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PATHOLOGY OF WOODEN BREAST MYOPATHY IN BROILER CHICKENS

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ACADEMIC DISSERTATION

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ABSTRACT

Abnormally hard breast fillet consistency began to emerge in commercial broiler chickens approximately around 2010. Due to the remarkable muscle hardness, the condition acquired a vernacular name ‘wooden breast myopathy’ (WB). This thesis includes studies of WB morphology and pathogenesis on field samples obtained from slaughterhouses in 2012 and experimental rearing studies of broilers, conducted in 2012 and 2014. The first substudy of this thesis describes the chronic morphology of WB and resulted in the first peer-reviewed publication on WB. The second and third substudies include further analyses on the morphology and pathogenesis of WB.

This work characterizes WB as an abnormally firm muscle consistency that is restricted to the pectoralis major muscle in broiler chickens. Additional macroscopic features include pale color, outbulging appearance, occasional hemorrhage and a layer of clear fluid on the muscle surface. WB starts to develop after two weeks of age at the earliest and typically proceeds into a chronic myodegeneration in three to four weeks of age. The lesion begins focally and typically develops into a diffuse lesion that involves the major pectoral muscle completely.

Microscopically, WB manifests as a polyphasic myodegenerative disease. This rules out single injuries to the muscle tissue as etiologies for wooden breast myopathy. Ongoing myodegeneration is accompanied by regeneration, and at chronic stages prominent secondary changes such as fibrosis develop. Lymphocytic phlebitis is strongly associated with the myodegeneration, but its role in the pathogenesis of wooden breast is currently disputable.

The restricted location of the wooden breast lesion in the pectoralis major muscle distinguishes it from several other myodegenerative diseases that widely affect the skeletal muscle system and often the cardiac and smooth muscle systems too. Other skeletal muscles, such as thigh or dorsal muscles, may occasionally exhibit wooden breast-like lesions, but the syndrome primarily affects the major pectoralis muscle.

Relatively reduced microvessel density contributes to the development of wooden breast myopathy. Either the affected birds initially exhibit lower capillary densities, compared to the birds that never succumb to wooden breast, or a relative reduction in the vascular supply, which occurs before the initiation of the degenerative lesion.

Decreased dietary selenium does not affect the prevalence of wooden breast myopathy. The morphology of polyphasic myodegeneration renders selenium deficiency as one possible causative factor for wooden breast myopathy, but decreased selenium content in the diet had no effect on the prevalence of wooden breast myopathy in our experimental rearing studies.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals (I – III):

I Sihvo HK, Immonen K, Puolanne E. 2014 (Electronic version 2013). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology* 51:619-23.
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II Sihvo HK, Lindén J, Airas N, Immonen K, Valaja J, Puolanne E. 2017. Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. *Veterinary Pathology* 54:119-128.
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III Sihvo HK, Airas N, Lindén J, Puolanne E. 2018. Pectoral vessel density and early ultrastructural changes in broiler chicken wooden breast myopathy. *Journal of Comparative Pathology* 161:1-10.
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ABBREVIATIONS

ADP	adenosine diphosphate
AE	avian encephalitis
ALD	anterior latissimus dorsi
ANOVA	analysis of variance
ART	avian rhinotracheitis
ATP	adenosine triphosphate
CAV	chicken anemia virus
CD	cluster of differentiation
CI	confidence interval
DMD	Duchenne muscular dystrophy
ECM	extracellular matrix
HE	hematoxylin and eosin
IB	infectious bronchitis
IBD	infectious bursal disease
ILT	infectious laryngotracheitis
IU	international unit
MyHC	myosin heavy chain
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMJ	neuromuscular junction
PAS-D	periodic acid-Schiff reaction with diastase
PSE	pale soft exudative
RyR	ryanodine receptor
SERCA	sarco/endoplasmic reticulum Ca^{2}+-ATPase
SR	sarcoplasmic reticulum
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
WB	wooden breast myopathy

1 INTRODUCTION

Humans and chickens share a lengthy common history, originating from the domestication of the chicken approximately five to ten thousand years ago. The large-scale commercial production of broiler chicken meat started to develop in the 1920s and currently involves 65 billion birds annually worldwide.

During the evolution of the chicken meat production industry, occasional cases of abnormal meat consistency may have occurred, but after 2010, a dramatic emergence of abnormally hardened consistency and pale color of breast fillets in commercial broiler chickens arose in Finland. According to the remarkable breast fillet hardness, the condition acquired a vernacular name 'wooden breast myopathy' (WB). The occurrence of WB in Finland was published in 2013, but soon it became evident that WB represents a worldwide phenomenon.

At the start of WB emergence, it remained unclear whether WB represents a real during-life disease or whether it was purely a meat quality issue that appeared in the breast fillet after the bird's death. No specific clinical signs were associated with WB at the farm level. The macroscopic appearance of WB differed from the previously described avian myopathies or quality impairments, such as deep pectoral myopathy or pale-soft-exudative meat, raising the question as to whether a novel type of myopathy had emerged in broiler chickens.

As WB damages breast fillet quality and appearance, the affected fillets are either downgraded or rejected at meat inspection or later during meat processing. As the breast fillets constitute the most valuable part of the broiler carcass, their rejection from human consumption causes remarkable economic losses. Other important concerns include the potential reduction in the animal welfare of the affected birds and the ethical issues related to the rejection of remarkable amounts of broiler meat.

This dissertation thesis stems from the research project that studied the effect of husbandry conditions and other external factors on the prevalence of WB in the Faculty of Agriculture and Forestry of University of Helsinki, Finland, led by Research Director, Professor Emeritus Eero Puolanne. As WB appeared to be a novel myopathic condition with unknown etiology, it soon became apparent that its basic morphology needs to be described first in order to proceed with studies on the pathomechanism, and this thesis developed on that ground.

This dissertation includes studies on the morphology of acute and chronic WB, the temporal development of WB and some aspects of the possible pathogenesis of WB. The first sets of samples were breast fillets of commercial broiler chickens, received from slaughterhouses, and the myodegenerative nature of the WB lesion was first revealed in those samples. In the slaughter-age broilers, the chronic lesions were remarkable, which hindered the determination of the nature and onset-time of the initial primary lesion and raised the need to study younger birds in order to determine the onset period of the lesion and to describe the acute lesions. Fortunately, several rearing experiments of broiler chickens were carried out in Professor Puolanne's project in order to study the effect of dietary and husbandry conditions on WB, and some of those experiments also provided valuable material for the pathologic studies of this dissertation.

2 LITERATURE REVIEW

2.1 SKELETAL MUSCLE

Structure and function

Skeletal muscles enable posture, locomotion and breathing under voluntary control. Although the muscle tissue type varies according to the function, both avian and mammalian skeletal muscle share very similar tissue structure (Rome et al., 1988).

Skeletal muscles are composed of bundles of long, multinucleated cells called myofibers – also known as myocytes, muscle fibers or muscle cells – supported by a connective tissue framework, nerves and blood vessels (Hill and Olson, 2012). The counterpart of cytoplasm in other cell types is called sarcoplasm in muscle tissue. Additionally, there are two distinct membrane systems in the myofiber: the transverse tubule system (t-tubules) and sarcoplasmic reticulum (SR). T-tubules are invaginations of the cell membrane (sarcolemma) into the myofiber at regular intervals and enable rapid spread of calcium ions throughout the myofiber, whereas the SR is an internal membrane system within the myofiber and effectively collects calcium ions from the sarcoplasm (Franzini-Armstrong and Engel, 2012).

In most animals, the typical myofiber cross-sectional diameter is 30-70 μm and the length varies from one millimeter up to several centimeters, depending on the muscle type and location (Cooper and Valentine, 2016). The myofibers consist of myofibrils that are formed from parallel actin (thin) and myosin (thick) filaments. Sarcomere denotes one unit of these filamentous proteins. The sliding of thin and thick filaments along each other results in contraction and relaxation of the muscle fibers (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954; Hill and Olson, 2012), enabling body movements when the muscle contracts across a joint. The movements occur under neuronal control; the neuronal message is transmitted via the neuromuscular junction (NMJ), where the motor neuron meets the myofiber. Each α-motor neuron innervates a group of myofibers of similar fiber type (Edström and Kugelberg, 1968; Burke et al., 1971) and together they form a motor unit (Buchthal and Schmalbruch, 1980).

When an action potential arrives at the neuromuscular junction along the motor nerve, the action potential spreads through the myofiber via t-tubules, resulting in release of Ca^{2+} ions from the SR into the sarcoplasm (Sandow, 1952; Smith et al., 1986). The Ca^{2+} ions are released mainly via ryanodine receptor channels, starting the process called excitation-contraction coupling (Sandow, 1952; Smith et al., 1986). The binding of Ca^{2+} to troponin uncovers the myosin-binding site on actin, allowing the myosin head to form a cross-bridge with the actin filament (Snellman and Tenow, 1954; Ebashi, 1974). The change in the angle of the myosin head consumes adenosine triphosphate (ATP) as a source of energy and results in the movement of actin and myosin in relation to each other and a subsequent shortening of the sarcomere (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). In the presence of ATP, new cycles of myosin-actin sliding begin until the cytosolic Ca^{2+} concentration is reduced as a result of the withdrawal of Ca^{2+} back into the SR via the sarco/endoplasmic

reticulum Ca^{2+}-ATPase (SERCA) pump and no further neuronal stimulation occurs (Franzini-Armstrong, 1980; Martonosi et al., 1982).

Muscle fibers are divided into several types according to their properties, such as contraction velocity (Table 1). The traditional classification of myofibers is based on myosin ATPase histochemical reactions in variable pH and divides the fibers into type 1 (slow) and type 2A and 2B (fast) fibers (Bárány, 1967). A more recent classification is based on immunohistochemical identification of myosin heavy chain (MyHC) isoform composition and includes an additional fast fiber class 2X (Schiaffino et al., 1989). The contraction velocity progressively increases from type 1 to type 2A, 2X and 2B fibers (Schiaffino and Reggiani, 2012). In addition to contraction velocity, the metabolic properties constitute another major difference between the fiber types.

The MyHC isoforms can be identified by several techniques, such as immunohistochemical stainings, in situ hybridization or electrophoretic separation of MyHCs (Termin et al., 1989; Schiaffino et al., 1989; DeNardi et al., 1993). In addition to the four major subclasses of myofiber type, minor fiber type populations exist in the skeletal muscle of the head, such as extraocular and jaw muscles (Schiaffino and Reggiani, 2012).

Skeletal muscle develops during embryogenesis from mononucleated precursor cells called myoblasts, which fuse into multinucleated myotubes (Pytel and Anthony, 2015). The embryonic skeletal muscle forms in successive waves of primary and secondary generation fibers; in the primary wave the muscle fiber types develop independent of innervation, whereas the secondary wave is more dependent on innervation (Stockdale and Miller, 1987; Harris et al., 1989; Schiaffino and Reggiani, 2012). In adult skeletal muscle, the fiber type contraction velocity phenotype can change in response to different hormonal stimulation, such as glucocorticoids or thyroid hormones, or changes in motor neuron firing pattern, such as denervation (Schiaffino and Reggiani, 2012). Progressive fiber atrophy and decrease in myofiber and motor neuron number occur during aging (Larsson et al., 1991).

Table 1. *Properties of different myofiber types*. Compiled from Schiaffino and Reggiani, 2012; Hill and Olson, 2012.

	Type 1	Type 2A	Type 2X	Type 2B
Contraction velocity	Slow	Moderately fast	Fast	Very Fast
Capillarization	High	Intermediate	Low	Low
Mitochondrial density	High	High	Medium	Low
Energy production	Aerobic	Long-term anaerobic	Short-term anaerobic	Short-term anaerobic
Myosin heavy chain gene	MYH7	MYH2	MYH1	MYH4
Main energy source	Triglycerides	Phosphocreatine, Glycogen	Phosphocreatine, Glycogen	Phosphocreatine, Glycogen
Fatigue resistance	High	Moderately high	Intermediate	Low
Motor neuron size	Small	Medium	Large	Very Large

In addition to the myofilaments actin and myosin that compose the core of sarcomere, several other proteins are needed to regulate and support the sarcomere. Support proteins connect the sarcomere to the sarcolemma, the cell membrane of skeletal muscle cells (Hill and Olson, 2012). Dystrophin is an essential support protein that connects the myofilaments to the sarcolemma by linking the outermost actin layer to a complex of several other transmembrane proteins on the sarcolemma (Hoffman et al., 1987; Ervasti and Campbell, 1993). This dystrophin-associated glycoprotein complex further connects to the extracellular matrix via laminin protein (Ervasti and Campbell, 1993). In addition to its mechanical support, dystrophin mediates intracellular signaling of mechanical force and cell adhesion (Gao and McNally, 2015). Defects in the dystrophin-associated protein complex are collectively named as muscle dystrophies, of which Duchenne muscular dystrophy (DMD) is one of the most common (Monaco et al., 1986). Naturally occurring DMD is well known in humans and dogs, but experimental models have been developed in several animal species varying from mouse to zebrafish (McGreevy et al., 2015). In DMD, a loss-of-function mutation in the dystrophin encoding genes and the subsequent deficiency of dystrophin lead to severe functional aberrations and myofiber pathology, such as variation of fiber size, increased number of internal nuclei, degeneration, necrosis, regeneration and connective tissue proliferation (Monaco et al., 1986; Pytel and Anthony, 2015). In addition to skeletal muscle, cardiac muscle and other organs, such as the brain are also affected by DMD (Pytel and Anthony, 2015).

The supporting extracellular matrix (ECM) that surrounds the myofibers is divided into three layers: the endomysium surrounding each myofiber, the perimysium surrounding a bundle of

myofibers, and the epimysium ensheathing the complete muscle (Chapman et al., 2016). The ECM constitutes only 1-9% of the cross-sectional area of muscle, but serves many crucial functions, since it transmits force from myofibers to tendons and provides mechanical stability to myofibers, vessels and nerves (Light and Champion, 1984; Kjaer, 2004). In humans, endomysium contains mainly type I and type IV collagens, whereas the main collagen in peri- and epimysium is type I with lesser amount of type III collagen (Light and Champion, 1984). Fibroblast is the main cell type in ECM and the producer of ECM proteins in response to upregulation of certain transcription factors, such as TGF-β, NF-κβ and TNF-α (Chapman et al., 2016). In addition to fibroblasts, myoblasts are also able to produce some ECM proteins, but fibroblasts are needed for the proper assembly of the proteins (Kühl et al., 1982).

Mitochondria are double-membrane cell organelles where cellular respiration occurs. The outer membrane encloses the whole mitochondrion, whereas the coiled inner membrane forms cristae and separates the intermembrane space from the mitochondrial matrix inside the inner membrane. In cellular respiration, energy is stored in ATP, which is formed from ADP and phosphorus by oxidative phosphorylation. ATP synthesis is coupled to an electrochemical gradient change created by proton H^+ concentration differences across the mitochondrial membrane in the electron transport chain (Kennedy and Lehninger, 1949). Two categories of mitochondria exist in the skeletal muscle: 1) intermyofibrillar mitochondria located between the myofibrils and 2) subsarcolemmal mitochondria that appear in clusters under the sarcolemma (Franzini-Armstrong and Engel, 2012). The functional significance of the two different locations is controversial, but intermyofibrillar mitochondria express higher respiratory chain complex activity and appear to uptake Ca^{2+} ions in proximity to the calcium release sites (Ferreira et al., 2010; Franzini-Armstrong and Engel, 2012). Under normal physiologic conditions, the contribution of mitochondria to cellular Ca^{2+} homeostasis is insignificant, but the Ca^{2+} uptake has a role in respiratory chain activation (Franzini-Armstrong and Engel, 2012). In pathologic conditions, Ca^{2+} ions accumulation in mitochondria can occur, particularly in association with oxidative stress, which may lead to mitochondrial degeneration (Duchen, 2000).

Humans and most animal species carry two types of DNA in their cells; linear-shaped nuclear DNA and circular-shaped mitochondrial DNA. Mitochondrial DNA contains fewer than 40 genes, all maternally inherited, compared to the approximately 20,000 genes in the nuclear DNA that are derived from both parents (Birky, 1978; Wolff and Gemmell, 2013). The mitochondrial DNA unit is located in the mitochondrial matrix within the inner mitochondrial membrane and encodes several components of the respiratory chain (Anderson et al., 1981; Friedman and Nunnari, 2014). All the other structures of the mitochondrion are coded by the nuclear DNA (Friedman and Nunnari, 2014). Dysfunctional mitochondria, typically arising from genetic defects in the respiratory chain components, causes several myopathies with muscular weakness and various other clinical symptoms (Di Mauro, 2010).

Physiologic and pathologic responses

Hypertrophy refers to increased myofiber diameter at cellular level, or to enlarged volume at the level of a whole muscle, which may originate from muscle cell hypertrophy or other causes, such as increased amount of extracellular matrix. In adult skeletal muscle, the increase in myofiber size typically results from the synthesis of new myofibrils and other cellular

components. Incorporation of myoblasts into a pre-existing myofibers is a more common mechanism for increasing fiber diameter during muscle development. Increased workload is a physiologic cause of hypertrophy and fiber diameter may increase by up to 100 µm (Cooper and Valentine, 2016). Pathologic hypertrophy may occur as a compensatory response to loss of other myofibers or as a specific primary hypertrophy, such as in 'double muscling' of cattle and some other mammals, caused by defective myostatin genes (Grobet et al., 1997). In addition to increased diameter, pathologically hypertrophic fibers can exhibit several other histologic changes, such as internal nuclei, fiber splitting, ring fibers or whorled fibers (Cooper and Valentine, 2016).

Atrophy occurs when cellular catabolism exceeds cellular component synthesis. It refers to a reduction of muscle volume at the level of a whole muscle, whereas atrophy at the cellular level is a decrease in the myofiber diameter. No sarcolemmal damage or leakage of muscle proteins into plasma occur in atrophy because myofibrils and other cell components are recycled by the ubiquitin-proteasome system or autophagy-lysosome system. Atrophy may affect specific fiber types or be generalized, depending on the cause. For example, hypothyroidism and disuse cause selective atrophy of type 2 myofibers, whereas all fiber types are affected in denervation atrophy. Atrophy can affect small or large groups of fibers, which is indicative of denervation etiology (Cooper and Valentine, 2016; Valentin, 2017).

A variety of degenerative lesions occur in skeletal muscle, ranging from local sarcolemmal injuries to complete myofiber necrosis (Yin et al., 2013). Etiologies for the injuries are various, such as trauma, excessive physical activity, toxic injury and genetic defects, among others. Due to the extensive length of myofibers as cells, necrosis can affect only a part of the fiber, which is termed segmental necrosis. Degeneration or necrosis of skeletal muscle can be classified based on the distribution (focal or multifocal) and temporal pattern (monophasic or polyphasic) of the injury (Cooper and Valentine, 2016). Minor lesions such as local plasma membrane damage can be restored by fusion of subsarcolemmal membrane vesicles (Galbiati et al., 1999; Bansal et al., 2003), but more severe damage leads to compromised sarcolemmal integrity, increased myofiber permeability and disturbance of the ion and osmotic balance of the cell (Yin et al., 2013). In particular, the uncontrolled release of Ca^{2+} ions leads to activation of calcium-dependent proteases, such as the calpains, which degrade the myofiber proteins (Dourdin et al., 1999). Muscle proteins and microRNAs leak into the circulation due to myofiber disruption and can be measured from the plasma as markers of muscle injury (Angelini et al., 1968; Laterza et al., 2009). Inflammatory cells are recruited to the site of injury; granulocytes arrive within the first hours after injury, followed by macrophages, which become the major inflammatory cell population within the first 24 hours after the injury (Fielding et al., 1993; Chazaud et al., 2009; Yin et al., 2013). Macrophages phagocytize the cellular debris of degraded myofibers and secrete both pro- and anti-inflammatory cytokines that regulate the inflammatory regenerative processes (Chazaud et al., 2009).

Degeneration or necrosis of an extensive number of myofibers often appears macroscopically as increased paleness of the muscle area, but less severe lesions may be difficult to observe macroscopically. In light microscopy, the earliest histologic change of degenerative myofibers include hypercontraction in longitudinal orientation or hypereosinophilic fibers with increased diameter in cross-sectional view. However, similar changes are often seen as artifacts due to sampling procedures and more reliable histologic changes of myofiber necrosis include loss of striation and nuclei with myofiber fragmentation and infiltration of

inflammatory cells, mainly macrophages, into the degraded myofiber. Necrotic myofibers are prone to mineralization, which appears as basophilic granular or crystalline material within the myofiber (Valentin, 2017).

Skeletal muscle tissue is able to partially regenerate in response to injury. Satellite cells are resident stem cells between the sarcolemma and the basal lamina of muscle fibers (Mauro, 1961) and remain quiescent until stimulated to proliferate (Bischoff, 1986). Cytokines excreted during inflammation in response to myofiber degeneration and necrosis strongly stimulate the activation of satellite cells. Satellite cell activation leads to its asymmetric division where one daughter cell remains as stem cell and the other differentiates into a myoblast, which fuses to a damaged myofiber and then matures as myofiber (Yin et al., 2013). Regeneration begins within a couple of days, peaks at two weeks after injury and gradually fades until approximately one month after injury (Gharaibeh et al., 2012). All satellite cells of a myofiber are activated also in the case of local injury in one end of the fiber and the activated satellite cells migrate to the regeneration site (Schultz et al., 1985). Regeneration is rarely observable macroscopically, unless covering remarkable areas of tissue. Histologically regeneration appears first as plump satellite cell nuclei along the basal lamina, which then arrange in rows and start to produce basophilic cytoplasm. The cytoplasm later acquires cross-striated appearance when the sarcomeres are formed (Valentin, 2017).

Fibroblasts seem to play an important role in muscle regeneration by prevention of premature differentiation of satellite cells, allowing sufficient proliferation of satellite cells to occur before their maturation into myocytes (Murphy et al., 2011).

In addition to regulative function in regeneration, fibroblasts respond to muscle injury with extracellular matrix proliferation. The primary muscle lesion typically involves the activation of an inflammatory response and the release of cytokines such as TGF-β and TNF-α that promote ECM production by fibroblasts (Mann et al., 2011; Chapman et al., 2016). In acute and reparable injury of a healthy skeletal muscle, a transient inflammatory infiltration and mild collagen deposit occurs before resolution and muscle regeneration, whereas in chronic injury the inflammatory response persists and collagen deposition accumulates (Mann et al., 2011). Excessive collagen-rich ECM accumulation (fibrosis) leads to decrease both in force production and passive motion range of the affected skeletal muscle (Zumstein et al., 2008; Klingler et al., 2012; Pytel and Anthony, 2015). In addition to fibroblasts, the fibrotic scar is often infiltrated with adipocytes, but the cellular mechanism of this fatty degeneration is currently controversial (Natarajan et al., 2010).

2.2 MODERN BROILER CHICKEN

The chicken (*Gallus gallus domesticus*) originates from the red junglefowl (*Gallus gallus*) and the grey junglefowl (*Gallus sonneratii*) and was domesticated approximately 5,000 to even 10,000 years ago (Eriksson et al., 2008; Siegel, 2014). Chickens have been actively bred from at least 1873, when the American Poultry Association was founded (Ekarius, 2016). Creation of hybrids particularly intended for meat production by crossing different chicken breeds became more systematic in the 1940s (Thomas et al., 1958; Warren, 1958).

The popularity of poultry meat has increased dramatically over the last several decades. The worldwide consumption of poultry meat in 2016 was 115 million tons, of which both the USA and China account for about 18 million tons each and the EU 13 million tons (OECD, 2017). In Finland, 71 million broilers were slaughtered in 2017, producing 119 million kilograms of meat (Natural Resources Institute Finland, 2017). In the chain of broiler breeding, the grandparent generation of broilers lay hatching eggs that become the parent (breeder) generation, which then provide rise to the broiler generation utilized for human consumption (Suomen Siipikarjaliitto, 2017). A national health-monitoring program has been available since 1989 in Finland in order to follow the vaccination efficacy and prevalence of several important poultry diseases (Finnish Food Safety Authority, 2016). The program follows the prevalence of avian encephalitis (AE), chicken anemia virus (CAV), infectious bursal disease (IBD), infectious bronchitis (IB), infectious laryngotracheitis (ILT), avian rhinotracheitis (ART), *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from the parent stocks in Finland. Although participation in the monitoring program is voluntary, it is popular and carried out by testing the antigen titers in the blood. In addition, all broiler generations are monitored for *Salmonella* bacteria, which has a very low prevalence in Finland. The Finnish grow-out farms employ all-in-all-out rearing method, whereas thinning out is more common in many other countries (Suomen Siipikarjaliitto, 2017).

The modern broiler chickens of the 21st century are the result of an intentional genetic selection towards better meat yield and relatively lower feed consumption (Havenstein et al., 1994; Hunton, 2006). During the last sixty years, the growth rate has multiplied, breast muscle weight proportional to the total body weight has doubled (Fig. 1) and the intestine is longer, whereas the amount of adipose tissue and the relative size of the heart have decreased (Schmidt et al., 2009; Zuidhof et al., 2014). However, many undesirable traits have emerged concurrently, such as ascites syndrome, poor reproduction, skeletal abnormalities and impairements of the immune system (Warren, 1958; Thomas et al., 1958; Griffin and Goddard, 1994; Lilburn, 1994; Scheele, 1997; Cheema et al., 2003).

The selection for rapid growth decreased the slaughter age of broiler chickens from 12-16 weeks posthatch in the 1950s to 7 weeks by the 1990s, and currently the typical slaughter age varies between 5 and 7 weeks of age posthatch, depending on the production method and broiler hybrid (Griffin and Goddard, 1994; Schmidt et al., 2009; The National Chicken Council, 2015). The modern broiler chicken gains weight on average 20-40 g daily for the first two weeks of life, then approximately 100 grams daily until slaughter age, when the broiler is approximately three kilograms in terms of liveweight (Zuidhof et al., 2014; The National Chicken Council, 2015). Pectoralis major muscle represents the most valuable part of the broiler carcass and weighs approximately one fifth of the total body weight (Zuidhof et al., 2014; Kuttappan et al., 2016).

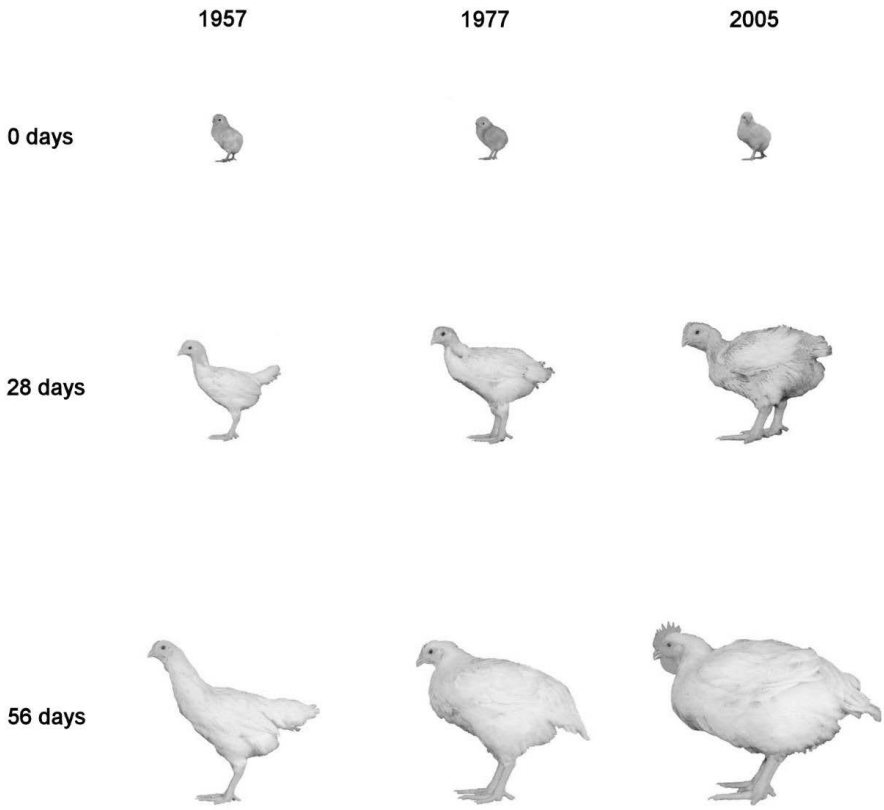


Figure 1. *The development of broiler chicken size with age. Alberta Meat Control strains from 1957 and 1977, Ross 308 in 2005.* Modified from Zuidhof *et al.*, 2014.

Pectoral muscles enable flight movements and shorten over a larger fraction of their resting length than many other avian muscles. The large pectoralis major muscle depresses the wings and the smaller pectoralis minor (supracoracoideus) elevates the wing (Biewener, 2011). The pectoralis major muscle originates both from the keel, furcular (wishbone) and dorsally from the ribs and inserts to the humerus of the wing (Biewener, 2011). In broiler chickens, the pectoralis major muscle consists almost entirely of type 2B myofibers, which are fast-twitch and glycolytic (Remignon et al., 1995; Papinaho et al., 1996; MacRae et al., 2006). These fibers exhibit increased diameter (hypertrophy) in rapidly growing healthy broiler chickens, compared to slower-growing broilers or layer chickens (Remignon et al., 1995; Soike and Bergmann, 1998a; MacRae et al., 2006; Velleman and Clark, 2015; Clark and Velleman, 2016). A typical myofiber diameter in adult slaughter-age broiler equals approximately 60-70 μm (Hoving-Bolink et al., 2000). The myofiber hypertrophy increases the diffusion distance between blood vessels and the myofiber center, which has been speculated to be a cause for the high incidence of muscle pathologies in the rapidly growing broiler chickens due to the

metabolic distress (Soike and Bergmann, 1998). Even in macroscopically unaltered pectoralis muscles, meat-type chickens express a higher amount of disseminated degenerative fibers than layer-type chickens (Soike and Bergmann, 1998b). In poultry muscles, centrally located nuclei are also common and considered to be a normal finding, although the majority of myofiber nuclei remain peripherally similar to mammals (MacRae et al., 2006; Barnes et al., 2016). In addition to the initial myofiber hypertrophy, the myofiber diameter further increases with age in the high-yielding broilers, whereas the total vessel density decreases (Joiner et al., 2014; Radaelli et al., 2017).

2.3 AVIAN MYOPATHIES AND MEAT QUALITY DEFECTS

Similar to other animals, avian species are affected by many different myopathies, which vary in both in their prevalence as well as in their clinical and economic significance. In addition to myopathies that occur during life, also meat quality defects that mainly develop post mortem are encountered in birds.

Deep pectoral myopathy

Deep pectoral myopathy was first recognized in adult turkeys (Dickinson et al., 1968), later in broiler chickens (Richardson, 1980) and characterized as a focal, well demarcated and centrally located necrosis (Fig. 2) in one or both deep pectoral (pectoralis minor or supracoracoideus) muscles (Siller and Wight, 1978; Wight and Siller, 1980). Also referred to as Oregon disease and green muscle disease, deep pectoral myopathy currently remains a significant disease in meat-type broiler chickens at present. Some of the latest prevalence data state an average prevalence of approximately 1-2%, whereas the flock prevalence may rise up to 16% (Bianchi et al., 2006; Kijowski and Konstanczak, 2009).

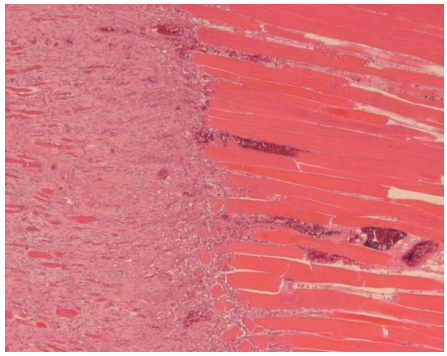


Figure 2. *Histology of deep pectoral myopathy. Necrosis and fibrosis on the left side of the micrograph abruptly demarcates from the viable muscle tissue on the right side. HE stain.* (Photo: Hanna-Kaisa Sihvo)

The pathogenesis of deep pectoral myopathy is explained by ischemia secondary to compression of swollen muscle between the keel bone and inelastic fascia or the major

pectoral muscle (Siller et al., 1978; Siller et al., 1979; Klasing et al., 2008). Muscle swelling usually originates from vigorous exercise of pectoral muscles and handling practices that induce extensive wing flapping may increase the prevalence of deep pectoral myopathy (Siller et al., 1978; Klasing et al., 2008).

Deficiency of selenium and vitamin E

Deficiency in the mineral selenium, vitamin E, or both, causes nutritional myopathy, encephalomalacia and exudative diathesis in poultry (Klasing et al., 2008). Both selenium and vitamin E function as antioxidants or precursors for antioxidants and they may partially complement each other, since selenium has been shown to prevent some pathologic changes of vitamin E deficiency (Scott, 1980; Eggermont, 2006; Klasing et al., 2008). Vitamin E is a lipid-soluble antioxidant that incorporates to the plasma membrane (Cardoso et al., 1999). Due to the lower antioxidant capacity and high amount of unsaturated lipids, the fast-twitch glycolytic type 2 myofibers are more susceptible to nutritional myopathy than the oxidative slow-twitch type 1 fibers (M. E. Murphy and Kehrer, 1986; Avanzo et al., 2001). Dietary levels of selenium under 0.1 mg/kg of diet have been associated with nutritional myopathy (Bains et al., 1975). The broiler breeding companies recommend dietary selenium levels of 0.30-0.35 mg and vitamin E levels of 50-80 IU per kg of diet, varying according to the dietary base and the age of the birds (Aviagen, 2014; Cobb-Vantress, 2015).

The fiber type composition in chicken pectoralis major muscle is almost entirely of fast, type 2B myofibers (Remignon et al., 1995; Papinaho et al., 1996; MacRae et al., 2006), which likely renders that muscle as the most common site of nutritional myopathy in chickens (Klasing et al., 2008). Macroscopic changes of nutritional myopathy include pale patches or stripes of variable width in skeletal muscles at approximately four weeks of age and onwards (Dam et al., 1952; Machlin and Shalkop, 1956). Histologically the pale stripes manifest as degeneration of myofibers, interstital edema and separation of myofibers, regeneration and finally fibrosis (Dam et al., 1952; Machlin and Shalkop, 1956; Klasing et al., 2008). In addition to skeletal muscles, smooth muscle, especially in gizzard wall, and cardiac muscle are also often affected in nutritional myopathy (Klasing et al., 2008).

Clinical signs of encephalomalacia include ataxia, paresis, muscular contractions, head retraction, collapse and death (Bains and Watson, 1978; Klasing et al., 2008). The cerebellum is the most commonly affected area of the brain, but the striatum, medulla oblongata and mesencephalon can also be affected (Pappenheimer, 1939; Klasing et al., 2008). Histologically, the main lesions consist of ischemic necrosis, demyelination and neuronal degeneration (Wolf and Pappenheimer, 1931; Klasing et al., 2008).

Exudative diathesis denotes subcutaneous edema associated with increased permeability of blood vessels, notably capillaries (Goldstein and Scott, 1956; Creech et al., 1958). The blood albumin ratio is reduced in the affected birds (Goldstein and Scott, 1956) and sudden death may occur (Klasing et al., 2008). Although vitamin E deficiency alone was initially thought responsible for exudative diathesis, selenium is now also considered important in the pathogenesis, as both selenium and vitamin E protect the capillary membrane against oxidative damage (Noguchi et al., 1973).

Ionophore toxicity

Ionophore compounds impair the normal transportation of ions across surface membranes in several different stages of coccidian parasites (Dowling, 1992). Therefore, ionophores are widely used as coccidiostats to chemically control coccidiosis in avian species (Dowling, 1992; Klasing et al., 2008). Ionophores are fermentation products of *Streptomyces* and other fungi, and some commonly used ionophore compunds include monensin, narasin, lasalocid and maduramicin (Dowling, 1992; Dutton et al., 1995). Ionophores have narrow safety margins and toxicity results from the abnormally increased outflux of K^+ and influx of Ca^{2+} and other ions in host cells (Dowling, 1992).

Clinical signs of ionophore toxicity include anorexia, weakness, dyspnea, increased mortality, paresis and paralysis (Dowling, 1992). Subchronic toxicity causes decreased liver weight, fibrin accumulation on the pericardium and hemorrhage in coronary adipose tissue (Wagner et al., 1983). Acute toxicity especially affects type 1 myofiber skeletal muscles and cardiac muscle, which exhibit hyalinization, necrosis and degeneration, as well as satellite cell proliferation and infiltration of macrophages (Hanrahan et al., 1981; Klasing et al., 2008). The posture-maintaining muscles typically contain more type 1 myofibers, whereas the pectoral muscles of broilers are composed almost entirely of type 2 fibers (Remignon et al., 1995; Papinaho et al., 1996; MacRae et al., 2006).

Many ionophore compounds are incompatible with other drugs, particularly antibiotics, because their concurrent administration leads to enhanced toxicity. Examples of incompatible drugs include tiamulin, erythromycin and chloramphenicol (Umemura et al., 1984; Dowling, 1992).

Senna toxicity

Senna occidentalis, also known as coffee senna or coffeweed, is a pantropical plant and a common contaminant within fodder in tropical regions and nearby. Coffee senna is toxic to birds and mammals and causes clinical signs of reduced feed intake, reduced body weight gain and increased mortality (Haraguchi, Gorniak et al., 1998; Barnes et al., 2016). In birds, additional clinical signs of reduced egg production, diarrhea, paralysis and ataxia have been described (Fulton, 2008). Necropsy findings include pale and edematous pectoral and semitendinous muscles that histologically exhibit necrosis and degeneration, primary axonal damage and decreased numbers of lymphoid cells in the spleen and bursa (Fulton, 2008). In addition to skeletal and cardiac muscle, the liver can also be affected (Haraguchi et al., 2003). Affected skeletal muscles are pale and atrophic and histologically exhibit myofiber swelling and fragmentation, satellite cell proliferation and atrophy of both type 1 and 2 myofibers (Haraguchi et al., 1998; Haraguchi, Calore et al., 1998).

Inherited muscular dystrophy

Hereditary muscular dystrophy of chickens first appeared in New Hampshire chickens (Asmundson and Julian, 1956) but also affects other chicken breeds and turkeys, Pekin ducks and Japanese quails (Rigdon, 1966; Schmitz and Harper, 1975; Braga et al., 1995; Tanaka et al., 1996a; Barnes et al., 2016). Clinical features of inherited muscular dystrophy include the inability to rise from dorsal recumbency, myotonia and a broad pectoral area (Holliday et al.,

1965; Julian, 1973). Pectoral muscles are particularly affected, but it can also affect other fast-twitch muscles. The lesions begin from proximal muscles and later descend into distal muscles. Macroscopically, the lesions are characterized by pale color and white striations parallel to muscle fibers (Asmundson and Julian, 1956; Asmundson et al., 1966; Mcmurtry, 1972; Wilson et al., 1979). The lesion begins as muscle hypertrophy, followed by irregular atrophy (Barnes et al., 2016). Degeneration, incerased number of nuclei, ring-fibers, variable cross-sectional diameter of myofibers and adipose tissue replacement of the degraded myofibers are typical histologic features (Julian, 1973; Tanaka et al., 1996b; Barnes et al., 2016).

In avian hereditary muscular dystrophy, a missense mutation of WWP1 gene causes replacement of an amino acid from arginine to glutamine (Matsumoto et al., 2008). WWP1 is a multifunction protein that takes part in many cellular functions, such as cell proliferation, survival, apoptosis in several cell types (Chen and Matesic, 2007; Zhi and Chen, 2012). The mutant WWP1 protein is unable to ubiquitinate and degrade caveolin-3 protein adequately, resulting in impaired breakdown of caveolae and accumulation of caveolin-3 protein in myocytes (Costello and Shafiq, 1979; Matsumoto et al., 2008; Matsumoto et al., 2010; Imamura et al., 2016). Caveolae are invaginations of the plasma membrane in several cell types, in which they take part in endocytosis, signaling and many other cell functions (Palade, 1953; Stan, 2005).

Exertional myopathy

Necrosis of skeletal and cardiac muscle is associated with prior excessive activity, usually resulting from capture or restrain (Barnes et al., 2016). The condition is called exertional or capture myopathy or exertional rhabdomyolysis and has been observed at least in turkeys and several wild avian species (Spraker et al., 1987; Hanley et al., 2005; Ruder et al., 2012; A. G. Hill and Miller, 2013).

Pectoral and leg muscles are most commonly affected and macroscopically show large pale and edematous areas that correspond to necrosis, fragmentation, increased numbers of satellite cell nuclei and macrophages histologically (Barnes et al., 2016). Accumulation of lactic acid in the muscle is the most likely cause for the muscle necrosis (Barnes et al., 2016).

Heat-stress-induced myopathy

In contrast to mammals, birds have no sweat glands and elevated environmental temperatures easily lead to hyperthermia (Klasing et al., 2008). Birds control their elevated body temperature by increased respiratory rate and open-mouth breathing, but if they fail to prevent hyperthermia, metabolic and circulatory imbalances and death occur (Swain and Farrell, 1975; Sandercock et al., 2001; Klasing et al., 2008).

Pectoral muscles may exhibit necrosis and interstitial edema in chickens that die of heatstroke and histology is peracute to acute, without any reparative or regenerative features (Sandercock et al., 2001; Barnes et al., 2016). A constant mild elevation in the environmental temperature causes no clinical signs, but results in reduced myofiber diameter (Joiner et al., 2014).

Glycogen storage disease

Type II glycogenosis due to acid maltase enzyme deficiency occurs in inbred lines of Japanese quail (Matsui et al., 1983). Acid maltase enzyme degrades glycogen into glucose and its deficiency causes intralysosomal glycogen accumulation that affects the liver, heart, brain and skeletal muscle (Barnes et al., 2016).

Myopathies of obscure etiology

Acute myopathy mainly affecting the pectoralis major muscle is described in broiler breeders between 12 and 20 weeks of age and in good body condition (Randall, 1982; Barnes et al., 2016). The characteristic histological feature is severe myodegeneration without significant cellular response. This acute condition of an unknown cause is associated with the rapid death of the bird.

In Brazil, a severe bilateral myodegeneration of the anterior latissimus dorsi (ALD) muscle in broiler chickens has been observed (Zimermann et al., 2012). The macroscopic changes include swelling and yellow discoloration of the skin that covers ALD muscles, which are hemorrhagic, pale and adherent to the adjacent muscles. The affected muscles are also thick and express increased density. Histologically, the lesions consist of myodegeneration, necrosis, regeneration, lymphohistiocytic cellular infiltration and mild fibrosis. Mild histologic changes also occur in visceral organs, such as bursal lymphoid depletion and kidney tubular epithelial degeneration. The etiology and pathomechanism of this condition are currently unknown, but ALD myopathy is associated with heavy body weight and rapid growth rate. A vaccine-induced pathomechanism has been speculated, but no proof for or against is available. ALD is composed only of type 1 myofibers (Geyikoglu et al., 2005).

Occasional degenerative myofibers have been described in several skeletal muscles of clinically asymptomatic turkeys in the 1990s and the condition has obtained variable names, including focal myopathy (Wilson et al., 1990; Sosnicki et al., 1991). The degenerative condition was associated with increased levels of creatine kinase in plasma and rapid growth of the turkeys (Wilson et al., 1990). Histologically, the degenerative changes included necrotic and hypercontracted myofibers, connective and adipose tissue proliferation and mononuclear cell infiltration (Sosnicki et al., 1989; Sosnicki et al., 1991).

Recently, an acute mortality syndrome associated with hyperthermia was described in broiler chickens in Europe (Niewold, 2013). The syndrome affected the heaviest male broilers at approximately three weeks of age, resulting in increased mortality with hyperthermia, cortical blindness, enlarged liver and degenerated breast muscles.

Pale soft exudative meat quality defect

Pale soft exudative meat defect was first defined in porcine meat (Wismer-Pedersen and Briskey, 1961; McLoughlin and Goldspink, 1963), but the condition has also been described in the meat of broiler chickens and other avian species (Pietrzak et al., 1997; Van Laack et al., 2000; Desai et al., 2016). The pale color, soft consistency and poor water-holding capacity are caused by the denaturation of myofibrillar proteins due to rapid post-mortem decline of pH when meat is still warm, before the temperature lowers during processing (Bendall and Swatland, 1988; Boles et al., 1992; Pietrzak et al., 1997). In pigs, stress and swine malignant

hyperthermia are main causes that lead to excessive ante-mortem heat and lactic acid production, resulting in high carcass temperature and acidic pH of the meat in early post-mortem stages (Boles et al., 1992).

In pigs, malignant hyperthermia is caused by defective ryanodine receptor (RyR) gene (O'Brien, 1987; Fujii et al., 1991). RyR is the main release-channel of calcium-ions on the sarcoplasmic reticulum (Jenden and Fairhurst, 1969) and defective RyR leads to increased release of Ca^{2+} ions into the sarcoplasm of resting muscle (MacLennan, 2000). Sequestration mechanisms for Ca^{2+} reuptake, such as the ATP-dependent calcium pumps, activate in response to the supraphysiological Ca^{2+} concentrations in the sarcoplasm, consuming ATP to adenosine diphosphate (ADP) and increasing oxygen consumption and carbon dioxide production (O'Brien, 1987; Hopkins, 2017). The sustained increase in Ca^{2+} ion concentration stimulates excessive and uncoordinated sarcomere contractions, which leads to heat production (Hopkins, 2017). Increased membrane permeability leads to hyperkalemia, which triggers cardiac symptoms (Hopkins, 2017). Prior clinical signs are absent until exposure anesthesia or other stressors that trigger malignant hyperthermia in animals and humans (Mitchell and Heffron, 1982; Hopkins, 2017). Some anesthetics, such as halothane in the past, directly trigger the RyR channel complex to open (Hopkins, 2017). Clinical signs in pigs include sudden rise in body temperature, muscle rigidity, arrytmia, tacycardia, myoglobinuria and even death (Lucke et al., 1979; Mitchell and Heffron, 1982).

Mammals express three different isoforms of RyR, namely 1, 2 and 3, of which RyR1 is the most abundant in skeletal muscle (McPherson and Campbell, 1993; Hopkins, 2017). Avian species express α and β isoforms of RyR (Airey et al., 1990), both being present in the skeletal muscle and αRyR most similar to the mammalian skeletal muscle RyR1 (Ottini et al., 1996). Although avian species seem not to share an exactly similar genetic defect in RyR as swine, the pathomechanism associated to rapid post-mortem pH decline and high remperature is characterized in broiler chickens and possibly arises from altered expression pattern of splice variants of RyRs (Pietrzak et al., 1997; Van Laack et al., 2000; Alvarado and Sams, 2003; Strasburg and Chiang, 2009).

Wooden breast, white striping and spaghetti meat

The reports on the emergence of wooden breast myopathy and white striping arose around the same time; white striping was first noted in 2009 and wooden breast myopathy in 2013 (Bauermeister et al., 2009; Kuttappan et al., 2012; Kuttappan et al., 2013; Petracci et al., 2013; Russo et al., 2015). Further description and discussion of wooden breast myopathy is provided in the subsequent parts of this thesis.

White striping refers to pale or white stripes, up to a few millimetres wide, that run parallel to the muscle fibers (Bauermeister et al., 2009; Kuttappan et al., 2012; Kuttappan et al., 2013; Petracci et al., 2013; Russo et al., 2015). The microscopic features and the composition of white stripes have been described in several reports. Some of them suggest that white striping is composed of adipose tissue, since increased fat content of the fillets with white striping has been observed both histologically as well as with methods assessing the proximate composition of the muscle (Kuttappan et al., 2013; Papah et al., 2017; Baldi et al., 2018). Other studies find no histological differences between WB and white striping (Soglia et al., 2016; Radaelli et al., 2017).

In addition to white striping, another meat quality defect called ‘spaghetti meat’ has been described during the last three years (Bilgili, 2015; Baldi et al., 2018). The main macroscopic change of spaghetti meat is loose structural integrity of the muscle, characterized by muscle bundles that easily separate from each other. Histological changes observed in spaghetti meat overlap with lesions of WB and white striping, but accumulation of loose connective tissue in the endo- and perimysial area seems to be more extensive in spaghetti meat, and likely is responsible for the extensively friable muscle consistency (Baldi et al., 2018).

3 AIMS OF THE STUDY

The aims of this thesis were as follows:

1. To characterize the macroscopic and microscopic morphology of WB. (I, II, III)

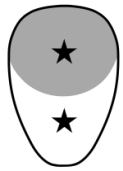
2. To describe the temporal development of WB. (II)

3. To study the WB pathomechanism and the effect of dietary selenium on WB. (II, III)

4 MATERIALS AND METHODS

Animals included in the studies of this thesis originated from three sources: field cases from slaughterhouses and experimental cases from two rearing experiments. A summary of the cases included in this thesis is represented in Table 2 below.

Table 2. *Summary of cases and studies included in this thesis. Pectoralis major muscles are visualised by schematic drawings and the grey color represents the area of the macroscopic WB lesion and asterisks the sampling site for histology. The roman numerals following each study in parentheses refer to the original articles to which this thesis is based on.*

Origin of samples	Case type, n			Total, n	Study
	Unaffected	Focal WB	Diffuse WB		
					
Field cases	2	-	10	12	Chronic morphology (I)
Experimental rearing 1	133	46	47	226	
	133 (61)[h]	46 (27)[h]	47 (23)[h]	226 (111)[h]	Acute WB morpholgy; temporal WB development; dietary selenium on WB (II)
	14	14	-	28	Pectoral vessel density (III)
Experimental rearing 2	222	53	55	330	
	3	3	3	9	Ultrastructural WB morphology (III)

[h] Histologically evaluated

4.1 ANIMALS AND STUDY DESIGN

Field cases (I)

The field study was a cross-sectional observation of commercial broiler chicken cases. A total of 12 pectoralis major muscles from 5- to 6-week-old commercial broiler chickens were retrieved from two high-throughput slaughterhouses in Finland in 2012. From both slaughterhouses, six single pectoralis major muscles, either from the right or left side, were selected: five affected with WB and one unaffected muscle from one flock of broiler chickens. The flocks originated from two farms, which claimed typical in-farm mortality rates and participated in the national health surveillance program for broiler chickens.

According to the routine process at the slaughterhouse, the birds were stunned in a carbon dioxide chamber, euthanized by jugular phlebotomy and the pectoralis major muscles were cut off and cooled to 5˚C. Due to the process at the slaughterhouse, the pectoralis major muscles were separated from the other organs of the body, rendering their co-evaluation impossible. The slaughterhouse personnel preliminarily evaluated the cooled pectoralis major muscles for consistency change by palpation, before the muscles were transported to the laboratory.

Experimental rearing 1 (II, III)

The study included 240 unvaccinated high-yielding male broiler chickens. At one day of age, the broilers were divided into 24 pens, 10 birds per pen, in one room of an experimental facility of the University of Helsinki. Each woodchip-bedded pen included two water nipples and a centrally located feeder. Heating lamps provided continuous lighting during the first seven days, which followed a daily 6-hour period of darkness until the end of the experiment. A routine clinical observation and maintenance of the husbandry conditions were performed daily.

The experimental rearing 1 was conducted as a temporal observation of a flock of broiler chickens. The birds were euthanized at the ages of 10, 18, 24, 35, 38 or 42 days. To compensate for the decrease in the proportional space per bird during their growth and to maintain the density of birds per square meter in each pen close to that used in the conventional breeding, the birds were randomly selected for euthanasia as follows: two birds per pen at 10 days of age, two birds per pen at 18 days, one bird per pen at 24 days, then all remaining birds from 10 pens at 35 days and 10 pens at 38 days, and the last 4 pens at 42 days.

Dietary treatment in experimental rearing 1 (II)

The pens were randomly allocated to two dietary treatments, SeLow and SeNorm, which differed in the content and quality of selenium. Half of the birds, 12 out of the 24 pens, received SeLow feed that contained inorganic selenium (sodium selenite, Na_2SeO_3; Anima Ltd., Poland) 0.11 mg/kg for the first seven days and then 0.13 mg/kg, whereas the remaining 12 pens received SeNorm feed that contained organic selenium yeast (Selsaf; Lesaffre Feed Additives, France); 0.32 mg/kg for the first seven days and then 0.30 mg/kg. The wheat- and soya-based feed included 0.007% of narasin as coccidiostat (Monteban; Elanco, USA) and

except for selenium, contained similar amounts of nutrients adjusted according to the dietary recommendations (Aviagen, 2014). Feed and water were provided *ad libitum*.

Experimental rearing 2 (III)

The study included 350 unvaccinated high-yielding male broiler chickens. At 1 day of age, the broilers were divided in 25 pens, 14 birds per pen, in one room of an experimental facility. The husbandry, lighting and heating procedures were as in experimental rearing 1.

The rearing experiment 2 was conducted as a temporal observation of a flock of broiler chickens. The birds were euthanized at the ages of 15, 19, 22, 32, 36, 39, 43 or 49 days; the numbers of euthanized birds were 17, 37, 47, 50, 50, 56, 63 and 10, respectively. Due to study objectives other than this thesis, the rearing experiment 2 focused on birds near slaughter-age and the majority of the birds were euthanized between ages 32 and 43. The number of euthanized birds per age group also varied due to the need to balance the animal density per pen.

The feed was based on wheat and soya and the nutrients were adjusted according to the dietary recommendations with 0.007% of narasin as coccidiostat (Monteban; Elanco, USA). Feed and water were provided *ad libitum* and were similar for all birds.

Study protocol in experimental rearing studies

For euthanasia, each bird was individually picked from the pen by random fashion, weighed and transferred to adjacent room, where the bird was euthanized by mechanical cervical dislocation, followed by jugular phlebotomy. The personnel operating in the animal room, aware of the dietary treatment per pen in the experiment 1, handed the birds with an identification number to the necropsy and sampling crew blind to the dietary treatment and pen number.

The experimental rearings were conducted in an experimental setting but without any ante mortem procedures to the animals. Due to the lack of ante mortem procedures, the experimental studies followed the legislation applicable to conventional breeding, instead of legislation applicable to animal experiments. The methods of rearing and euthanasia were approved by the Laboratory Animal Centre of the University of Helsinki.

4.2 MACROSCOPIC EVALUATION AND NECROPSY

Field cases

At the laboratory, approximately 24-hours post mortem, each pectoralis major muscle was photographed on top of a paper sheet labelled with the individual case identification number. After visual evaluation of the macroscopic appearance, the muscle was palpated and identified as macroscopically unaffected (normal palpatory muscle consistency) or affected with WB (abnormally hardened muscle consistency).

Experimental rearing 1 and 2

Immediately after the euthanasia, the birds were necropsied. The necropsy was conducted blind to the dietary treatment in experiment 1. The skin, head, oral cavity, wings, legs and footpads were evaluated, followed by a blunt dissection of the pectoral skin to reveal the pectoralis major muscles. The preliminary WB assessment included visual evaluation and palpation of both major pectorals, in order to evaluate the presence and bilateral occurrence of the changes. Due to the bilateral nature of the lesion, the right major pectoral was cut off for a second, definitive WB evaluation, while the necropsy was continued with the opening of the ribcage and inspection of the internal organs, brachial nerve plexi and muscles of the thigh area and the opening of the joints of the left hip, knee and ankle, breakage of the right femur to test the bone strength and visually evaluate the bone marrow.

In the second WB evaluation, the right major pectoral muscle was photographed on top of a paper sheet labelled with the individual case identification number, assessed visually, palpated and identified as macroscopically unaffected (normal palpatory muscle consistency) or affected (abnormally hardened muscle consistency) with WB. In addition, the severity of the consistency change (mildly increased consistency hardness or hard) and the location of the WB lesion (focal in the cranial or caudal area, or diffusely affected), as well as the color, the presence of white stripes and the exudate were recorded.

4.3 HISTOPATHOLOGIC ASSESMENT

Field cases

All 12 pectoralis major muscles were sampled for histology in the middle third of the cranio-caudal direction, approximately one centimeter deep from the ventral surface originally facing the skin. The 1x1x2-cm samples were immersion fixed in 10% buffered formalin solution (VWR Ltd., UK), embedded in paraffin in longitudinal and transverse orientation and cut into 4-µm sections that were stained with hematoxylin and eosin stain (HE, all cases) and Masson trichrome stain (2 WB cases and both unaffected cases). The tissue sections were evaluated under a light microscope, blind to the macroscopic results.

Experimental rearing 1 and 2

After the macroscopic WB evaluation, each right pectoralis major muscle was sampled for histology, although not all were processed histological slides. A 1x1x2-cm tissue sample was excised in the middle area of the unaffected cases and diffuse WB cases, whereas the focally affected cases were sampled in the most severely affected area. Additionally, a subset of focal WB cases was also sampled in the unaffected area of the muscle (15 cases in experimental rearing 1, and all 9 cases from experimental rearing 2 that were included in this thesis). All samples were immersion fixed in 10% buffered formalin solution (Oy FF-Chemicals Ab, Finland).

For experimental rearing 1, a total of 111 cases out of the 226 birds included in the macroscopic study were histologically evaluated. The 111 cases included all birds of age groups 18 and 24 days, and stratified random selection of other age groups balanced on the

dietary treatment and the presence of macroscopic WB. Out of the 330 cases of the experimental rearing 2, 9 cases were included in this thesis and those were all histologically evaluated. The tissue samples were embedded in paraffin, dehydrated with an ascending series of alcohol and cut into 4-µm sections that were stained with HE. The stained sections were evaluated under a light microscope, blind to the macroscopic diagnosis and dietary treatment.

Histologic scoring criteria

Histopathologic changes in terms of myodegeneration, perivascular cell accumulation and the amount of adipose tissue were scored according to the criteria described in Table 3. The degenerative myofibers were defined as hyalinized fibers with loss of cross striation and fragmented fibers with surrounding or infiltrating macrophages or heterophilic granulocytes. Exemplary micrographs of the histological changes corresponding to each score are included in the original article II. In addition to the scored features, other histologic changes, such as regenerative myofibers, necrosis, inflammatory cell infiltration, loose connective tissue accumulation and fibrosis were evaluated.

Table 3. *Histologic scores assigned for myodegeneration, perivascular cell accumulation and intramuscular amount of adipose tissue.*

	0	1	2	3
Myodegeneration	Absent or minimal	Mild	Moderate	Severe to excessive
Perivascular cell accumulation	Normal vessel walls without surrounding cell infiltration	Perivascular infiltrations of lymphocytes with or without mild intramural infiltration	Marked perivascular infiltration of lymphocytes with intramural infiltration sometimes obliterating the vascular wall	-
Adipose tissue amount	Absent	Mild (thickness of the adipose tissue bed approximately similar to the largest diameter measure of the blood vessel)	Moderate (≤ 2x blood vessel diameter)	Marked (≥ 3x blood vessel diameter)

Histological evaluation of other tissues

In experimental rearing 1, a tissue sample for histology was obtained from the biceps femoris muscle of five broilers: two unaffected cases and two WB cases with macroscopically normal muscles and one WB case with WB-like changes in the biceps femoris muscle. Tissue samples were formalin-fixed and processed into HE-stained histological slides similarily as the tissue samples from the pectoralis major muscles. The histolocigal scoring criteria presented in Table 3 were applied also for these samples.

Internal organs, including the heart, spleen, liver, kidney, lung, gizzard, duodenum and pancreas, of 12 birds were sampled for histology in the experimental rearing 1. These birds represented one WB case and one unaffected case randomly selected per each age group.

4.4 IMMUNOHISTOCHEMISTRY

Immunohistochemistry with a CD3 antibody (polyclonal rabbit anti-human A0452; DAKO, Denmark) to visualize T cells and with a CD79a antibody (monoclonal mouse anti-human HM57 M7051; DAKO, Denmark) to visualize B cells was performed with the streptavidin-biotin method. Spleen served as positive control tissue and exhibited intense staining in the corresponding anatomic areas (Lund-Johansen and Browning, 2017).

Immunohistochemistry was performed on two WB cases out of the 12 field cases and on three cases from the experimental rearing 1 (10- or 18-day-old birds with V1 or V2).

4.5 ELECTRON MICROSCOPY

Cases and sample collection

From the rearing experiment 2, nine broiler chickens were retrieved from the 62 broilers that were euthanized at the age of 22 days. The nine broilers were selected in a stratified random fashion: three unaffected cases, three focal WB cases and three diffuse WB cases.

After necropsy and macroscopic WB evaluation as described above, samples for electron microscopy were collected during the same process and from the same location as the histological samples. Several 2x2x2-mm tissue samples were collected as follows: from the middle area of the pectoralis major muscle in unaffected and diffuse WB cases, whereas the focal WB cases were sampled from the lesion area and the macroscopically unaffected area. The sampling was performed within 5 minutes of the post mortem.

Sample preparation and electron microscopy

The 2x2x2-mm tissue samples for electron microscopy were immersion fixed in 2.5% glutaraldehyde (Sigma-Aldrich, USA) for two hours in room temperature and then transferred into 2% paraformaldehyde (Sigma-Aldrich, USA) at +4°C until further processing. Two of the tissue samples were post-fixed in 1% osmiumtetroxide (Electron Microscopy Sciences, England) for one hour at room temperature, dehydrated in an ethanol series of 70%, 96% and

100%, incubated in transtitional solvent acetone and embedded gradually in Epon (TAAB Laboratories Equipment Ltd., England). Longitudinally oriented semithin tissue sections of 500 nm were cut, stained with toluidine blue and evaluated under a light microscope. From the semithin tissue sections, the least degenerated areas were selected for processing into ultrathin sections on copper grids with an oval hole.

Each sample was screened with Jeol JEM-1400 electron microscope (Jeol Ltd., Tokyo, Japan). In addition to general evaluation of the tissue, the presence and morphology of specific structures such as sarcolemma, basal lamina, intermyofibrillar and subsarcolemmal mitochondria, satellite cells, myofibrils, sarcoplasmic reticulum, T tubules or triads, myocyte nuclei, endothelium and the extracellular matrix were evaluated.

Electron micrographs were acquired using a Gatan Orius SC 1000B bottom mounted CCD-camera (Gatan Inc., USA), approximately 30 micrographs per each sample area. The electron micrographs were further evaluated on a computer screen. From the electron micrographs, the diameter size of all visible transversal sarcoplasmic reticulum pouches was measured and rounded to the nearest hundred nanometers. The burnt-in scale bar of the electron micrographs served as the calibration source for the measurements that were obtained with ZEN Pro software (Carl Zeiss, Germany). The rounded values were ranked without statistical analysis.

4.6 MICROVESSEL DENSITY

Cases

A case-control study included a total of 28 broiler chickens (14 unaffected and 14 focal WB cases) that were retrieved from the rearing experiment 1 as follows: three broilers at 18 days of age; five at 24 d; four at 35 d; and two at 38 d. After the macroscopic evaluation of the right major pectoral muscle and histologic evaluation of the sample areas, described above, 4-µm tissue sections were cut from the paraffin-embedded tissue blocks and stained with periodic-acid Schiff reaction with diastase (PAS-D) for visualization of the blood vessels and endomysium. In summary, the unaffected cases were sampled in the middle of the major pectoral muscle and the focal WB cases were sampled in two locations: in the unaffected area and in the lesion area, which typically is located in the cranial half of the major pectoral muscle.

Vessel and myofiber count

The PAS-D stained sections were photographed (AxioLabA1 microscope and Axiovision software, Carl Zeiss, Germany) at 8 microscopic fields, 0.15 mm^2 each, representing the typical morphology of the tissue section and which is devoid of large blood vessels. From each photograph, the number of myofibers (incomplete myofibers on the photograph edge counted as half a fiber and degenerated myofibers excluded) and microvessels (up to 50-70 µm in diameter) were manually counted.

The photographs featuring the lowest and the highest number of myofibers were excluded and two out of the remaining six photographs were randomly selected. The randomization was

performed by assigning random numbers for the remaining six photographs, from which two photographs with the smallest random number were then selected. Only the selected two photographs were further analysed for vessel number and myofiber area.

The myofiber area was determined from each set of two photographs as follows: the cross-sectional area of each complete myofiber was calculated with the spline contour function of ZEN Pro software (Carl Zeiss, Germany) and totaled into one number (total myofiber area in the two photographs). The manually determined vessel number from each set of two photograps was totaled into one number (total vessel number in the two photographs). The ratio of the two summed parameters was calculated (total myofibre area per total vessel number).

4.7 STATISTICAL ANALYSES

For the cases retrieved from the experimental rearing 1, the statistical analysis of the association between histological grades and WB or white striping (II) was performed by the Fisher exact test. The test was first performed for the birds aged 18 and 24 days together, then including all histologically evaluated birds pooled together. The Fisher exact test was also utilized to determine relationship between WB severity and WB location or white striping. The association between bird weight and the presence of WB or dietary treatment were assessed by the analysis of variance (ANOVA). The effects of dietary treatment and age on WB prevalence were assessed by logistic regression analysis. The effects of the dietary treatment and age of the birds in experimental rearing 1 were statistically analyzed by logistic regression with WB as the outcome variable. Analysis of variance was performed in order to analyze the association between weight and dietary treatment.

The coefficient of variation was calculated for each set of eight micrographs (III), both for vessel number and myofiber number, in order to evaluate the representativeness of the further selected two micrograps. For further statistical analyses, only the sets of two microscopic fields were included. The differences in vessel number or myofiber number between the unaffected, focal WB unaffected area and focal WB lesion area were analyzed with one-way ANOVA and Bonferroni post-hoc analyses. Based on the ANOVA results, two pooled age groups were created: birds of 18 and 24 days of age and birds of 35 and 38 days of age. Differences in the total myofiber area, total vessel number and the ratio of those two between the different case types (unaffected, focal WB unaffected area and focal WB lesion area) were analysed with t-tests.

All statistical analyses were performed with IBM SPSS Statistics (IBM Corp., NY) v.23 (II) or v.24 (III). P-values less than 0.05 were considered significant.

5 RESULTS

5.1 ANIMALS

Field cases

Out of the 12 pectoralis major muscles, ten were identified as diffuse WB cases and two as unaffected in the final macroscopic evaluation at the laboratory. No increased in-farm mortality rates or clinical signs were reported for the flocks that the samples originated from.

Rearing experiment 1

Out of the 240 broilers, 14 birds (5.8%), seven for each dietary treatment, died between the planned ages of euthanasia. The post mortem findings of these birds were consistent with sudden death syndrome, ascites syndrome or other changes. These 14 birds were excluded from further studies due to the development of post mortem changes and rigor mortis. Average daily weight gain during 42 days of rearing was 65 g, all birds included.

The remaining 226 broiler chickens exhibited no clinical signs in routine daily observation, except for one bird with walking difficulties but no macroscopic lesions or post mortem findings.

Rearing experiment 2

Out of the 350 broilers, 20 birds (5.7%) died or were euthanised between the planned ages of euthanasia. The post mortem findings included dehydration, sudden death syndrome, ascites syndrome, spraddle legs and other changes. Including all birds of the study, the average daily weight gain was 71 g.

Since a different number of birds were euthanized at several ages during the experimental rearing studies, the comparison of the average daily weight gain between the experimental flocks or with commercial slaughter-age flocks may be biased. Other publications not included in this thesis will provide more detailed analysis of the weight progression of the birds in the experimental rearing studies 1 and 2.

5.2 MACROSCOPIC MORPHOLOGY OF WB (I, II)

Hardened consistency and pale color

An abnormally hardened consistency and pale color of the pectoralis major muscle was the macroscopic hallmark change of WB (Fig. 3). The lesion affected the pectoralis major muscle completely or extensive areas of it (diffuse WB) or a smaller area surrounded by normal muscle consistency (focal WB). The lesion was typically present both in the right and left pectoralis major muscle simultaneously. The focal WB lesion typically occured in the cranial part or in the caudal tip of the pectoralis major muscle. An out-buldging ridge was sometimes

present in the caudal WB lesion. In diffuse WB, the lesion typically extended through the complete thickness of pectoralis major muscle. Focal WB lesion more commonly affected the outer portion of the muscle cross-section (Fig. 4).

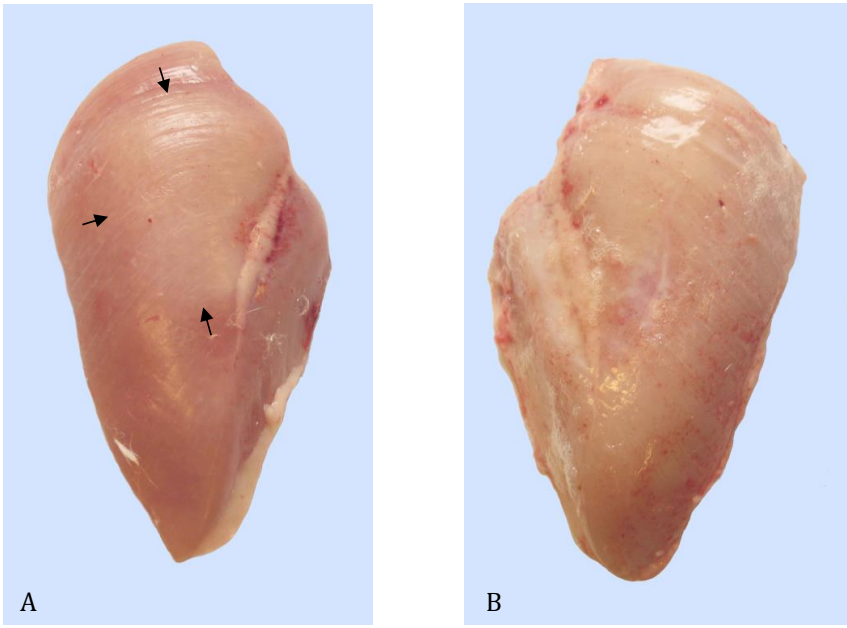


Figure 3. *A) A focal WB lesion (lined by arrows) surrounded by unaffected muscle tissue. Pectoralis major muscle of a 22-day-old broiler. B) A diffuse WB lesion in the pectoralis major muscle of a 43-day-old broiler chicken. Both cases exhibit also thin white stripes along the myofiber direction. Rearing experiment 2.* (Photos: Kaisa Immonen)

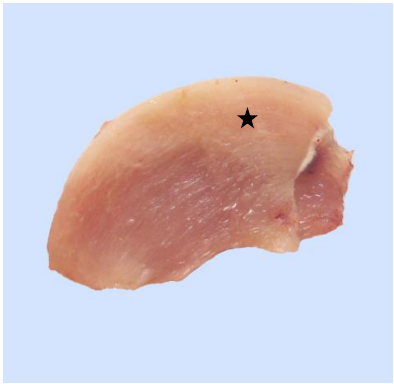


Figure 4. *Cross-sectioned pectoralis major muscle shows an outer rim of pale and palpatorily hardened muscle (asterisk), whereas the inner area remains of normal color and consistency. A 35-day-old broiler chicken with a cranial focal WB lesion. Rearing experiment 2.* (Photo: Kaisa Immonen)

White striping and other changes

White stripes of up to several millimeters, parallel to the muscle fibers, often existed together with WB (Fig. 3). However, both WB and white stripes occured also separately, without concomitant presence of the other in the same muscle. In rearing experiment 1, white striping data was available from 224 out of 226 birds. White striping and WB were significantly associated ($P < .001$), analysed with Fisher's exact test (Table 4). When present, white striping typically appeared throughout the pectoralis major and in focal cases white striping was not restricted to the WB area. No statistical analysis of the association was performed on field cases or rearing experiment 2.

Table 4. *Association of white striping and WB in the rearing experiment 1.*

	White striping absent, n	White striping present, n	Total	
WB absent, n	79	53	132	224
WB present, n	6	86	92	
Total	85	139		
	224			

Clear or slightly yellowish, moderately viscous material (exudate or transudate) covered the muscle surface of some WB cases. Superficial hemorrhages on the muscle surface were observed in many WB cases, but those were typically very acute.

WB lesion in biceps femoris muscle

In the rearing experiment 1, one 38-day-old broiler exhibited a WB lesion in the biceps femoris muscle (Fig. 5). The poorly demarcated lesion area was slightly paler than the surrounding muscle and the palpatory muscle consistency was mildly hardened. In the pectoralis major muscle, the bird exhibited a diffuse WB lesion.

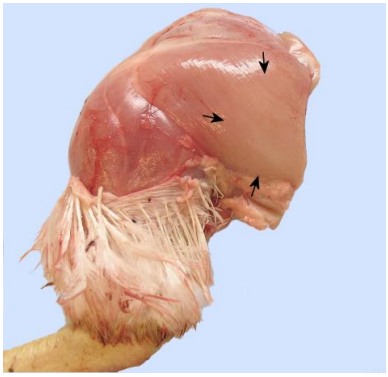


Figure 5. *A pale area (indicated by arrows) with hardened consistency in the left leg of a 38-day-old broiler chicken from the rearing experiment 1. The upper portion of the leg is skinned, with feathers and skin remaining in the lower part.* (Photo: Kaisa Immonen)

5.3 HISTOLOGIC MORPHOLOGY OF WB (I, II)

WB lesion

Myodegeneration was a consistent histologic finding in all WB lesions. Myodegeneration and macroscopic WB lesions were significantly associated ($P < 0.001$, Table 5). The macroscopically unaffected cases exhibited either absent to minimal (score 0, all age-groups) or mild (score 1, only in groups 18 and 24 days of age) myodegeneration. The WB severity subclasses mild and hard differed in the presentation of myodegeneration; all myodegeneration scores were present within the mild category, whereas the majority (75%) of the hard WB cases exhibited severe (score 3) myodegeneration. In addition to the evident myodegeneration with ilfiltration of macrophages phagocytizing the fragmented myofiber material, many intact myofibers expressed marked variation in myofiber shape and diameter in the cross-sectional orientation (Fig. 6). Moderate or marked interstitial edema and loose connective tissue accumulation accompanied moderate (score 2) and severe or excessive (3) myodegeneration (Fig. 7). Fibrosis frequently accompanied score 2 and 3 myodegeneration in older WB cases (35, 38 and 42 days of age), but significant fibrosis was absent in the younger age groups, even when severe myodegeneration was present. The interstitial thickening would sometimes also include the epimysium covering the pectoralis major muscle in chronic WB cases.

Regeneration accompanied myodegeneration in all age-groups and myodegeneration scores, consistent with polyphasic myodegeneration. A few WB cases exhibited focal areas abundant in regenerative myofibers, but in general the regeneration was scattered throughout the lesion.

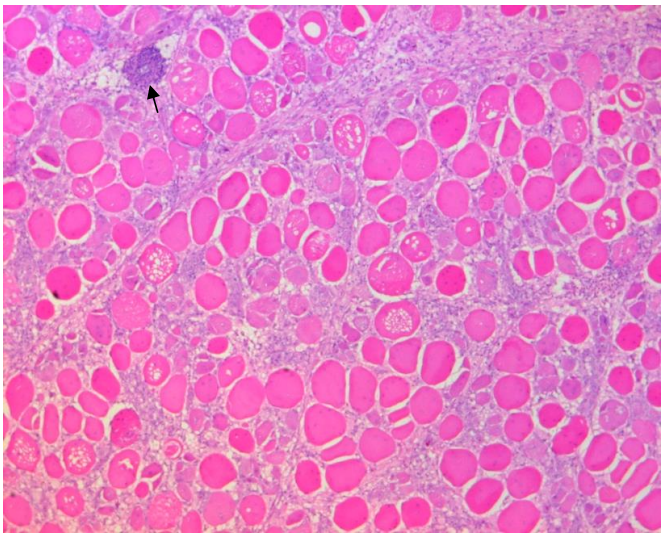


Figure 6. *Degeneration and variation of size and shape of myofibers with mildly increased volume of the interstitium. Moderate accumulation of inflammatory cells surround a small vein (arrow). HE staining. WB lesion, broiler chicken, rearing experiment 1.*

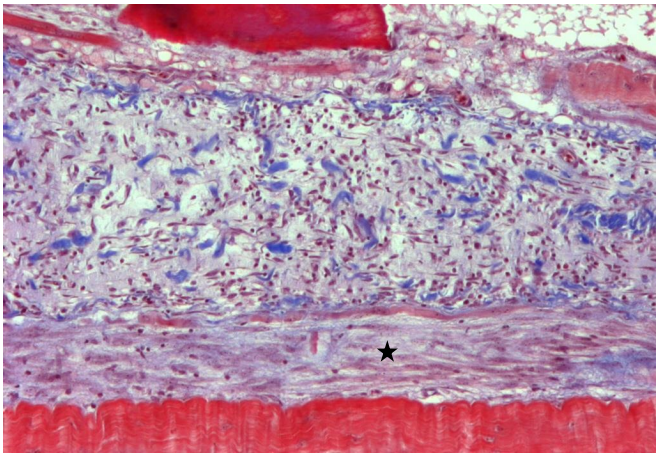


Figure 7. *Interstitial thickening by loose connective tissue with occasional strands of mature collagen (blue), above the myoregeneration (asterisk). Masson trichrome staining. WB lesion, broiler chicken, rearing experiment 1.*

Perivascular accumulation of inflammatory cells was significantly associated with WB ($P < 0.001$, Table 5) and with the severity of myodegeneration ($P < 0.001$). The inflammatory cells were identified as lymphocytes both by morphology and positive immunohistochemistry against CD3 antigen and the infiltration typically formed highly cellular cuffs around the veins, often eccentrically (Fig. 8). Lymphocyte aggregates were especially frequent in WB lesions in which the macroscopic WB lesion was hard in terms of severity; lymphocyte accumulation was lacking in only one of these cases, whereas in 75% of cases the accumulation was severe (vasculitis score 2).

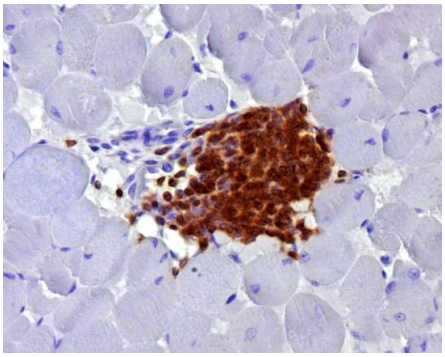


Figure 8. *The perivascular accumulation of inflammatory cells exhibit positivity for CD3 antibody in immunohistochemical staining. Broiler chicken, rearing experiment 1.*

Adipose tissue concentrated around the blood vessels in both unaffected and WB cases. When all age-groups were pooled, the amount of adipose tissue was associated with WB ($P = 0.01$) and myodegeneration ($P = 0.004$). However, when only ages 18 and 24 days were included, no association was detected ($P > 0.05$).

The studies of this thesis were not specifically designed to evaluate the histology of white striping. However, 53 cases exhibited white striping only, without macroscopic WB changes, and they represented age groups 18, 24, 35, 38 and 42. Out of the 53 cases, 21 were evaluated histologically and they exhibited absent or mild myodegeneration, absent or mild perivascular lymphocyte infiltration and absent, mild or moderate adipose tissue amount.

Table 5. *Histologic myodegeneration and perivascular cell accumulation in unaffected and WB pectoralis major muscles. Rearing experiment 1. Percentage: of the row total.*

Macroscopic appearance	Histologically evaluated cases, n	Myodegeneration, n (%)				Perivascular cell accumulation, n (%)		
		0	1	2	3	0	1	2
Unaffected	61	50 (82)	11 (18)	0	0	52 (85)	9 (15)	0
Wooden breast	50	5 (10)	6 (12)	12 (24)	27 (54)	10 (20)	11 (22)	29 (58)
Total	111	55	17	12	27	62	20	29

Myodegeneration scores: 0 minimal; 1 mild; 2 moderate; 3 severe to excessive. Perivascular cell accumulation scores: 0 no infiltration; 1 perivascular lymphocytes with or without mild intramural infiltration; 2 marked perivascular and intramural lymphocyte infiltration, for details see Table 3.

Unaffected area of focal WB cases

The macroscopically unaffected area that was sampled from 15 focal WB cases exhibited absent to minimal (score 0), mild (1) or moderate (2) myodegeneration histologically. Perivascular lymphocyte aggregates or vasculitis were absent (n = 8), mild (n = 6) or marked (n = 1). Adipose tissue in perivascular area scored either 1 or 2 in all unaffected areas of focal WB cases.

5.4 TEMPORAL DEVELOPMENT OF WB (II)

In rearing experiment 1, the 10-day-old broiler chickens exhibited no hardened consistency of the pectoralis major muscle post mortem by palpaltion. The first macroscopic WB cases were observed in 18-day-old broilers and the prevalence peaked at 35 days of age (Table 6). In the rearing experiment 2, the first macroscopic WB cases were observed in broilers of the youngest age group, at 15 days (Table 7, unpublished data). Overall, the most common location was a diffuse distribution, but in the younger birds a focal lesion was more common (Table 8). The WB location and severity was associated ($P < 0.001$): 2/13 (15%) with a caudal location, 12/33 (36%) with a cranial location, and 38/47 (81%) with a diffuse location in WB cases classified as hard in terms of severity.

Table 6. *The occurrence of macroscopic wooden breast in the age groups of rearing experiment 1. Percentage: of the row total.*

Age, days	Birds, n	Unaffected, n (%)	Wooden breast, n (%)
10	46	46 (100)	0
18	47	34 (72)	13 (28)
24	21	11 (52)	10 (48)
35	44	12 (27)	32 (73)
38	51	24 (47)	27 (53)
42	17	6 (35)	11 (65)
Total	226	133 (59)	93 (41)

Table 7. *The occurrence of macroscopic wooden breast in the age groups of rearing experiment 2. Percentage: of the row total.*

Age, days	Birds, n	Unaffected, n (%)	Wooden breast, n (%)
15	17	15 (88)	2 (12)
19	37	33 (89)	4 (21)
22	47	39 (83)	8 (17)
32	50	32 (64)	18 (36)
36	50	32 (64)	18 (36)
39	56	34 (61)	22 (39)
43	63	29 (47)	34 (53)
49	10	8 (80)	2 (20)
Total	330	222 (67)	108 (33)

Table 8. *Wooden breast location and severity macroscopically in the age groups of rearing experiment 1.*

Age, days	Wooden breast, n	Wooden breast location, n (%)			Wooden breast severity, n (%)	
		Focal		Diffuse	Mild	Hard
		Cranial	Caudal			
10	0	0	0	0	0	0
18	13	4 (31)	8 (62)	1 (8)	10 (77)	3 (23)
24	10	0	5 (50)	5 (50)	4 (40)	6 (60)
35	32	12 (38)	0	20 (63)	8 (25)	24 (75)
38	27	10 (37)	0	17 (63)	10 (37)	17 (63)
42	11	7 (64)	0	4 (36)	9 (82)	2 (18)
Total	93	33 (35)	13 (14)	47 (51)	41 (44)	52 (56)
		46 (49)				

5.5 EFFECT OF SELENIUM ON WB (II)

No significant difference in WB prevalence was observed between the two dietary treatments containing either conventional or reduced level of selenium (Table 9). For the birds that received the conventional level of selenium, the odds ratio for WB was not significant (OR 0.78; 95% CI 0.42-1.46; P = 0.44). No significant difference in the body weight of the broilers was observed between the two dietary treatments (P > 0.05).

Table 9. *Wooden breast occurrence in the two dietary groups of rearing experiment 1. Percentage: of the row total.*

Diet	Birds, n	Unaffected, n (%)	Wooden breast, n (%)
Low selenium	113	64 (57)	49 (43)
Conventional selenium	113	69 (61)	44 (39)
Total	226		

5.6 ULTRASTRUCTURE OF WB (III)

Mitochondria and sarcoplasmic reticulum

In the ultrastructural study comparing the unaffected, focal WB and diffuse WB cases, the most remarkable changes were observed in the sarcoplasmic reticulum (SR) and mitochondria. The diameter of sarcoplasmic reticulum was enlarged in the areas affected with WB (Table 10) and the enlargement was observed in both forms of SR: free sarcoplasmic reticulum and terminal cisternae that surround the T-tubules.

Table 10. *Diameter of the sarcoplasmic reticulum in unaffected, focal WB and diffuse WB cases.*

	Typical diameter, nm	Maximal observed diameter, nm
Unaffected	≤ 100	280
Focal WB, unaffected	≤ 100	400
Focal WB, WB lesion	200-300	400
Diffuse	200-300	600

Granular material was observed in the lumen of the terminal cisternae, whereas the lumen of the free sarcoplasmic reticulum typically appeared completely electron-lucent. However, occasional electron-lucent empty lumens of terminal cisternae were occasionally detected, especially when their diameter was enlarged. The triads, composed of one T-tubule flanked by terminal cisternae on both sides, were typically located at the Z-line, but some triads were closer to the junction of A- and I-bands, irrespective of the WB status.

In the unaffected cases and in the unaffected areas of focal WB cases, the intermyofibrillar mitochondria typically appeared singly or in rows of two to three mitochondria, alingned along the myofibrils. Aggregations of 10 to 25 mitochondria (mitochondrial hyperplasia) were observed between the myofibrils of diffuse WB cases and the lesion area of focal WB cases. Occasionally, the aggregations included over 40 mitochondria in the diffuse WB cases. Loss of cristae, swollen matrix chambers or vacuolization were present in the mitochondria in all cases, but these changes were the most prominent and frequent in diffuse WB cases and in the lesion area of focal WB cases. Both unaltered and altered mitochondria were observed in all cases and the degree of mitochondrial changes varied from minimal to severe even within one myofiber. The morphologically altered mitochondria typically appeared concomitant with swollen SR, but also unaltered mitochondria were present among areas with altered SR, and vice versa. No marked differences in the number or morphology of subsarcolemmal mitochondria were observed between the unaffected, focal WB or diffuse WB cases by subjective assessment.

Other cellular components

Within the WB lesion areas, the majority of myofibers were well preserved and exhibited no indication of sarcomere-originating diseases. Occasional myofibers exhibited early degenerative changes, such as reduced width of sarcomeres, even though the least degenerated areas within the lesion had been selected from the semithin sections. No features of some hereditary myopathies, such as nemalin rods or inclusion bodies were observed in our samples. All evaluated samples contained glygocen granules in the typical locations. Invaginations of the plasma membrane approximately 60-nm in diameter, resembling caveolae, were present in the sarcolemma of all groups.

5.7 PECTORAL MICROVESSEL DENSITY AND WB (III)

The median coefficient of variation was 11.2% for myofiber number and 9.2% for vessel number when all sets of eight microscopic fields were included. Further stastistical analyses were performed on the total myofiber number and total vessel number of the two selected microscopic fields. The total vessel number and total myofiber area in the two selected microscopic fields were further statistically analysed. A significant difference in vessel number ($P < 0.001$) and in myofiber area ($P = 0.002$) between the age groups were detected using one-way ANOVA, when the unaffected, focal WB unaffected and focal WB lesion area were pooled per each age group. However, after Bonferroni post-hoc analysis, no significant difference between the ages 18 and 24 days and between the ages 35 and 38 days of age were

present, but the former two significantly differed from the latter two ages: the total vessel number of the 18-day-old birds versus 35 d, $P < 0.001$ and versus 38 d, $P = 0.001$; 24-day-old birds versus 35 d, $P = 0.001$ and versus 38 d, $P = 0.007$; and the total myofiber area of the 18-day-old birds versus 35 d, $P = 0.017$ and versus 38 d, $P = 0.036$; 24-day-old birds versus 35 d, $P = 0.018$, and versus 38 d, $P = 0.049$. Therefore, the birds were pooled into two age groups, younger (18 and 24 days) and older (35 and 38 days) based on the results of ANOVA and further statistical analyses were performed inside these groups.

The total microvessel number and the total myofiber area were the highest in the young (18 and 24 days of age) unaffected cases (Tables 11 and 12). The ratio of myofiber area to microvessel number of unaffected cases significantly differed from the unaffected area of focal WB in both age groups. The majority of microvessels were capillaries. Other types of microvessels (arterioles and venules) were observed in all microscopic fields but constituted the minority.

Table 11. *The mean of total number of microvessels, total myofiber area and their ratio in 18- and 24-day-old unaffected and focal WB broiler chicken pectoralis major muscles.*

	Broilers 18 and 24 days of age		
	Unaffected	Focal WB	
		Unaffected	Lesion
Vessel number	84[a]	60[b]	57[b]
Myofiber area (μm^2)	205,427[a]	181,947[b]	160,596[b]
Myofiber area (μm^2) per vessel number	2,471[a]	3,041[b]	2,833[ab]

Means with different letters indicate significant differences ($P \leq 0.05$) between groups on the same row.

Table 12. *The mean of total number of microvessels, total myofiber area and their ratio in the 35- and 38-day-old unaffected and focal WB broiler chickens.*

	Broilers 35 and 38 days of age		
	Unaffected	Focal WB	
		Unaffected	Lesion
Vessel number	49[a]	40[a]	42[a]
Myofiber area (μm^2)	163,440[a]	154,906[a]	118,852[b]
Myofiber area (μm^2) per vessel number	3,385[a]	3,913[b]	2,874[a]

Means with different letters indicate significant differences ($P \leq 0.05$) between groups on the same row.

6 DISCUSSION

The human consumption of animal meat for food has been increasing globally for several decades (Sans and Combris, 2015; The Food and Agriculture Organization, 2016). Consumers consider the relatively low fat content and the competitive price as favourable features of poultry meat compared to other meat products (Magdelaine et al., 2008). However, the consumer awareness of ecological and ethical issues related to meat production is growing and demands attention of the meat industry. In addition, novel plant-based options as protein source for human consumption have been developed and compete on the market as alternatives for meat products of animal origin (Mäkinen et al., 2017).

Syndromes resulting in meat quality impairments, such as white striping and abnormal consistency of breast fillets have emerged recently (Kuttappan et al., 2013; Petracci et al., 2014). Although they do not impose microbiological or other food safety risks, these quality issues render the products less attractive to the consumer (Kuttappan et al., 2012). This is avoided by downgrading or rejecting the affected fillets, resulting in a considerable waste of poultry meat.

The name 'wooden breast myopathy' (WB) was established by the poultry meat industry to describe the hardened consistency of the major pectoral muscle in broiler chickens at slaughter-age, before the nature of the disease was known. As a term, WB currently covers the pathologic changes seen in the major pectoral muscles, possible clinical signs in live animals, as well as the meat quality impairments associated with the changes. To date, the majority of published observations have focused on the chronic and post-mortem changes of WB and also on meat quality issues. In the future, the early lesions, pathogenesis and the possible reduction of animal welfare associated with WB are likely to gain more attention.

This dissertation focused on the morphological and pathological aspects of WB, thus a large amount of data on bird growth, diet, husbandry and other such aspects are presented and discussed in other forums. Many of the pathological lesions of WB, such as myodegeneration, represent pathological changes that have been familiar to pathologists for decades. However, taking all aspects together, WB can be considered to be a novel myopathic entity, which may provide information on the development of muscle pathologies in rapidly growing animals, and possibly translational to humans as well.

Macroscopic WB denotes abnormally firm muscle consistency

Altogether, over 500 broiler chickens were evaluated in the studies included in this dissertation. The morpholocigal features of birds affected with WB were very consistent throughout the studies and have since been further confirmed and defined in other reports (Mudalal et al., 2015; Mutryn et al., 2015; Velleman and Clark, 2015; de Brot et al., 2016; Soglia et al., 2016; Papah et al., 2017; Wold et al., 2017)

The macroscopic hallmarks of WB include the abnormally hardened consistency and pale color of the major pectoral muscle. Additional features include outbuldging appearance of the affected areas and sometimes hemorrhage and a layer of clear, slightly gelatinous material covering the muscle surface. The macroscopic lesions typically occur simultaneously in both

the left and right major pectoral muscle. Some of the previously described myopathies, such as nutritional myopathy of selenium and vitamin E deficiency exhibit some macroscopic changes similar to WB, such as pale color (Klasing et al., 2008). However, the hardened muscle consistency seems to be a distinctive macroscopic feature of WB.

The hardened muscle consistency in WB could be explained by the prominent fibrosis often observed in the chronic phase of WB, but contradictory to that hypothesis, our studies indicate that there is a lack of fibrosis, particularly in early WB cases, despite their hardened muscle consistency. Others have similar observations and suggest myofiber degeneration and swelling together with fibrosis to be responsible for the hardened consistency of WB muscles (Papah et al., 2017).

WB restricts to the major pectoral muscle

WB lesions typically affect only the major pectoral muscle, without the involvement of other skeletal muscles, cardiac muscles or smooth muscle, as shown both in our studies and others (Papah et al., 2017). However, an occasional involvement of other muscles may occur. One WB-affected bird in our first experimental rearing study exhibited both macroscopic and histological lesions similar to WB in a thigh muscle. Very similar changes have also been published to occur in the anterior latissimus dorsi (ALD) muscle of broiler chickens in Brazil (Zimermann et al., 2012). A recent report also described WB-like histological changes in the minor pectoral muscle of Ross 308 broilers in Japan, but hardened muscle consistency and other macroscopic changes characteristic of WB were lacking (Kawasaki et al., 2018).

Almost invariably, only type 2B fibers are found in the major pectoral muscle of broiler chickens (Remignon et al., 1995; Papinaho et al., 1996; MacRae et al., 2006; Papah et al., 2017). Compared with layer chickens or slower-growing broilers, the type 2B myofibers of rapidly-growing broilers exhibit increased diameter, i.e. hypertrophy (Remignon et al., 1995; Soike and Bergmann, 1998; MacRae et al., 2006; Velleman and Clark, 2015; Clark and Velleman, 2016). Due to hypertrophy, the diffusion distance for oxygen and muscle metabolites between the vasculature and and the center or the myofiber increases. Thus, the large diameter of myofibers in the major pectoral muscle may represent one predisposing factor for myodegenerative lesions.

White striping associates with WB

White striping was associated to WB with statistical significance in our studies, but both features also appeared independently. We defined no distinct histological pattern for white striping, although the sampling methods were not specifically designed to differentiate WB and white striping. Previous reports suggest that white striping is composed of adipose tissue (Kuttappan et al., 2013; Papah et al., 2017; Baldi et al., 2018), whereas other studies find no histological differences between WB and white striping (Soglia et al., 2016; Radaelli et al., 2017), in accordance with our findings.

Polyphasic myodegeneration dominates histologically

The macroscopic WB lesion was strongly associated with a polyphasic myodegeneration histologically in our studies. The classification of myodegenerative lesions into monophasic

and polyphasic refers to the temporal pattern of lesion development in the muscle (Cooper and Valentine, 2016). The polyphasic lesion type denotes the occurrence of both degenerative and regerative changes simultaneously within the lesion area. This indicates a repeated or progressing damage to the muscle cells and rules out a single pathologic insult as an etiopathogenesis for WB.

Skeletal muscle of broilers is known to exhibit a minimal degree of myodegeneration, usually occasional single degenerative fibers, compared to layer chickens or other animal species (Soike and Bergmann, 1998b; Harvey and Marshall, 2000; Williams et al., 2008; Russo et al., 2015). It was true also in our studies; in the macroscopically unremarkable pectoral muscles, occasional myodegenerative fibers were observed through all age groups. We employed four scores to classify the severity of the myodegenerative lesions; absent-minimal, mild, moderate, and severe. Excluding the absent-minimal, in all other scores the myodegeneration was accompanied with a variable amount of regenerative fibers.

The contradictory role of phlebitis in WB

In addition to the polyphasic myodegeneration, a prominent histological feature of WB was lymphocytic vasculitis, i.e. phlebitis, and perivascular infiltrations focused on the small-caliber veins. Recently, phlebitis has been suggested as a primary initiator of the WB lesion, via impaired vascular flow and subsequent formation of congestion and edema (Papah et al., 2017). However, despite the concurrent or sequential existence of phlebitis and myodegeneration, respectively, the presence of myodegeneration without any vascular involvement in some of our WB cases challenges the primary role of phlebitis. On the other hand, some claim that perivascular accumulation of lymphocytes is a common finding in severe muscle necrosis, regardless of the etiology (Barnes et al., 2016).

Vascular inflammation restricted to the venular vessels is rare in all species in general, especially when it occurs in one organ system without systemic involvement. One of the best described is the human enterocolic lymphocytic phlebitis, where the vascular lesion leads to development of thrombosis and subsequent problems arising from that (Saraga and Costa, 1989; Ngo and Chang, 2007). In WB, no thrombosis has been detected in our studies or by others (Papah et al., 2017) and it is unlikely that thrombosis has a role in the pathogenesis of WB.

The lymphocytes in WB were positive for the T cell marker CD3 by immunohistochemistry. The CD3 is expressed as a co-receptor on the surface of both helper CD4+ T cells, as well as on cytotoxic CD8+ T cells (Kumar et al., 2010). Although histologically similar, the two subclasses of T cells, helper and cytotoxic, differ functionally. Notably simplified, the main function of CD4+ cells is to assist macrophages and B cells to combat infections, whereas the CD8+ cells directly attack the host cells that harbor infective agents within them (Kumar et al., 2010). Thus, a further definition of the perivascular lymphocytes population in WB could provide more insights into the pathomechanism. In fact, our unpublished results of immunohistochemistry yielded positive staining against the CD8 antigen in the perivascular lymphocytes of WB cases, but due to unselective staining of some blood cells the results were not further reported.

Focal onset at two weeks of age progresses to diffuse WB

In our experimental rearing studies, the first macroscopic WB changes were detected in broilers of 15 or 18 days of age, depending on the examination schedule. Since none of the 10-day-old birds exhibited macroscopic WB changes, it can be concluded that the lesion starts to develop between 10 and 15 days of age. The early macroscopic lesions were usually focal and exhibited mild muscle firmness compared to the more severe and typically diffuse lesions in the birds older than 22 or 24 days. This indicates that WB starts to develop as a focal lesion and progresses into a diffuse, severe lesion by the conventional slaughter age of the broilers at approximately 5-6 weeks. A similar course of WB progression has been reported recently, with the first macroscopic changes observed in birds by three weeks of age (Papah et al., 2017).

Of the focal WB lesions of our experimental rearing 1, over 70% were located in the cranial part of the major pectoral muscle. In addition to the similar appearance of lesions that first appear in the cranial area, more progressed histological lesions such as fibrosis have been detected in the cranial area than in the caudal portion of the muscle (Bailey et al., 2015; Clark and Velleman, 2016). The cranial portion of the muscle is anatomically thicker compared to the caudal end, and it can be speculated whether the thickness has any effect on the disease course.

We observed changes consistent with polyphasic myodegeneration in broilers at 15 or 18 days of age at the earliest, some of them concurrently exhibiting macroscopic WB lesions. Since a few days is required for regeneration to develop (Hawke and Garry, 2001), this indicates that the first degenerative muscle lesions begin approximately at 12 to 15 days of age. This is in accordance with other studies, which have reported the emergence of histological myodegenerative lesions at 2-3 weeks of age, followed by a remarkable increase in severity with the progression of macroscopic WB in birds approaching 5-6 weeks of age (Radaelli et al., 2017; Papah et al., 2017).

Despite the strong link between macroscopic WB and polyphasic histological degeneration, some of the young broilers between 15 and 24 days of age exhibited mild polyphasic myodegeneration, but no macroscopic WB lesions. The lesions in this group likely represent a pre-macroscopic phase of WB, which progresses into macroscopically detecteable WB lesion in the course of time. This is supported by the prevalence data of macroscopic WB; the occurrence increases until the peak at approximately 35 days of age, meaning that some of the younger unaffected birds will develop WB, even though it is not macroscopically visible at the time. Pre-macroscopic myodegeneration was also observed in the unaffected area of focal WB cases, providing further evidence for the WB development through a focal lesion towards a diffuse form. Thus, the appearance of regeneration may represent a distinctive feature between the ‘usual’ occasional degeneration of all rapid-growing broilers and the early WB lesion before it is macroscopically detectable.

Heavy weight – predisposing or secondary feature?

A high number of studies have shown a link between the occurrence of WB and rapid growth rate, high breast meat yield, or both (Mudalal et al., 2015; Mutryn et al., 2015; Soglia et al., 2016; Papah et al., 2017; Tasoniero et al., 2017; Kawasaki et al., 2018). However, the vast majority of the studies on the effect of weight on WB have been conducted on slaughter-age

broilers, or breast muscles from the slaughterhouse production line. Such a setting is unable to differentiate whether the increased weight is a preceding feature or a secondary effect of WB. Contradictory views, proposing an insignificant role of growth rate or high breast meat yield in WB development represent the minority (Bailey et al., 2015).

There was no significant difference in the mean live weight between the macroscopically unaffected and WB broilers in our first experimental rearing. This may partially result from the relatively high proportion of young birds within the study population, since many of the affected young birds, from 15 to 24 days of age, exhibit the focal phase of WB development.

Feed restriction between 2 and 3 weeks of age in rapidly growing broilers has been shown to temporarily restrict or delay the development of myodegenerative lesions, but the effect was lost with the reversion of diet that enabled birds to express their full growth potential (Radaelli et al., 2017). In our experimental rearing studies, the birds received feed ad libitum, which likely promoted the relatively high live weight of the birds and the high prevalence of WB.

Clinical signs and welfare issues related to WB

The routine daily observation of the birds in our experimental flocks revealed no clinical signs associated with WB. However, only a short visual observation was performed, without a physical examination of the birds or prolonged observation of their ability to move. A reduced mobility of the wings has been linked to the myodegenerative lesions of WB in some studies (Kawasaki et al., 2016; Papah et al., 2017). In addition, inability to arise from dorsal recumbency and decreased activity to move has been associated with WB (Papah et al., 2017). This is in accordance with reduced ability to stand and walk associated with WB in the birds of our studies (Norring et al., submitted). These findings indicate that WB reduces the welfare of the affected animals. Although the amount of pain associated with WB is difficult to demonstrate from the above studies, severe myodegenerative lesions in humans have been reported to cause substantial pain (Silva et al., 2016)

Detection of WB lesions in live birds was not attempted in our studies. However, each individual bird was manually handled during weighing and euthanasia and the caretakers reported no obvious changes in the pectoral muscle consistency. Studies with a specific attempt to evaluate the WB lesion ante mortem, the distinction between unaffected and abnormally hardened consistency has been possible in birds at 4 or 6 weeks of age at the earliest (Clark and Velleman, 2016; Papah et al., 2017; Griffin et al., 2018).

The decreased ability of the WB-affected birds to lift their wings has also been proposed as a method for WB detection from live animals, although that represents a rather labour-demanding method (Kawasaki et al., 2016). In a meat processing line setting, automated on-line systems for screening for fillets with WB have been developed recently, such as the near-infrared spectroscopy, which will enable more detailed data on the prevalence and severity of the disease (Wold et al., 2017).

Ultrastructural findings point to osmotic imbalance

Our ultrastructural study was targeted on the intact myofibers flanking the degenerative fibers within the WB lesion, in order to evaluate the early lesions before the progressed

degeneration. The 22-day-old broiler chickens exhibited both focal and diffuse WB without prominent fibrosis. The main ultrastructural findings included increased diameter of the sarcoplasmic reticulum and aggregation and morphologic changes of intermyofibrillar mitochondria.

Ultrastructural changes in the affected myofibers of WB cases include myofilament disruption and loss, Z-line aberrations, endomysial fibrosis, and mitochondrial degeneration (Papah et al., 2017). This is in conjuction with the previously described common findings of myodegeneration in other species (Ghadially, 1997).

The cellular mechanism for sarcoplasmic dilatation include entrance of excess water into the cell due to osmotic imbalances, such as primary sarcoplasmic defects or secondary to myodegeneration, fatigue or hypoxia (Fujisawa, 1975; Ghadially, 1997a; Duhamel et al., 2004; Frías et al., 2005). Downregulation of SERCA (sarco-endoplasmic reticulum Ca^{2+}-ATPase) in hypoxia leads to a reduced transfer of calcium ions from the cytoplasm into the SR (Revuelta-López et al., 2015). SR enlargement in muscle fatigue likely stems from the same mechanism of defective calcium-ion transfer, but it remains to be more throughly charactirezed (Frías et al., 2005; Duhamel et al., 2004). The role of muscle fatigue in the early phase of WB is currently unknown, but it likely enhances the progression of an advanced WB lesion with prominent degeneration, due to increased workload directed to the remaining functional myofibers. The reduced luminal electron density and occasional granularity of enlarged terminal cisternae in WB lesions may suggest defective calcium-ion regulation. The intraluminal granular material in terminal cisternae presumably is calsequestrin protein that augments the calcium-binding capacity of the SR (Franzini-Armstrong and Engel, 2012; Cheville, 2009).

The intermyofibrillar mitochondria of all three groups in our study – unaffected, focal WB and diffuse WB cases – expressed morphological changes that were variable in terms of severity. These changes included vacuolisation, loss of cristae and swelling of the mitochondria, but were more frequent and pronounced in the diffuse WB cases and lesion areas of focal WB cases than in the unaffected areas. Osmotic imbalances and hypoxia typically lead to such morphologic changes, especially in cells with high metabolic needs (Cheville, 2009; Sewry, 2002). The findings indicate the WB lesion areas may encounter ante mortem stress, such as hypoxia, or are more liable to post mortem alterations.

Aggregations of intermyofibrillar mitochondria in the WB lesion areas of our study is consistent with mitochodrial hyperplasia. Various pathologies as well as increased functional burden to the skeletal muscle provoke mitochondrial hyperplasia (Ghadially, 1997b). Density and volume of intermyofibrillar mitochondria increase in hypoxic conditions, whereas myofibrillar loss provokes also aggregation of mitochondria (Sewry, 2002; Jacobs et al., 2016). In the WB areas, the nondegenerate myofibers that we studied were surrounded by degenerative fibers, which may have an effect on the mitochondria. However, as both hypoxia and myodegeneration may induce similar changes, the impact of those two factors cannot be differentiated ultrastructurally.

The triad complex, consisting of a T-tubule flanked by the terminal cisternae of sarcoplasmic reticulum, typically appeared at the sarcomere Z-lines in our study, but occasionally the location varied. The triad location in humans is fixed at the junction of A- and I-bands and displacement occurs in some pathologic conditions (Ghadially, 1997a; Wrede et al., 2012).

The triad location is reported to vary in birds, but data on the typical location in the broiler skeletal muscle are sparse (Ghadially, 1997a; Martonosi, 2000).

Hypoxia represents a potential pathomechanism of WB

The microvessel density of major pectoral muscle was examined in unaffected birds and at two sites of focally affected WB birds in our study. Two parameters, the number of small vessels and the area of myofibers, and their ratio, were analyzed in two age populations: young (18- and 24-day-old) and slaughter-age (35- and 38-day-old) birds.

In unaffected birds, the total vessel number of the older group was lower compared to the younger birds, which is in conjuction with previous studies that report decreases in vessel number with the age of birds (Joiner et al., 2014; Radaelli et al., 2017). In both sites of the focal WB cases, i.e. the unaffected and lesion site, the vessel number was significantly decreased compared to unaffected cases of the same age group. The significant decrease from unaffected cases to unaffected area of focal WB cases is especially important, since it indicates that a reduction in blood vessel number precedes the macroscopic WB lesion. Another explanation for this finding is that the number of pre-existing blood vessels could be initially lower in birds that will succumb to WB, acting as a predisposing factor for WB development.

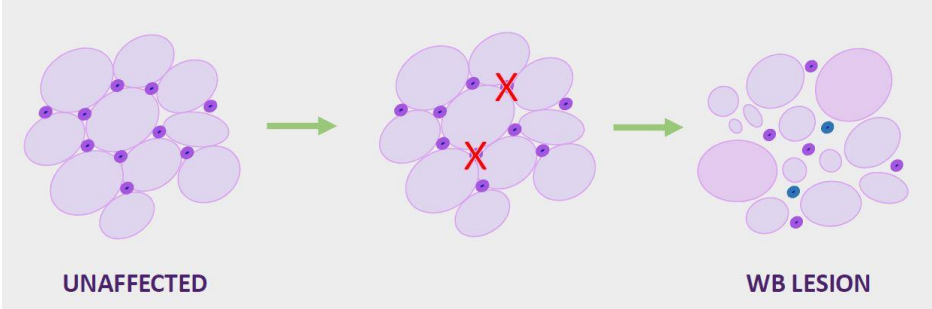


Figure 9. *A hypothesis of vascular impairment initiating the WB lesion. In the middle: reduction in the vessel number, either due to their destruction or initially insufficient number, results in myofiber hypoxia and defective metabolism. On the right: Myodegenerative lesion has developed and the total vessel number includes the pre-exisisting vessels and neovascularization (in blue).*

The total myofiber area, including all complete myofibers within the evaluated micrographs, was highest in the unaffected cases and respectively decreased with the the lesion development towards the lowest area in the lesion site of the focal WB cases. This directly links to the amount of myodegeneration that destructs the myofibers.

Previosly, the supply of small blood vessels to the muscle tissue has been evaluated as capillary density, i.e. number of capillaries per area, or as the capillary-to-myofiber ratio, i.e. the number of capillaries per number of myofibers (Hudlická, 1985; Hoving-Bolink et al., 2000). As our studies and others have shown, degeneration and regeneration remarkably affect the myofiber number and diameter in WB lesions (Velleman and Clark, 2015; de Brot

et al., 2016; Soglia et al., 2016; Papah et al., 2017; Baldi et al., 2018). This would likely bias the results when unaffected and degenerative areas are compared. Therefore, we analyzed the ratio of myofiber area to vessel number in order to more accurately evaluate the suffifiency of blood supply to the intact muscle mass.

The highest ratio of myofiber area to vessel number in both age groups, young and slaughter-age birds, was detected in the unaffected site of focal WB cases and it was significantly higher compared to the unaffected cases. This means that each small blood vessel supplies a higher area of myofiber mass in the unaffected area of focal WB cases than in the unaffected cases – further evidence for relatively insufficient vascular supply as possible pathomechanism for WB. Within the focal WB cases, the myofiber area to vessel number ratio was decreased in the lesion site compared to the unaffected site, although this was only statistically significant in the older age group. The more numerous vessels per myofiber area in the WB affected site of the focal cases arises from the decreases myofiber area due to myodegeneration.

Some excluded etiopathogeneses

In humans and other species, there are several intrasarcoplasmic myopathies with typical ultrastructural features, such as nemaline rods, inclusions, rimmed or autophagic vacuoles, central cores or excessive accumulation of glycogen or other substances within the myofibers (Fernandez et al., 2005). No above-mentioned morphologic features were observed in the samples of our study, indicating that WB is not morphologically consistent with these previously described conditions.

Caveolae are present in the plasma membrane of several cell types and take part in endocytosis, exocytosis, signalling and many other cell functions (Palade, 1953; Stan, 2005). Caveolin-3 is muscle-specific and its deficiency leads to muscular dystrophy in mice and humans (Hagiwara et al., 2000; Minetti et al., 2002). However, overexpression of caveolin-3 and increased numbers of caveolae are also associated with muscular problems, such as Duchenne muscular dystrophy in humans and muscular dystrophy in chickens (Repetto et al., 1999; Matsumoto et al., 2010). The sarcolemmal invaginations consistent with caveolae were present both in the the unaffected and WB cases of our study, indicating that caveolin deficiency is an unlikely cause of WB.

Some viral infections may affect the muscular system in poultry. Infectious bronchitis (IB) virus variant 793/B has been associated with myopathic lesions in the pectoral muscles of broilers and the muscle lesions are assumed develop secondary to immune-complex mediated vascular changes (Dhinakar Raj and Jones, 1996). IB virus belongs to coronaviruses and the most common clinical problems focus on the kidneys, respiratory and reproductive systems, leading to poor egg quality and impaired production (Cavanagh and Gelb, 2008). No lymphocytic phlebitis has been linked to IB in chickens, but vasculitis is a usual feature in some coronaviral infections in other animal species, such as in feline infectious peritonitis. However, with the granulomatous nature of the vasculitis and the systemic, fatal disease course, the lesions in feline infectious peritonitis (Kipar et al., 2005) are rather distinctive from the WB lesions.

In addition, the parent flocks of the birds of this study were included in the national health surveillance program for poultry and tested negative for IB virus, which makes the impact of

IB infection in WB unlikely. Finnish poultry was free of clinical IB infections for several decades, until its recent emergence (Pohjola et al., 2014). However, the genotypes detected in Finland included QX, D274-like and 4/91 and may represent escaped vaccine viruses (Pohjola et al., 2014).

Dietary effects on WB

In nutritional myopathy caused by the deficiency of selenium, vitamin E, or both, typical macroscopic changes in skeletal muscle include pale color and dry appearance, histologically consistent with myodegeneration (Ruth and Van Vleet, 1974; Van Vleet and Valentine, 2007; Klasing et al., 2008). However, lesions in other organs, such as degenerative lesions in cardiac muscle, exudative diathesis and encephalomalacia are often observed, which do not occur in WB. Deficiency of selenium and vitamin E reduce the antioxidant capacity against oxidative stress, especially in the pectoralis muscles of chickens (Avanzo et al., 2001).

In our study, the reduced selenium concentration in the feed provoked no increase in the prevalence of WB. Similar findings have been reported regarding white striping; increasing dietary supplementation of vitamin E has nonexistent or very limited effects on the occurrence of nutritional myopathy or white striping in chickens (Guetchom et al., 2012; Kuttappan et al., 2012).

In addition to selenium, the amino acid composition of the broiler feed has gained interest concerning WB. Increasing levels of dietary lysine was associated with higher body weight and breast fillet weight in broilers, as well as higher occurrence of WB (Cruz et al., 2017). Hence, the effect of lysine on WB seems to be indirect through the higher weight gain.

Animal model for human disease

The chicken has been used to model several human diseases and genetic factors (Dodgson and Romanov, 2004). Birds exhibiting WB may also have some potential as an animal model for myodegenerative diseases in humans and other animal species. In that regard, several advantages can be associated with WB myopathy: high natural prevalence of the disease with low occurrence of other ailments, a single affected muscle in easily-accessible location, an optimal-sized animal species with rapid generation turnover, and early manifestation of the changes. Additionally, the growth rate can be controlled to some extent with feed content modifications (Cruz et al., 2017).

The healthy, non-affected broilers also exhibit naturally hypertrophic pectoral muscles, but without the degenerative disease, and might serve as a competitor for rodent models of muscle hypertrophy. They are medium-sized animals and are large compared to rodents, with large muscles located conveniently for example biopsy procedures. Tens of different techniques and models in rodents have been developed to imitate the muscle hypertrophy of resistance training in humans, but the non-spontaneous nature of the muscle hypertrophy creates many difficulties, for example the motivating and training of the animals is often challenging (Cholewa et al., 2014).

Sarcopenia denotes the age-related atrophy of type 2 fibers in non-postural muscles and it represents a major cause of physical disability in elderly humans (Dhillon and Hasni, 2016). Although to some extent sarcopenia can be considered as a physiologic consequence of aging,

means to decrease its prevalence and severity would have positive economic and human welfare effects. The same mechanisms that cause hypertrophy of the type 2 fibers in broilers could possibly be used to stimulate the muscles in aging humans, to prevent the atrophy.

Limitations of the study

Apart from the first samples obtained from slaughterhouses, the birds of our studies were reared in experimental conditions. Although the animals of the experimental studies were conventional hybrids from a commercial hatchery, the conditions may have had an effect on the birds and the prevalence of WB. The small group size compared to commercial conditions may have promoted birds' access to feed and water, possibly enhancing the growth rate.

Both rearing studies of this thesis included hundreds of birds, which resulted in relatively high number of both unaffected and affected cases even when the birds were divided in different age groups. Due to the lack of any preliminary experimental data before the first rearing study, sample size calculations and power analysis were based on rough estimates of WB prevalence data from the industry.

Electron microscopy examines a very restricted tissue area at a time in the ultra-thin sections. Among all tissue types, muscle tissue can be considered particularly special due to the extensive length of myofibers, which makes the capture of a complete myofiber impossible by electron microscopy. Additionally, in our study, the sample number was rather low. Semi-thin tissue sections, required for preparation of ultra-thin sections, provide more detailed morphologic view compared to traditional sections for light microscopy, but require less processing and are less time consuming to evaluate than ultrathin sections, thus offering an attractive alternative for morphologic evaluation of muscle.

Secondary changes, such as accumulation of fibrous tissue and collagen, are prominent features in WB cases at slaughter age. In order to discover the primary changes and etiology, it is necessary to study the lesions in younger birds. However, the definition of a biologically coherent control group of unaffected young birds can be challenging due to phenotypic changes later with increasing age.

7 CONCLUSIONS

In this thesis dissertation, the morphology and development of a novel type of degenerative muscle disease in broiler chickens were defined. Additionally, some aspects on the pathogenesis of the myopathy were revealed. The results provide the ground knowledge for further studies on the pathogenesis and preventive efforts of the disease.

Wooden breast myopathy is characterized by abnormally hardened consistency of the major pectoral muscles of broiler chickens. Additional macroscopic features include pale color, outbuldging appearance, occasionally hemorrhage and a layer of clear fluid on the muscle surface. The lesion begins focally in birds approximately two weeks of age and typically develops into a diffuse lesion that involves the major pectoral completely by the birds' slaughter-age at five to six weeks.

Microscopically, wooden breast myopathy manifests as a polyphasic myodegeneration. This rules out single injuries to the muscle tissue as etiologies for wooden breast myopathy. Ongoing myodegeneration is accompanied by regeneration, and at chronic stages prominent secondary changes such as fibrosis develop. Lymphocytic phlebitis is strongly associated with the myodegeneration, but its role in the pathogenesis of wooden breast is currently disputable.

Wooden breast myopathy is limited to the major pectoralis muscle. This distinguishes wooden breast myopathy from several other myodegenerative diseases, which affect the skeletal muscle system widely, and often also cardiac and smooth muscle. Other skeletal muscles, such as thigh or dorsal muscles, may occasionally exhibit wooden breast-like lesions, but the syndrome affects the major pectoralis.

Relatively reduced microvessel density contributes to the development of wooden breast myopathy. Either the affected birds exhibit lower capillary density initially, compared to the birds that never succumb to wooden breast, or a relative reduction in the vascular supply occurs before the initiation of the degenerative lesion.

Decreased dietary selenium does not affect the prevalence of wooden breast myopathy. The morphology of polyphasic myodegeneration renders selenium deficiency as one possible causative factor for wooden breast myopathy, but decreased selenium content in the diet had no effect on the prevalence of wooden breast myopathy in our experimental rearing studies.

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REFERENCES

Airey JA, Beck CF, Murakami K, Tanksley SJ, Deerinck TJ, Ellisman MH, Sutko JL (1990) Identification and localization of two triad junctional foot protein isoforms in mature avian fast twitch skeletal muscle, *The Journal of Biological Chemistry,* **265**, 14187-14194.

Alvarado CZ, Sams AR (2003) Injection marination strategies for remediation of pale, exudative broiler breast meat, *Poultry Science,* **82**, 1332-1336.

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome, *Nature,* **290**, 457-465.

Angelini C, Di Mauro S, Margreth A (1968) Relationship of serum enzyme changes to muscle damage in vitamin E deficiency of the rabbit, *Lo Sperimentale,* **118**, 349-369.

Asmundson VS, Julian LM (1956) Inherited muscle abnormaity in the domestic fowl, *Journal of Heredity,* **47**, 248-252.

Asmundson VS, Kratzer FH, Julian LM (1966) Inherited myopathy in the chicken, *Annals of the New York Academy of Sciences,* **138**, 49-58.

Avanzo JL, De Mendonça Jr, C X, Pugine SMP, De Cerqueira Cesar M (2001) Effect of vitamin E and selenium on resistance to oxidative stress in chicken superficial pectoralis muscle, *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology,* **129**, 163-173.

Aviagen (2014) *Ross Broiler Management Handbook*, Aviagen Ltd., Newbridge, UK.

Aviagen (2014) *Ross 308 Broiler: Nutrition Specifications*, Aviagen Ltd., Newbridge, UK, pp. 1-10.

Bailey RA, Watson KA, Bilgili SF, Avendano S (2015) The genetic basis of pectoralis major myopathies in modern broiler chicken lines, *Poultry Science,* **94**, 2870-2879.

Bains BS, Watson AR (1978) Nutritional myopathy- a cause of ataxia in broiler chickens, *New Zealand Veterinary Journal,* **26**, 31-32.

Bains BS, MacKenzie MA, McKenzie RA (1975) Selenium-responsive myopathy in broiler breeder hens in Queensland, *Australian Veterinary Journal,* **51**, 140-145.

Baldi G, Soglia F, Mazzoni M, Sirri F, Canonico L, Babini E, Laghi L, Cavani C, Petracci M (2018) Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers, *Animal,* **12**, 164-173.

Bansal D, Miyake K, Vogel SS, Groh S, Chen C, Williamson R, McNeil PL, Campbell KP (2003) Defective membrane repair in dysferlin-deficient muscular dystrophy, *Nature,* **423**, 168-172.

Bárány M (1967) ATPase activity of myosin correlated with speed of muscle shortening, *The Journal of general physiology,* **50**, 218.

Barnes J, Abdul-Aziz T, Fletcher O (2016) Muscular system. In: *Avian Histopathology*, T Abdul-Aziz, J Barnes, O Fletcher, H Shivaprasad, D Swayne, Eds., AAAP, Florida, US, pp. 107-142.

Bauermeister L, Morey A, Moran E, Singh M, Owens C et al. (2009) Occurrence of white striping in chicken breast fillets in relation to broiler size, *Poult Sci,* **88**, 33.

Bendall JR, Swatland HJ (1988) A review of the relationships of pH with physical aspects of pork quality, *Meat Science,* **24**, 85-126.

Bianchi M, Petracci M, Franchini A, Cavani C (2006) The Occurrence of Deep Pectoral Myopathy in Roaster Chickens, *Poultry Science,* **85**, 1843-1846.

Biewener AA (2011) Muscle function in avian flight: achieving power and control, *Philosophical Transactions of the Royal Society B: Biological Sciences,* **366**, 1496-1506.

Bilgili S (2015) Broiler Chicken Myopathies IV: Stringy/mushy breast.

Birky CW (1978) Transmission genetics of mitochondria and chloroplasts, *Annual Review of Genetics,* **12**, 471-512.

Bischoff R (1986) A satellite cell mitogen from crushed adult muscle, *Developmental Biology,* **115**, 140-147.

Boles JA, Parrish FC, Huiatt TW, Robson RM (1992) Effect of porcine stress syndrome on the solubility and degradation of myofibrillar/cytoskeletal proteins, *Journal of Animal Science,* **70**, 454-464.

Braga IS, Oda K, Kikuchi T, Tanaka S, Shin Y, Sento M, Itakura C, Mizutani M (1995) A new inherited muscular disorder in Japanese quails (Coturnix coturnix japonica), *Veterinary Pathology,* **32**, 351-360.

Buchthal F, Schmalbruch H (1980) Motor unit of mammalian muscle, *Physiological Reviews,* **60**, 90-142.

Burke RE, Levine DN, Zajac I, Tsairis P, Engel WK (1971) Mammalian motor units: Physiological-histochemical correlation in three types in cat gastrocnemius, *Science,* **174**, 709-712.

Cardoso SM, Pereira C, Oliveira R (1999) Mitochondrial function is differentially affected upon oxidative stress, *Free Radical Biology & Medicine,* **26**, 3-13.

Cavanagh D, Gelb J (2008) Infectious Bronchitis. In: *Diseases of Poulty*, K Klasing, R Crespo, H Shivaprasad, F Hoerr, R Fulton, Eds., Wiley-Blackwell, Ames, Iowa, USA, pp. 117-135.

Chapman MA, Meza R, Lieber RL (2016) Skeletal muscle fibroblasts in health and disease, *Differentiation; Research in Biological Diversity,* **92**, 108-115.

Chazaud B, Brigitte M, Yacoub-Youssef H, Arnold L, Gherardi R, Sonnet C, Lafuste P, Chretien F (2009) Dual and beneficial roles of macrophages during skeletal muscle regeneration, *Exercise and Sport Sciences Reviews,* **37**, 18-22.

Cheema MA, Qureshi MA, Havenstein GB (2003) A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets, *Poultry Science,* **82**, 1519-1529.

Chen C, Matesic LE (2007) The Nedd4-like family of E3 ubiquitin ligases and cancer, *Cancer Metastasis Reviews,* **26**, 587-604.

Cholewa J, Guimarães-Ferreira L, da Silva Teixeira T, Naimo MA, Zhi X, de Sá, Rafaele Bis Dal Ponte, Lodetti A, Cardozo MQ, Zanchi NE (2014) Basic models modeling resistance training: an update for basic scientists interested in study skeletal muscle hypertrophy, *Journal of Cellular Physiology,* **229**, 1148-1156.

Clark DL, Velleman SG (2016) Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers, *Poultry science,* **95**, 2930-2945.

Cobb-Vantress (2015) Cobb500 Broiler Performance & Nutrition Supplement. Cobb-Vantress, Arkansas, US, pp. 1-14.

Cooper B, Valentine B (2016) Muscle and tendon. In: *Jubb, Kennedy, and Palmer's Pathology of domesti animals, Vol. 1*, G Maxie, Ed., Elsevier, US, pp. 164-249.

Costello BR, Shafiq SA (1979) Freeze-fracture study of muscle plasmalemma in normal and dystrophic chickens, *Muscle & Nerve,* **2**, 191-201.

Creech BG, Rahman MM, Reid BL, Couch JR (1958) Exudative diathesis in chicks, *The Journal of Nutrition,* **64**, 55-65.

Cruz RFA, Vieira SL, Kindlein L, Kipper M, Cemin HS, Rauber SM (2017) Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels, *Poultry Science,* **96**, 501-510.

Dam H, Prange I, Søndergaard E (1952) Muscular degeneration (white striation of muscles) in chicks reared on vitamin E deficient, low fat diets, *Acta Pathologica Et Microbiologica Scandinavica,* **31**, 172-184.

de Brot S, Perez S, Shivaprasad HL, Baiker K, Polledo L, Clark M, Grau-Roma L (2016) Wooden breast lesions in broiler chickens in the UK, *The Veterinary Record,* **178**, 141.

DeNardi C, Ausoni S, Moretti P, Gorza L, Velleca M, Buckingham M, Schiaffino S (1993) Type 2X-myosin heavy chain is coded by a muscle fiber type-specific and developmentally regulated gene, *The Journal of Cell Biology,* **123**, 823-835.

Desai MA, Jackson V, Zhai W, Suman SP, Nair MN, Beach CM, Schilling MW (2016) Proteome basis of pale, soft, and exudative-like (PSE-like) broiler breast (Pectoralis major) meat, *Poultry Science,* **95**, 2696-2706.

Dhillon R, Hasni S (2016) Pathogenesis and Management of Sarcopenia, *Clinics in Geriatric Medicine,* **33**, 17-26.

Dhinakar Raj G, Jones RC (1996) Immunopathogenesis of infection in SPF chicks and commercial broiler chickens of a variant infectious bronchitis virus of economic importance, *Avian Pathology,* **25**, 481-501.

Di Mauro S (2010) Pathogenesis and treatment of mitochondrial myopathies: recent advances, *Acta Myologica,* **29**, 333-338.

Dickinson E, Stevens J, Helfer D (1968) A degenerative myopathy in turkeys, *Proceedings of the 17th Western Poultry Disease Conference,* 6.

Dodgson JB, Romanov MN (2004) Use of Chicken Models for the Analysis of Human Disease, *Current Protocols in Human Genetics,* **40**, 15.5.12.

Dourdin N, Balcerzak D, Brustis JJ, Poussard S, Cottin P, Ducastaing A (1999) Potential m-calpain substrates during myoblast fusion, *Experimental Cell Research,* **246**, 433-442.

Dowling L (1992) Ionophore toxicity in chickens: a review of pathology and diagnosis, *Avian pathology: journal of the W.V.P.A,* **21**, 355-368.

Duchen MR (2000) Mitochondria and calcium: from cell signalling to cell death, *The Journal of Physiology,* **529**, 57-68.

Dutton CJ, Banks BJ, Cooper CB (1995) Polyether ionophores, *Natural Product Reports,* **12**, 165-181.

Ebashi S (1974) Regulatory mechanism of muscle contraction with special reference to the Ca-troponin-tropomyosin system, *Essays in Biochemistry,* **10**, 1-36.

Edström L, Kugelberg E (1968) Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat., *Journal of Neurology Neurosurgery and Psychiatry,* **31**, 424-433.

Eggermont E (2006) Recent advances in vitamin E metabolism and deficiency, *European Journal of Pediatrics,* **165**, 429-434.

Ekarius C (2016) *Storey's illustrated guide to poultry breeds*, Storey Publishing, North Adams, MA.

Eriksson J, Larson G, Gunnarsson U, Bed'hom B, Tixier-Boichard M, Strömstedt L, Wright D, Jungerius A, Vereijken A, Randi E, Jensen P, Andersson L (2008) Identification of the Yellow Skin Gene Reveals a Hybrid Origin of the Domestic Chicken*, PLoS Genetics,* **4**.

Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin*, The Journal of Cell Biology,* **122**, 809-823.

Fernandez C, Figarella-Branger D, Meyronet D, Cassote E, Tong S, Pellissier J (2005) Electron microscopy in neuromuscular disorders*, Ultrastructural Pathology,* **29**, 437-450.

Ferreira R, Vitorino R, Alves RMP, Appell HJ, Powers SK, Duarte JA, Amado F (2010) Subsarcolemmal and intermyofibrillar mitochondria proteome differences disclose functional specializations in skeletal muscle*, Proteomics,* **10**, 3142-3154.

Fielding RA, Manfredi TJ, Ding W, Fiatarone MA, Evans WJ, Cannon JG (1993) Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle*, The American Journal of Physiology,* **265**, 166.

Finnish Food Safety Authority (2016) Siipikarjan terveystarkkailuohjelma (Poultry Health Surveillance Programme).

Franzini-Armstrong C (1980) Structure of sarcoplasmic reticulum*, Federation Proceedings,* **39**, 2403-2409.

Franzini-Armstrong C, Engel A (2012) Skeletal Muscle: Architecture of membrane systems. In: *Muscle*, EN Olxon, J Hill, Eds., Academic Press, London, UK, pp. 763-772.

Friedman JR, Nunnari J (2014) Mitochondrial form and function*, Nature,* **505**, 335-343.

Fujii J, Otsu K, Zorzato F, De L, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia*, Science,* **253**, 448-451.

Fulton R (2008) Other Toxins and Poisons. In: *Diseases of Poultry*, Y Saif, A Fadly, J Glisson, L McDougald, L Nolan et al., Eds., Blackwell Publishing, Ames, Iowa, USA, pp. 1231-1260.

Galbiati F, Volonte D, Minetti C, Chu JB, Lisanti MP (1999) Phenotypic behavior of caveolin-3 mutations that cause autosomal dominant limb girdle muscular dystrophy (LGMD-1C). Retention of LGMD-1C caveolin-3 mutants within the golgi complex*, The Journal of Biological Chemistry,* **274**, 25632-25641.

Gao QQ, McNally EM (2015) The Dystrophin Complex: Structure, Function, and Implications for Therapy, *Comprehensive Physiology,* **5**, 1223.

Geyikoglu F, Vuraler Ö, Temelli A (2005) The histochemical and ultrastructural structures of avian latissimus dorsi muscle fiber types and changes in them caused by water copper level, *Turkish Journal of Veterinary and Animal Sciences,* **29**, 131-138.

Ghadially FN (1997) Intracytoplasmic filaments. In: *Ultrastructural Pathology of the Cell and Matrix*, 4th Edit., Butterworth-Heinemann, Newton, MA, USA, pp. 887-982.

Gharaibeh B, Deasy B, Lavasani M, Cummins J, Li Y et al. (2012) Musculoskeletal tissue injury and repair: Role of stem cells, their differentiation, and paracrine Effects. In: *Muscle: Fundamental biology and mechanisms of disease*, Academic Press, pp. 881-897.

Goldstein J, Scott ML (1956) An electrophoretic study of exudative diathesis in chicks, *The Journal of Nutrition,* **60**, 349-359.

Griffin HD, Goddard C (1994) Rapidly growing broiler (meat-type) chickens: their origin and use for comparative studies of the regulation of growth, *The International Journal of Biochemistry,* **26**, 19-28.

Griffin JR, Moraes L, Wick M, Lilburn MS (2018) Onset of white striping and progression into wooden breast as defined by myopathic changes underlying Pectoralis major growth. Estimation of growth parameters as predictors for stage of myopathy progression, *Avian Pathology,* **47**, 2-13.

Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Ménissier F, Massabanda J, Fries R, Hanset R, Georges M (1997) A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle, *Nature Genetics,* **17**, 71-74.

Guetchom B, Venne D, Chénier S, Chorfi Y (2012) Effect of extra dietary vitamin E on preventing nutritional myopathy in broiler chickens, *Journal of Applied Poultry Research,* **21**, 548-555.

Hagiwara Y, Sasaoka T, Araishi K, Imamura M, Yorifuji H, Nonaka I, Ozawa E, Kikuchi T (2000) Caveolin-3 deficiency causes muscle degeneration in mice, *Human Molecular Genetics,* **9**, 3047-3054.

Hanley CS, Thomas NJ, Paul-Murphy J, Hartup BK (2005) Exertional myopathy in whooping cranes (Grus americana) with prognostic guidelines, *Journal of Zoo and Wildlife Medicine: Official Publication of the American Association of Zoo Veterinarians,* **36**, 489-497.

Hanrahan LA, Corrier DE, Nagi SA (1981) Monensin toxicosis in broiler chickens, *Veterinary Pathology,* **18**, 665-671.

Haraguchi M, Calore EE, Dagli ML, Cavaliere MJ, Calore NM, Weg R, Raspantini PC, Górniak SL (1998) Muscle atrophy induced in broiler chicks by parts of Senna occidentalis seeds, *Veterinary Research Communications,* **22**, 265-271.

Haraguchi M, Dagli MLZ, Raspantini PC, Górniak SL (2003) The effects of low doses of Senna occidentalis seeds on broiler chickens, *Veterinary Research Communications,* **27**, 321-328.

Haraguchi M, Gorniak SL, Calore EE, Cavaliere MJ, Raspantini PC, Calore NM, Dagli ML (1998) Muscle degeneration in chicks caused by Senna occidentalis seeds, *Avian Pathology: Journal of the W.V.P.A,* **27**, 346-351.

Harris AJ, Fitzsimons RB, McEwan JC (1989) Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles, *Development,* **107**, 751-769.

Harvey AI, Marshall IG (2000) Skeletal Muscle. In: *Sturkie's Avian Physiology*, 5th Edit., G Causey Whittow, Ed., Academic Press, California, pp. 123-139.

Havenstein GB, Ferket PR, Scheideler SE, Larson BT (1994) Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets, *Poultry Science,* **73**, 1785-1794.

Hawke TJ, Garry DJ (2001) Myogenic satellite cells: physiology to molecular biology, *Journal of applied physiology (Bethesda, Md.: 1985),* **91**, 534-551.

Hill AG, Miller R (2013) Exercise-induced deep pectoral necrosis in white-headed pigeons (Columba leucomela), *Journal of Zoo and Wildlife Medicine: Official Publication of the American Association of Zoo Veterinarians,* **44**, 990-995.

Hill JA, Olson EN (2012) An Introduction to Muscle. In: *Muscle*, JA Hill, EN Olson, Eds., Academic Press, Boston/Waltham, pp. 3-9.

Hoffman EP, Brown RH, Kunkel LM (1987) Dystrophin: The protein product of the duchenne muscular dystrophy locus, *Cell,* **51**, 919-928.

Holliday TA, Van Meter JR, Julian LM, Asmundson VS (1965) Electromyography of chickens with inherited muscular dystrophy, *The American Journal of Physiology,* **209**, 871-876.

Hopkins PM (2017) Malignant hyperthermia, *Anaesthesia and Intensive Care Medicine,* **18**, 321-323.

Hoving-Bolink AH, Kranen RW, Klont RE, Gerritsen CL, de Greef KH (2000) Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites, *Meat Science,* **56**, 397-402.

Hudlická O (1985) Development and adaptability of microvasculature in skeletal muscle., *Journal of Experimental Biology,* **115**, 215-228.

Hunton P (2006) 100 Years of poultry genetics, *World's Poultry Science Journal,* **62**, 417-428.

Huxley AF, Niedergerke R (1954) Structural Changes in Muscle During Contraction: Interference Microscopy of Living Muscle Fibres, *Nature,* **173**, 971-973.

Huxley H, Hanson J (1954) Changes in the Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation, *Nature,* **173**, 973-976.

Imamura M, Nakamura A, Mannen H, Takeda S (2016) Characterization of WWP1 protein expression in skeletal muscle of muscular dystrophy chickens, *Journal of Biochemistry,* **159**, 171-179.

Jenden DJ, Fairhurst AS (1969) The pharmacology of ryanodine, *Pharmacological Reviews,* **21**, 1-25.

Joiner KS, Hamlin GA, Lien AR, Bilgili SF (2014) Evaluation of capillary and myofiber density in the pectoralis major muscles of rapidly growing, high-yield broiler chickens during increased heat stress, *Avian Diseases,* **58**, 377-382.

Julian LM (1973) Animal model: hereditary muscular dystrophy of chickens, *The American Journal of Pathology,* **70**, 273-276.

Kawasaki T, Iwasaki T, Yamada M, Yoshida T, Watanabe T (2018) Rapid growth rate results in remarkably hardened breast in broilers during the middle stage of rearing: A biochemical and histopathological study, *PLoS ONE,* **13**, e0193307.

Kawasaki T, Takashi Y, Watanabe T (2016) Simple method for screening the affected birds with remarkably hardened pectoralis major muscles among broiler chickens, *J. Poult. Sci.,* **53**, 291-297.

Kennedy EP, Lehninger AL (1949) Oxidation of fatty acids and tricarboxylic acid cycle intermediates by isolated rat liver mitochondria, *The Journal of Biological Chemistry,* **179**, 957-972.

Kijowski J, Konstanczak M (2009) Deep pectoral myopathy in broiler chickens, *Bull Vet Inst Pulawy,* **53**, 487-491.

Kipar A, May H, Menger S, Weber M, Leukert W, Reinacher M (2005) Morphologic features and development of granulomatous vasculitis in feline infectious peritonitis, *Veterinary pathology,* **42**, 321-330.

Kjaer M (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading, *Physiological Reviews,* **84**, 649-698.

Klasing K, Crespo R, Shivaprasad H, Hoerr F, Fulton R (2008) Noninfectious Diseases. In: *Diseases of Poultry*, 12th Edit., YM Saif, AM Fadly, JR Glisson, LR McDougald, LK Nolan et al., Eds., Wiley-Blackwell, pp. 1121-1258.

Klingler W, Jurkat-Rott K, Lehmann-Horn F, Schleip R (2012) The role of fibrosis in Duchenne muscular dystrophy, *Acta Myologica: Myopathies and Cardiomyopathies: Official Journal of the Mediterranean Society of Myology,* **31**, 184-195.

Kühl U, Timpl R, von der Mark K (1982) Synthesis of type IV collagen and laminin in cultures of skeletal muscle cells and their assembly on the surface of myotubes, *Developmental Biology,* **93**, 344-354.

Kumar V, Abbas A, Fausto N, Aster J (2010) Diseases of the immune system. In: *Robbins and Cotran Pathologic Basis of Disease*, V Kumar, A Abbas, N Fausto, J Aster, Eds., Saunders, Philadelphia, PA, USA, pp. 183-258.

Kuttappan VA, Brewer VB, Mauromoustakos A, McKee SR, Emmert JL, Meullenet JF, Owens CM (2013) Estimation of factors associated with the occurrence of white striping in broiler breast fillets, *Poultry science,* **92**, 811-819.

Kuttappan VA, Goodgame SD, Bradley CD, Mauromoustakos A, Hargis BM, Waldroup PW, Owens CM (2012) Effect of different levels of dietary vitamin E (DL-a-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets, *Poultry science,* **91**, 3230-3235.

Kuttappan VA, Hargis BM, Owens CM (2016) White striping and woody breast myopathies in the modern poultry industry: a review, *Poultry Science,* **95**, 2724-2733.

Kuttappan VA, Lee YS, Erf GF, Meullenet J-C, Mckee SR, Owens CM (2012) Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping, *Poultry science,* **91**, 1240-1247.

Kuttappan VA, Shivaprasad Hl, Shaw DP, Valentine BA, Hargis BM, Clark FD, McKee SR, Owens CM (2013) Pathological changes associated with white striping in broiler breast muscles, *Poultry science,* **92**, 331-338.

Larsson L, Ansved T, Edström L, Gorza L, Schiaffino S (1991) Effects of age on physiological, immunohistochemical and biochemical properties of fast-twitch single motor units in the rat, *The Journal of Physiology,* **443**, 257-275.

Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE (2009) Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury, *Clinical Chemistry,* **55**, 1977-1983.

Light N, Champion AE (1984) Characterization of muscle epimysium, perimysium and endomysium collagens, *The Biochemical Journal,* **219**, 1017-1026.

Lilburn MS (1994) Skeletal growth of commercial poultry species, *Poultry Science,* **73**, 897-903.

Lucke JN, Hall GM, Lister D (1979) Malignant hyperthermia in the pig and the role of stress, *Annals of the New York Academy of Sciences,* **317**, 326-337.

Lund-Johansen F, Browning MD (2017) Should we ignore western blots when selecting antibodies for other applications?, *Nature Methods,* **14**, 215.

Machlin LJ, Shalkop WT (1956) Muscular degeneration in chickens fed diets low in vitamin E and sulfur, *The Journal of Nutrition,* **60**, 87-96.

MacLennan DH (2000) Ca2+ signalling and muscle disease, *European Journal of Biochemistry,* **267**, 5291-5297.

MacRae VE, Mahon M, Gilpin S, Sandercock DA, Mitchell MA (2006) Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (Gallus domesticus), *British poultry science,* **47**, 264-272.

Magdelaine P, Spiess MP, Valceschini E (2008) Poultry meat consumption trends in Europe, *World's Poultry Science Journal,* **64**, 64.

Mäkinen OE, Sozer N, Ercili-Cura D, Poutanen K (2017) Chapter 6 - Protein From Oat: Structure, Processes, Functionality, and Nutrition. In: *Sustainable Protein Sources*, SR Nadathur, JPD Wanasundara, L Scanlin, Eds., Academic Press, San Diego, pp. 105-119.

Mann CJ, Perdiguero E, Kharraz Y, Aguilar S, Pessina P, Serrano AL, Muñoz-Cánoves P (2011) Aberrant repair and fibrosis development in skeletal muscle, *Skeletal Muscle,* **1**, 21.

Martonosi AN, Dux L, Terjung RL, Roufa D (1982) Regulation of membrane assembly during development of sarcoplasmic reticulum: the possible role of calcium, *Annals of the New York Academy of Sciences,* **402**, 485-514.

Matsui T, Kuroda S, Mizutani M, Kiuchi Y, Suzuki K, Ono T (1983) Generalized glycogen storage disease in Japanese quail (Coturnix coturnix japonica), *Veterinary Pathology,* **20**, 312-321.

Matsumoto H, Maruse H, Inaba Y, Yoshizawa K, Sasazaki S, Fujiwara A, Nishibori M, Nakamura A, Takeda S, Ichihara N, Kikuchi T, Mukai F, Mannen H (2008) The ubiquitin ligase gene (WWP1) is responsible for the chicken muscular dystrophy, *FEBS Letters,* **582**, 2212-2218.

Matsumoto H, Sasazaki S, Fujiwara A, Ichihara N, Kikuchi T, Mannen H (2010) Accumulation of caveolin-3 protein is limited in damaged muscle in chicken muscular dystrophy, *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology,* **157**, 68-72.

Mauro A (1961) Satellite cell of skeletal muscle fibers, *The Journal of Biophysical and Biochemical Cytology,* **9**, 493-495.

McGreevy JW, Hakim CH, McIntosh MA, Duan D (2015) Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy, *Disease Models & Mechanisms,* **8**, 195-213.

McLoughlin JV, Goldspink G (1963) Post-mortem changes in the colour of pig longissimus dorsi muscle, *Nature,* **198**, 584-585.

McMurtry SL (1972) Hereditary muscular dystrophy of the chicken. Quantitative histopathological findings of the Pectoralis at 6 weeks of age, *Archives of Pathology,* **94**, 217.

McPherson PS, Campbell KP (1993) Characterization of the major brain form of the ryanodine receptor/Ca2+ release channel, *Journal of Biological Chemistry,* **268**, 19785-19790.

Minetti C, Bado M, Broda P, Sotgia F, Bruno C, Galbiati F, Volonte D, Lucania G, Pavan A, Bonilla E, Lisanti MP, Cordone G (2002) Impairment of caveolae formation and T-system disorganization in human muscular dystrophy with caveolin-3 deficiency, *American Journal of Pathology,* **160**, 265-270.

Mitchell G, Heffron JJ (1982) Porcine stress syndromes, *Advances in Food Research,* **28**, 167-230.

Monaco AP, Neve RL, Colletti-Feener C, Bertelson CJ, Kurnit DM, Kunkel LM (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene, *Nature,* **323**, 646-650.

Mudalal S, Lorenzi M, Soglia F, Cavani C, Petracci M (2015) Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat, *Animal: an international journal of animal bioscience,* **9**, 728-734.

Murphy ME, Kehrer JP (1986) Free radicals: a potential pathogenic mechanism in inherited muscular dystrophy, *Life Sciences,* **39**, 2271-2278.

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration, *Development,* **138**, 3625-3637.

Mutryn MF, Brannick EM, Fu W, Lee WR, Abasht B (2015) Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing, *BMC genomics,* **16**, 399.

Natarajan A, Lemos DR, Rossi FMV (2010) Fibro/adipogenic progenitors: a double-edged sword in skeletal muscle regeneration, *Cell Cycle,* **9**, 2045-2046.

Natural Resources Institute Finland (2017) Total meat production.

Ngo N, Chang F (2007) Enterocolic lymphocytic phlebitis: clinicopathologic features and review of the literature, *Archives of Pathology & Laboratory Medicine,* **131**, 1130-1134.

Niewold T (2013) About hot chicks, a new acute mortality syndrome most likely caused by fatal hyperthermia as a consequence of mitochondrial uncoupling, *Poultry Science,* **92**, 847-848.

Noguchi T, Cantor AH, Scott ML (1973) Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks, *The Journal of Nutrition,* **103**, 1502-1511.

O'Brien PJ (1987) Etiopathogenetic defect of malignant hyperthermia: Hypersensitive calcium-release channel of skeletal muscle sarcoplasmic reticulum, *Veterinary Research Communications,* **11**, 527-559.

OECD (2017) Meat consumption 2017.

Ottini L, Marziali G, Conti A, Charlesworth A, Sorrentino V (1996) Alpha and beta isoforms of ryanodine receptor from chicken skeletal muscle are the homologues of mammalian RyR1 and RyR3, *The Biochemical Journal,* **315** (Pt 1), 207-216.

Palade GE (1953) Fine structure of blood capillaries, *J. Appl. Phys.,* **24**, 1424.

Papah MB, Brannick EM, Schmidt CJ, Abasht B (2017) Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens, *Avian Pathology,* **46**, 623-643.

Papinaho PA, Ruusunen MH, Suuronen T, Fletcher DL (1996) Relationship between Muscle Biochemical and Meat Quality Properties of Early Deboned Broiler Breasts, *The Journal of Applied Poultry Research,* **5**, 126-133.

Pappenheimer AM (1939) Nutritional encephalomalacia in chicks and certain related disorders of domestic birds.

Petracci M, Mudalal S, Bonfiglio A, Cavani C (2013) Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens, *Poultry Science,* **92**, 1670-1675.

Petracci M, Mudalal S, Cavani C (2014) Meat quality in fast-growing broiler chickens, 221.

Pietrzak M, Greaser ML, Sosnicki AA (1997) Effect of Rapid Rigor Mortis Processes on Protein Functionality in Pectoralis Major Muscle of Domestic Turkeys, *Journal of Animal Science,* **75**, 2106-2116.

Pohjola LK, Ek-Kommonen SC, Tammiranta NE, Kaukonen ES, Rossow LM, Huovilainen TA (2014) Emergence of avian infectious bronchitis in a non-vaccinating country, *Avian Pathology: Journal of the W.V.P.A,* **43**, 244-248.

Pytel P, Anthony D (2015) Peripheral nerve and skeletal muscle. In: *Pathologic basis of disease*, V Kumar, A Abbas, J Aster, Eds., Elsevier Saunders, US, pp. 1227-1250.

Radaelli G, Piccirillo A, Birolo M, Bertotto D, Gratta F, Ballarin C, Vascellari M, Xiccato G, Trocino A (2017) Effect of age on the occurrence of muscle fiber degeneration associated with myopathies in broiler chickens submitted to feed restriction, *Poultry science,* **96**, 309-319.

Randall CJ (1982) Acute pectoral myopathy in broiler breeders, *Avian Pathology: Journal of the W.V.P.A,* **11**, 245-252.

Remignon H, Gardahaut MF, Marche G, Ricard FH (1995) Selection for rapid growth increases the number and the size of muscle fibres without changing their typing in chickens, *Journal of muscle research and cell motility,* **16**, 95-102.

Repetto S, Bado M, Broda P, Lucania G, Masetti E, Sotgia F, Carbone I, Pavan A, Bonilla E, Cordone G, Lisanti MP, Minetti C (1999) Increased number of caveolae and caveolin-3 overexpression in Duchenne muscular dystrophy, *Biochemical and Biophysical Research Communications,* **261**, 547-550.

Richardson JA (1980) Deep pectoral myopathy in seven-week-old broiler chickens, *Avian Diseases,* **24**, 1054-1059.

Rigdon RH (1966) Hereditary myopathy in the white Pekin duck, *Annals of the New York Academy of Sciences,* **138**, 28-48.

Rome LC, Funke RP, Alexander RM, Lutz G, Aldridge H, Scott F, Freadman M (1988) Why animals have different muscle fibre types, *Nature,* **335**, 824-827.

Ruder MG, Noel BL, Bednarz JC, Keel MK (2012) Exertional myopathy in pileated woodpeckers (Dryocopus pileatus) subsequent to capture, *Journal of Wildlife Diseases,* **48**, 514-516.

Russo E, Drigo M, Longoni C, Pezzotti R, Fasoli P, Recordati C (2015) Evaluation of White Striping prevalence and predisposing factors in broilers at slaughter, *Poultry science,* **94**, 1843-1848.

Ruth GR, Van Vleet JF (1974) Experimentally induced selenium-vitamin E deficiency in growing swine: selective destruction type I skeletal muscle fibers, *American Journal of Veterinary Research,* **35**, 237-244.

Sandercock DA, Hunter RR, Nute GR, Mitchell MA, Hocking PM (2001) Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: implications for meat quality, *Poultry Science,* **80**, 418-425.

Sandow A (1952) Excitation-contraction coupling in muscular response, *The Yale Journal of Biology and Medicine,* **25**, 176-201.

Sans P, Combris P (2015) World meat consumption patterns: An overview of the last fifty years (1961–2011), *Meat Science,* **109**, 106-111.

Saraga E, Costa J (1989) Idiopathic Entero-colic Lymphocytic Phlebitis: A Cause of Ischemic Intestinal Necrosis, *American Journal of Surgical Pathology,* **13**, 303-308.

Scheele CW (1997) Pathological changes in metabolism of poultry related to increasing production levels, *The Veterinary Quarterly,* **19**, 127-130.

Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, LØmo T (1989) Three myosin heavy chain isoforms in type 2 skeletal muscle fibres, *Journal of Muscle Research & Cell Motility,* **10**, 197-205.

Schiaffino S, Reggiani C (2012) Chapter 60 - Skeletal Muscle Fiber Types. In: *Muscle*, JA Hill, EN Olson, Eds., Academic Press, Boston/Waltham, pp. 855-867.

Schmidt CJ, Persia ME, Feierstein E, Kingham B, Saylor WW (2009) Comparison of a modern broiler line and a heritage line unselected since the 1950s, *Poultry Science,* **88**, 2610-2619.

Schmitz JA, Harper JA (1975) Histopathology of hereditary muscular dystrophy of the domestic turkey (Meleagris gallopavo), *Canadian Journal of Comparative Medicine: Revue Canadienne De Medecine Comparee,* **39**, 389-396.

Schultz E, Jaryszak DL, Valliere CR (1985) Response of satellite cells to focal skeletal muscle injury, *Muscle & Nerve,* **8**, 217-222.

Scott ML (1980) Advances in our understanding of vitamin E, *Federation Proceedings,* **39**, 2736-2739.

Siegel PB (2014) Evolution of the modern broiler and feed efficiency, *Annual Review of Animal Biosciences,* **2**, 375-385.

Siller WG, Martindale L, Wight PAL (1979) The Prevention of Experimental Deep Pectoral Myopathy of the Fowl By Fasciotomy, *Avian Pathology,* **8**, 301-307.

Siller WG, Wight PA (1978) The pathology of deep pectoral myopathy of turkeys, *Avian pathology: journal of the W.V.P.A,* **7**, 583-617.

Siller WG, Wight PAL, Martindale L (1978) Exercise-induced deep pectoral myopathy in broiler fowls and turkeys, *Veterinary Science Communications,* **2**, 331-336.

Silva TDd, Massetti T, Monteiro, Carlos Bandeira de Mello, Trevizan IL, Arab C, Caromano FA, Voos MC, Oliveira ASB, Favero FM (2016) Pain characterization in Duchenne muscular dystrophy, *Arquivos De Neuro-Psiquiatria,* **74**, 767-774.

Smith JS, Coronado R, Meissner G (1986) Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic reticulum. Activation by Ca2+ and ATP and modulation by Mg2+, *The Journal of General Physiology,* **88**, 573-588.

Snellman O, Tenow M (1954) A contractile element containing tropomyosin (actotropomyosin), *Biochimica Et Biophysica Acta,* **13**, 199-208.

Soglia F, Mudalal S, Babini E, Di Nunzio M, Mazzoni M, Sirri F, Cavani C, Petracci M (2016) Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality, *Poultry Science,* **95**, 651-659.

Soike D, Bergmann V (1998a) Comparison of Skeletal Muscle Characteristics in Chicken Bred for Meat or Egg Production: II. Histochemical and Morphometric Examination, *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine,* **45**, 169-174.

Soike D, Bergmann V (1998b) Comparison of Skeletal Muscle Characteristics in Chicken Bred for Meat or Egg Production. I. Histopathological and Electron Microscopic Examination, *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine,* **45**, 161-167.

Sosnicki A, Cassens RG, McIntyre DR, Vimini RJ, Greaser ML (1989) Incidence of microscopically detectable degenerative characteristics in skeletal muscle of turkey, *British poultry science,* **30**, 69-80.

Sosnicki A, Cassens RG, Vimini RJ, Greaser ML (1991) Histopathological and ultrastructural alterations of turkey skeletal muscle, *Poultry Science,* **70**, 349-357.

Spraker TR, Adrian WJ, Lance WR (1987) Capture myopathy in wild turkeys (Meleagris gallopavo) following trapping, handling and transportation in Colorado, *Journal of Wildlife Diseases,* **23**, 447-453.

Stan RV (2005) Structure of caveolae, *Biochimica Et Biophysica Acta,* **1746**, 334-348.

Stockdale FE, Miller JB (1987) The cellular basis of myosin heavy chain isoform expression during development of avian skeletal muscles, *Developmental Biology,* **123**, 1-9.

Strasburg GM, Chiang W (2009) Pale, soft, exudative turkey--The role of ryanodine receptor variation in meat quality, *Poultry Science,* **88**, 1497-1505.

Suomen Siipikarjaliitto (2017) Näin kasvatetaan broileri.

Swain S, Farrell DJ (1975) Effects of different temperature regimens on body composition and carry-over effects on energy metabolism of growing chickens, *Poultry Science,* **54**, 513-520.

Tanaka S, Braga IS, Kimura T, Ochiai K, Itakura C, Mizutani M (1996a) Inherited muscular disorder in mutant Japanese quail (Coturnix coturnix japonica): an immunohistochemical study, *Journal of Comparative Pathology,* **115**, 139-150.

Tanaka S, Braga IS, Kimura T, Ochiai K, Itakura C, Mizutani M (1996b) Inherited muscular disorder in mutant Japanese quail (Coturnix coturnix japonica): an ultrastructural study, *Journal of Comparative Pathology,* **114**, 325-337.

Tasoniero G, Bertram HC, Young JF, Dalle Zotte A, Puolanne E (2017) Relationship between hardness and myowater properties in Wooden Breast affected chicken meat: A nuclear magnetic resonance study, *LWT - Food Science and Technology,* **86**, 20-24.

Termin A, Staron RS, Pette D (1989) Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle, *Histochemistry,* **92**, 453-457.

The Food and Agriculture Organization (2016) FAOSTAT.

The National Chicken Council (2015) U.S. Broiler Performance.

Thomas CH, Blow WL, Cockerham CC, Glazener EW (1958) The Heritability of Body Weight, Gain, Feed Consumption, and Feed Conversion in Broilers, *Poultry Science,* **37**, 862-869.

Umemura T, Nakamura H, Goryo M, Itakura C (1984) Histopathology of monensin-tiamulin myopathy in broiler chicks, *Avian Pathology: Journal of the W.V.P.A,* **13**, 459-467.

Valentin B (2017) Skeletal muscle. In: *Pathologic basis of veterinary disease*, J Zachary, Ed., Elsevier, US, pp. 908-953.

Van Laack RL, Liu CH, Smith MO, Loveday HD (2000) Characteristics of pale, soft, exudative broiler breast meat, *Poultry science,* **79**, 1057-1061.

Van Vleet JF, Valentine BA (2007) Muscle and tendon. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 5th Edit., MG Maxie, Ed., Saunders Ltd., pp. 185-277.

Velleman SG, Clark DL (2015) Histopathologic and Myogenic Gene Expression Changes Associated with Wooden Breast in Broiler Breast Muscles, *Avian Diseases,* **59**, 410-418.

Wagner DD, Furrow RD, Bradley BD (1983) Subchronic toxicity of monensin in broiler chickens, *Veterinary Pathology,* **20**, 353-359.

Warren DC (1958) A Half Century of Advances in the Genetics and Breeding Improvement of Poultry, *Poultry Science,* **37**, 3-20.

Wight PAL, Siller WG (1980) Pathology of Deep Pectoral Myopathy of Broilers, *Veterinary pathology,* **17**, 29-39.

Williams S, Fletcher O, Abdul-Aziz T (2008) Muscular System. In: *Avian Histopathology*, 3rd Edit., O Fletcher, Ed., American Association of Avian Pathologists, Florida, pp. 80-95.

Wilson BW, Nieberg PS, Buhr RJ, Kelly BJ, Shultz FT (1990) Turkey muscle growth and focal myopathy, *Poultry Science,* **69**, 1553-1562.

Wilson BW, Randall WR, Patterson GT, Entrikin RK (1979) Major physiologic and histochemical characteristics of inherited dystrophy of the chicken, *Annals of the New York Academy of Sciences,* **317**, 224-246.

Wismer-Pedersen J, Briskey EJ (1961) Rate of anaerobic glycolysis versus structure in pork muscle*, Nature,* **189**, 318-320.

Wold JP, Veiseth-Kent E, Høst V, Løvland A (2017) Rapid on-line detection and grading of wooden breast myopathy in chicken fillets by near-infrared spectroscopy*, PLoS One,,* **12**, e0173384.

Wolf A, Pappenheimer AM (1931) The histopathology of nutritional encephalomalacia of chicks, *The Journal of Experimental Medicine,* **54**, 399-405.

Wolff JN, Gemmell NJ (2013) Mitochondria, maternal inheritance, and asymmetric fitness: Why males die younger*, BioEssays,* **35**, 93-99.

Yin H, Price F, Rudnicki MA (2013) Satellite Cells and the Muscle Stem Cell Niche*, Physiological Reviews,* **93**, 23-67.

Zhi X, Chen C (2012) WWP1: a versatile ubiquitin E3 ligase in signaling and diseases*, Cellular and molecular life sciences: CMLS,* **69**, 1425-1434.

Zimermann FC, Fallavena LCB, Salle CTP, Moraes HLS, Soncini RA, Barreta MH, Nascimento VP (2012) Downgrading of heavy broiler chicken carcasses due to myodegeneration of the anterior latissimus dorsi: Pathologic and epidemiologic studies*, Avian Diseases,* **56**, 418-421.

Zuidhof MJ, Schneider BL, Carney VL, Korver DR, Robinson FE (2014) Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005*, Poultry Science,* **93**, 2970-2982.

Zumstein MA, Jost B, Hempel J, Hodler J, Gerber C (2008) The clinical and structural long-term results of open repair of massive tears of the rotator cuff*, The Journal of Bone and Joint Surgery. American Volume,* **90**, 2423-2431.

ORIGINAL PUBLICATIONS

I Sihvo HK, Immonen K, Puolanne E. 2014 (Electronic version 2013). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology* 51:619-23.
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II Sihvo HK, Lindén J, Airas N, Immonen K, Valaja J, Puolanne E. 2017. Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. *Veterinary Pathology* 54:119-128.
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III Sihvo HK, Airas N, Lindén J, Puolanne E. 2018.
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