Parameters of Broilers Affected by Wooden Breast Myopathy. Animals, 14(10), 1-9. https://doi.org/10.3390/ani14101499 [S1] [M.kr.: A002] [Citav. rodiklis: 2.7, bendr. cit. rod.: 2.145, kvartilis: Q1 (2023. InCites JCR SCIE)]

LIST OF SCIENTIFIC CONFERENCE ABSTRACTS

1. **Lebednikaitė, E.**, Šiugždaitė, J., Balčiauskienė, Žana, & Pockevičius, A. (2022). Bacteria Isolated from Broilers Subcutaneous Tissue of Myopathy Affected Area. International Conference "Microbiota and Animal: Interaction, Health, Welfare and Production", the Conference Dedicated to the 30th Anniversary of the Research Center of Digestive Physiology and Pathology of the Department of Anatomy and Physiology of LSMU Veterinary Academy : Programme and Abstracts : Kaunas, 29 September, 2022 Lithuanian Academy of Sciences. Lithuanian University of Health Sciences. Veterinary Academy. Vilnius : Lithuanian Academy of Sciences, 2022. ISBN 9789986080893., 49-50. https://hdl.handle.net/20.500.12512/115742 [T1d] [M.kr.: A002]
2. **Lebednikaitė, E.**, Klementavičiūtė, J., Bartkienė, E., Klupšaitė, D., Mockus, E., Anskienė, L., Balčiauskienė, Žana, & Pockevičius, A. (2023). Fatty acid profile of broilers pectoralis major muscle affected by myopathy. 2nd International PhD Student's Conference Environment - Plant - Animal - Product (ICDSUPL) at the University of Life Sciences in Lublin : 19-20 April 2023, Lublin, Poland : Volume 2 Editors: Karazyna Ognik, Anna Stępniowska ; Doctoral School of the University of Life Sciences in Lublin. Doctoral Students Council of the University of Life. Sciences in Lublin. Lublin : Publishing House of the University of Life Sciences in Lublin, 2023. ISBN 9788372593900., 1-1. https://doi.org/10.24326/ICDSUPL2 [T1e] [M.kr.: A003, A002]
3. **Lebednikaitė, E.**, Bartkienė, E., Mockus, E., Klupšaitė, D., Klementavičiūtė, J., Anskienė, L., Balčiauskienė, Z., & Pockevičius, A. (2023). Volatile compounds profile characterization of pectoralis major muscle of broilers affected by myopathy. XXIInd Congress of the WVPA (World Veterinary Poultry Association) : Verona (Italy), September 4-8, 2023 : Book of Abstracts, 647-647. https://hdl.handle.net/20.500.12512/241059 [T2] [M.kr.: A002, A003]
4. **Lebednikaitė, E.**, Gudas, T., Cikanavičiūtė-Pučinskienė, G., & Pockevičius, A. (2024). Histomorphometry of broilers' pectoralis major muscle affected by myopathies. 3rd International PhD Student's Conference at the University of Life Sciences in Lublin, Poland: ENVIRONMENT – PLANT – ANIMAL – PRODUCT : 24 April 2024 Editors: Katarzyna Ognik, Anna Stępniowska, 1-1. https://doi.org/10.24326/ICDSUPL3.A017 [T1e] [M.kr.: A002]

CURRICULUM VITAE

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2020–2024	Doctoral (PhD) Postgraduate studies, Lithuanian University of Health Sciences, Faculty of Veterinary Medicine
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2012–2018	Master's degree of Veterinary Medicine, Lithuanian University of Health Sciences, Faculty of Veterinary Medicine

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PADĖKA

Dėkoju disertacijos moksliniam vadovui prof. dr. Aliui Pockevičiui už galimybę studijuoti doktorantūroje, už tikslingą ir nuoseklų tyrimų kuravimą.

Nuoširdžiai dėkoju AB „Vilniaus paukštyno“ veterinarijos gydytojai gerb. Žanai Balčiauskienei už geranoriškumą, konsultacijas, vertingus patarimus, sudarytas sąlygas ir nepamainomą pagalbą renkant mėginius disertacijos tyrimams.

Dėkoju visiems Patologijos centro dėstytojams už nuolatinį palaikymą ir įžvalgas. Už kraujo tyrimų atlikimą ir konsultacijas dėkoju Gyvūnų reprodukcijos laboratorijos vadovei dr. Neringai Sutkevičienei. Taip pat dėkoju doc. dr. Linai Anskienei už pagalbą atliekant statistinius skaičiavimus. Dėkoju Šarūnui už profesionalų ir kruopštų anglų kalbos redagavimą.

Ypatingai dėkoju mylimai mamai, kuri visad buvo šalia manęs, suteikė nuolatinį palaikymą, padrąsinimą ir emocinę paramą, kuri man ypatingai svarbi, suteikė stiprybės įveikti visus iššūkius. Tavo palaikymas, meilė ir tikėjimas tebepadeda man įveikti visus sunkumus.

Didžiulė padėka Kazimierui už gebėjimą visada praskaidrinti mintis, už kantrybę, tikėjimą ir palaikymą, už didžiulę empatiją ir širdį.

Esu dėkinga visiems savo draugams. Ypatingai doktorantėms Agnei, dr. Emilijai ir Kotrynai, nes doktorantūros kelią patyręs žmogus geriausiai supranta jo iššūkius. Draugų gebėjimas motyvuoti ir skatinti nepasiduoti buvo labai svarbus. Džiaugiuosi, kad Jus turiu. Ačiū, kad visad džiaugėtės mano pasiekimais ir padėjote, kai buvo sunku.

Dėkoju visiems, kurie buvo su manimi doktorantūros kelionėje, skatino nepasiduoti ir tikėti.