

A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles

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ABSTRACT In the past decade, the poultry industry has faced the occurrence of growth-related muscular abnormalities that mainly affect, with a high incidence rate, the Pectoralis major of the fast-growing genotypes selected for their production performances (high growth rate and breast yield). These myopathies are termed as White Striping, Wooden Breast, and Spaghetti Meat and exhibit distinctive phenotypes. A spatiotemporal distribution has been demonstrated for these disorders as in the early stage they primarily affect the superficial area in the cranial portion of the muscle and, as the birds grow older, involve the entire tissue. Aside from their distinctive phenotypes, these myopathies share common histological features. Thus, it might be speculated that common causative mechanisms might be responsible for the physiological and structural perturbations in the muscle associated with these conditions and might underpin their occurrence. The present review paper aims to represent a critical survey of the outcomes of all the histologic and ultrastructural observations carried out on White Striping, Wooden Breast, and Spaghetti

Meat affected muscles. Our analysis has been performed by combining these outcomes with the findings of the genetic studies, trying to identify possible initial causative mechanisms triggering the onset and the time-series of the events ultimately resulting in the development and progression of the growth-related myopathies currently affecting broilers Pectoralis major muscles. Several evidences support the hypothesis that sarcoplasmic reticulum stress, primarily induced an accumulation of misfolded proteins (but also driven by other factors including altered calcium homeostasis and accumulation of fatty acids), may be responsible for the onset of these growth-related myopathies in broilers. At the same time, the development of hypoxic conditions, as a direct consequence of an inadequate vascularization, triggers a time-series sequence of events (i.e., phlebitis, oxidative stress, etc.) resulting in the activation of response mechanisms (i.e., modifications in the energetic metabolism, inflammation, degeneration, and regeneration) which are all strictly related to the progression of these myopathic disorders.

Key words: white striping, wooden breast, spaghetti meat, initial causative mechanisms, time-series of events

2021 Poultry Science 100:101180

<https://doi.org/10.1016/j.psj.2021.101180>

INTRODUCTION

The occurrence of abnormalities affecting the *Pectoralis major* muscle (**PM**) of the fast-growing and high-breast yield broilers selected for meat production has been observed and studied for around 10 y leading to hypothesize a connection between the breeding procedures carried out in the past 50 y and the development of these myopathic conditions (Velleman, 2015; Papah et al., 2017; Petracci et al., 2019). Among them, the latest abnormalities are termed as White Striping

(**WS**), Wooden Breast (**WB**), and Spaghetti Meat (**SM**). Each myopathy shows distinctive phenotypes with the appearance of white striations of variable thickness (up to few mm) running parallel to the fiber direction in WS, the presence of out-bulging and pale areas of hardened consistency either in the cranial and/or in the caudal area of the muscle often associated with the presence of petechial hemorrhages in WB, exudate and viscous material, and an overall impaired integrity of the muscle whose fiber bundles tend to detach one from the others in SM, respectively (Petracci et al., 2019).

In a time-based approach, the appearance of white striations was the first condition observed by people working in commercial slaughterhouses all over the World followed by the macroscopic traits associated with WB and, only later on, by SM (Soglia et al., 2019a). This progression in the rise of those that are currently termed as growth-related abnormalities also reflects the topic of the research papers published in recent years. Indeed, the

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Received June 22, 2020.

Accepted March 22, 2021.

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Table 1. Temporal trend of peer reviewed papers published per year concerning White Striping, Wooden Breast and Spaghetti Meat myopathies affecting broilers' *Pectoralis major* muscles retrieved in Scopus¹ (original calculations based on data available in Scopus on March 14, 2021).

Year	Myopathy		
	White Striping	Wooden Breast	Spaghetti Meat
2009	1	-	-
2012	2	-	-
2013	4	-	-
2014	4	1	-
2015	6	6	-
2016	10	12	1
2017	8	17	-
2018	12	27	2
2019	27	33	8
2020	35	64	10
2021	9	22	0
Total	118	182	21

¹Including the terms “white striping”, “wooden breast” or “woody breast”, “spaghetti meat” either in the title or in the abstract of the paper.

outputs of the first study dealing with the histological characteristics of WS and their impact on meat quality have been published in 2009 (Kuttappan et al., 2009). Five years later, the microscopic features of the WB-affected muscles (which often exhibit also the appearance of white striations) have been deeply investigated (Sihvo et al., 2014), whereas the incidence of PM exhibiting SM defect has been described for the first time in 2016 (Sirri et al., 2016). From their onset, a significant number of studies have been carried out to improve the knowledge concerning PM affected by these growth-related myopathies as proven by the temporal trend of peer-reviewed papers retrieved in Scopus (Table 1).

As recently reviewed by Petracci et al. (2019), several studies have been carried out to establish the impact of

WS, WB, and SM on the quality traits and technological properties of the forthcoming meat. Researchers investigated whether the adoption of nutritional strategies and processing solutions may be useful in reducing the occurrence of these myopathic conditions or, at least, limit their detrimental effects on the quality of processed products. Furthermore, either microscopic and ultrastructural investigations have been performed trying to characterize the distinctive features associated with the occurrence of WS, WB, and SM and to identify eventual aberrations in the muscle structure or in its components which may play a role in their onset (Sihvo et al., 2014, 2017, 2018; Velleman, 2015; Velleman and Clark, 2015; Soglia et al., 2016b, 2020a; Papah et al., 2017; Baldi et al., 2018; Chen et al., 2019). Based on the findings of the histological studies, the existence of a common causative biological network of events triggering the myodegenerative processes found within the muscles affected by these conditions and underpinning their development might be speculated. Indeed, despite their distinctive phenotypes, WS, WB, and SM muscles share common histological features (Soglia et al., 2019a), which are summarized in Figure 1. These include a profoundly altered muscular architecture, the presence of hypercontracted fibers having rounded profile, nuclei internalization, and the occurrence of multifocal myofibril degeneration (up to necrosis) associated with occasional regeneration, splitting, and fragmentation of the myofibers (Kuttappan et al., 2009; Sihvo et al., 2014, 2017; Velleman and Clark, 2015; Baldi et al., 2018). In addition, a compromised connective tissue composing either the perimysial or the endomysial compartment has been observed along with fat and inflammatory cells infiltrations (Sihvo et al., 2014; Soglia et al., 2016b; Papah et al., 2017; Chen et al., 2019). Aside from sharing these common histological traits, WS, WB and SM affected muscles also exhibit peculiar


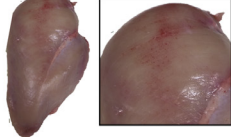

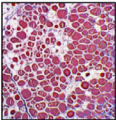
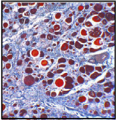
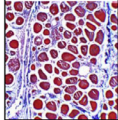
	White Striping	Wooden Breast	Spaghetti Meat
Phenotype			
Description	White striations of variable thickness running parallel to the myofiber direction	Out-bulging and pale areas having hardened consistency often exhibiting petechial hemorrhages	Loss of integrity and separation of the fiber bundles composing the tissue
Microscopic appearance			
Pathognomonic microscopic features	Increased deposition of lipid at perimysial level (lipidosis)	Proliferation of connective tissue at perimysial level up to fibrosis	Progressive rarefaction of the connective tissue composing the perimysial septa
Common histological traits	Profound modifications in the muscle architecture including the presence of fibers having rounded profile, nuclear rowing and internalization, hypercontracted fibers, degeneration up to lysis along with occasional regeneration, inflammatory cells infiltration, compromised perimysial septa		

Figure 1. Phenotype and microscopic features of the *Pectoralis major* muscles affected by White Striping, Wooden Breast, and Spaghetti Meat myopathic conditions.

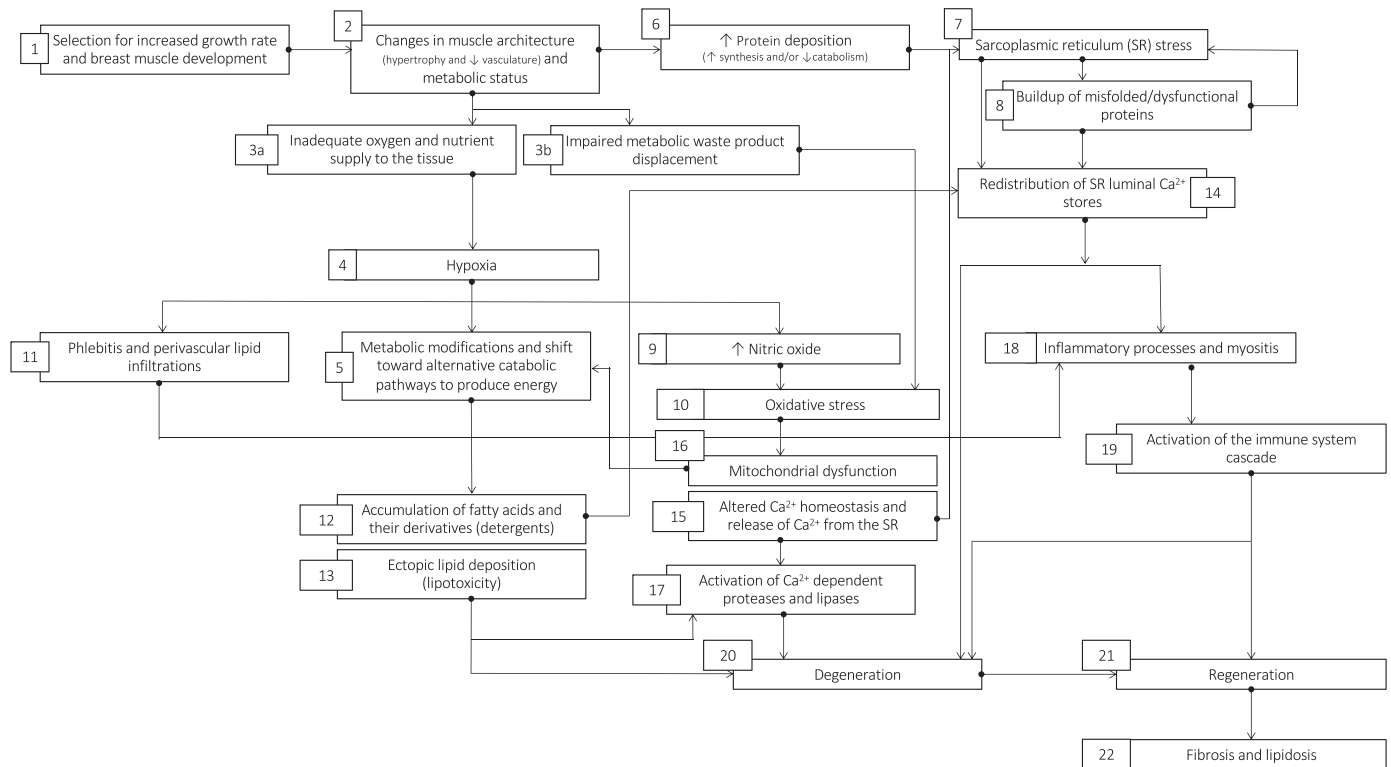


Figure 2. Schematic representation of the possible time series of events, molecular pathways and biological processes involved in the onset and triggering the occurrence of the White Striping, Wooden Breast, and Spaghetti Meat myopathies affecting broilers' *Pectoralis major* muscles.

microscopic features at the perimysial level (i.e., an abnormal deposition of adipose tissue, proliferation and thickening of the connective tissue, and a progressive rarefaction of the connective tissue) which may account for their respective distinctive phenotypes (Soglia et al., 2019a).

At the same time, several genetic studies were carried out through different approaches (i.e., RNA-sequencing, real-time quantitative PCR, microarray, heritability, and genetic correlation estimation, Genome-Wide Association study) to assess the presence of differentially expressed genes or to identify markers and genes involved or associated with the onset and the progression of these myopathic conditions (Mutryn et al., 2015; Velleman and Clark, 2015; Alnahhas et al., 2016; Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018, 2019; Brothers et al., 2019; Marchesi et al., 2019; Papah and Abasht, 2019; Soglia et al., 2020a). However, the outcomes of these studies did not reveal the existence of a major causative gene but rather support a polygenic inheritance of these defects (Pampouille et al., 2018) in substantial agreement with previous researches that analyzed the heritability and genetic correlation of these myopathies (Bailey et al., 2015; Alnahhas et al., 2016). In addition, it is worth mentioning that the majority of the genetic studies focused on WS and WB whereas, to the best of our knowledge, only one paper has been published by Soglia et al. (2020a) concerning gene expression in SM affected muscles.

Within this context, in order to provide a more comprehensive and clear representation of the complex etiology associated with the development of the myopathic conditions affecting broilers PM (i.e., WS, WB, and

SM), an overview of molecular pathways and biological processes on the whole involved in their occurrence has been delineated based on the outcomes available in the literature and reported in Figure 2. Thus, relying on the time sequence biological processes reported in the aforementioned scheme, the present review paper aims to reconsider in deep the complex etiology associated to the occurrence of these defects. Moreover, this review represents a critical survey of the outcomes of all the phenotypic, histologic, and ultrastructural characteristics described by different Authors for WS, WB, and SM affected muscles. Our analysis has been performed by combining these outcomes with the findings of the genetic studies, trying to identify possible initial causative mechanisms triggering the onset and the time-series of the events ultimately resulting in the development and progression of the growth-related abnormalities currently affecting fast-growing chickens' PM.

MUSCLE DEVELOPMENT UNDER NORMAL PHYSIOLOGIC CONDITIONS

Skeletal muscle development begins at embryonal level with the separation of mesoderm (i.e., the germinal layer from which the skeletal muscles originate) into blocks, called somites. Somites are transient structures (50 in chicks) producing the cells that will form vertebrae and ribs, dermis of the dorsal skin, and skeletal muscles (Gilbert, 2000). After differentiation, the somite cells result in the development of muscle cells precursors, the myoblasts (Grim, 1970). Embryonic myoblasts then migrate and

populate myoblast beds where they continue to proliferate. Primary myofibers are formed from the alignment of myoblasts leading to the development of multinucleated myotubes around which the remaining myoblasts continue to proliferate and fuse to generate secondary myofibers (Velleman, 2019). It is worth mentioning that muscle fiber formation, and as a consequence their number, is completed at hatching (Smith, 1963). Within this context, skeletal muscle growth taking place post-hatch is primarily the result of a satellite cells-mediated hypertrophic growth of the pre-existing fibers, as recently reviewed by Velleman (2015, 2019).

Changes in Muscle Architecture and Metabolic Status Induced by Genetic Selection

The selection practices carried out since 1950 to develop fast-growing and high-breast yield chicken strains allowed to achieve astonishing production performances (Figure 2-1) (Tixier-Boichard, 2020). Indeed, the current hybrids intended for meat production reach an average live weight of 3.5 kg (5 times larger if compared to that of the genotypes not selected for this purpose) with a breast yield of 25.8% in one-third of the time required at the 1957 ARBC Random Bred (Havenstein et al., 2003a, 2003b; Aviagen, 2019). This substantial progress has been obtained by inducing a hypertrophic growth of the fibers composing the PM (Dransfield and Sosnicki, 1999; Velleman, 2019) (Figure 2-2). In detail, if compared to those found in slow-growing genotypes, the fibers within the PM of fast-growing and high-breast yield broilers exhibit a remarkably enlarged diameter (Rémignon et al., 1996; Dransfield and Sosnicki, 1999). In agreement with that, significant differences have been found by comparing the expression profile of the genes involved in muscle growth and development in meat-type chickens divergently selected for body weight (Yin et al., 2014) thus supporting the genetic basis of the improved production performances in broiler chickens. Selection practices carried out to increase the development of the muscle and achieve fast growth-rates, distinctive traits of the modern broiler hybrids, have also affected the anabolic and catabolic processes of the muscular proteins which represent an important regulatory mechanism for defining muscle growth (Goll et al., 1992; Dransfield and Sosnicki, 1999). Concurrently, a reduction in muscle vascularization (Figure 2-2), as depicted by a decrease in heme pigment content along with a lower expression level of Myocardin (*MYOCD*) which may play a role in the differentiation of the smooth muscle cell lineage and vascularization, has been observed in fast-growing hybrids in comparison to slow-growing lines (Berri et al., 2001, 2007; Le Bihan-Duval et al., 2001; Pampouille et al., 2019; Tixier-Boichard, 2020). Intriguingly, contrasting results were previously found by assessing the fiber composition of PM in chickens belonging to different genotypes (Ashmore et al., 1972; Barnard et al., 1982; Horák et al., 1989). Indeed,

although some years ago no differences in fiber-typing have been reported by Rémignon et al. (1994), who hypothesized that this trait is only marginally affected by growth-rate, a shift toward a more glycolytic activity, resulting from a conversion from type IIA to IIB fibers, has been demonstrated to occur as a consequence of the selection practices for an increased mass accretion in pigs, double-muscled cattle and chickens (Ashmore et al., 1972; Hendricks et al., 1973; Ono et al., 1993). Besides that, alterations in muscle structure and biochemistry, as depicted by an increased plasma activity of the intracellular enzyme creatine kinase, have been observed in broiler chickens selected for their production performances (Rémignon et al., 1996; Dransfield and Sosnicki, 1999; Sandercock et al., 2006, 2009; Berri et al., 2007). In detail, the release of creatine kinase into the circulation may be a direct consequence of muscle damage (Sandercock et al., 2006) but may also follow the protein turnover closely related to the fast growth-rate of meat-type chickens (Berri et al., 2007). Within this context, impaired regulation of the cell cations level (i.e., increased sodium and calcium content along with an overall reduction in magnesium and potassium) have been observed in fast-growing birds and considered to underlie the initiation of the degenerative processes detectable in skeletal muscles affected by spontaneously arising abnormalities (Sandercock and Mitchell, 2004).

Aside from the remarkable changes in muscle architecture, the selection progress has also affected the energetic metabolism of the PM (Figure 2-5). Indeed, as previously mentioned, a shift toward type IIB fibers, using carbohydrates as a main source of energy, has been associated with the selection for mass accretion. In addition, a negative genetic correlation between glycogen reserves within the tissue and breast muscle development has been found in fast-growing broilers (Berri et al., 2001, 2007). These concurrently exhibit a reduced use of glycogen and a lower expression of genes (i.e., Protein phosphatase 1 regulatory subunit 3A, *PPP1R3A*) involved in glycogen metabolism (Pampouille et al., 2019). Taken together, these findings support the hypothesis that inducing a hypertrophic growth of the fibers, shifting their type toward those having glycolytic metabolism and concurrently depleting the glycogen stores within the tissue, genetic selection ultimately resulted in PM being more prone to the development of myopathic conditions.

TIME-SERIES EVENTS INVOLVED IN THE ONSET AND PROGRESSION OF MYOPATHIC CONDITIONS

Muscle Hypertrophy and Metabolism

As previously stated, the profound changes in muscle architecture and metabolism induced by the selection practices carried out to develop fast-growing and high-breast yield hybrids are currently considered among the

Table 2. Summary of the main histological evidences and differential expression of genes involved in determining muscle hypertrophy and metabolism in *Pectoralis major* muscles (PM) exhibiting myopathic conditions.

Main findings	References
Histological evidences	
correlation (+) between severity of myopathic conditions and body weight	Kuttappan et al., 2012; Petracci et al., 2013; Chen et al., 2019
correlation (+) between severity of myopathic conditions and breast muscle yield	Alnahhas et al., 2016;
↓ microvessel density and ↑ myofiber area per vessel number	Sihvo et al., 2018
↑ weight gain one or two weeks after hatch ↑ susceptibility to develop wooden breast	Papah et al., 2017
spatio-temporal distribution of myopathies primarily affecting the cranial portion of the muscle	Soglia et al., 2016b; Baldi et al., 2018; Chen et al., 2019; Soglia et al., 2019a
↓ histological lesions from the skin-facing surface towards the inner section of the PM	Soglia et al., 2016b; Clark and Velleman 2017; Baldi et al., 2018
Gene expression	
↑ oxygen carriers	Pampouille et al., 2019

main predisposing factors responsible for the onset of muscle disorders including WS, WB, and SM in broilers (Alnahhas et al., 2016; Chen et al., 2019). The histological traits and the main findings concerning the differential expression of genes involved in determining hypertrophy and metabolism in PM exhibiting myopathic conditions are summarized in Table 2.

Myofiber Density and Hypertrophy It has been demonstrated that those birds having particularly high weight gain 1 or 2 wk after hatch are more prone to develop WB thus suggesting a role of the rapid increase in body weight in the pathogenesis of this defect (Papah et al., 2017). It is noteworthy to highlight that, if compared to other intramuscular positions, a higher myofiber density has been observed in the cranial region of the PM (Smith and Fletcher, 1988), which is the portion primarily and more severely affected by WS, WB, and SM defects (Chen et al., 2019; Soglia et al., 2019a). This increase in myofiber number may further exacerbate the insufficient oxygen and nutrient supply to the tissue (which readily occur as a consequence of the hypertrophic growth of the fibers and of the reduced vasculature within the muscle), and this condition, leading to poor drainage and impaired displacement of metabolic waste products, may trigger the development of myopathic disorders (Figure 2-3a,b). In agreement with this hypothesis, a spatiotemporal distribution of these defects has been demonstrated as WS, WB and SM primarily affect the skin-facing surface of the cranial portion of the muscle and, only in a later phase, involve the entire muscular tissue (Soglia et al., 2016b; Baldi et al., 2018; Chen et al., 2019).

Vascular Development A study has been carried out to assess microvessels density in unaffected muscles as well as in those exhibiting focal WB (i.e., hardened consistency enclosed in the cranial portion of the PM with the caudal region being unaffected) in young (18 and 24 days old) and market-age broilers (Sihvo et al., 2018). These Authors observed a significant reduction in the blood vessel number along with a relevant increase in the myofiber area per vessel number in birds exhibiting focal WB. This result suggests a direct, or eventually an indirect, involvement of capillaries and blood supply in the progression of the WB condition. In agreement with that, an overexpression of genes encoding for

oxygen carriers has been observed in WS and WB thus corroborating the hypothesis of insufficient oxygen supply to the muscle tissue (Figure 2-3a) (Pampouille et al., 2019). In addition, the physiology of the PM itself (i.e., its thickness in correspondence to the cranial area) may further reduce the oxygenation and nutrients supply to the tissue. This hypothesis is supported by the evidence that the severity of the histological lesions associated with the occurrence of myopathic disorders, gradually decreases moving from the skin-facing surface toward the inner section of the muscle (Soglia et al., 2016b, 2017; Clark and Velleman, 2017; Baldi et al., 2018; Petracci et al., 2019). Taken together, these findings highlight the relevant role of the profound changes in muscle architecture induced by the selection in triggering an insufficient oxygen and nutrient supply to tissue (Figure 2-3a) along with an ineffective metabolic waste product removal (Figure 2-3b), which ultimately can lead to hypoxic condition (Figure 2-4).

Hypoxia

Hypoxia is considered among the main conditions triggering either the onset or the progression of myodegeneration affecting broilers' PM (Figure 2-4). Several studies have been conducted to better understand its consequences on muscle histology and functionality as well as on the differential expression of genes triggering and related to the development of hypoxia in PM exhibiting myopathic conditions (Table 3).

Studies carried out to assess the metabolic features and the eventual differences in the transcriptome profile have revealed clear evidences of hypoxia in market-age broilers exhibiting myopathic disorders (Mutryn et al., 2015; Abasht et al., 2016). In detail, several genes regulated by the Hypoxia-inducible factor 1 alpha (*HIF1A*) have been found differentially expressed in WB and/or WS affected muscles (Mutryn et al., 2015; Malila et al., 2019; Marchesi et al., 2019). Indeed, once activated by the occurrence of a low-oxygen condition, this transcription factor is responsible for regulating many other genes involved in different biological pathways (i.e., energy metabolism, cell proliferation, apoptosis, angiogenic, and proangiogenic

Table 3. Summary of the main histological evidences, molecular traits and differential expression of genes triggering and related to the development of hypoxia in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
neovascularization (i.e., presence of tubular structures)	Sihvo et al., 2017
mitochondrial hyperplasia and ultrastructural modifications, sarcoplasmic reticulum enlargement	Sihvo et al., 2018
phlebitis and venous damage	Papah et al., 2017; Papah et al., 2018; Chen et al., 2019; Lake et al., 2020
Molecular traits	
↑ synthesis of nitric oxide, ↑ taurine content,	Boerboom et al., 2018
↑ long- and ↑ medium-chain fatty acids	Soglia et al., 2016a; Boerboom et al., 2018; Gratta et al., 2019
Gene expression	
↑ HIF-1 alpha and ↑ HIF-1-dependent genes involved in ECM remodeling, glycolytic pathway, energy metabolism, angiogenesis and apoptosis	Mutryn et al., 2015; Malila et al., 2019; Marchesi et al., 2019; Bordini et al., 2021
↑ genes in response to ROS	Zambonelli et al., 2016

Abbreviations: ECM, extracellular matrix; ROS, reactive oxygen species.

processes) to counteract the development of hypoxic conditions within the tissue (Mutryn et al., 2015; Malila et al., 2019; Marchesi et al., 2019). According to this, evidences of neovascularization, as depicted by the presence of tubular structures, have been observed at histological level in WB, thus supporting the hypothesis that hypoxia promotes the development of new vessels (Sihvo et al., 2017). In addition, the downregulation of Interleukin 1 beta (*IL-1β*) observed in WS/WB muscles belonging to marketable age broilers (Zambonelli et al., 2016) might corroborate the hypothesis of a reduced vascularization underpinning the occurrence of these myopathic conditions. Indeed, being directly involved in the synthesis of proinflammatory cytokines, *IL-1β* exerts a relevant role in inducing and supporting angiogenesis (Voronov et al., 2014). In addition, the increased expression level of 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 (*PFKFB4*), L-lactate dehydrogenase A chain (*LDHA*) and Phosphorylase kinase regulatory subunit beta (*PHKB*) (all genes encoding enzymes involved in the glycolytic pathway) observed in WS and WB strongly suggest the activation of a response mechanism of the PM against hypoxia involving an attempt to catalyze glycogen breakdown (Malila et al., 2019; Zhang et al., 2020) (Figure 2-5). In addition, the HIF1-dependent upregulation of Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 (*PLOD2*) found in WB correlates with the microscopic features (i.e., fibrosis) observed within the muscles affected by this condition (Mutryn et al., 2015).

It is interesting to highlight that, based on the findings of a time-series evaluation of the histological features in WB, the development of hypoxic conditions has been hypothesized to take place as early as during the first week of age of the broilers, and that the severity of the lesions resulting from it gradually increases during animals' growth (Papah et al., 2017).

Mitochondrial and Sarcoplasmic Reticulum Alterations

Intriguingly, the first ultrastructural changes detected in the early phase of WB (in 22-day-old broilers) involve

the mitochondria and the sarcoplasmic reticulum (Sihvo et al., 2018). Mitochondrial hyperplasia and changes in their morphological traits have been observed in either focal or diffuse WB cases and ascribed to the development of hypoxic conditions and osmotic imbalances (Papah et al., 2017; Sihvo et al., 2018) (Figure 2-4). Similarly, the remarkable enlargement of the sarcoplasmic reticulum observed in myopathic muscles is likely the result of an excessive entrance of water into the cell to counteract the osmotic imbalances. This ultrastructural change may be deemed as a secondary effect of hypoxia (Sihvo et al., 2018) (Figure 2-4) or concurrently attributed to the potential buildup of deleterious misfolded proteins within this cellular compartment (Figure 2-8) (Papah et al., 2018). Indeed, the impressive development of the breast muscles achieved by the modern chicken hybrids surely needs to be supported by an increased protein deposition (Figure 2-6) which is the result of either an increased anabolism itself or of a reduction in the catabolic processes affecting the polypeptide chains (Dransfield and Sosnicki, 1999; Vignale et al., 2017; Papah et al., 2018). This increase in protein synthesis potentially overburdens the sarcoplasmic reticulum (Figure 2-7), which is responsible for monitoring and optimizing protein folding (Kaufman, 1999; Osowski and Urano, 2011). Within this context, the downregulation of genes involved in the EIF2 signaling pathway (considered a regulatory response to inhibit protein synthesis which overcomes the capability of the sarcoplasmic reticulum to manage), along with reduced expression of those involved in the ubiquitin-proteasome pathway observed in WB as early as three weeks post-hatch, suggests the buildup of deleterious misfolded or dysfunctional proteins (Papah et al., 2018) (Figure 2-8). It may thus be speculated that the presence of unfolded proteins within the sarcoplasmic reticulum may play a role in impairing its functionality and inducing sarcoplasmic reticulum stress (Figure 2-7) (Boncompagni et al., 2020), which may represent one of the most important underpinning and causative factors responsible for the early ultrastructural changes affecting this cellular compartment in WB.

As a response mechanism against hypoxic conditions, the muscle tries to increase the blood perfusion to the

tissue, as depicted by the enhanced arginine conversion to citrulline, leading to the production of nitric oxide observed in WS (Boerboom et al., 2018) (Figure 2-9). However, this compensatory mechanism is limited since molecular oxygen is needed for nitric oxide synthesis and broilers cannot synthesize arginine but are dependent on its dietary supplementation (Galkin et al., 2007; Khajali and Wideman, 2010). In addition, it has to be mentioned that, because of its free radical nature and its ability to inhibit mitochondrial respiration (by binding to cytochrome c oxidase in competition with oxygen) (Galkin et al., 2007), the increased synthesis of nitric oxide may be considered among the factors triggering the development of oxidative stress in myopathic muscles (Figure 2-10). Furthermore, it has been demonstrated that nitric oxide can promote mitochondrial biogenesis (Nisoli and Carruba, 2006; Galkin et al., 2007; Wadley and McConell, 2007). As mitochondrial hyperplasia has been mentioned among the first ultrastructural changes associated with the development of myopathic disorders (i.e., WB) (Sihvo et al., 2018), it seems reasonable, therefore, to ascribe it to the increased synthesis of this compound within the tissue taking place as a response mechanism to counteract hypoxia.

At molecular level, the increased taurine content observed in WS (Boerboom et al., 2018), exerting a role in protecting the tissue against the damages induced by hypoxic conditions, may further compromise the blood perfusion to the muscle itself (Mankovskaya et al., 2000) (Figure 2-3a). Indeed, taurine is a compound with distinctive osmotic properties, and its accumulation may result in tissue swelling as well as in a compression of the blood vessels.

Phlebitis and Vascular Damage

Vascular involvement in the progression of the myopathic disorders affecting broilers PM has been demonstrated at marketable age through either microscopic (Sihvo et al., 2014, 2017; Chen et al., 2019) and gene expression studies (Papah et al., 2018; Brothers et al., 2019) (Figure 2-11). A summary of the main findings is reported in Table 4. Intriguingly, evidences of hemodynamic perturbations, as depicted by the presence of multifocal perivascular and perivenous macrophages infiltrations, have been observed as early as 18 d of age

and in 1-week-old broilers (Papah et al., 2017; Sihvo et al., 2017). As phlebitis is the first event taking place before the development of any other microscopic lesion in young broilers, vascular involvement has been hypothesized to play a relevant role in triggering the time-series of events ultimately resulting in the development of myopathic disorders. However, it has to be mentioned that the occurrence of lymphocytic phlebitis has been identified even in an unselected chicken strain (Chen et al., 2019). In addition, the same Authors have found evidence of extensive phlebitis without any microscopic lesion associated with WB. Hence, it could be speculated that, in PM already prone to these tissue changes, the impaired venous drainage resulting from phlebitis and edema may induce perturbations to the physiological and structural functions of the tissue (i.e., accumulation of metabolic wastes) ultimately triggering the development of inflammatory processes and muscle damage (Velleman, 2015; Papah et al., 2017). In detail, a high expression of the gene encoding for lipoprotein lipase (a rate-limiting enzyme catalyzing triglyceride hydrolysis) has been observed in capillaries and within the venous endothelia in WB (Lake et al., 2019; Papah and Abasht, 2019). This enhanced hydrolytic activity has been hypothesized to increase the permeability of the venous endothelia to either free fatty acids and/or inflammatory cells that, ultimately, contribute to the development of lymphocytic phlebitis associated with the development of myopathic disorders affecting the PM (Papah and Abasht, 2019). It is noteworthy to point out that, through RNA-seq analysis it has been possible to demonstrate that, if compared to females, male broilers, which grow faster, are more prone to the development of vascular damages and, concurrently, exhibit a remarkably reduced angiogenic activity (Brothers et al., 2019). In detail, the upregulation of several genes involved in angiogenic processes observed in myopathic muscles highlights the attempt of the tissue to maintain vascularization following muscle damage (Brothers et al., 2019; Marchesi et al., 2019; Pampouille et al., 2019).

Taken together, these observations further highlight the importance of a proper functionality of the vascular system perfusing the PM and the need for a more adequate vascularization supporting the impressive growth of the muscle tissue itself.

Table 4. Summary of the main histological evidences and differential expression of genes triggering and related to the development of phlebitis and vascular damage in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
hemodynamic perturbations including multifocal perivascular and perivenous macrophages infiltrations in young broilers (18 d of age and 1-week-old)	Papah et al., 2017; Sihvo et al., 2017
oedema, perivenous to transmural infiltrations of lymphocytes and inflammatory cells along with endothelial cells hypertrophy in marketable age broilers	Sihvo et al., 2017; Papah et al., 2017; Chen et al., 2019
Gene expression	
↓ interleukin 1 beta involved in angiogenesis	Zambonelli et al., 2016
↑ genes related to atherosclerosis and arteriosclerosis processes	Papah et al., 2018
↑ lipoprotein lipase (<i>LPL</i>) in capillaries and within the venous endothelia	Lake et al., 2019; Papah and Abasht, 2019
↑ genes involved in angiogenic process	Brothers et al., 2019; Marchesi et al., 2019; Pampouille et al., 2019
↑ antiangiogenic genes	Brothers et al., 2019

Table 5. Summary of the main molecular traits and differential expression of genes triggering and related to perturbations in the energetic metabolism in *Pectoralis major* muscles (PM) exhibiting myopathic conditions.

Main findings	References
Molecular traits	
↓ intermediates and ↓ end products of the glycolytic pathway	Abasht et al., 2016; Malila et al., 2019; Soglia et al., 2019b; Baldi et al., 2020
≠ enzymes related to glycolysis: ↑ lactate dehydrogenase, ↑ glyceraldehyde dehydrogenase ↑ glycogen phosphorylase ↓ magnesium-dependent enzymes	Zambonelli et al., 2016; Zhang et al., 2020
↓ proteins related to glycogen degradation, pyruvate to lactate conversion, tricarboxylic acid cycle	Kuttappan et al., 2017
shunt from the glycolytic pathway toward amino acids catabolism and lipid oxidation to produce energy	Abasht et al., 2016
↑ synthesis of molecules composing ECM including proteoglycans, glycosaminoglycans and glycoproteins	Abasht et al., 2016; Zambonelli et al., 2016; Papah et al., 2018
↑ metabolites involved in pentose phosphate pathway	Abasht et al., 2016
↑ long- and ↑ medium-chain and ↑ monounsaturated fatty acids	Soglia et al., 2016a; Abasht et al., 2016; Boerboom et al., 2018; Gratta et al., 2019
Gene expression	
shunt from the glycolytic pathway toward amino acids catabolism and lipid oxidation to produce energy	Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2019; Brothers et al., 2019; Lake and Abasht, 2020
↑ glycolytic activity in WS affected muscle	Malila et al., 2019
↓ genes encoding for glycolytic enzymes in WB and WS/WB	Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018; Malila et al., 2019
↑ genes involved in the pentose phosphate pathway	Zambonelli et al., 2016; Pampouille et al., 2019
↓ bioenergetic capacity of the PM	Papah et al., 2018
≠ Fiber-typing	Mutryn et al., 2015; Zambonelli et al., 2016; Lake et al., 2019; Papah and Abasht, 2019;
↑ genes involved in lipid uptake and transport	Papah et al., 2018; Brothers et al., 2019; Papah and Abasht, 2019; Marchesi et al., 2019
≠ lipid metabolism	Brothers et al., 2019; Papah and Abasht, 2019; Lake et al., 2019
↑ genes involved in adipogenesis	Marchesi et al., 2019

Abbreviations: ECM, extracellular matrix; WS, White Striping; WB, Wooden Breast.

Metabolic Modifications

Evidences of relevant perturbations in the energy metabolism have been observed in myopathic PM either at molecular level (Abasht et al., 2016; Boerboom et al., 2018; Soglia et al., 2019b) and by assessing the eventual differential expression of the genes involved in energy-generating pathways (Papah et al., 2018; Brothers et al., 2019; Marchesi et al., 2019; Pampouille et al., 2019; Lake and Abasht, 2020). The available information on the molecular traits and the main findings concerning the differential expression of genes triggering and related to perturbations in the energetic metabolism of PM exhibiting myopathic conditions are summed up in Table 5.

Glycolysis and Glucose Metabolism An overall reorientation of the energy metabolism (Figure 2-5), shunting from the glycolytic pathway, toward the use of amino acid catabolism and lipid oxidation to produce energy has been found (Abasht et al., 2016; Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2019; Lake and Abasht, 2020). This re-routing may reflect the progressive reduction in glycogen (as well as in the ability of the muscle to store it) associated with the selection practices for fast growth-rate and increased breast-yield (see paragraph Changes in Muscle Architecture and Metabolic Status Induced by Genetic Selection) (Beauclercq et al., 2017; Pampouille et al., 2019), which are further exacerbated in myopathic muscles. Alternatively, as previously

mentioned, these modifications in muscle metabolism may be ascribed to the development of hypoxic conditions (Figure 2-4), and thus to the expression of *HIF1A*, which, in turn, is able to affect the transcription of genes involved in energy-generating pathways (i.e., glycolysis and gluconeogenesis) (Malila et al., 2019). Aside from a *HIF1A*-induced increased transcription of *PFKFB4*, *LHDA*, and *PHKB* in WS, the same Authors have observed an opposite result in WB. Thus, it can be speculated that the progression of WB condition may trigger a different response mechanism involving a shunt of glucose toward other metabolic pathways rather than the attempt of the PM to enhance the glycolytic activity of the tissue itself. Consistent with an overall reduced glycolytic activity of the muscle, a relevant decrease in the expression of genes encoding for glycolytic enzymes has been observed in PM exhibiting myopathic disorders (i.e., WB and WS/WB) (Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018; Malila et al., 2019). However, different results come out when the outcomes of gene expression are compared to the translation level of the enzymes involved in the glycolytic pathway. Indeed, a higher content of lactate dehydrogenase, glyceraldehyde dehydrogenase, and glycogen phosphorylase along with an overall reduction in the synthesis of magnesium-dependent enzymes have been observed in PM concurrently affected by WS and WB (Zambonelli et al., 2016; Zhang et al., 2020). On the other hand, a downregulation of proteins related to glycogen degradation, pyruvate to lactate conversion, and tricarboxylic acid

cycle has been observed in WB (Kuttappan et al., 2017). Aside from differences that may be ascribed to the progression of one myopathic disorder rather than another, this discrepancy may also be attributed to the analytical approach adopted. Beyond that, an overall reduction in the content of both intermediates and end products of the glycolytic pathway has been observed in marketable age broilers (Abasht et al., 2016; Malila et al., 2019; Soglia et al., 2019b; Baldi et al., 2020). However, the higher carbohydrate (i.e., glucose and fructose) level found in 3-week-old broilers along with the upregulation of genes involved in the hexosamine biosynthesis pathway and glycosylation (Papah et al., 2018) support the hypothesis of an increased glucose flux toward the synthesis of molecules composing the Extracellular Matrix (ECM) including proteoglycans, glycosaminoglycans and glycoproteins (Abasht et al., 2016; Zambonelli et al., 2016; Papah et al., 2018) (Figure 2-5). In that sense, it is worth mentioning that high glucosamine levels (an intermediate product of the hexosamine biosynthesis pathway) have been demonstrated to induce sarcoplasmic reticulum stress (Figure 2-7), ultimately leading to the activation of the Unfolded Protein Response (UPR) as a self-defense mechanism to maintain homeostasis (Schröder and Kaufman, 2005) (see paragraph Sarcoplasmic reticulum stress and UPR activation). In addition to that, an increase in the metabolites or in the expression of genes involved in the pentose phosphate pathway has been observed (Abasht et al., 2016; Zambonelli et al., 2016; Pampouille et al., 2019).

Sarcoplasmic Reticulum Stress and UPR Activation The UPR activation and alterations in the sarcoplasmic reticulum are among the first ultrastructural changes taking place in the early phase of WB. These evidences shed the light on the potential role of stressful conditions (Figure 2-7) within this cellular compartment in triggering the onset and progression of myopathic changes affecting broilers' PM. Within this context, activation of UPR signaling network may be a direct consequence of the accumulation/aggregation of misfolded or defective proteins at luminal level leading to endoplasmic reticulum stress (see paragraph Antioxidant systems, Mitochondrial and sarcoplasmic reticulum alterations). A similar cellular response, intended to maintain proteostasis and preserve the functionality of the endoplasmic reticulum, has been previously demonstrated in different organs and considered as an important factor triggering fibrotic remodeling in various tissues (Tanjore et al., 2013; Hetz and Papa, 2018; Kropski and Blackwell, 2018). Furthermore, UPR activation was found to affect several cellular mechanisms by activating proinflammatory transcription factors, modifying the metabolic pathways involved in energy production, and inhibiting protein synthesis (van der Harg et al., 2017; Kropski and Blackwell, 2018; Afroze and Kumar, 2019). In detail, the inhibition of glycolytic pathway along with the alterations in mitochondrial metabolism found in association with a prolonged activation of UPR signaling (van der Harg et al., 2017) overlaps with the aforementioned metabolic alterations

observed in broilers' PM affected by growth-related myopathies (see paragraph Glycolysis and glucose metabolism).

Mitochondrial Functionality Aside from the aforementioned rerouting of the energy metabolism, evidences of alterations in the mitochondrial cellular respiration, as depicted by the expression profile of genes involved in the synthesis of electron transport chain components in mitochondria, has been found in myopathic muscles (Zambonelli et al., 2016; Pampouille et al., 2019). In agreement with that, the downregulation of citrate synthase and oxoglutarate dehydrogenase observed in 3-week-old broilers exhibiting WB myopathy allowed to hypothesize an overall reduced bioenergetic capacity of the PM itself (Papah et al., 2018) (Figure 2-16). Also, as citrate synthase is considered a reliable biomarker of the mitochondrial content in the muscle, the same Authors ascribed its reduced expression in WB to a lower presence of these intracellular organelles. However, this hypothesis is questionable as mitochondrial hyperplasia has been recognized among the first ultrastructural alterations associated with the development of WB (Sihvo et al., 2018). Therefore, the lower expression of citrate synthase observed within WB muscles is likely the result of the profound ultrastructural perturbations observed within the mitochondria (i.e., degeneration of cristae) (Figure 2-16) impairing their functionality, rather than to an overall reduction in their content.

Fiber Type Switching Based on the results obtained through gene expression and proteomics, the occurrence of fiber type switching has been observed in myopathic muscles belonging to broilers at marketable age (Mutryn et al., 2015; Zambonelli et al., 2016) or at 3 wk of age (Lake et al., 2019; Papah and Abasht, 2019). To the best of our knowledge, it is not clear whether this phenomenon can be considered a response mechanism toward the onset of the abnormalities, or rather plays a relevant role in triggering the muscle energy metabolism dysregulations responsible for the progression of the degenerative processes associated with the myopathic conditions affecting the PM (i.e., WS, WB, and SM). In detail, the appearance of type I fibers within the PM has been readily observed in 3-week-old broilers and the expression of the genes related to this type of muscle fibers was proved to progressively increase with the age, as the severity of the myopathic condition worsens (Papah and Abasht, 2019). As hypothesized by Papah & Abasht (2019), the overexpression of the slow-twitch myofiber-related genes may be induced by a stretch overload occurring as a consequence of the fast growth-rate of the PM over a short time. Intriguingly, this hypothesis may be further corroborated by the longer sarcomeres observed within the myofibers composing either the lesion or non-lesion site of WB muscles (Tijare et al., 2016; Sun et al., 2018; Tonniges et al., 2018; Soglia et al., 2020b) that have been ascribed to the development of an increased tear within the sarcomeric structure ultimately resulting in overly-stretched sarcomeres (Soglia et al., 2020b).

Lipid Metabolism Along with the presence of slow-twitch fibers, a concurrent upregulation of the genes involved in lipid uptake and transport has been observed in either 3-week-old (Papah et al., 2018; Brothers et al., 2019; Papah and Abasht, 2019) and marketable age broilers (Marchesi et al., 2019). Functional analysis of these genes suggests that an active intracellular mobilization of lipid and their intracellular storage/accumulation may be involved in the early pathogenesis of WB (Papah et al., 2018; Brothers et al., 2019; Lake et al., 2019) (Figure 2-12 and 13). Among the differentially expressed genes, fatty acid translocase (*CD36*, primarily involved in free fatty acids transportation into the cytoplasm), fatty acid binding protein 4 (*FABP4*, a fatty acid carrier playing a major role in fat accumulation), lipoprotein lipase (*LPL*, a biomarker of adipogenesis) and perilipin 2 (*PLIN2*, responsible for intracellular lipid storage) seem to play a major role in triggering a dysregulation in the lipid metabolism associated with the myopathic disorders. Intriguingly, the expression of the aforementioned genes (i.e., *CD36*, *FABP4*, *LPL*, and *PLIN2*) is strictly regulated by the Peroxisome proliferator-activated receptor alpha that, on its turn, has been predicted to be upregulated by ingenuity pathway analysis (Brothers et al., 2019). As this ligand-dependent nuclear receptor also plays a key role in regulating peroxisomal beta-oxidation (Kersten et al., 2001), its upstream activation in myopathic muscles further corroborates the hypothesis of a metabolic shift and fiber type switching from type IIB toward the slow myofibers which use lipids as the main source of energy (Figure 2-5). In addition to that, it is worth noting that the increased expression of genes involved in adipogenesis (including Rho associated coiled-coil containing protein kinase 2, *ROCK2*) perfectly matches with the appearance of white striations representing a distinctive trait of WS condition (Marchesi et al., 2019). However, despite the upregulation of genes involved in lipid mobilization within the intracellular compartments, evidences of failure in mitochondrial beta-oxidation as depicted by the accumulation of long- and medium-chain fatty acids have been observed in myopathic muscles (i.e. WS and/or WB) (Soglia et al., 2016a; Boerboom et al., 2018; Gratta et al., 2019). The accumulation of these compounds and their derivatives likely occurs as a consequence of the development of hypoxic conditions which inhibit the mitochondrial beta-oxidation as well as the activity of the respiratory chain (Van der Vusse et al., 1992; Boerboom et al., 2018). Indeed, mitochondrial dysfunctions reasonably resulting from the profound alterations observed in their ultrastructure (Sihvo et al., 2018), likely contribute to impair beta-oxidation taking place within these organelles. In detail, the acute accumulation of free fatty acids in myopathic muscles and, in particular, of monoacylglycerols and long-chain fatty acids (Soglia et al., 2016a; Abasht et al., 2016; Boerboom et al., 2018; Gratta et al., 2019), may exert detrimental effects on the cellular membrane due to their strong detergent properties and may affect its integrity and functionality

(Tumova et al., 2016) (Figure 2-12). Furthermore, an excessive accumulation of triglycerides (i.e. ectopic lipid deposition) within the PM in young chickens, which under physiological conditions contain only a small amount of stored fat, may exert a lipotoxic activity, thus contributing to the development of myopathic disorders affecting fast-growing broilers (Lake et al., 2019) (Figure 2-13). In detail, a substantial redistribution of the luminal Ca^{2+} stores within the sarcoplasmic reticulum (i.e. from the sarcoplasmic reticulum to the sarcoplasm and among other subcompartments of this organelle itself) has been demonstrated to take place as a consequence of the chronic exposure to elevated free fatty acids concentrations in different cell types (Wei et al., 2009) (Figure 2-14). Thus, considering the remarkable increase in monounsaturated fatty acids observed in myopathic muscles (Soglia et al., 2016a; Abasht et al., 2016; Boerboom et al., 2018; Gratta et al., 2019), it might be speculated that a similar mechanism may underlie the development of sarcoplasmic reticulum stress (Figure 2-7) thus contributing to the cascade of events ultimately responsible for the inflammatory response and degenerative processes taking place within the PM.

Oxidative Stress

Oxidative stress (Figure 2-10) results from the accumulation of Reactive Oxygen Species (**ROS**) generated through metabolic reactions outpacing the antioxidant defenses of the PM. In addition, mitochondrial dysfunctions have also been associated with increased ROS content within the tissue (Balaban et al., 2005) (Figure 2-16), thus corroborating the hypothesis of an impaired functionality of these organelles in myopathic PM.

Biomarkers related to oxidative stress have been identified in myopathic PM either at protein (Abasht et al., 2016; Boerboom et al., 2018; Soglia et al., 2019b) and gene levels (Mutryn et al., 2015; Zambonelli et al., 2016; Malila et al., 2019; Pampouille et al., 2019), and the main findings are summarized in Table 6.

Overall, an accumulation of ROS is detrimental to the muscle tissue as it may result in impaired sarcolemmal integrity, affect the contractile ability of the myofibers, and induce the activation of stress response-related pathways (Allen et al., 2008). At molecular level, evidences of the exposure to free radical compounds, as depicted by the presence of lipid peroxidation products, have been observed in WS and WB (Abasht et al., 2016; Salles et al., 2019). In agreement with that, an upregulation of Transient receptor potential cation channel, subfamily A, member 1 (*TRPA1*) observed in myopathic muscles evidences the attempt of PM to counteract the accumulation of reactive compounds responsible for oxidative stress (Mutryn et al., 2015). Similarly, given the higher copper content observed in these muscles, an increased activity of the enzyme superoxide dismutase has been hypothesized in WS (Boerboom et al., 2018).

Table 6. Summary of the main molecular traits and differential expression of genes triggering and related to oxidative stress in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Molecular traits	
↑ lipid peroxidation products	Abasht et al., 2016; Salles et al., 2019
↑ copper content supporting ↑ superoxide dismutase activity	Boerboom et al., 2018
↓ anserine and ↓ carnosine content	Abasht et al., 2016; Sundekilde et al., 2017; Soglia et al., 2019b
↑ cysteine-glutathione disulfide and ↑ glutathione degradation	Abasht et al., 2016; Brothers et al., 2019
Gene expression	
↑ genes involved in hindering accumulation of ROS	Mutryn et al., 2015
↑ genes involved in the response to ROS	Zambonelli et al., 2016
≠ expression of genes encoding for the two classes of glutathione S-transferase	Malila et al., 2019
≠ expression of stress response-related genes	Mutryn et al., 2015; Zambonelli et al., 2016; Brothers et al., 2019; Pampouille et al., 2019

Abbreviation: ROS, reactive oxygen species.

Antioxidant Systems Histidine-containing dipeptides, anserine and carnosine, are among the most efficient free-radical scavengers in avian muscles (Yanai et al., 2008). However, the remarkable reduction in their content associated with the occurrence of WS and WB (Abasht et al., 2016; Sundekilde et al., 2017; Soglia et al., 2019b) suggests that their depletion may have occurred in the attempt to neutralize the accumulation of ROS, thus endorsing the development of oxidative stress within myopathic muscles. Besides, the glutathione detoxification pathway represents the main mechanism responsible for removing harmful products (Wu et al., 2004). In detail, glutathione has been found to exert several biological functions within the skeletal muscle, including regulating the redox state of the cell, providing a reservoir of cysteine for protein synthesis, and protecting the tissue against ROS and oxidants (Sen, 1998). Indeed, glutathione S-transferase catalyzes the conjugation of glutathione and electrophiles having the potential for cytotoxic damage thus converting them into less toxic forms and leading to detoxification (Ketterer et al., 1983). Within this context, the down-regulation of Glutathione S-transferase A4 (*GSTA4*) along with the increased expression of Glutathione S-transferase Mu 2 (*GSTM2*), encoding for the two classes of glutathione S-transferase, observed in myopathic muscles (Malila et al., 2019), strongly suggests the onset of a defense mechanism within the PM against oxidative stress. In agreement with that, an increased content of cysteine-glutathione disulfide, formed upon the S-glutathionylation of cysteine exposed to oxidative stress, has been observed in WB muscles (Abasht et al., 2016). In addition, the differential expression of several stress-response related genes further substantiates the development of phenomena associated with oxidative stress (Mutryn et al., 2015; Zambonelli et al., 2016; Brothers et al., 2019; Pampouille et al., 2019; Zhang et al., 2020). Among them, some are involved in oxidative stress response (such as *HIF1A*, Alpha-crystallin B chain -*CRYAB*, Adiponectin, C1Q and collagen domain containing -*ADIPOQ*, Thioredoxin -*TXN*, Guanine nucleotide-binding protein G(q) subunit alpha -*GNAQ*, and Glutathione Specific Gamma-Glutamylcyclotransferase 1 - *CHAC1*), whereas others are members of the heat-shock family, whose related proteins exert

several biological functions including acting as chaperones, playing a role in controlling protein folding and stimulating a pro-survival response against oxidative stress (Mungrue et al., 2009; Crawford et al., 2015). Interestingly, the increased expression of *CHAC1* observed in male broilers (Brothers et al., 2019) has been previously associated with sarcoplasmic reticulum stress and may be harmful to the PM as the protein encoded by the *CHAC1* gene degrades glutathione (Crawford et al., 2015). This finding supports the hypothesis of an enhanced glutathione degradation taking place within the PM of male broilers thus highlighting their higher susceptibility to oxidative stress, which likely plays a relevant role in the development of myopathic disorders (Brothers et al., 2019). In addition, as *CHAC1* is also a member of the Unfolded Protein Response (Mungrue et al., 2009), its increased expression observed in myopathic muscles suggests the activation of this pathway, thus further supporting the hypothesis of sarcoplasmic reticulum stress (Figure 2-7) due to the accumulation of misfolded and dysfunctional proteins at luminal level (Figure 2-8).

Altered Ca^{2+} Homeostasis

The histological evidences, the molecular results and the main findings concerning the differential expression of genes triggering and related to an altered Ca^{2+} homeostasis in myopathic PM are reported in Table 7. It is worth mentioning that both accumulation of ROS and sarcoplasmic reticulum stress may affect calcium homeostasis (Görlach et al., 2015; Kropski and Blackwell, 2018) (Figure 2-15). On this basis, the relevant increase in Ca^{2+} concentration measured in myopathic muscles (Soglia et al., 2016b; Tasoniero et al., 2016; Zambonelli et al., 2016) strengthens the hypothesis of the occurrence of an intracellular calcium overload within the affected muscles with the potential to affect the sarcolemmal integrity (Sandercock and Mitchell, 2003) and trigger the onset of myopathic disorders. In agreement with that, the differential expression of several genes involved in Ca^{2+} transport has been detected either in 3-week-old (Papah et al., 2018) and in marketable age broilers (Zambonelli et al., 2016;

Table 7. Summary of the main histological evidences, molecular traits and differential expression of genes triggering and related to an altered Ca^{2+} homeostasis in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
hypercontracted fibers	Kuttappan et al., 2013; Sihvo et al., 2014; Velleman and Clark, 2015
≠ anchoring of the contractile filaments	Liu et al., 2020
↓ Ca^{2+} sensitivity of the fibers	Liu et al., 2020
Molecular traits	
↑ Ca^{2+} concentration	Soglia et al., 2016b; Tasoniero et al., 2016; Zambonelli et al., 2016
↑ calpain activity and ↑ post-mortem degradation of myofibrillar proteins	Soglia et al., 2018; Hasegawa et al., 2020
Gene expression	
≠ expression of genes involved in Ca^{2+} transport either in 3-weeks old or in marketable age broilers	Zambonelli et al., 2016; Papah et al., 2018; Marchesi et al., 2019
↑ genes involved in calcium signaling pathway and ↑ cytosolic Ca^{2+} concentration	Marchesi et al., 2019
↑ genes as a response mechanism to counteract ↑ intracellular Ca^{2+} and stop hypercontraction	Mutryn et al., 2015; Zambonelli et al., 2016; Marchesi et al., 2019
↓ genes suggesting a ≠ Ca^{2+} metabolism and ↓ excitation-contraction coupling	Pampouille et al., 2019

Marchesi et al., 2019). This, together with the overabundance of creatine kinase observed in WB (Zhang et al., 2020), suggests an essential role of an eventual Ca^{2+} release from the sarcoplasmic reticulum in altering the sarcolemmal integrity thus triggering the myodegenerative processes associated with WS, WB, and SM myopathies. In detail, the upregulation of Calcium/calmodulin dependent protein kinase II alpha (*CAMK2A*), possibly interacting with other genes involved in the calcium signaling pathway, may result in an increased cytosolic calcium concentration triggering the onset of sarcoplasmic reticulum stress (Marchesi et al., 2019). Moreover, a higher transcription level of the gene ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (*ATP2A2*) has been observed in WS and/or WB (Mutryn et al., 2015; Zambonelli et al., 2016; Marchesi et al., 2019). Encoding for the protein SERCA2, an essential component of the sarcoplasmic reticulum acting as a calcium pump and involved in the contraction and relaxation of the myofibrils (Calderón et al., 2014), the increased expression of this gene likely results in a faster Ca^{2+} ions reuptake from the cytoplasm to the sarcoplasmic reticulum, and to a faster release of it (Mutryn et al., 2015) which, in turn, may affect the contractile ability of the tissue. In agreement with that, the same Authors detected an upregulation of Parvalbumin (*PVALB*). This transcriptional alteration has been considered as a compensatory change aiming at buffering the calcium ions and stop the hypercontraction of the fibers. In that respect, it is worth mentioning that a high proportion of hypercontracted myofibers has been observed in WB (Kuttappan et al., 2013; Sihvo et al., 2014; Velleman and Clark, 2015), along with a remarkable increase in calcium concentration (Soglia et al., 2016b; Tasoniero et al., 2016). However, it is worth noting that in the complex context of muscle perturbations related to PM myopathies, these changes may be both a compensatory response of functional cells and an early marker of abnormal functionality, predicting a possible future degeneration. Despite it could be hard to distinguish between compensatory and pathologic changes in tissue samples, the evidences in the cited literature

support the hypothesis of an impaired contraction-relaxation coupling resulting from a faster than normal uptake and release of Ca^{2+} ions taking place in myopathic muscles.

In agreement with these results, the downregulation of Sarcalumenin (*SRL*) observed in myopathic muscles suggests an altered calcium metabolism with the potential to affect the contractility and the excitation-contraction coupling within these muscles (Papah et al., 2018). Furthermore, the lower Ca^{2+} sensitivity of the fibers along with the profound changes in the mechanical anchoring of the contractile filaments observed in WB likely affect the contractile properties of the tissue (Liu et al., 2020).

Proteases and lipases

The increased calcium overload observed in myopathic muscles (Figure 2-15) may result in the activation of proteases and/or lipases (Figure 2-17) (as depicted by the upregulation of membrane-associated phospholipase genes) (Mutryn et al., 2015; Abasht et al., 2019), which may ultimately lead to the onset of degenerative processes affecting the myofibers (Figure 2-20). The enhanced calpain activity and increased postmortem degradation of myofibrillar proteins observed in WB further support this hypothesis (Soglia et al., 2018; Hasegawa et al., 2020). Taken together, these phenomena may induce the activation of a downward spiral: as an excessive calcium concentration can impede mitochondrial performances, this would reduce the energy available for pumping calcium out of the cell, further exacerbating the damage to the fibers and resulting in an overall altered membrane integrity.

Inflammatory Processes and Activation of the Cascade of the Immune System

Inflammation According to previous sections, Table 8 provides a summary of the studies dealing with the inflammatory processes and the activation of the immune system cascade in PM exhibiting myopathic

Table 8. Summary of the main histological evidences, molecular traits and differential expression of genes triggering and related to the inflammatory processes and activation of the cascade of the immune system in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
myositis and presence of heterophils	Sihvo et al., 2017; Papah et al., 2017; Chen et al., 2019
mild focal or multifocal inflammatory events surrounding the vessel wall in young chickens	Papah et al., 2017; Sihvo et al., 2017
inflammatory cells infiltrations involving the entire vasculature wall in marketable age broilers	Velleman and Clark, 2015; Papah et al., 2017; Chen et al., 2019
Molecular traits	
↑ taurine content	Boerboom et al., 2018
Gene expression	
myositis	Papah et al., 2018; Chen et al., 2019;
↑ genes involved in the complement system	Papah et al., 2018
↑ genes related to the acute phase of the inflammatory reaction and ↑ genes involved in the production of inflammatory molecules	Papah et al., 2018; Pampouille et al., 2019
≠ expression of genes related to chronic inflammatory events	Mutryn et al., 2015; Zambonelli et al., 2016; Marchesi et al., 2019
↑ response mechanism to counteract inflammatory events	Mutryn et al., 2015; Zambonelli et al., 2016

conditions. The presence of tissue inflammation (i.e., myositis) and the evidences of an early inflammatory response has been detected in 2-week-old broilers either at histological level or through the assessment of gene expression profile (Papah et al., 2017, 2018; Sihvo et al., 2017; Chen et al., 2019) (Figure 2-18). In detail, mild, focal, or multifocal inflammatory events mainly surrounding the vessel wall along with a concurrent upregulation of several genes involved in the complement system have been found as early as 2 to 3 wk post-hatch (Papah et al., 2017, 2018; Sihvo et al., 2017). These events, together with the presence of heterophils (Papah et al., 2017), evidence the activation of an acute inflammatory response in myopathic muscles (Papah et al., 2018) thus supporting the hypothesis of the involvement of an early (and surely earlier than expected) inflammatory reaction in the progression of the myopathic disorders affecting broilers' PM. This hypothesis is further supported by the upregulation of several genes and proteins related to the acute phase of the inflammatory reaction as well as of those involved in the production of inflammatory molecules (i.e., cytokines, chemokines) (Papah et al., 2018; Pampouille et al., 2019). In virtue of these observations along with the evidences of a vascular involvement before the onset of myopathic lesions (Figure 2-11), the early inflammatory events likely involve the venous wall. At later developmental stages, this early subclinical onset of the myopathic conditions is then followed by structural and physiological perturbations to the muscle bedewed by the affected veins, leading to the activation of the immune system response (Figure 2-19) along with a progressive increase in the severity of the lesions (Sihvo et al., 2014; Mutryn et al., 2015; Soglia et al., 2016b; Zambonelli et al., 2016; Papah et al., 2017; Baldi et al., 2018).

Immune System in Response to Muscle Damage

Several genes related to the response toward chronic inflammatory events have been found differentially expressed in myopathic muscles belonging to marketable age broilers (Mutryn et al., 2015; Zambonelli et al., 2016; Marchesi et al., 2019). The upregulation of genes

encoding for members of the large ADAMTS family of zinc-dependent proteases and corticotrophin-releasing hormone observed in myopathic muscles support the occurrence of chronic inflammatory events (Figure 2-18) taking place within the tissue and the activation of response mechanisms aiming at counteracting them (Figure 2-19) (Mutryn et al., 2015; Zambonelli et al., 2016). In agreement with that, the increased concentration of taurine observed at molecular level in WS (Boerboom et al., 2018), acting as a means to improve the inflammatory response, might be considered as a compensatory mechanism of the PM to hinder inflammation (Terrill et al., 2016). At histological level, in marketable age broilers, inflammatory cell infiltrations (i.e., macrophages) involve the entire vasculature wall (Velleman and Clark, 2015; Papah et al., 2017; Chen et al., 2019). It is worth mentioning that macrophages, being predominant during chronic inflammatory events, are not only involved in phagocytosis of cellular debris but also exert a relevant role in controlling and promoting muscle regeneration and ECM remodeling by acting on myogenic and fibroadipogenic precursors (FAPs) (Saclier et al., 2013; Pakshir and Hinz, 2018) (see paragraph ECM remodeling).

Myodegeneration and Regeneration

The polyphasic myodegenerative processes (Figure 2-20) observed in myopathic muscles are the direct consequence of the aforementioned time-series events resulting in impaired sarcolemma integrity. Table 9 summarises the main findings from studies related to myodegeneration and regeneration in PM exhibiting myopathic conditions.

Fiber Degeneration Myofiber lysis and polyphasic degeneration have been found to develop in PM belonging to 2-week-old broilers and either the severity or the diffusion of the lesions gradually increased over time and achieved impressive incidence rates (Papah et al., 2017; Sihvo et al., 2017; Chen et al., 2019). Indeed, the prevalence of segmental myofiber degeneration and necrosis

Table 9. Summary of the main histological evidences, molecular traits and differential expression of genes triggering and related to myodegeneration and regeneration in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
myofiber lysis and polyphasic degeneration in 2-wk old broilers	Papah et al., 2017; Sihvo et al., 2017; Chen et al., 2019
attempts of tissue repair (i.e., myofiber regeneration, myotube formation and nuclear rowing) starting from week 3 post-hatch	Papah et al., 2017; Sihvo et al., 2017
prevalence of the regenerative processes gradually ↑ over time	Kuttappan et al., 2013; Sihvo et al., 2014, 2017; Velleman and Clark, 2015; Soglia et al., 2016b; Papah et al., 2017; Baldi et al., 2018
↓ satellite cells number and ↓ ability to proliferate and differentiate	Daughtry et al., 2017
↓ satellite cell-mediated regeneration (↓ diameter of regenerated myofibers)	Velleman and Clark, 2015
≠ heterogeneity of satellite cells population	Ferreira et al., 2020
Molecular traits	
molecular activities preceding the histological evidences of regeneration observed in 2 weeks old broilers	Papah et al., 2018
↓ satellite cell-mediated regeneration (↑ decorin, ↑ transforming growth-factor beta and ↑ myostatin)	Velleman and Clark, 2015
↑ desmin in WS	Soglia et al., 2020a
↑ vimentin in WB	Soglia et al., 2020a
Gene expression	
≠ expression of genes evidencing caspases activation	Mutryn et al., 2015; Marchesi et al., 2019
↑ genes encoding for nuclear factors	Zambonelli et al., 2016
↑ myogenic regulatory factors	Mutryn et al., 2015; Velleman and Clark, 2015; Malila et al., 2019
↑ genes encoding for vimentin and desmin	Soglia et al., 2020a

Abbreviations: WS, White Striping; WB, Wooden Breast.

attains more than 95% and 70%, respectively, in 7-week-old broilers (Papah et al., 2017). Intriguingly, evidences of myofiber degeneration are also detected in PM that does not exhibit any macroscopic features associated with the development of myopathic conditions (Sihvo et al., 2017). These findings further corroborate the hypothesis of an early (likely as early as one week of age) occurrence of microscopic and molecular perturbations preceding the visible traits ascribable to WS and WB. In agreement with the histological examinations, the differential expression of the genes Endothelin receptor type A (*EDNRA*) and Caspase recruitment domain-containing protein 9 (*CARD9*) observed in myopathic PM evidences the activation of caspases, thus resulting in apoptosis (Figure 2-20) (Mutryn et al., 2015; Marchesi et al., 2019). On the other hand, the overexpression of the genes encoding for nuclear factors (i.e., Nuclear factor, interleukin 3 regulated -*NFIL3*, and Snail family zinc finger 2 -*SNAI2*) found in myopathic muscles is likely a response mechanism of the tissue to limit the apoptotic processes and counteract fiber necrosis (Zambonelli et al., 2016). Beyond that, it is worth mentioning the upregulation of the gene Matrix metalloproteinase 2 (*MMP2*) found in WB affected muscles (Mutryn et al., 2015). An enhanced expression of this gene, which is typically associated with the degradation of collagen type IV (Sand et al., 2013; Jabłońska-Trypuć et al., 2016; Bhan et al., 2019), suggests a role of this component of the basement membrane in triggering the development of myopathic disorders. Indeed, the secretion of matrix metalloproteinase by the inflammatory cells infiltrating the tissue as a consequence of inflammation has the potential to profoundly damage this cellular component (Cheng et al., 2006; Bhan et al., 2019). At the same time, as collagen type IV exerts a relevant role in the migration of inflammatory cells across the basement membrane taking place in the acute phase of

inflammation, its degradation by MMP2 can impair the migration process (Monaco et al., 2006) thus inhibiting an effective response mechanism to counteract the acute inflammation taking place in myopathic muscles. At present, it has not been clarified whether collagen type IV and its degradation are secondary changes resulting from myofiber degeneration or rather represent primary events able to trigger the development of the myopathic conditions affecting broiler PM. However, genes encoding for this non-fibrillar collagen (i.e., *COL4A1* and *COL4A2*) have been recently detected as hubs and highly associated to the phenotypic traits of broilers' myopathic PM (Bordini et al., 2021). As a consequence, these Authors, hypothesized that collagen type IV accumulation may underlie and trigger the onset of sarcoplasmic reticulum stress responsible for the development of myopathic disorders in fast-growing chickens.

Regenerative Processes The presence of degenerating or necrotic myofibers within the myopathic muscle can induce satellite cell-mediated repair mechanisms to regenerate the fibers back to their initial condition (Figure 2-21) (Velleman, 2019). At histological level, attempts of tissue repair (i.e., evidences of myofiber regeneration, myotube formation, and nuclear rowing) concomitantly to myositis have been observed starting from week 3 post-hatch (Papah et al., 2017; Sihvo et al., 2017). From this time point, the prevalence of regenerative processes progressively increases over time and it becomes prominent in marketable age broilers (Kuttappan et al., 2013; Sihvo et al., 2014, 2017; Velleman and Clark, 2015; Soglia et al., 2016b; Papah et al., 2017; Baldi et al., 2018). Notwithstanding, molecular activities preceding the distinctive histological traits associated with regeneration are demonstrated to be activated as early as week 2, thus indicating an earlier occurrence of myoregeneration than previously observed (Papah et al., 2018). Overall, the differential expression

of genes involved in muscle development, cell growth, and cell differentiation, corroborate the activation of regenerative processes as an attempt to counteract myodegeneration taking place within the PM exhibiting myopathic conditions. In detail, the increased transcription level of myogenic regulatory factors (i.e. Myogenic differentiation 1 -*MYOD1*, Myogenin -*MYOG*, Sarcoglycan delta -*SGCD*, Calpain 3 -*CAPN3*, Myosin light chain kinase -*MYLK*) evidence the activation of a satellite cell-mediated regeneration process of the tissue in the attempt to maintain sarcolemma integrity (Mutryn et al., 2015; Velleman and Clark, 2015; Malila et al., 2019). Besides that, it is worth noting the overexpression of the gene Cysteine and glycine rich protein 3 (*CSR3*) (Mutryn et al., 2015; Zambonelli et al., 2016). In detail, aside from its relevant role in myogenic differentiation, the increased expression of this gene, mainly translated in slow-twitch skeletal muscles, further corroborates the hypothesis of a fiber type-switching (see par. Fiber type switching) as a secondary response to myofiber regeneration taking place under altered redox homeostasis (Mutryn et al., 2015). It is worth remembering that in satellite cell-mediated regeneration process these pluripotent cells must be activated and, after re-entering the cell cycle, undergo proliferation and differentiation (Velleman, 2019). However, the satellite cell niche and its components (including ECM, growth factors, and vascularization) can strongly affect the satellite cells activity (Christov et al., 2007; Bi and Kuang, 2012; Velleman, 2019). Within this context, the reduced vascularization and higher inter-capillary distance observed in fast-growing hybrids (Figure 2-2) (as a result of hypertrophy, see paragraph Muscle growth and hypertrophy, Vascular development), may profoundly impair regeneration of the PM in response to damage (Velleman, 2015). In agreement with that, a compromised ability of the satellite cells to proliferate and differentiate (hypothesized to result from a telomeric shortening following division), along with a reduction in their number, have been associated with a hypertrophic growth of the PM (Daughtry et al., 2017). In addition, differences in the satellite cells population have been observed by comparing WB muscles (either focal or severe cases) with their unaffected counterpart (Ferreira et al., 2020). Based on the protein expression of myogenic regulatory factors (i.e., Myogenic factor 5 -*MYF5*, *MYOD*, and Paired box 7 -*PAX7*), these Authors hypothesized that, in PM focally affected by WB, satellite cells are still trying to repair the tissue whereas this process has already ended in severe cases (Ferreira et al., 2020). Within this context, satellite cell activity in myopathic muscles may be further impaired by the proliferation of collagen and connective tissue, up to fibrosis, which distinctively affects WS and WB muscles. It is worth noting that, despite the evidences of an activation of the satellite cell-mediated regeneration process, if compared to their unaffected counterpart, a remarkably lower myofiber diameter has been found in WB muscles (Velleman and Clark, 2015). As regeneration should not reduce the diameter of the fibers, this

finding further corroborates the hypothesis of an altered repair process resulting from reduced satellite cell activity in PM exhibiting myopathic conditions. This may likely be ascribed to the increased synthesis of Decorin (*DCN*), Transforming growth factor beta (*TGF- β*), and Myostatin (*MSTN*), which are strong inhibitors of muscle cell proliferation and differentiation, along with the overall reduced vascularization associated with their onset (Velleman and Clark, 2015; Velleman, 2019). Within this context, it is worth mentioning that a different progression of the regenerative processes has been found in myopathic muscles depending on the onset of WS, WB, or SM (Soglia et al., 2020a). Indeed, based on the expression and distribution of Vimentin (*VIM*) and Desmin (*DES*), widely considered as reliable markers of regeneration, these Authors hypothesized the occurrence of intense repair processes at the earliest stages in WB whereas these have been found in a late developmental stage in WS, as depicted by the increased amount of vimentin and desmin found within the PM affected by these conditions, respectively. On the other hand, as vimentin plays a relevant role in regulating fibroblasts proliferation (Cheng et al., 2016), the lack of correspondence between the upregulation of the gene and the low amount of its related protein found in SM likely result in an overall reduced fibroblasts number, thus determining the distinctive phenotype associated with this myopathic condition (Soglia et al., 2020a).

When the lesions resulting from the myodegenerative processes associated with the development of myopathic disorders in broilers become severe and persistent, the regenerative processes aiming at restoring the fibers to their original condition may be ineffective, thus inducing the differentiation of pluripotent stem cells to adipocytes or fibroblasts (Figure 2-22) (Velleman, 2015). Within this context, the activation of UPR signalling following endoplasmic reticulum stress may exert a relevant role in defining the fate of the pluripotent stem cells population (Hetz and Papa, 2018). Indeed, UPR activation has been demonstrated to impair the function and activation of fibroblasts that ultimately result in a direct profibrotic effect involving an abnormal accumulation of collagen (Lenna and Trojanowska, 2012; Kropski and Blackwell, 2018).

ECM Remodeling

The remodeling of the ECM up to fibrosis, involving degeneration of the connective tissue in which an increased deposition of fibrillar collagen along with ECM replace the functional tissue, represents one of the most distinctive features ascribable to the myopathic conditions affecting broilers PM. The histological traits and the main findings concerning the differential expression of genes triggering and related to ECM remodeling in PM exhibiting myopathic conditions are summarized in Table 10. Evidences of the progression in fibrosis from molecular to microscopic changes have been observed in WB affected muscles. Indeed, the first signs of fibrosis have been detected at histological level from week 4

Table 10. Summary of the main histological evidences and differential expression of genes triggering and related to ECM remodeling in *Pectoralis major* muscles exhibiting myopathic conditions.

Main findings	References
Histological evidences	
↑ deposition of fibrillar collagen (from week 4 onwards)	Kuttappan et al., 2013; Sihvo et al., 2014, 2017; Soglia et al., 2016b; Papah et al., 2017
Gene expression	
↑ genes belonging to the collagen family	Mutryn et al., 2015; Papah et al., 2018; Bordini et al., 2021
↑ genes involved in ECM remodeling and fibroblasts proliferation and function	Mutryn et al., 2015; Papah et al., 2018; Brothers et al., 2019; Pampouille et al., 2019; Bordini et al., 2021
↑ <i>PDGFRA</i> supporting the activation of FAPs	Papah et al., 2018; Pampouille et al., 2019
↑ Wnt signaling	Philips et al., 2020; Bordini et al., 2021

Abbreviations: ECM, extracellular matrix; FAPs, fibro-adipogenic precursors.

onwards (with the severity of the fibrosis gradually increasing with age), whereas the earliest molecular signs have been demonstrated to occur as early as week 3 (Kuttappan et al., 2013; Sihvo et al., 2014, 2017; Soglia et al., 2016b; Papah et al., 2017, 2018). Differences in either the distribution or the crosslinking of the collagen fibers, ascribable to a different expression of *DCN*, have been observed in connective tissue fibrosis in fast-growing broilers having a different genetic background (Velleman and Clark, 2015; Tonniges et al., 2018; Velleman et al., 2018). Besides that, the overexpression of the genes within the collagen family has been found (Mutryn et al., 2015; Papah et al., 2018). Overall, these findings demonstrate that fibrosis (and eventually lipidosis) represent a common time-series event following muscle repair taking place under hypoxic conditions (Figure 2-22) (Lopes-Ferreira et al., 2001; Mutryn et al., 2015; Zambonelli et al., 2016). Several genes directly involved in the ECM remodeling (including platelet-derived growth factor receptor alpha -*PDGFRA*, Fibronectin 1 -*FN1*, Tenascin C -*TNC*, and Connective tissue growth factor -*CTGF*) and fibroblasts proliferation and function (i.e., Fibroblast activation protein alpha (*FAP*), Fibroblast growth factor receptor like 1 -*FGFRL1*, and Fibroblast growth factor binding protein 1 -*FGFBP1*) are differentially expressed in PM with myopathic conditions (Mutryn et al., 2015; Papah et al., 2018; Brothers et al., 2019; Pampouille et al., 2019). The upregulation of these genes is surely involved in initiating ECM remodeling and, enhancing the synthesis and deposition of collagen, glycosaminoglycans, and proteoglycans, ultimately resulting in the development of fibrosis. Within this context, it is worth mentioning the activation of Wnt signalling observed in WB (Phillips et al., 2020). This pathway is directly involved in the activation of satellite cells and, interacting with TGF- β may exert a profibrotic effect (Cisternas et al., 2014) by exhausting the satellite cells population which is designed for a limited number of divisions (Sacco et al., 2010). In addition, it is interesting to highlight the increased expression of the gene *PDGFRA* observed in myopathic muscles (Papah et al., 2018; Pampouille et al., 2018, 2019). Indeed, the increased expression of this gene is a marker of those that are termed as FAPs, multipotent progenitors that, once activated, support the satellite cell-mediated

regeneration processes (Joe et al., 2010; Heredia et al., 2013; Biferali et al., 2019). Besides that, under pathologic conditions, according to their plasticity, FAPs are able to differentiate into fibroblasts or adipocytes involved in infiltrating dystrophic muscles (Uezumi et al., 2011). Therefore, the upregulation of *PDGFRA* observed in myopathic muscles strongly supports the activation of FAPs and correlates with the distinctive fibrosis and lipidosis associated with the development of WB and WS, respectively (Papah et al., 2018; Pampouille et al., 2018, 2019). Thus, it is possible to hypothesize that, identifying the molecular signals responsible for the differentiation of FAPs to a fibrogenic rather than to an adipogenic lineage would be of relevant importance to further understand the predisposition of the PM to develop WS or WB condition.

CONCLUSIONS

In conclusion, myopathic disorders affecting broilers' PM have a complex etiology and several biological pathways, as well as response mechanisms, are involved in their progression. Although the primary cause responsible for the onset of the growth-related abnormalities (WS, WB, and SM) has not been identified yet, critically examining all the results available in the literature, our review highlights the probable main involvement of sarcoplasmic reticulum stress and hypoxia in initiating the cascade of events ultimately resulting in the development of these myopathic conditions. Indeed, sarcoplasmic reticulum stress is primarily induced by the accumulation of misfolded/dysfunctional proteins but may also be exacerbated by other phenomena such as ectopic lipid deposition, altered calcium homeostasis, and build-up of misfolded or dysfunctional proteins taking place within the PM. At the same time, the hypertrophic growth of the PM induced by genetic selection is associated with a reduced vascularization of the tissue that predisposes it to the development of hypoxic conditions. Then, once established, hypoxia triggers a time-series sequence of events (i.e., phlebitis, oxidative stress, altered calcium homeostasis, etc.) resulting in the activation of response mechanisms (i.e., modifications in the energetic metabolism, inflammation, degeneration, and

regeneration) which are all strictly related to the progression of these myopathic disorders.

ACKNOWLEDGEMENTS

The research has been partially funded by a PRIN National Grant 2017 (Ministry of Education, University and Research) entitled “Use of local chicken breeds in alternative production chain: welfare, quality and sustainability” (Prot. 2017S229WC).

DISCLOSURES

The authors declare no conflicts of interest.

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