REVIEW

Energy metabolism and sources of oxidative stress in wooden breast - a review [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract
Modern broilers show dramatic growth over a short interval and contribute directly to the success of the poultry meat industry. The growth performance of commercial broilers is a result of genetic selection for "performance traits", such as body size, meat yield, and feed conversion rate. However, due to the rapid growth rate of modern commercial broilers, several growth-related conditions have arisen, increasing economic losses and consumer concerns. Among the most economically consequential is the muscle disorder called wooden breast. Together with associated myopathies such as white striping and spaghetti meat, wooden breast is causing losses of $200 million a year in the U.S. alone and occurs worldwide. No causative factors are known for wooden breast to date. Wooden breast can affect over 80% of broilers in a flock, yet no methods of amelioration are currently available. Overall, the evidence suggests that wooden breast is a genetic, age-dependent condition associated with fast growth rate. The primary features of wooden breast are muscle degeneration and fibrosis, high levels of oxidative stress, hypoxia, and altered energy metabolism. Recent work has also implicated reduced pectoral vessel density in the pathogenesis of wooden breast. This review examines the history of myopathies in commercial broilers and the relationship of myopathies to metabolism and oxidative performance. This review summarizes the foundational knowledge of wooden breast and provides a platform for further investigation of wooden breast.

Keywords
wooden breast, broiler chicken, production efficiency, myopathy, meat quality
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Introduction

Throughout the last 60 years the poultry industry has worked to increase growth rate, muscle development, and maturation of chickens in order to maximize production efficiency and keep poultry meat prices low (Bohren, 1953; Chambers et al., 1981; Elwing et al., 2016; Fairfull & Chambers, 1984; Jaap, 1963; Merritt et al., 1962; Paxton et al., 2010; Remignon et al., 1994; Skoglund et al., 1966; Tallentire et al., 2018). These improvements have been accomplished through selective breeding and carefully maintaining multiple generations of broiler breeder flocks. However, through these efforts, the genetic diversity of current commercial flocks has become limited, allowing for numerous health concerns to arise (Julian 2005; Paxton et al., 2010; Tallentire et al., 2018). These concerns include weak immunity, vascular insufficiency, skeletal impairment, heat intolerance and a class of disorders termed myopathies which are generally considered meat quality issues rather than bird health concerns (Chen et al., 2019; Clark & Velleman, 2016; Cruz et al., 2017; Hubert et al., 2018; Kuttappan et al., 2012; Kuttappan et al., 2017a; Kuttappan et al., 2017b; MacRae et al., 2006; Malan et al., 2003; Mazzoni et al., 2015; Scheele, 1997; Siller, 1985; Sihvo et al., 2014; Tarrant et al., 2017; Tallentire et al., 2018; Tijare et al., 2016; Trocino et al., 2015; Velleman et al., 2018; Wideman et al., 2013).

Abridged history of myopathies in the poultry industry

Generally, a myopathy constitutes a disorder of the skeletal muscle, often localized to a specific muscle tissue, in which impaired cell structure and metabolism generate macroscopic symptoms and muscle dysfunction. Myopathies can occur due to inheritance, chronic immune and endocrine disruptions, and environmental stimuli. For example, wing-flapping induces deep pectoral myopathy (DPM) (Siller, 1985). Additionally, antibiotics such as monensin (Umemura et al., 1984), hormone dysregulation such as the inhibition of prostaglandin (McLennan, 1985) and degeneration of mitochondria due to feed ingredients such as Senna seeds (Cavaliere et al., 1997) have been observed to cause myopathies in poultry. Inherited (Asmundson & Julian, 1956; Asmundson et al., 1966) and nutritional muscular dystrophy (Weinstock et al., 1955) were the first myopathies to be investigated and are still arising in poultry over 60 years later. Due to the different modalities of inducing myopathies, it is difficult to pinpoint the pathophysiology of myopathies. However, myopathies have become a prevalent consumer issue and economic concern in today’s fast growth commercial broiler lines, necessitating intensive investigation. Recent literature has focused on the three most commonly observed broiler breast myopathies, DPM, white striping (WS) and wooden breast (WB), and a newly observed myopathy termed “spaghetti meat” (SM).

Although they are all classified as myopathies of the breast, DPM impacts the pectoralis minor while WS, WB, and SM impact the pectoralis major. DPM was first observed in broilers in 1980 (Richardson et al., 1980) and is commonly referred to as green muscle disease due to a characteristic green coloring of the pectoralis minor in affected birds. Since then, numerous investigators have concluded that the massive pectoralis major of commercial broiler suffocates the pectoralis minor, resulting in hemorrhage and necrosis of the muscle (Bailey et al., 2015; Grunder et al., 1984; Kuttappan et al., 2016; Lien et al., 2012; Siller, 1985; Velleman, 2015; Wight & Siller, 1980). The green coloring is caused by the release of iron from the hemorrhaged erythrocytes as they break down. Histological characterization of DPM by Wight & Siller (1980) showed anucleated myofibers surrounded by fibrous and adipose tissues, and disintegration of the sarcoplasmic reticulum, mitochondria, and Z-lines. These studies suggest that DPM is a result of artificial selection for high growth rate and increased breast yield.

WS is so named based on the appearance of the affected pectoralis major, marked by white striations that run parallel to the muscle fibers. Recent studies have determined that the manifestation of white striping is not only altered appearance but also a decrease in mineral and protein content, water holding capacity, and an increase in fat and collagen content (Bailey et al., 2015; Kuttappan et al., 2013b; Kuttappan et al., 2013c; Kuttappan et al., 2012; Kuttappan et al., 2013a; Mazzoni et al., 2015; Mudalal et al., 2015; Owens, 2014; Petracci et al., 2013; Petracci et al., 2014; Sihvo et al., 2014; Trocino et al., 2015; Vignale et al., 2017). At the histological level, variable fiber size, necrosis, loss of cross striations, multinucleated cells, interstitial inflammation and fibrosis, lipidosis, and mononuclear cell infiltration are seen (Kuttappan et al., 2013c; Kuttappan et al., 2013a; Mazzoni et al., 2015; Trocino et al., 2015). Few studies have investigated the molecular mechanisms of WS. Vignale et al. (2017) focused on elucidating the activity of genes related to protein synthesis and degradation, such as IGF-1, atrogin-1, insulin receptor, and MuRF1. Their results indicated that MuRF1 and atrogin-1 are significantly upregulated in WS birds, while IGF-1 was significantly down-regulated. Vignale et al. (2017) suggested that these changes in gene expression indicated that fast growth commercial broilers have a higher rate of muscle degradation, which leads to the development of WS. Furthermore, Pampouille et al. (2018) utilized a genome-wide association study of quantitative trait loci, which resulted in the identification of possible genetic and molecular markers for WS and provided evidence for polygenic inheritance of the condition. These include several genes involved in muscle structure and metabolism, some known to be involved in neuromuscular disorders (Pampouille et al., 2018).

One of the recently identified myopathies known as WB is a significant concern for the poultry industry due to its worldwide occurrence high incidence (Bailey et al., 2015; Clark & Velleman, 2016; Cruz et al., 2017; Mutryn et al., 2015; Petracci et al., 2019; Sihvo et al., 2017). WB is characterized by an abnormally hard or “wooden” breast, with hardness originating at the cranial region in the least severe cases and extending the full length of the breast, from the cranial to the caudal region in the most severe cases. Breasts affected by WB often appear to have a medial ridge extending from the cranial to caudal region and meat quality factors such as texture, shear force, pH and water holding capacity are all impacted (Bailey et al., 2015; Clark & Velleman, 2016; Coble et al., 2014; Cruz et al., 2017; Kuttappan et al., 2016; Kuttappan et al., 2017b; Mazzoni et al., 2015; Sihvo et al., 2014; Sihvo et al., 2017; Soglia et al., 2016;
SM, the most recently described myopathy impacting the poultry industry, also primarily affects the pectoralis major. It manifests as impaired muscle structure, resulting in the separation of muscle fiber bundles, and generally impacts the cranial portion of the breast fillet (Baldi et al., 2018). WS and SM co-occur and share histological features such as increased perimysial and endomysial degradation of connective tissues, infiltration of inflammatory cells, and thin and split fibers surrounded by loose connective tissue (Baldi et al., 2018). Collagen of SM affected breasts is immature and has fewer cross-links compared to normal breast fillets (Baldi et al., 2019). Furthermore, SM is higher in moisture content, but lower in protein and fat content than WS affected breasts (Baldi et al., 2018; Baldi et al., 2019). SM affected breasts are downgraded for quality and used in further processed products, resulting in economic losses (Baldi et al., 2018; Baldi et al., 2019). Nutritional interventions for SM through altered arginine:lysine ratios were investigated by Zampiga et al. (2019), resulting in significantly reduced incidence at the highest arginine:lysine ratio. This reduction in SM incidence was hypothesized to be due to enhanced vasodilation and better blood flow to the muscle through increased production of nitric oxide via the arginine-nitric oxide pathway (Zampiga et al., 2019). However, the authors noted that the reduction in lysine could also be responsible for the decreased incidence of SM, as other researchers have shown increases in breast muscle myopathies due to increased dietary lysine concentrations (Cruz et al., 2017; Zampiga et al., 2019).

Significance of myopathies to food production, quality, and safety
Chicken breast is the most consumed meat in the U.S., and the quality and safety of this food commodity are significant both from an economic and food security standpoint. Broiler breast myopathies decrease meat quality by altering the muscle ultrastructure, which in turn impacts texture, tenderness, and in some cases, flavor. With high incidence rates, the annual economic losses due to broiler breast myopathies in the U.S. are roughly $200 million and increasing (Kuttappan et al., 2016). The pectoralis major is known as the breast when discussed as a meat product and is the most purchased cut, while the pectoralis minor is known as the tender. Chicken breast meat is consumed widely due to the high protein content and the pectoralis minor is known as the tender. Chicken breast meat is consumed widely due to the high protein content and low-fat content. However, due to these myopathies, this desirable protein and fat ratio is altered. WB affected meat, for instance, has higher fat content and lower protein content (Kuttappan et al., 2017a; Mazzoni et al., 2015; Mudalal et al., 2015; Owens, 2014; Petracchi et al., 2014; Saglia et al., 2016; Tasoniero et al., 2017; Tijare et al., 2016). The percentage of fat increase in breast meat affected by WB is 1.2-1.3% (Saglia et al., 2016; Tasoniero et al., 2016).

Furthermore, as consumers have become aware of these myopathies, there is an increasing rejection of WB affected meat, as well as discussion on social media (Burginger, 2019; Crews, 2017; Elder, 2017; Gee, 2016; Johnson, 2018; Jones, 2016; Keiger, 2017; Ngo, 2017; Pellegrini, 2017; Petreyčik, 2019; Picchi, 2016; Prescott, 2017; Rainey, 2016; Versace, 2019; Walansky, 2017). Often, the public is misinformed on the source of these undesirable characteristics and misattribute them to questionable production practices or confuse them with genetically modified organisms. Although such views are incorrect, they contribute to misinformation about animal agriculture and exacerbate food waste. Thus, it is imperative to improve education for the public on these crucial issues while solutions are found and implemented.

Literature review
Chicken breast muscle characteristics
Although selective breeding of production animals developed around specific phenotypes, scientific advances in genetics and nutrition have created the ability to maximize the efficiency of selection using genotype data. However, trait heritability has been the basis of selection since roughly the 1980s (Chambers et al., 1981; Fairfull & Chambers, 1984), and the implementation of genetic and genomic data in poultry selection is still in its infancy. Le Bihan-Duval et al. (2008) reported that growth and body composition traits such as body weight and abdominal fat were heritable (h² = 0.49 and 0.48 respectively), as were muscle characteristics such as glycolytic potential and muscle fiber cross-sectional area (h² = 0.43 and 0.41 respectively) in broilers. This study also reports that glycolytic potential was negatively correlated to meat quality traits such as color, drip loss and shear force, and overall breast muscle weight (Le Bihan-Duval et al., 2008).

Chicken breast muscle physiology is well studied as it is the most valuable portion of the carcass and has been for more than 30 years. In 1994, Remignon et al. evaluated the muscle characteristics of slow-growth and fast-growth chicken lines to isolate muscle characteristics specific to growth-rate. Their study found no differences in muscle fiber type at 55 weeks (Remignon et al., 1994). The breast was composed only of Type IIB fast-twitch fibers, and cross-sectional areas were larger and more numerous in the fast-growth lines compared to the slow growth line. However, some studies have identified a small percentage (0.5-10%) of Type IIA fibers in slow-growth and laying type chickens (Branciari et al., 2009; Clark & Velleman, 2016; Dransfield & Sosnicki, 1999; MacRae et al., 2006; Remignon et al., 1995; Scheuermann et al., 2004; Velleman et al., 2018; Velleman, 2007).
Specifically, water holding capacity (WHC) impacts the flavor and toughness of the breast through the amount of water retained or lost during cooking, as well as the ability of the meat to absorb marinades and flavorings. WHC is directly related to the post-mortem pH, with a low pH resulting in reduced WHC and a high pH resulting in increased WHC. However, pH only accounts for about one-third of WHC, with the remainder due to the steric effects of muscle proteins. During rigor, the myofibrillar proteins form irreversible bonds, and muscle contraction occurs, reducing space for water storage. As rigor resolves, the contraction reduces, and several other muscle proteins such as z-lines have degraded, allowing the charged actomyosin complex to recruit water molecules and thus contributing to WHC. The specific combination of fat, protein, pH, and WHC is mainly responsible for being associated with the composition of chicken in comminuted products such as chicken nuggets. Deviations of these attributes result in downgrades in product quality, reduced consumer acceptance, and economic losses (Betti et al., 2009; Kato et al., 2018; Kuttappan, 2012; Pellegrini, 2017; Petracci et al., 2019).

**Known mechanisms of WB**

First identified roughly 15 years ago and intensely investigated during the last five years, many etiologies for WB have been proposed. The most commonly investigated etiologies include nutritional deficiencies and toxicities, exercise induction, and hypoxia of the pectoralis major (Bailey et al., 2015; Bodle et al., 2018; Chen et al., 2019; Clark & Velleman, 2016; Cruz et al., 2017; Guetchom et al., 2012 Lilburn et al., 2019; Livingston et al, 2019a; Meloche et al., 2018; Papah et al., 2017; Soglia et al., 2016; Siervo et al., 2018; Trocino et al., 2015; Velleman & Clark, 2015; Velleman et al., 2018). Despite this intense study, the molecular mechanisms and associated disease pathways driving WB remain unknown. There is, however, a consensus that WB is associated with the selection for fast growth-rate, occurring at highest frequency in the largest birds; slow growth varieties are not impacted (Hubert et al., 2018; Kong et al., 2017; Meloche, et al., 2018; Muralal et al., 2015; Mutryn et al., 2015; Velleman & Clark 2015). Although similarities exist between WB and well-known myopathies such as WS, the histological characteristics of WB are well described. They include muscle fiber damage, interstitial fibrosis, infiltration of macrophages and an increase in fat and collagen content (Bowker et al., 2019; Clark & Velleman, 2016; Lilburn et al., 2019; Mazzoni et al., 2015; Meloche et al., 2018; Siervo et al., 2017; Soglia et al., 2016; Siervo et al., 2014; Siervo et al., 2018; Velleman et al., 2018; Velleman & Clark, 2015).

At the molecular level, gene expression and metabolomics studies have shown that oxidative stress is one of the main features of WB tissue (Abasht et al., 2016; Clark & Velleman, 2016; Hubert et al., 2018; Kong et al., 2017; Mutryn et al., 2015; Papah et al., 2018; Siervo et al., 2017). Reactive oxygen species (ROS), the free radicals produced in vivo by specialized enzymes such as NADPH-oxidase, nitric oxide synthase and myeloperoxidase, mitochondrial respiration and the monoxygenase activity of cytochrome p450, act as modulators of gene expression, increased cell proliferation, prevention of cell division, apoptosis, neocrosis and cell death, and are responsible for the damage occurring (Weidinger & Kozlov, 2015) in oxidative stress (Bonnard et al., 2008; Halliwell, 2007; Mutryn et al., 2015; Netzer et al., 2015). In healthy tissues, ROS production is combated by antioxidants, preventing cellular damage. However, in tissues under oxidative stress, the ratio of ROS to antioxidants is highly imbalanced, and long term cellular damage can occur. An example of this damage is direct modulation of the sodium/potassium-ATPase channels, resulting in changes in ion balance, which trigger changes in cellular calcium metabolism, causing increased concentrations of intracellular free calcium and subsequent cellular impairment (Sims & Muyderman, 2010). This characterization provides tremendous insight into the physiological occurrences of WB and provides an excellent foundation for the development of further investigations.

**The role of selection for performance in development of WB**

The poultry industry has made immense progress at increasing growth rate, breast yield, and feed efficiency of commercial broilers through careful selective breeding regimens and maintenance of pedigree flocks (Clark & Velleman, 2016; Collins et al., 2014; Dransfield & Sosnicki, 1999; Fanatico et al., 2007; MacRae et al., 2007; MacRae et al., 2006; Mazzoni et al., 2015 Petracci & Cavani, 2012; Siller, 1985; Velleman & Clark, 2015). However, the quality of the meat produced is directly related to the morphological structure of the muscle, and growth-related selection has changed this structure. Intense selection has resulted in decreased capillary blood supply to the pectoralis major and minor, reduced connective tissue spacing between myofibers and muscle fiber bundles, and increased degeneration of myofibers (Baldi et al., 2018; Berri et al., 2007; Dransfield & Sosnicki, 1999; Fanatico et al., 2007; Kuttappan et al., 2012; Lilburn et al., 2019; MacRae et al., 2007; MacRae et al., 2006; Mahon, 1999; Mazzoni et al., 2015; Mitchell, 1999; Petracci & Cavani, 2012; Scheele, 1997; Siervo et al., 2014; Siervo et al., 2018; Soglia et al., 2019; Trocino et al., 2015). Furthermore, myofibers of fast-growth commercial birds are three to five times larger than those of slower-growing birds (Dransfield & Sosnicki, 1999; Velleman, 2015).

These muscular changes associated with selection for rapid growth in early life are exacerbated in WB (Clark & Velleman, 2016; MacRae et al., 2007; MacRae et al., 2006; Mazzoni et al., 2015; Petracci & Cavani, 2012; Soglia et al., 2016; Trocino et al., 2015; Velleman & Clark, 2015; Velleman et al., 2018). Some researchers hypothesized as early as 1999 (Mahon, 1999) that poultry growth rates might be hitting a maximum threshold at which myofiber metabolism would be compromised by its increased size and the inability of oxygen, nutrients, and waste to diffuse across the fiber. This idea is supported through the demonstration of increased numbers of necrotic, basophilic, and hyaline fibers, and fibers with
NADH rich rims or negative cores. These features are indicative of mitochondrial dysfunction and altered oxidative metabolism in fast growth rate commercial broilers when compared to laying hens (MacRae et al., 2007; MacRae et al., 2006). Multiple recent histological studies of WB have demonstrated that the same lesions are observed in both affected and unaffected birds, but affected birds show significantly more lesions (Clark & Velleman, 2016; Mazzoni et al., 2015; Sihvo et al., 2017; Sihvo et al., 2018; Trocino et al., 2015; Velleman et al., 2018; Velleman & Clark, 2015). Three of these investigations have surveyed and found an absence of the histological characteristics of WB in the slow-growth broiler or layer lines (Clark & Velleman, 2016; Velleman & Clark, 2015; Velleman et al., 2018).

Others have investigated the pathophysiology of WB using transcriptomics, metabolomics, and proteomics approaches. When investigating affected and unaffected birds of the same lines, these studies have identified pathways showing differential regulation to include those associated with cellular movement, proliferation, assembly, function and maintenance, protein synthesis, post-translational modification, protein folding and carbohydrate metabolism (Abasht et al., 2016; Cai et al., 2018; Hubert et al., 2018; Kong et al., 2017; Kuttappan et al., 2017b; Kuttappan et al., 2017a; Mutryn et al., 2015; Papah et al., 2018; Schilling et al., 2017). When comparing both affected and unaffected fast-growth commercial broilers against slow-growth broilers, they have observed differential regulation of the same pathways as discussed above. However, they also observed differential regulation of disease pathways involved in organismal injury or abnormalities and abnormal development and morphology of muscle (Hubert et al., 2018; Kong et al., 2017; Velleman & Clark, 2015). Additionally, comparison of fast-growth and slow-growth varieties indicated that mitochondrial activities such as oxidative phosphorylation and the tricarboxylic acid cycle are downregulated in breast muscles of fast-growth broilers (Kong et al., 2017). These observations support the histological characteristics of WB. Furthermore, the molecular signatures of WB are suggestive of several diseases and disorders. The most significant included gastrointestinal disease, cardiovascular disease, hepatic system disease, neurological disease, and cancer (Abasht et al., 2016; Hubert et al., 2018; Kong et al., 2017; Kuttappan et al., 2017a; Mutryn et al., 2015).

Although differences in gene expression and the subsequent alterations to physiological pathways can be detected when comparing unaffected and affected WB samples, the resolution is weak and cannot provide a definitive pathology. Unaffected samples may not have developed the condition at the time of collection or are asymptomatic (Abasht et al., 2016; Hubert et al., 2018; Kuttappan et al., 2017a; Kuttappan et al., 2017b; Sihvo et al., 2017). The utilization of slow-growth varieties as a negative control for WB investigations can provide a clearer representation of the associated changes. Additionally, such a comparison would increase our understanding of genes and pathways that are concurrently important for WB and production traits such as growth rate and feed efficiency. Data from these investigations suggest that unaffected WB tissue may not be an adequate negative control for the determination of the molecular characteristics and pathophysiology of WB.

**Role of mitochondria and oxidative metabolism in WB**

The mitochondrion has crucial roles in cellular respiration, as well as several other factors specific to WB. As determined by Mutryn et al. (2015), gene expression studies of WB affected muscle samples have indicated intracellular calcium accumulation that has the potential to impair cell membrane integrity. Interestingly, the mitochondria regulate cytosolic calcium concentrations, which in turn regulate the cellular reduction-oxidation reactions of a variety of transcription factors and cellular enzymatic reactions, control cellular, and mitochondrial metabolic pathways, and manage mitochondrial ROS production (de Oliveira et al., 2017; Wallace, 2013). Furthermore, calcium overload has the potential to activate proteases and lipases within the cell, resulting in myofiber degeneration, a commonly observed histological feature of WB tissue (Halliwell, 2007; Mutryn et al., 2015). To this end, the role of mitochondria in WB occurrence or severity remains unclear. This gap in knowledge is surprising due to the mitochondrion’s significance for muscle energetics, cell signaling, and death, which are all known features in WB progression.

It is well known that mitochondria are commonly called the powerhouse of the cell and that they function in oxidative metabolism to provide energy in the form of ATP to the body as well as facilitate the removal of wastes and act in cell signaling, differentiation and programmed cell death (Angelini et al., 2009; Bonnard et al., 2008; de Oliveira et al., 2017; Irwin et al., 2008; Koch, 2016; Latorre-Pellicer et al., 2016; Liao et al., 2015; Muir et al., 2016; Moreno-Losuertos et al., 2011; Netzer et al., 2015; Sims & Muyderman, 2010; Tuppen et al., 2010; Velarde, 2013; Wallace & Chalkia, 2013). However, the mitochondrion is the only organelle in the cell with a genome (Koch, 2016; Latorre-Pellicer et al., 2016; Tuppen et al., 2010; Wallace & Chalkia, 2013; Yarham et al., 2010). Mitochondrial DNA (mtDNA) is small, circular, and clonally inherited from the mother. It consists of two regions; the non-coding region, which controls mtDNA, and the coding region, which codes for tRNAs, rRNAs, and 13 cellular energy production genes, and contains no introns (Latorre-Pellicer et al., 2016; Moreno-Losuertos et al., 2011; Muir et al., 2016; Takemoto et al., 1999; Tuppen et al., 2010; Wai et al., 2008; Wallace & Chalkia, 2013). Mitochondrial DNA is haploid and does not recombine; it is typically stable over time, but due to its few repair mechanisms, it tends to have a much higher mutation rate than nuclear DNA, and mutations are commonly deleterious.

The number of mitochondria in a cell varies significantly by type and tissue, and they often have more than one genome sequence, a common condition known as heteroplasmay (Abbott et al., 2014; Carelli et al., 2015; DiMauro & Schon, 2001; Lehmann et al., 2015; Luo et al., 2018; Payne et al., 2013; Tuppen et al., 2010; Wallace & Chalkia, 2013; Wai et al., 2008). This heteroplasmay often acts as a sort of shield for an individual carrying mutant mtDNA mutations. Over a lifetime, the percentage of mutant mtDNA increases, resulting in a decline in the individuals bioenergetic capacity below the minimum
threshold and symptoms of the pathogenic mtDNA mutation ensue (Abbott et al., 2014; Carelli et al., 2015; Lehmann et al., 2015; Latorre-Pellicer et al., 2016; Wallace & Chalkia, 2013). Due to this unique nature, pathogenic mtDNA mutations and the conditions caused by them are widespread, continually arising, can be localized to a specific tissue, are familial, and often age-related (Lehmann et al., 2015; Moreno-Loshuertos et al., 2011; Tuppen et al., 2010; Wai et al., 2008; Weber et al., 1997; Zeviani et al., 1991). This description fits the developmental stages observed in WB, as it arises at different ages, affects a high percentage, and occurs in varying severities throughout a broiler flock.

A critical trait of WB posing a dilemma for determining its pathology is its apparent localization to the breast muscle only. However, diseases caused by mitochondrial mutations typically localize to a tissue or region of the body, and the observation of both histological and molecular markers of oxidative stress and myocyte degeneration reveals their role in the pathogenesis of WB. We can determine the role of mitochondrial activity in WB through the use of next generation sequencing technologies and bioinformatics. However, investigations into human diseases in which mitochondria have recently been implicated such as diabetes, obesity, metabolic syndrome, stroke, Alzheimer’s, and cancer have demonstrated that an understanding of the molecular characteristics of the disease is instrumental in determining the involvement of mitochondrial-related bioenergetic alterations (Angelini et al., 2009; Barja & Herrero, 2000; Bonnard et al., 2008; D’Souza et al., 2011; Glancy et al., 2017; Latorre-Pellicer et al., 2016; Long et al., 2012; Maurya et al., 2018; Moreno-Loshuertos et al., 2011; Muir et al., 2016; Sims & Muyderman, 2010; Velarde, 2013; Wallace & Chalkia, 2013; Yarham el al., 2010). It is through these mechanisms that mitochondrial mutations can have significant physiological impacts; therefore, it is necessary to characterize the mitochondrial structure and gene expression related to WB.

**Genetic and nutritional interactions as potential drivers of WB**

In contrast, based on the evidence for oxidative stress and hypoxia in WB, some hypotheses have suggested a genomic and nutritional interaction based on the high energy, soy-based diet typical of the commercial broiler (Cruz et al., 2017; Haug et al., 2007; Koppenol et al., 2015; Li et al., 2019; Livingston et al., 2019b). Although many nutritional interventions were assessed, none have managed to decrease the incidence of WB (Bodic et al., 2018; Cruz et al., 2017; Guetchom et al., 2012; Livingston et al., 2019b; Livingston et al., 2019a; Lilburn et al., 2019; Livingston et al., 2019c; Sobotik et al., 2018; Tricino et al., 2015). Still, other hypotheses have gone further to implicate endocrine disruptors such as phytoestrogens from soybean meal in poultry feed, as a potential driver of genomic/nutritional interaction. Phytoestrogens are naturally occurring, plant-derived, biologically active compounds that both structurally and functionally mimic estrogens when in humans and animals (Bacciottini et al., 2007; Cederroth & Nef, 2009; Patisaul & Jefferson, 2010; Stevenson et al., 2014).

Soybean meal, one of the main ingredients in industrial poultry feed, has one of the highest concentrations of phytoestrogens among plant-based feed sources (Cederroth & Nef, 2009; Gjorgovska et al., 2014; Haug et al., 2007; National Research Council et al., 1994; Patisaul & Jefferson, 2010; Payne et al., 2001; Stein et al., 2008; USDA ARS, 2016). Due to their similarity to estrogen and the ability to bind to both estrogen receptor α and β, phytoestrogens act as endocrine-disrupting compounds (Cederroth & Nef, 2009; Gjorgovska et al., 2014; Patisaul & Jefferson, 2010). As estrogen regulates various vital physiological processes such as lipid and glucose metabolism, bone development, sexual maturation, and reproduction, perturbations of this suite of endocrine functions can potentially alter critical system-wide processes. Therefore, the nature and extent to which phytoestrogens drive occurrence and severity of WB deserves further attention.

The implications of high dietary concentrations of phytoestrogens are also relevant to mitochondrial cell signaling, and oxidative metabolism (Irwin et al., 2008; Liao et al., 2015; Mauvais-Jarvis, 2011; Sarkar et al., 2015; Sims & Muyderman, 2010; Velarde, 2013). One pathway involves alteration of mitochondrial cell signaling and mitochondrial hormonal regulation due to increased exposure to phytoestrogens. Recent work has shown the role of mitochondrial estrogen receptor-β and its implications for mitochondrial bioenergetics and tumorigenesis (Liao et al., 2015; Velarde, 2013). Additionally, human studies have determined that oxidative stress in skeletal muscle is often induced by dysregulation of the metabolism of energy fuel substrates such as lipids and glucose. The resulting damage to the mitochondria causes an increase in the buildup of ROS and fuel substrates (Bonnard et al., 2008; de Oliveira et al., 2017; Irwin et al., 2008; Liao et al., 2015; Marco et al., 1961; Netzer et al., 2015; Velarde, 2013). The endocrine-disrupting actions of phytoestrogens are capable of inducing metabolic dysregulation, and this is a possible element in the pathogenesis of WB warranting further investigation.

**Impacts of dietary omega-6 (ω-6) and omega-3 (ω-3) supplementation to oxidative stress and inflammation on WB gene expression**

Due to the many vital physiological activities of estrogens, the regulators of estrogen metabolism have become a popular topic in human and animal health. Currently, the most studied are the ω-3 and ω-6 polyunsaturated fatty acids. They boast a myriad of physiological effects including reduction of cholesterol, reduction of circulating levels of 17β-estradiol, reduced binding of estradiol to the estrogen receptor, stabilization of electrical activity of cardiac myocytes, increased apoptosis of cancerous cells, increased 2-hydroxylation of estradiol, and protection for mitochondria (Behling et al., 2015; Cao et al., 2012; de Oliveira et al., 2017; Dikshit et al., 2015; Gómez Candela et al., 2011; Jeromson et al., 2015; Lord et al., 2002; Marco et al., 1961). These effects provide benefits such as reduced risk of cardiovascular and metabolic diseases, and reduced risk of multiple cancer types (Behling et al., 2015; Betti et al., 2009; Cao et al., 2012; Dikshit et al., 2015; Gómez Candela et al., 2011; Jeromson et al., 2015; Lorente-Cebrián et al., 2015; Lord et al., 2002; O’Keefe et al., 1995; Simopoulos, 2002).

Multiple studies have investigated increasing ω-3 fatty acid concentrations in chicken feed, as an indirect way to increase human ω-3 consumption (Betti et al., 2009;
Carragher et al., 2016; Haug et al., 2007; Koppenol et al., 2015; Konieczka et al., 2017; Moghadam et al., 2017; Newkirk & Classen, 2002; O’Keefe et al., 1995; Rymer & Givens, 2005; Ratnayake et al., 1989; Zuidhof et al., 2009). A majority of these studies utilized fish-based additives as the source of ω-3s. The fish-based ω supplement did increase the ω-3 content of the muscle, but also caused a fishy taste and decreased the shelf life of the product (Koppenol et al., 2015; O’Keefe et al., 1995; Ratnayake et al., 1989; Rymer & Givens, 2005).

More recent studies have investigated plant-based sources of ω-3s such as flax and canola; however, most of these investigations demonstrated reduced growth rates and final carcass weights (Betti et al., 2009; Carragher et al., 2016; Haug et al., 2007; Konieczka et al., 2017; Newkirk & Classen, 2002; Parveen et al., 2013). Currently, the soy- and corn-rich standard poultry industry diet is very high in ω-6s but very low in ω-3s (Dikshit et al., 2015; Haug et al., 2007; O’Keefe et al., 1995; Rymer & Givens, 2005). For humans, the recommended ratio of ω-6:3 is roughly 5:1. Ratios of 2:3:1 have demonstrated improvements in patients with cardiovascular disease, rheumatoid arthritis, asthma and multiple types of cancers, while ratios of 10:1 have shown adverse effects (Gómez Candela et al., 2011; Jeromson et al., 2015; Rymer & Givens, 2005; Simopoulos, 2010; Simopoulos, 2002; Simopoulos, 2016).

Balancing the ω-6:3 content of industry broiler feed could have a positive impact on several conditions, which are currently causing tremendous losses to the poultry industry, including WB, WS, green muscle disease, and sudden death syndrome (SDS) to name a few. Furthermore, none of these studies have attempted to explain the physiological processes which result in the observed changes due to supplemented ω-3s, and few have considered differences in gene expression. Investigation of these processes utilizing multi-disciplinary approaches will help determine the extent to which nutritional modulation of ω-6:3 fatty acid ratios can influence oxidative stress in the muscle.

Conclusions

As detailed previously, the most commonly observed theme in WB is a state of oxidative stress in the pectoralis major; the origins and implications of the offending ROS remain unknown to date. Furthermore, the intracellular calcium buildup and NADH displacement indicate dysfunction of the oxidative metabolism machinery and a severe threat to cellular health. Comparative analysis utilizing chicken from multiple varieties (differing growth profiles) to explore transcriptional and genomic changes due to WB in both nuclear and mtDNA would provide increased depth for understanding the pathogenesis of WB and the impact of artificial selection for commercial traits, as would investigating the effects of omega ratios on the molecular signatures inflammation between fast- and slow-growth varieties. Combining these data with further evaluation of WB through “omics” technologies can help determine both the origins and implications of this oxidative stress and its actions in the pathogenesis of WB. Through these observations, a clear view of nutritional, age, and genetic interactions could be developed and generate new solutions for WB.

Data availability

No data are associated with this article.

References


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Bruno Ronchi
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The review is lacking in the following aspects:
1. The effects of the disease on meat quality of commercial broiler.
2. The effects of some disease control programs (e.g. against coccidiosis).
3. The impact on productive efficiency and profitability.
4. The protective effects of some antioxidants administered with the diet; biochemical signatures potentially useful for diagnosis.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal nutrition and metabolism

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Casey Owens

Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, USA

- The review is well written. Addition of review on gene expression work would be beneficial and the last part regarding Omegas is not that strong as there is no work to date that shows any relationship to WB. I would suggest deleting that portion or strengthen it.

- Please use "Pectoralis major" (or minor) italicized throughout.

- Please use another term other than "varieties" such as "breeds". Varieties is not commonly used for poultry (more so for plants).

- Please provide a reference for "First identified roughly 15 years ago...".

- WB characteristics have also been observed in thigh meat so please reword statement about WB localizing in the breast only (ie, maybe change to primarily in the breast) ("A critical trait of WB.....).

- Omega-6 and Omega-3 review is weak in terms of showing no direct relationship to WB. Tie these together more, condense significantly, or delete.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** poultry meat science/myopathies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Comments on this article**

**Version 1**

Reader Comment 25 May 2020

Mohammed Gagaoua, Teagasc, Dublin, Ireland

One aspect that the authors did not consider in their review is muscle type and muscle fibers. I think that it is important to include this aspect and detail it accordingly, as we know that the distribution of mitochondria differ between oxidative and glycolytic fibers, and also differ among muscles.

The other aspect I recommend to the authors, is to check among the proteomics studies in this field, if oxidative stress proteins were identified. In other species we have different proteins such as Peroxiredoxins, SOD1 & 2, PARK7 known as DJ-1 and many others. A literature review about this would help improve this very nice review.

**Competing Interests:** I have no conflict of interest with this work.

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