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Prediction and Evaluation of Breast Myopathy

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Prediction and Evaluation of Breast Myopathy

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Poultry Sciences

by

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ABSTRACT

Broiler breast myopathies, including woody breast, white striping, and spaghetti breast, negatively impact the industry. Therefore, evaluation, prediction, and frequency of these muscle abnormalities on modern birds are important for decision making. Modern broilers are not highly active and often sit with the breast of the bird resting on the floor. Therefore, the first experiment was to promote bird movement and explore the impact on the breast myopathies. The movement was stimulated by human interaction (walking through pen) and higher light intensity so that birds walked around the pen more often throughout the day. The control group had normal low light intensity and minimal human interaction. However, the results were not different between the treatments on performance or breast myopathies. In other studies, broilers were palpated for woody breast (WB) and the breast region and/or fillets were measured for width, length, thickness, etc. in efforts to develop prediction of WB during growout and in the plant. Differences on the thickness and other part of the measurements on the fillets were found between WB categories (normal to severe) and it was determined that these features were good predictors for the differentiation on the severe WB and normal categories. Furthermore, palpation and measurements of the breast on the live bird throughout the growing period were also well correlated and some measurements were good predictors, like measurements on the cranial region of the breast with a cloth tape. There was observed that the different strains were different between each other as well the WB myopathy. Furthermore, the broiler breast myopathies demographic chapter to summarize all the data collected in 3 years with the most utilized strains in the market. Generally, myopathies increase as the birds get older and larger and they also increase AS breast yield increases to higher levels (breast yield > 30%).

Additionally, males generally had greater WB than females and females had greater SM than males.

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INTRODUCTION

Introduction

Chicken meat consumption has increased over the years. According to USDA (2018), the global chicken production will increase at least 2% reaching to 97.8 million tons this year due to the low cost, diversity of preparation, dietary and sensory properties, and religious and cultural principles. The poultry industry has improved the selection and the growth of the birds for breast yield and fast performance in order to meet the high demand for chicken meat (Petracci et al., 2015). The increase of the bird growing and consequently muscle growing levels and the selection on the broiler genetics along the improvement in the nutrition and environmental aspects resulted in extraordinary achievements on the chicken growth, but also cause a big improvement on the breast muscle growth. The studies done by Havenstein et al. (2003) and Zuidhof et al. (2014) evaluated the growth performance of antique birds (1950's broiler lines) compared with modern chickens. The evaluation shows the difference between broiler studied and how the lines by the genetic selection and the nutrition could affect the growth, feed conversion, and the carcass/parts yield. They presented that fast growing birds were masterpiece on feed efficiency, growth rate, and muscle yields than the other old lines, improving in time, sustainability, and economically.

The improvement of the growth rates causes some consequences for the bird, such as muscle myopathies. These abnormalities result in economic losses on the carcass or condemnation, especially on the breast muscle being one of the most valuable and important chickens cut. According to FAO, (2019), condemnation is increasing due to the myopathies, thus affects the industry worldwide, especially USA and Brazil who are the top producers (USDA, 2019). However, there is no specific category for condemnation that fits myopathies; the carcasses are condemned because of the repugnant aspect. Even on the Brazilian inspection guide RIISPOA, 2000, article 172, they affirm, "Repulsive meats that are considered are going to be totally condemned all the carcasses that present bad aspect, abnormal coloration or that exalt medicine, excreta smell or other that are not considered normal”. On the other hand, USDA-FSIS (2014), only for the breast muscle with “green atrophy, green breast, and green muscle degeneration” mostly associated with turkeys as of 2014. However, in July of 2017, a directive 35-17 was released for the meat that shows: “The presence of inflammatory tissue requires trimming of all affected tissue (…) overall shiny surface indicating excess fluid in the tissues” (USDA-FSIS, 2017). At the end, this directive was not focused specifically on woody breast just because the exudative and hemorrhagic does not appear in all the cases. Based on these reasons the breast myopathies need to be deeply understand in order to decrease the impact on the economy and on the bird health. The aim of this dissertation is to understand better the breast myopathies and to try to find a valid methodology to use in live birds to try to prevent the myopathies in the processing plant, moreover could support research and selection with an earlier detection.

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LITERATURE REVIEW

Broiler Improvement

The birds utilized at the poultry industry have changed over the years in terms of physiology and body composition. Many factors can influence these changes such as genetic selection, nutritional balance, sanitary, and environmental control (Huang and Ahn, 2018). The genetic selection prioritized economic characteristics such as body weight gain, carcass yield, and feed conversion (Gous, 1986). All these together with nutrition requirements and *ad libitum* feed to produce kilograms/meat make possible a chicken to reach around 4 kg in 42 days, meaning that the birds are processed with a younger age and higher body weight compared with birds of ‘50s, Athens-Canadian Randombred Control (ACRBC) and, two University of Alberta Meat Control strains unselected since 1957 and 1978 (Havenstein et al., 2003; Zuidhof et al., 2014).

The ACRBC birds are random bred, or have been not selected for particular traits, and the Ross 308 (males presented in Fig. 1) represents common modern broilers, those selected fir multiple attributes including growth performance trait. The muscular increase from the genetic selection illustrated at Figure 1. The carcasses of the ACRBC birds was not selected genetically compared with the other carcass of the lineage of Ross 308, a broiler strain commonly used that has be undergone selection over the year. Both lines were evaluated in 2001 and received the same feed composition according to the nutrient requirements of poultry (Pesti, 2003). In addition, they were compared by the ages of 43, 57, 71, and 85 days old showing the major differences between each line at a given age, for example, the ACRBC was reaching the same weight of the Ross 308 after 69 days. These differences are primarily attributed to genetics and nutrition requirements.

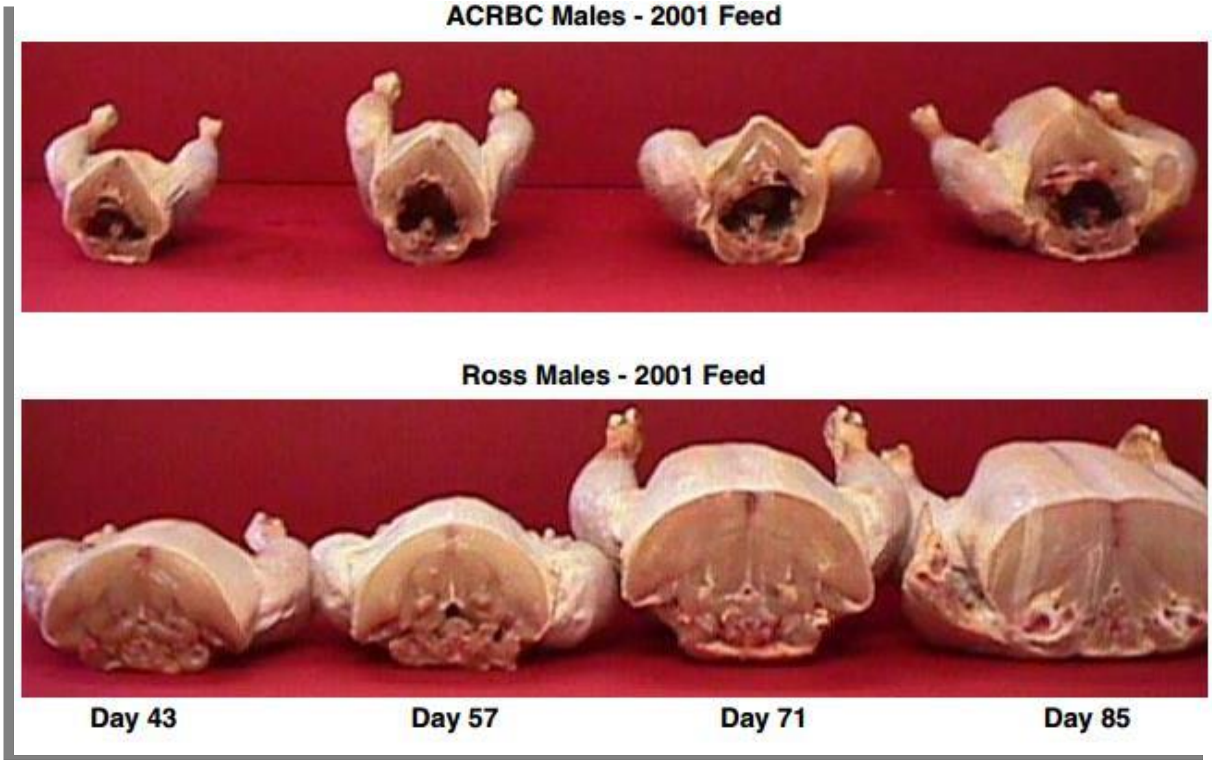

Figure 1. Comparison between broiler carcass ACRBC (control) and Ross 308 at 43, 57, 71, and 85 days old receiving the same feed (Havenstein et al., 2003).

Broilers pass by an intense selection for better carcass yield and mainly for the breast. The consumption of chicken meat and chicken breast fillet are related to the appearance, lower fat, easy to prepare, and cheap to buy (Kuttappan et al., 2012c). A study conducted 28 years ago already show the improvement on breast yield of 3% more than the 1957 ACRBC compared with the 1991 Arbor Acres that was around 11.9% (Havenstein et al., 1994), while nowadays the birds can even reach 30% of the whole chicken (Lilburn, 1994). Most of the breast fillets present the normal aspect. Although, the yield increase caused deep alterations on the muscle fibers and on the vessel that support this tissue increasing the muscle problems (Dransfield and Sosnicki, 1999; Hoving-Bolink et al., 2000). In the last decade, myopathies such as white striping and woody breast have been identified (Kuttappan et al., 2012a; Sihvo et al., 2017).

Muscle Physiology

The muscle structure is the basic unit that gives structure in all living animals (Johnson, 2018). The muscle formation starts in the embryo. The mesoderm cells in the somites are located on the notochord on both sides stem muscles (Velleman, 2019). The formation of them occurs when the mesoderm cells move from the somites to them specific places and then start the proliferation (Brandebourg, 2013). To form the multinucleated primary muscle fibers, the embryonic myoblast needs to fuse; then afterwards, the secondary muscle fibers form above the primary fibers. With this order of formation, they will organize with them proper function and orientation of the muscle fiber (Velleman et al., 2018). At the time of hatch, muscle fiber formation and organization is connected, and then the myoblast starts multiplication (hyperplasia) forming the multinucleated muscle fibers. Hence, myoblast act while still in embryo stage, and post hatch, the satellite cells are responsible to help the muscle growth and regeneration (Clark et al., 2017). Satellite cells are located in the basal lamina and sarcolemma at the skeletal muscle fiber. The activators of the satellite cell are the growth factor, extracellular matrix and the cytokine sequestered inside (Miao et al., 2016). They signal this cell to get out of the quiescent state to the activation, migration, myogenic differentiation and muscle development (Rhoads et al., 2009; Dodson et al., 2010; Murphy et al., 2011; Urciuolo et al., 2013)

Muscle is a highly organized tissue. The muscle fibers are surrounded by the endomysium that forms a thick and protective cover of a connective tissue. The perimysium engulfs the muscle fiber bundles (myofibers) between the epimysium and the endomysium consisted of connective tissue involve each myofibers (Lu et al., 2019). The cross-sectional diameter of mature myofibers is 30-70 µm and length varies depending on the location and the muscle type (Sihvo, 2019). The structure inside the muscle fiber consists of myofibrils that have cells called sarcomeres, the smallest contractile unit of the muscle (Brandebourg, 2013). The primary composition of them consists of two myofilaments: a thick one made up of myosin and a thin one made of actin. These two myofilaments are responsible for the muscle contraction and the striated appearance of the skeletal muscle due to the arranged parallel and overlapping with each other (Johnson, 2018). Troponin and tropomyosin are also contractile proteins associated with actin, and the Z disk or line in the outer side connects the thin filaments/actin, and the M line does the separation of the sarcomere (Ertbjerg and Puolanne, 2017).

The connective tissue that surrounds the muscle fibers is composed of fibronectin, ground substances components, proteoglycans, elastin, and collagen; and it is known as the extracellular matrix (ECM) (Purslow, 2014). The purpose of this tissue is to organize proteins associated muscle structure, mediate muscle to growth and connect muscles to the bone for locomotion (Sanes, 2003; Jenniskens et al., 2006). All these cells and organizations hold the muscle integrity and support together all the nerves, intramuscular adipocytes and blood vessels (Miao et al., 2016). The primary component of the connective tissue is the collagen, the most abundant protein in the body due to its function with structure (Archile-Contreras et al., 2010). There are more than 30 types that are associated collagen, found in skin, muscle, tendons, cartilage, etc (Myllyharju, 2004; Veit et al., 2006; Söderhäll et al., 2007). In the muscle, the dominant collagen is type I and III (Cruz et al., 2017). The amount of each of those collagens depends on the animal ages, muscle types, and location (Listrat et al., 1999). Collagen type I is on the perimysium of adult bovine muscle, and at the endomysium, the type III is more common (Mayne and Sanderson, 1985). Furthermore, the same species around six months of age the collagen type I increase the same occurs in rats during aging the type III collagen decreases and the type I increases (Kovanen and Suominen, 1989; Listrat et al., 1999). The characteristics of the collagen type III fibrils is that they have few disulfide bonds and are smaller in diameter compared with type I, indicating a shear force less resistant in cooked meat (Velleman et al., 2017).

Muscle increases the intramuscular connective tissue along the animal growth, increasing as well the toughness (Purslow, 2014). The intramuscular fibroblasts synthesize the intramuscular connective tissue derived from the mesenchymal generator cells in the early embryonic development (Miao et al., 2016). However, this type of cell can switch to do less fibrogenesis and more adipogenesis helping on the palatability, marbling, and tenderness of the meat. The fibroblast is the central cell to build up connective tissue. The regeneration of this cell is by the fibrogenesis, being responsible for the development of fibroblasts and protein synthesis and the connective tissue build up. The synthesis of connective tissue is essential for the epimysium and perimysium of the muscle bundles until the time of birth (Du et al., 2010). Fibrosis is an excessive collagen deposition in the extracellular matrix proteins normally formed after an healing process (Liu and Gaston Pravia, 2010). The collagen content and cross-linking correlate with fibrosis, but cross-linking are reduced by turnover of collagen (Archile-Contreras et al., 2010); the lysyl oxidase is the enzyme that does a rate-limiting catalyzing collagen fibrils cross-linking (Siegel et al., 1976).

Three helical polypeptide chains intertwined the collagen molecule, and the telopeptide regions are on both ends. The enzyme that regulates collagen cross-linking is the lysyl oxidase (Siegel and Fu, 1976). The metabolic function is oxidizing hydroxylysine or lysine in the non-helical portions of collagen molecules to aldehydes, and then they react with the collagen sides forming divalent bonds. The cross-linking development depends on the molecules of lysine and hydroxylysine in the non-helical regions. The degree of collagen cross-linking differs in animals of different breeds (Miao et al., 2016). The different types of muscle also present various degrees of cross-linking and could be a reason that some muscle present more connective tissue than others

(Velleman et al., 2017). The cross-linking of collagen occurs slowly, increasing with the age of the animal, resulting in older animals having more cross-linking, or insoluble collagen. The amount of collagen cross-linking, reduce the meat tenderness, the collagen turnover is regulated by the metalloproteinase when this enzyme is expressed and also the inhibitors the cross-linking and meat tenderness is affected by inflammation and oxidative stress being some of the factors that regulate (Woessner, 1991; Clark et al., 2008; Murphy, 2010; Purslow, 2014)

The growth rate on the collagen depends on the endogenous hormones and nutrition. Processing animals after the period of fast growth rate improve in tenderness, because of the characteristic of newly synthesized collagen, but this depends on the animal. Older animals tend to have more connective tissue because of protein replacement (Weston et al., 2002). Testosterone can influence the collagen synthesis and solubility, and toughness. Somatotropin also could increase the tenderness in pig muscle. Castrated animals have a lower concentration of testosterone, consisting of less tough meat compare with the ones that still intact (McCormick, 1994). However, market broiler chickens are relatively young, do not reach sexual maturity, and therefore are not impacted by effect of testosterone on collagen (Podisi et al., 2011).

Pectoralis major is the one of the biggest muscle in the broiler chicken, normally a broiler with 1.8kg younger than 39 days of age and have 19% breast meat yield. Depending on the age, the breast fillet could reach sometimes more than 30% of the whole carcass. It consists of white meat and predominantly fibers type IIB and IIA. These fibers are characterized by rapid contraction (fast twitch) lower capillary density, smaller fibers, glycolytic, and fewer mitochondria (Dalle Zotte et al., 2017). Therefore, Remignon et al. (1994) did a study with males broilers with different ages and live weight, concluded that the *Pectoralis major* is composed of glycolytic fibers type IIB, corroborating with the study that compares different birds species, affirming that chickens presents the breast muscle only with glycolytic fibers with fast contraction – type IIB. However, another species, for example ducks, has on the same muscle region oxidative-glycolytic fibers of fast contraction (type IIA), mostly for the reason that the other birds can fly for long distances requiring muscle fibers to have higher oxidative capacity to allow for long term function that does a fast contraction in an extended period.

The contraction and relaxation of the muscle fibers occur when the thin and thick filaments slide along each other (Huxley and Hanson, 1954; Huxley and Niedergerke, 1954). The motor neuron meets the fiber on the neuromuscular junction that receives the neuronal message from the neuronal control to be able to do the movement. Every group of similar fiber type myofibers is innervated by a motor neuron forming a motor unit (Edstrom and Kugelberg, 1968; Burke et al., 1971; Buchthal and Schmalbruch, 1980). The motor nerve passes the action potential releasing acetylcholine to the neuromuscular junction. Acetylcholine is a neurotransmitter that can bind or interact with surface receptor molecules at the sarcolemma (Kerth, 2013). When the interaction with the molecules happens, a depolarization occurs on the local muscle fiber. Then the signal is spreading through the myofibers via t-tubules releasing Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. The tropomyosin will leave the myosin heads binding site after Ca^{2+} ions bind to troponin C inside of the tropomyosin (Sandow, 1952; Smith et al., 1986; Hedrick et al., 1989). The process of excitation-contraction coupling uses adenosine triphosphate (ATP) and starts because the troponin exposes the myosin-binding site on actin (Snellman and Tenow, 1954; Ebashi, 1974). The myosin binding with actin pull them causing a myriad power strokes combined effects resulting in contraction of sarcomere and muscle fiber (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). The shortening of the sarcomere consumes the ATP hydrolyzing to ADP and inorganic phosphate. The contraction continues until the movement stop ceasing the action potential, then recovering the sarcolemma repolarization. The Ca^{2+} is requested by sarcoplasmic reticulum using ATPase pumps. Ca^{2+} releases from troponin C and tropomyosin moves and covers the actin-binding site realizing the process of muscle relaxation (McMahon, 1984).

Live muscle to meat

Post mortem process starts when the blood flow stops consequently the oxygen supply cease then an anaerobic metabolism starts reducing the pH to approximate 5.8 decreasing from 7.2 while the tissue was oxygenated (Dransfield and Sosnicki, 1999). The quick change on the pH is caused by the increase of lactic acid from the anaerobic glycolytic pathway (Braden, 2013). Not only lactic acid is building up, but also sarcoplasmic reticulum loss the ionic pump functions liberating the calcium. Since the pump is not functional anymore, there are not troponin to detach the cross-bridges formed by the actin-myosin. At last, with the absence of ATP, it is not possible to break the action-myosin bonds (Braden, 2013). This stage occurs rapidly in broilers but can take one hour after the chicken slaughter (Brewer et al., 2012b). The contracted state decrease with endogenous proteolytic enzymes over the myofibrillar proteins at the z-line over time, letting age the meat became softer (Bhat et al., 2018). However, actin-myosin bonds do not disassociate.

Myopathies in Production

Genetic and balanced nutrition improvements have created chickens that grow to around 4 kg in 42 days (Zuidhof et al., 2014). However, this rapid growth affects muscle development creating pathological issues such as green tenders, anterior *Latissimus dorsi* (ALD) muscle myodegeneration, and breast fillets with white striping (WS), hard meat called “wooden breast” (WB), and spaghetti meat (SM). More specifically, the breast muscles problems present histological characteristics like myodegeneration or/and a higher deposition of collagen and fat between muscular fibers (Sihvo et al., 2014). White striping is characterized by white stripes parallel of the same direction of the muscle fibers (Kuttappan et al., 2012c; a). Breast fillet presenting hard texture areas at the surface, pale coloration, and frequently covered by a transparent or yellowish and viscous exudate with multifocal petechial distributed characterize woody breast syndrome (Sihvo et al., 2014). The muscle fibers separated from themselves, rarefaction of the perimysium and endomisyum on the breast fillet characterize the spaghetti meat (Soglia et al., 2019).

An important aspect related to the musculoskeletal pathologies is the relation of fast-growing animals presenting less number of muscle fibers than the slow grow animals and hypertrophy of the muscle cells. Also, the muscle develops with the age and the genetic potential for the breast fillet (Dransfield and Sosnicki, 1999). Muscle fibers after birth only can be hypertrophied (Bechtel, 1986). The increasing on the diameter size of the muscle fiber occurs with decreasing of the blood capillaries since the fiber length and the diameter increases occurs a displacement of the vessel that surrounds the fibers. The oxygen support gets limited causing ischemia (Joiner et al., 2014).

A study from Hoving-Bolink et al. (2000) reported that chickens with higher breast fillet yield showed lower capillary density, indicating the larger breast fillet could be a health risk for the birds because of the reduction on the oxygen support for the muscle. According to Joiner et al. (2014), the muscle fiber diameter increases significantly with the age, while the number absolute of vessels and capillaries do not change with vascular support around of the myofibers of fast-growing animals. Besides that, the fiber type in the muscle also can influence the capillary density and the proportion. Red fibers (type I) have a small diameter, are rich in myoglobin, and use aerobic metabolism (oxidative). They also have a higher number of capillaries surrounding the muscle fibers compared with the white fibers, which have a larger diameter and use anaerobic metabolism (glycolytic) (Hudson et al., 1967; Santiago, 2001)

The *Pectoralis major* muscle in the broiler chicken is composed of white glycolytic fibers IIB (Remignon et al., 1994), therefore have lower capillary density in the fast twitch coupled with increased growth rate and size of the breast making this muscle more susceptible to abnormalities. The myopathy of white striping and woody breast occurs primarily in the *Pectoralis major*. However, it has also been observed in the *Pectoralis minor*. The microscopy lesions of meat with white striping are composed of myodegeneration with lipidosis and aggregation of connective tissue (Kuttappan et al., 2012b). Similar lesions are observed with woody breast where the researchers report polyphasic myodegeneration and tissue necrosis (Sihvo et al., 2014), and the replacement of this tissue is with fibrosis and fat in all over the muscle (Bilgili, 2016).

Breast Myopathies

White striping (WS)

The macroscopic characteristics of white striping myopathy are the presence of the parallel white striations that follows the muscle fibers (Petracci and Cavani, 2011; Kuttappan et al., 2012c, 2016; Dalle Zotte et al., 2014; Bailey et al., 2015). They are visible on ventral side of the muscle and frequently occur in the *Pectoralis major*, but eventually can also occur in the *Pectoralis minor* and the legs (Kuttappan et al., 2013a),

There is a classification method to score the severity of this myopathy. The normal breast fillets receive score 0 and do not present any white striation. Moderate scores show thin white striation with the thickness of less than 1 mm, and the severe score presents thicker striations higher than 1 mm, showing in Figure 2 (Kuttappan et al., 2013c).

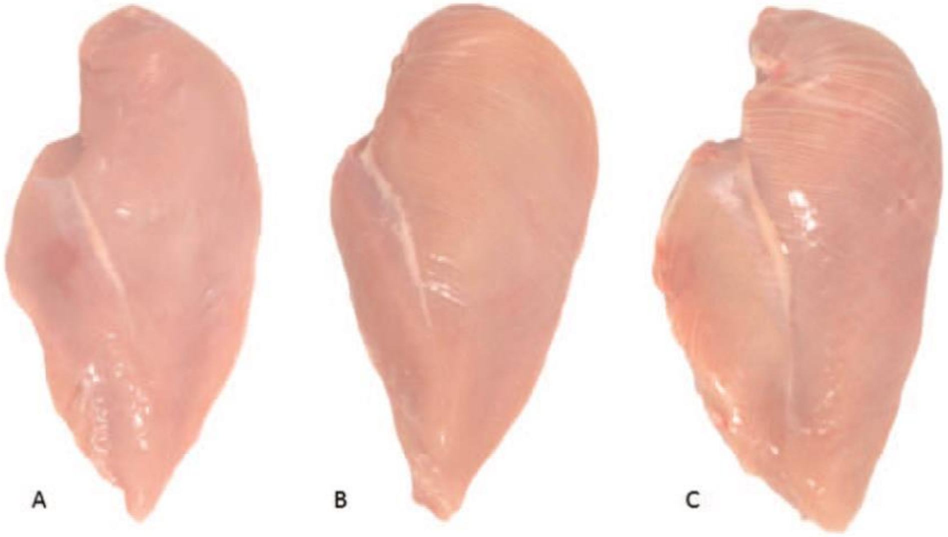

Figure 2. Representatives samples of breast fillets score A) Normal (without striations), B) Moderate (striations < 1 mm), and C) Severe (striations > 1mm) (Kuttappan et al., 2013c).

Higher scores are associated in breasts that had a higher cranial thickness and breast yield (Kuttappan et al., 2013a). The same study showed a positive and strong correlation (r_s =0.84) between the thickness and breast weight. The severe white striping breast fillets were associated with higher b* being more yellowish and researches suggested that a higher percentage of adipose tissue content could contribute to the color changes (Petracci et al., 2013b; Kuttappan et al., 2017).

Another study had focused on the histological state associated with the different scores (Kuttappan et al., 2013c). The moderate and severe presented loss on the transverse striations, variability on the muscle fiber size (Figure 3), vacuolar/ floccular degeneration, mild mineralization, lysis, interstitial inflammation along with fibrosis, and some regeneration (multinucleated cells and nuclear rowing). Masson’s trichrome stain also showed differences in the normal and severe score for white striping where severe white striped meat had higher degeneration, necrosis, lipidosis, and fibrosis. All of the different histological stains showed the increase of the lipids on the muscular tissue while the proteins decrease according to the score of the white striping.

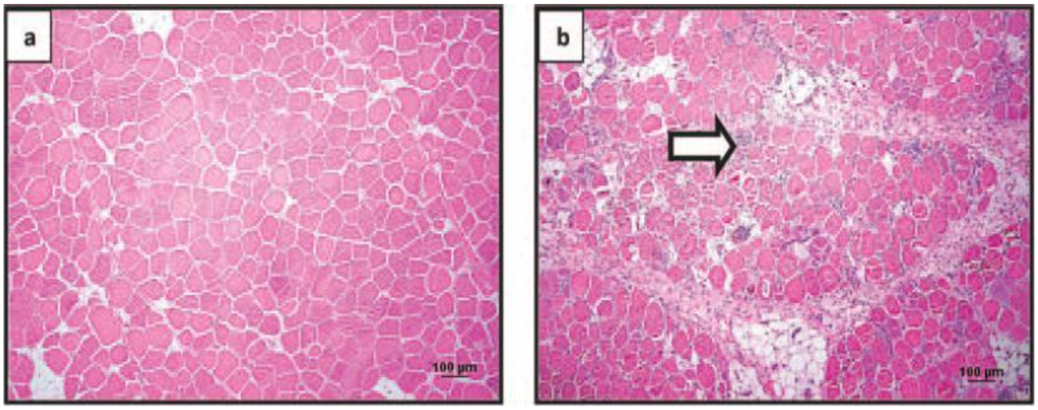

Figure 3. Histological samples of chicken breast fillets scored of a) normal and b) severe white striping stained with hematoxylin and eosin. The arrow represents degeneration of muscular fibers with inflammatory cells infiltrations (Kuttappan et al., 2013c).

Comparing the serological profile of the birds with severe and normal score of white striping resulted in difference in the hematological parameters, including the total number of leukocytes (Kuttappan et al., 2013b). Hence, birds with a higher score of white striping presented high serum levels of alanine transaminase, aspartate aminotransferase, lactate dehydrogenase, and creatine kinase. The serological and hematologic profiles of creatine kinase represent a muscle damage without presenting an inflammation or a systemic infection (Kuttappan et al., 2013c; Petracci et al., 2015). Some etiological causes have been already established in that males are more susceptible than females, a diet that contains low energy diet results in birds with fewer lesions than those fed the high energy diet, high genotype changes than the standard breast yield genotype, and for slaughter weight and growth rate weight has less lesion the lighter and lower than the heavier and higher (Kuttappan et al., 2012c, 2013a; Petracci et al., 2013a, 2015; Lorenzi et al., 2014).

It has been confirmed by most muscular research that modification is presented on the white striping. The poultry breast fillet with these lesions present a decrease of protein and ash content because of a higher percentage of collagen, moisture, and intramuscular fat compare with standard meats (Kuttappan et al., 2012a; Petracci et al., 2015). Besides, the birds with high performance, high breast yield, and diets with high energy are significantly related to the white striping (Petracci et al., 2015). There is any relation with infection or inflammation, and high enzyme levels to confirm the muscular damage caused by the regenerative myopathy (Kuttappan et al., 2013c; a).

Wooden/Woody breast (WB)

The high breast yield broilers type are a common and desirable characteristic selected in the poultry industry in most of the genetic broiler lines. A study done by Zuidhof et al. (2014) showed that the chicken breast from 1957 (AMC) to 2004 (Ross 308) had increased by 79% and 85% for females and males broilers, respectively. This could be one of the reasons of most of the broiler lines are presenting a higher incidence of breast myopathy, such as wooden breast (WB) (Petracci & Cavanni, 2012). This affects the *Pectoralis major* and eventually the *Pectoralis minor* of chicken breast fillets (Griffin et al., 2018).

The muscle presents hardness to the touch or with rigid areas and/or with texture on the ventral of the muscle, and transudate is sometimes observed around the thicker portion of the breast (Bilgili, 2016). The methodology of scoring woody breast fillets is rudimentary, and consists of a trained person tactile evaluation the hardness of the breast fillet. The scores vary from 0 to 3 based on (Tijare et al., 2016). Woody breast scores of 0 are flexible throughout the entire fillet, while scores of 3 are firm to the touch and very rigid throughout the entire fillet. Often the scores can be further divided into 0.5 increments (Table 1).

Table 1. Woody breast score palpation on breast fillets.

Woody breast scores	Characteristics
0	Normal breast fillet, no color alteration, flexible throughout.
0.5	Hardness on the cranial region, could present pale area compared with the other regions.
1	Hardness throughout cranial and caudal region, but flexible in medial/caudal region.

1.5	Hardness throughout cranial, medial, and caudal region.
2	Hard and rigid but with some flexibility in mid to caudal region
2.5	Hard and rigid, limited flexibility at mid to caudal region. It can be paler, could present exudate, hemorrhage, and a white fibrotic membrane over the breast fillet.
3	Hard and rigid throughout all fillet with no flexibility; It can be paler, could present exudate, hemorrhage, and a white fibrotic membrane over the breast fillet.

Based on Tijare et al., 2017 with modifications for expanded scale (Mallmann, 2019)

Woody breast live palpation were able to be detected in birds after four weeks old, besides were noticed that severe cases could not return themselves to the normal position when in accidental dorsal recumbence (Papah et al., 2017). Although, Griffin et al. (2018) first WB palpation lesions could possible in live chickens with 42 days old, besides already seen breast shape alteration in size at day 30.

The fast growing muscle present lesions such as pale and hardened areas along the breast fillet (Sihvo et al., 2014; Soglia et al., 2019). Heavier and thicker conformation of this muscle increase the probabilities of WB occurrence. Woody breast can also present exudate, hemorrhagic lesions, and petechiae can be present in severe cases, illustrated in Figure 4 (Sihvo et al., 2016; Kuttappan et al., 2016; USDA-FSIS, 2017; Sihvo, 2019). Mostly, the WB histological characteristics are with the fiber size variability, atrophic and degenerative fiber, necrosis, hyalinization, lipidosis, fibrosis, fragmented loss of striation, hypereosinophilic amorphous fibers, and inflammatory cells infiltration, mainly macrophages and heterophils, within and around the degenerative fiber (Figure 4) (Velleman and Clark, 2015; Soglia et al., 2016b; Sihvo, 2019). A moderate inflammatory infiltration with macrophages, heterophils and occasionally lymphocytes and fibroblast in the interstice region are found on the woody breast categories of chicken breast fillet. Histological parameters presenting on this myopathy are a polyphasic myodegeneration with an aggregation of connective tissue and/or fibrosis (Mudalal et al., 2015).

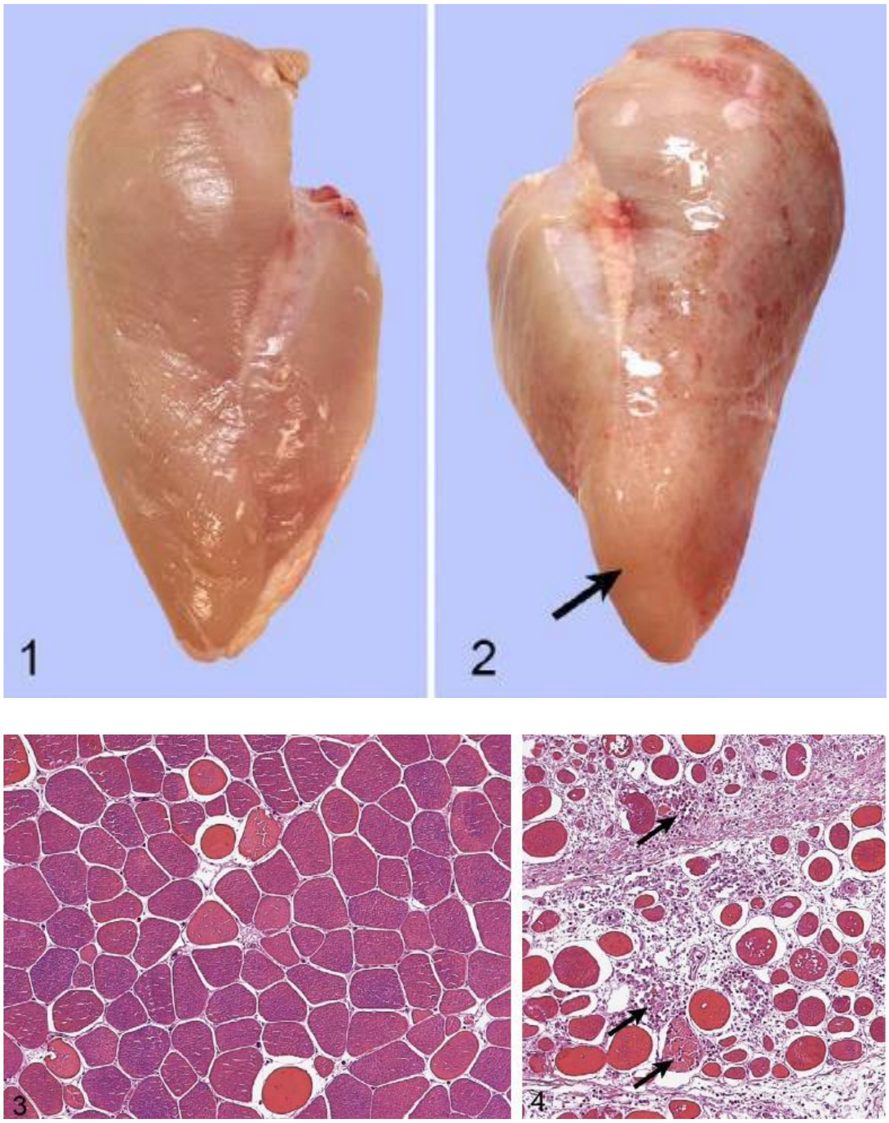

Figure 4. Comparison between a *Pectoralis major* of a normal breast fillet (1) with a muscle that shows hardness, pale and covered by a clear viscous fluid, with some texture over the caudal extremity (arrow) and multifocal petechiae. Normal histological slide (3) the muscular cells are polygonal with cohesion and uniformity. The next picture (4) is a breast muscle of a woody breast fillet, and the reduce the number of muscle fiber are visible, degeneration of them as well (arrows) the muscle are more round and separated/substituted of a loose connective tissue or with a slightly inflammatory infiltration mostly of heterophiles and macrophages. HE staining (Sihvo et al., 2014).

Data of the breast fillets footprint were also studied to be able to know more about these abnormalities. Higher the weight, yield, and thickness of the breast severe the lesions of WB will be (Mudalal et al., 2015; Chatterjee et al., 2016; Zambonelli et al., 2016; Dalle Zotte et al., 2017;

Kuttappan et al., 2017; Xing et al., 2017; Mallmann et al., 2018; Dalgaard et al., 2018). At the cranial part of the breast is where hardness presents most, diffusing along the breast, depending on the severity of wooden breast. Its color is ordinarily pale, and the surface of the chicken breast presents depressions that are visible and touchable (Dalle Zotte et al., 2014).

Not only the shape of the breast can have differences in the WB categories, the bird lines can also be an important factor for the WB incidence. Since breeder companies select directly for breast yield for certain strains this impacted on the severity of the myopathies. All the studies realized with the standard and the high yield line reported a higher incidence in WB for the high yield birds (Brewer et al., 2012b; Petracci et al., 2013b; Lorenzi et al., 2014; Bailey et al., 2015; Trocino et al., 2015; Alnahhas et al., 2016; Livingston et al., 2019a)

The RNA-sequence analysis indicates localized oxidative stress, hypoxia, higher levels of intracellular calcium and muscle fiber type switching related to the fast-growing broilers (Abasht et al., 2019). The studies related many statements of fast-growing being the main reason causing increasing muscle damage (Velleman and Clark, 2015); frequency on pale, soft and oxidative meat (Wang et al., 1999); greater metabolic stress and lower capillaries density (Hoving-Bolink et al., 2000; Macrae et al., 2006; Sihvo et al., 2018a); focal myopathy (WILSON et al., 1990); muscle cell cation regulation (Sandercock et al., 2009); phlebitis (Papah et al., 2017); lower glycogen content, increased fatty acid uptake, decreased glycolytic capacity, and mitochondrial oxidation (Abasht et al., 2019).

Strategies searches for decreasing the incidence of this abnormality bring to a more in depth study of the factors that are associated with this problem. Therefore, the need for research to be able to evaluate and predict the alterations that occur in the breast fillet with the bird ages.

Need for Research

The purpose of this research is finding causes that could induce the breast abnormalities, a way to predict the WB in live birds and a methodology to score more accurate, correlated and find a model to the most predictable factors for the myopathies.

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Chapter I. **EVALUATION OF INCREASED ACTIVITY AND LIGHT INTENSITY ON THE INCIDENCE OF MYOPATHIES IN MALE BROILER CHICKENS**

Abstract

Woody breast (WB) and white striping (WS) are significant myopathies in broiler chickens that resulted in decreased meat quality. Ischemia in the *Pectoralis major* muscle has been associated with the WS and WB. Increased activity raises blood flow and dilates blood vessels. Therefore, we hypothesize that increasing bird activity by increasing lighting intensity and human interaction could reduce the incidence of WS and WB. The present study consisted of two experimental groups, active and control, replicated across 3 room of 80 birds. The control group was managed similar to commercial setting with an average lighting intensity 6.5 lx and one person entering the room for daily checks. The active group had the average light intensity of 25.9 lx with a person walking through the room for 2 minutes either 3 times a day (week 1-3) or 6 times a day (week 4-7). At 0, 14, 35, and 56 days, all broilers were individually weighed, and feed intake was collected for each pen. At d57, all birds were processed and processing yields, WS score, WB score and compression force were measured. Data were analyzed by JMP® Pro 14 using fit model with chamber treated as a random effect and treatment as a fixed effect. Stimulation of activity with human interaction and light intensity did not alter ($P>0.05$) performance, processing yields, WS, WB, or compression force. These results suggest that simulated activity of the birds through the human interaction and increased lighting may not affect performance, meat quality, processing parameters and the severity of WS or WB in broiler chickens.

Keywords: Activity, light intensity, chickens, woody breast, white striping

Introduction

Modern fast-growing broilers were genetically selected for efficiently converting feed into muscle, particularly to the breast muscle, *Pectoralis major* (Zuidhof et al., 2014). However, the consequences of these improvements are associated to morphological abnormalities defects like the wooden breast and white striping (Radaelli et al., 2017).

Studies affirm that fast-growing broilers were spending less time eating and drinking, which means that they are ingesting more feed and water in one time instead of several times. This decrease of movements increase the body weight and also the prostrated behavior (Bizeray et al., 2000; Rutten et al., 2002; Bessei, 2006).

The two most prominent myopathies in broilers are white striping (WS) and woody breast (WB). White striping is characterized by white striations parallel to muscle fibers on breast, thigh, and tender muscles of broilers (Kuttappan et al., 2012c). Woody breast is characterized by a hard consistency of the raw breast fillets due to infiltration of hyaline cartilage (Dransfield and Sosnicki, 1999; Griffin et al., 2018; Zhuang and Bowker, 2018). Both myopathies are characterized by myodegeneration, necrosis, fibrosis, lipidosis, ischemia, and phlebitis in modern broiler chickens (Soglia et al., 2016b; Papah et al., 2017). A recent transcriptome analysis of *Pectoralis major* muscle affected with WS elucidated transcripts involved in angiogenesis, hypoxia, and cell death, suggesting WS may alter blood flow into the *Pectoralis major* (Marchesi et al., 2019). Ischemia and phlebitis are associated with the WS and WB, these myopathies may be the result of decreased circulation in the *Pectoralis major* muscle (Papah et al., 2017). Therefore, identifying strategies that increase blood flow to the *Pectoralis major* may provide a means of mitigating these myopathies.

Increased activity through increased light intensity or movement improve blood flow in broilers. It has been clearly recognized in broilers that increases in light intensity from 5 to 20 lx increases activity (Blatchford et al., 2009). Additionally, increases lighting, possibly due to increased activity has been shown to increase blood parameter (Olanrewaju et al., 2012).

Whereas in humans prolonged periods of sitting change the structure of the arteries on the lower limbs and hemodynamics increasing blood viscosity and blood pooling (Mcmanus et al., 2015). Vessel vasodilatation in the skeletal muscle is done by the increasing of the blood flow that the exercise caused (Duncker and Bache, 2008). Also, high pressure over the muscle could lead to Volkmann's contracture or more known by the compartment syndrome, that reduces the perfusion of the capillary causing a muscle ischemia and oxygen deprivation (Mubarak et al., 1989). It may be possible that the sitting/resting position of the birds leads to decreased blood flow and circulation. Hence, the objective of the present study was to evaluate the effect of increased activity of broilers on the incidence of WS and WB.

Material and Methods

Animal source and diets

In the present study, 480 day-of-hatch male Cobb 700 broiler chicks were obtained from Cobb-Vantress (Siloam Springs, AR, USA). All animal handling procedures were approved by with the Institutional Animal Care and Use Committee at the University of Arkansas (protocol 17044). Feed and water were provided *ad libitum* with all the treatments receiving a three phase commercial diet formulated to meet or exceed nutritional requirements of broiler chickens as recommended by the National research council (1994), and adjusted to breeder's recommendations (Cobb-Vantress Inc., 2013).

Experimental design

On Day of hatch, chicks were neck-tagged and randomly located to one of six environmentally controlled chambers (3.7 m long × 2.5 m wide × 2.5 m high). A total of 80 chicks were placed per chamber and the chamber was assigned to either the control or active group. All the chambers were started with the same light intensity for the first week illustrated on Figure 1.

The control group were checked once a day for mortality and feed levels. Additionally, room temperature, and light intensity were measured at that time (Figure 1). Based on commercial recommendations, the light intensity of the control group was reduced to close to 5 lx. The active group were also checked for mortality and feed levels as well as room temperature and light intensity measured once daily. In addition to this check, birds were encouraged to increase movement through human interaction. This was accomplished by having a person walk through the chambers for 2 minutes either 3 times a day (week 1-3) or 6 times a day (week 4-7). Additionally, the light intensity was maintained at close to 25 lx.

All birds followed the same temperature and lighting schedule. Temperatures were maintained at 34°C for the first 5 d and was then gradually reduced according to standard management practices until they reached 23°C (Figure 1). The lighting schedule was similar despite the difference in intensity. The first week the birds had 1 h of dark and 23h of light and the same light intensity. In the second week, they received 20 h of light and 4 h of dark until the end of the experiment.

Broilers weights were collected at d 0, 14, 35, and 56 days. Feed was weighed in at d 0, 14, and 35 with each diet phase change and orts were recorded on d 14, 35, and 56 prior to the diet change. From these measurements, body weight gain (BWG) per chamber was calculated by initial weight less the actual weight. Feed intake (FI) per chamber was calculated by weight of feed intake on the end of the phase from the feed on day one of the phase, and feed conversion ratio (FCR) was calculated as chicken growth by amount of feed intake.

Sample preparation

All fillets used in this study were collected from broilers processed at 56 d of age utilizing a commercial-style inline processing system at University of Arkansas Poultry Processing Pilot

Plant using standard procedures (Mehaffey et al., 2006). Briefly, feed, but not water, was withdrawn for 10 h before harvest. Broilers were weighed, electrically stunned, exsanguinated, de-feathering, and manual eviscerated. Carcasses were pre-chilled for 15 min at 12˚C in immersion tanks then chilled for three hours at 1˚C in immersion chill tanks until deboning. Following chill, carcass and cut-up parts were weighed for determination of yield performance. Whole breast fillets were evaluated for degree of hardness (WB) based on tactile evaluation using the scale developed by Tijare et al., (2016). Briefly, fillets were categorized as 0 if the fillets that were flexible throughout the fillet; 1 if the fillets were hard in the cranial region but flexible throughout the remainder of the fillet; 2 if the fillets that were hard throughout the fillet but remained flexible in mid to caudal region of the fillet; 3 if the fillets that were extremely hard and rigid throughout the fillet. White striping was evaluated based on the visual scale of 0 to 3 depending on the thickness of the white striations, 0 considered normal and 3 the most severe cases (Kuttappan et al., 2012c). Concisely, fillets were categorized as a 0 if there were no white striations, 1 whereas striations <1mm thick, and 2 when the striations were >1mm and mostly all over the breast surface. For both evaluations, fillets that fell between categories were scored by them, i.e. as a 0.5. To minimize variability in scoring, one experienced person carried out all scoring of fillets.

Compression force

Compression force (CF) test parameters were conducted on the right fillet using method published by Sun et al. (2018). Briefly, fillets were compressed to 20% of the fillet height three times on the cranial region in different areas using a 6-mm flat probe on a TA. XT Plus Texture Analyzer (model TAX-T2, Texture Technologies, Scarsdale, NY). No sample cutting was performed. The maximum force to compress the samples was set at 5 g, probe height set at 55 mm, pre and post-probe speeds were both 10 mm/s, and the test speed of the probe was 5 mm/s.

Statistical analysis

Data were using a general linear model in JMP® Pro 14 (SAS Institute, 2018) for performance parameters, carcass characteristics and myopathy incidence. For individual body weight and body weight gain, data were analyzed within phase (day 0-14; day 14-35; day 35-56) with treatment group fit as a fixed effect and bird within environmental chamber fit as a random effect. For pen based feed intake and feed conversion parameters, data were analyzed within phase (day 0-14; day 14-35; day 35-56) with treatment group fit as a fixed effect. For all data pertaining to carcass characteristics, data were analyzed with treatment group fit as a fixed effect and bird within environmental chamber fit as a random effect. For data pertaining to carcass quality (WS, WB, and CF), data were analyzed with treatment group fit as a fixed effect, breast fillet weight fit as a covariate and bird within environmental chamber fit as a random effect. Significance was set at $P < 0.05$.

As distribution of WS and WB can be different while the overall means may not be different, a chi-squared (χ^2) test was ran to test frequency distributions. Significance was set at $P < 0.05$.

Results and Discussion

Recent studies have shown that altered transcript abundance associated with angiogenesis, hypoxia, and cell death are associated with WS and WB (Marchesi et al., 2018; Kuttappan et al., 2017). In this study, we hypothesized that increasing activity of broilers through increased lighting and human interaction could increase blood flow and oxygen, hence reduce the incidence of WS and WB. To test this hypothesis, a group of broilers were subjected to elevated lighting and encouragement to move through human interaction and compared to a group of broilers subjected to commercial standards.

As lighting and temperature can alter movement of broilers, Figure 1 depicts the lighting and temperature changes by treatment. Each line represents the daily conditions of the environmental chamber within the treatment group. For the first week, all birds underwent similar lighting, average of 22.71 ± 0.07 lx, however, this was altered starting in week 2 as the control group had decrease in lighting intensity to the commercial recommendations of 5 lx, but actually 6.78 ± 0.03 lx. Broilers in the active group remained at approximately 25 lx (25.94 ± 0.18) for the duration of the study. It was not measured the activity of the birds during times without interaction to determine if this increase in lighting intensity elevated activity. However, Olanrewaju et al. (2012) and Deep et al., (2012) reported increased activity. Therefore, we anticipate this increase of 25 lx would increase the activity of our broiler without affecting the bird welfare (Collier et al., 2011).

Increased energy requirements for elevated actively can have adverse effects on growth performance. However, in this study, no differences were observed in body weight ($P>0.05$), feed intake ($P>0.05$), or feed conversion ($P>0.05$) between control and active groups (Table 1). However, control birds had numerically higher body weight than active birds, the same happens with the birds studied by Olanrewaju et al. (2012). As previous studies have found lowering light intensities improve production parameters (Olanrewaju et al., 2006; Olanrewaju et al., 2014), they also could not find significant differences in the body weight unless by the differences on sex.

As live weights were not different, it was not surprising that carcass yield and cut-up parts, breast, tender, wings, leg quarters, and rack yield, were not significantly different between the two treatments ($P>0.05$; Table 2). In this study, it was hypothesized that increasing activity of broilers would decrease the incidence of 2 of the most prominent myopathies in the industry today. However, WS and WB were not different between the means (Table 2) and also WB were not different for the frequency distribution across activity groups within severity (*P>0.05)*, but WS had a higher incidence of severe cases for the active treatment (Table 3). Since WB scores are a subjective methodology, the other way to be more objective to evaluate the meat hardness is doing the CF. The results of CF also were not significantly different among the treatments (*P>0.05*). These results suggest that increased activity of the birds through human interaction and increase lighting in this study does not impact performance, processing parameters and the severity of WS or WB in broiler chickens.

Conclusion

In conclusion, this study did not observe differences in growth performance, WS or WB with increased activity in broiler. However, this study was the first to test this hypothesis and therefore utilized a limited number of individuals. From this study, it was able to determine that a balance data with enough n for each category to be determine significant affects and if additional studies were conducted to test this hypothesis, since the variation of WB and WS are not controlled and the etiology not really understood.

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Figures and Tables

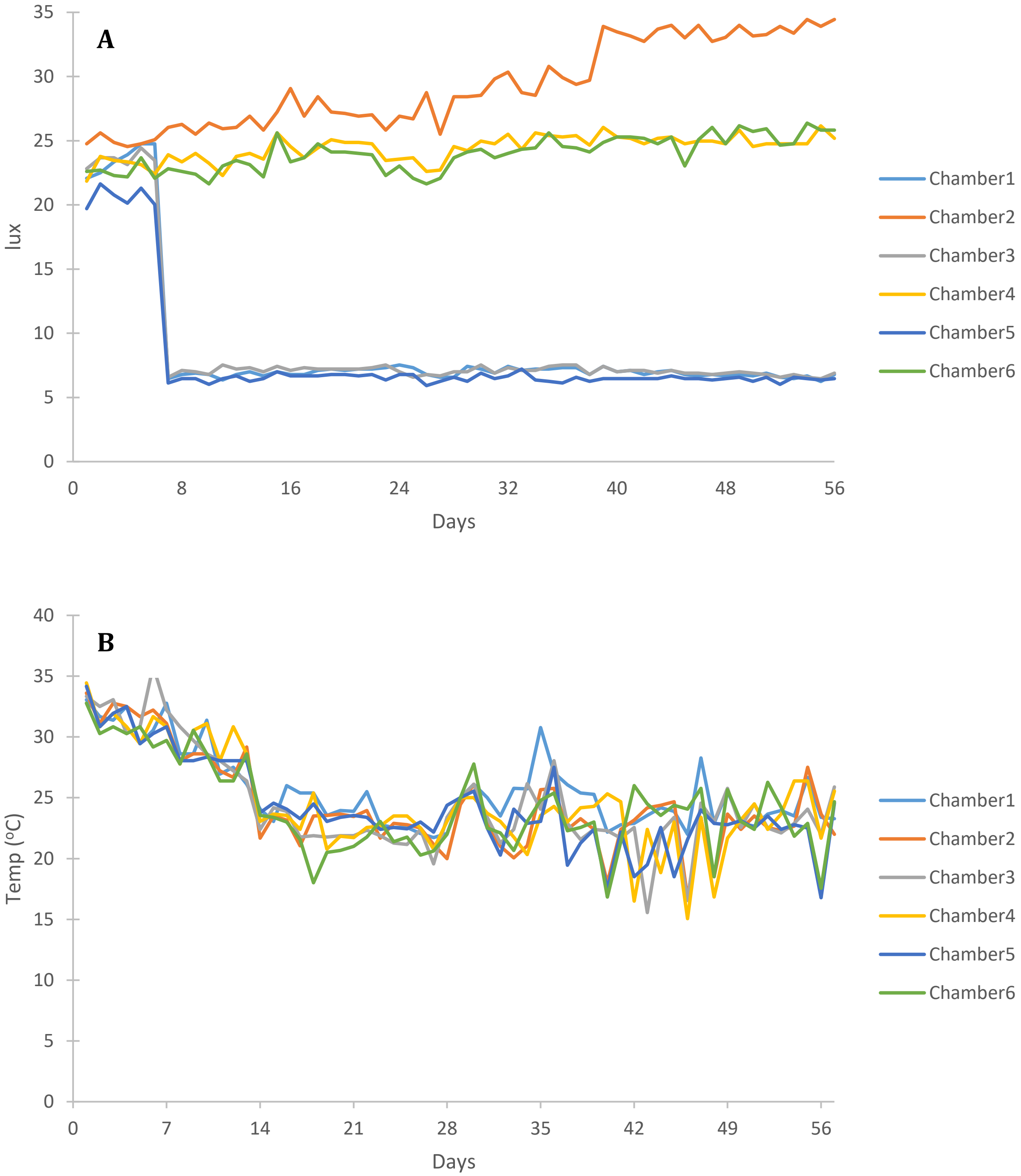

Figure 1. Chambers light intensity (A) and temperature (B) by day.

Table 1. Evaluation of control and active treatment on growth performance in broiler chickens.

Parameters	**Active**[1]	**Control**[1]	**SEM**	***P-value***
Phase 1[2]				
Body weight (kg)[3]; DOH[4]	0.043	0.043	0.001	*1.000*
Body weight (Kg)[3]; Day 14	0.384	0.375	0.015	*0.687*
Body weight gain (Kg)[3]	0.341	0.332	0.015	*0.688*
Feed consumption (Kg)[5]	0.512	0.589	0.065	*0.449*
Feed conversion[5]	1.51	1.52	0.211	*0.421*
Phase 2[2]				
Body weight (Kg)[3]; Day 35	2.18	2.10	0.085	*0.560*
Body weight gain (Kg)[3]	1.79	1.72	0.080	*0.585*
Feed consumption (Kg)[5]	2.11	2.08	0.319	*0.954*
Feed conversion[5]	1.88	1.89	0.159	*0.980*
Phase 3[2]				
Body weight (Kg)[3]; Day 56	3.97	3.89	0.107	*0.356*
Body weight gain (Kg)[3]	1.79	1.65	0.067	*0.2178*
Feed consumption (Kg)[5]	4.06	3.96	0.082	*0.235*
Feed conversion[5]	2.26	2.39	0.063	*0.189*

[1] Active: human activity stimulation (3 times for 2 min first 3 wk, 6 times for 2 min 4-7 wk) and light intensity of 25.94 ± 0.18. Control: mimic commercial setting with light intensity (6.78 ± 0.03 lx) and daily check with minimal activity encouraged.

[2] Phase 1: day of hatch through 14 days of age; Phase 2: 14 days of age through 35 days of age; Phase 3: 35 days of age through 56 days of age.

[3] Individual bird data.

[4] DOH; Day of hatch.

[3] Pen based data.

Table 2. Evaluation of activity and light intensity on meat quality and processing parameters in broiler chickens at 57 days old.

Parameters	**Active**[1]	**Control**[1]	**SEM**	***P-value***
Meat quality				
White Striping [2]	1.33	1.15	0.08	*0.22*
Woody Breast [3]	0.86	0.83	0.08	*0.86*
Compression force (N)	3.53	3.46	0.18	*0.74*
Processing				
Bird weight (Kg)	3.96	3.84	0.03	*0.47*
Carcass (Kg)	3.15	3.03	0.02	*0.45*
Breast yield[4] (%)	30.11	29.71	0.16	*0.16*
Tender yield[4] (%)	5.83	5.83	0.08	*0.97*
Wings yield[4] (%)	9.74	9.93	0.08	*0.16*
Leg quarters yield[4] (%)	28.02	28.25	0.22	*0.58*
Rack yield[4] (%)	26.00	26.15	0.17	*0.58*

[1] Active: human activity stimulation (3 times for 2 min first 3 wks, 6 times for 2 min 4-7 wks) and light intensity of 25.94 ± 0.18. Control: mimic commercial setting with light intensity 6.78 ± 0.03 lx and daily check with minimal activity encouraged.

[2] Scores 0 to 3 by Kuttappan et al. (2012). Score 0 is a non-affected bird and 3 the severe cases.

[3] Scores 0 to 3 by (Tijare et al., 2016). Score 0 is a non-affected bird and 3 the severe cases.

[4] yield percentage of carcass

Table 3. Incidence of Woody Breast (WB) and White striping (WS) between the active and control treatments.

	Active[1] (%)	**Control[1] (%)**	$\chi 2$
WB Scores			*0.501*
0	53	58	
1	35	30	
2	10	9	
3	3	4	
WS scores			$\chi 2$
0	15	20	*0.015*
1	49	54	
2	32	25	
3	3	0.5	

[1] Active: human activity stimulation (3 times for 2 min first 3 wk, 6 times for 2 min 4-7 wk) and light intensity of 25.94 ± 0.18. Control: mimic commercial setting with light intensity (6.78 ± 0.03 lx) and daily check with minimal activity encouraged.

Chapter II. **MEAT QUALITY ATTRIBUTES ASSOCIATED WITH WOODY BREAST AND EFFECT OF LOCATION AND FREEZING ON BREAST FILLET**

Abstract

Woody breast (WB) is a major myopathy in broilers characterized by hardness of the breast fillet and can be evaluated by human palpation with a severity scale of 0 (normal) to 3 (severe). The objective of this study was to determine instrumental and meat quality factors that are associated with WB scores that may potentially be used in sorting programs. Additionally, this study was to determine if there is a location effect (breast side) or effect of freezing on compression force (CF) of fillets. After commercial style processing and deboning (3 h postmortem), 206 breast fillets were collected and scored for WB. Thickness and length (overall, cranial, caudal, and keel regions) of the *Pectoralis major* were measured with a caliper. CF was measured using Texture Analyzer in four regions at the cranial part of the fillet on both the right (RS) and left (LS) sides. Color and pH were analyzed on the LS of the breast. The RS frozen at -20^{o}C for 48 h and thawed for 24 h, and then CF was measured, along with cook loss, MORS, and BMORS. Pearson correlation coefficients and ordinal logistic regression were used. Measurement responses were compared for the four categories of WB (0=normal, 1=mild, 2=moderate, and 3=severe) using Fit Model with JMP® Pro 14. The keel length measurement on the breast showed no difference ($P>0.05$) and small correlation. However, the thickness is correlated moderately (r = 0.67) and could differentiate between the scores. A thicker breast denotes a higher severity of WB. In addition, CF of LS and RS sides of the breast fillet were significantly different, with the RS of the breast showing higher force ($P<0.05$). Freezing significantly decreased ($P<0.05$) CF of thawed fillets compared to pre-frozen fillets. Cook loss increased ($P<0.05$) as severity for WB increased. Peak counts for MORS and BMORS were higher ($P<0.05$) for the severe compared to the lower levels of severity. In conclusion, the thickness of the breast fillet may potentially be used for sorting purposes, possibly in combination with other sorting criteria. This model of prediction of WB could be used in industry to select the different WB categories. Freezing the breast meat improves the softness of the WB and differences exist between right and left fillets. Future research should assess impact of freezing on sensory.

Key words: broiler breast, wooden breast, meat quality, myopathies, thawed meat

Introduction

Boneless breast meat is a popular meat choice in the United States and is considered a premium product. To meet the demand of this fast-growing industry, processors are adopting high breast-yielding strains of broilers that meet the needs in the growing heavy debone market segments. However, with these choices of demand, the incidence of myopathies such as Woody breast (WB) and White striping (WS) have also increased (Kuttappan et al., 2013; Kuttappan et al., 2016). Several issues affecting WB and WS, including the sex of the birds, high yielding genotypes, and higher growth rates have been reported to increase these myopathies (Petracci and Cavani, 2012; Sun et al., 2018; Mehaffey et al., 2006b). These myopathies affect the consumer acceptability and quality of poultry meat. Specifically, WB is characterized by hard consistency on the *Pectoralis major*, and pale color on raw fillets (Sun et al., 2018; Sihvo et al., 2013). Histological changes are associated with myopathy (Soglia et al., 2015; Soglia et al., 2017). This changes occurs on lower protein, higher collagen and fat content, resulting in increased drip loss, pH, cook loss, and water holding capacity and marinate uptake (Kuttappan et al., 2012b; Russo et al., 2015; Kuttappan et al., 2017). Severe WB fillets are poor quality meat that are often downgraded in the poultry industry causing economic losses. The objective of this study was to determine instrumental and meat quality factors that are associated with WB scores that may potentially be used in sorting programs. Additionally, this study was to determine if there is a location effect (breast side) or effect of freezing on compression force (CF) of fillets.

Material and Methods

Animal source and diets

In the present study, 240 d-of-hatch male Cobb 700 broiler chicks were obtained from Cobb-Vantress (Siloam Springs, AR, USA). Feed and water were provided *ad libitum* with all the treatments receiving a commercial feed depending on the phase. The feed change, and growth performance evaluation were done at 0, 14, 35, and 56 days. Diets were formulated to approximate the nutritional requirements of broiler chickens as recommended by the NRC, (1994) and adjusted to breeder's recommendations (Cobb-Vantress Inc., 2013). At 57 days, all birds were processed using a commercial in-line system at the University of Arkansas. All animal handling procedures complied with the Institutional Animal Care and Use Committee at the University of Arkansas, Fayetteville, USA.

Processing of birds

About 10 h before slaughter, feed was withdrawn, but the birds were given an *ad libitum* supply of water. A commercial-style processing in-line system was used where the birds were electrically stunned, manually slaughtered by severing the left carotid artery and jugular vein, bled out, soft scalded, and defeathered (Mehaffey et al., 2006). The carcasses were then manually eviscerated, prechilled at 12°C for 15 min followed by chilling for 90 min at 1°C in immersion chilling tanks. While prechilling and chilling, the carcasses were manually agitated frequently to prevent the thermal layer in the tank and to enhance the chilling efficiency. The carcasses were taken out of the tanks, packed in ice, and aged at 4°C until deboning at 4h postmortem, common industry deboning times. Ready-to-cook (RTC) weight of each carcass was measured before deboning. The *Pectoralis major* muscle was removed from each carcass by six trained people to avoid any alterations in fillet dimensions and other meat quality parameters due to the deboning procedures. The butterfly fillet from each bird was placed in resalable plastic bags and stored at 4°C until 24 h postmortem.

Carcass and meat quality parameters

Whole breast fillets were evaluated for degree of hardness (WB) based on tactile evaluation scale by Tijare et al., (2016) categorized as 0 = fillets that were flexible throughout (normal); 1 = fillets that were hard mainly in the cranial region but flexible otherwise (mild); 2 = fillets that were hard throughout but flexible in mid to caudal region (moderate); 3 = fillets that were extremely hard and rigid throughout from cranial region to caudal tip (severe). Additionally, fillets were scored in 0.5 increments, when necessary, and rounded down for classification purposes. To minimize variability in scoring, one person carried out all scoring of fillets. After scoring, to determine the fillet dimensions, keel length (middle of the butterfly fillet), fillet length (at the longest point), fillet width (at the widest point), cranial thickness (height at the thickest portion), and caudal length (1/3 of the fillet length) were measured using calipers adapted from Mehaffey et al. (2006). CF was measured using Texture Analyzer in four regions at the cranial part of the fillet on both the right (RS) and left (LS) sides. Each butterfly fillet was halved into left and right. Fillets were compressed to 20% of the fillet height using a 6-mm flat probe on a TA.XT Plus Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK). The trigger force was set at 5 g, pre and post-probe speeds were both 10 mm/s, and the test speed of the probe was 5 mm/s (Sun et al., 2018). The left fillets were used for measuring pH and color, whereas the texture analyses were estimated on the right fillets. Muscle pH was measured using a Testo spear tip probe and meter (Model Testo 205, Testo Inc., Sparta, NJ). To assess overall (generalized) color changes in the fillet, L*, a*, and b* color values were determined as an average of 3 different sites on the dorsal (bone side) of the fillet using a Minolta colorimeter

(CR-300, Konica Minolta, Ramsey, NJ). The right fillets were vacuum packed and stored at −20°C until the cook loss and Meullenet-Owens razor shear energy (MORSE) were measured as described below. Before cooking, the fillets were taken out of the freezer and thawed at 4°C for 24 h and realized CF as the same way mentioned with fresh fillets. All fillets were cooked on raised wire racks in covered aluminum-lined pans in an air convection oven to an internal end-point temperature of 76°C. The difference between fillet weights before and after cooking was taken, and cooking loss was expressed as percentage with respect to the initial weight. After cooking, the fillets were cooled to room temperature, individually wrapped in aluminum foil, and stored overnight at 4°C, to be used for the determination of tenderness by the MORS and Blunt MORS (BMORS) technique (Cavitt et al., 2004; Lee et al., 2008; Lee et al., 2016) of the cooked samples and the results are reported in terms of shear energy, or MORSE/BMORSE (N·mm). The method uses a texture analyzer (model TAX-T2, Texture Technologies, Scarsdale, NY) with a 5-kg load cell using a razor blade or blunt blade probe. Four shear readings, at different locations on the cranial region, were done perpendicular to the muscle fibers on each fillet and the mean was calculated. The crosshead speed was 5 mm/s along with a sample shear depth of 20 mm and a trigger force of 0.1 N. The instrumental data were collected using Texture Exponent 32 version 1.0.0.92, and the macro options texture exponent (Stable MicroSystems, Godalming, Surrey, UK) was employed to determine the force and energy values from the force-distance curves. The MORS force (N) and MORS energy (N × mm; MORSE) and the BMORS force (N) and BMORS energy (N × mm; BMORSE) were determined and used as instrumental predictors of meat tenderness.

Statistical analysis

The data were analyzed using ANOVA with the categories. Least square means were separated with t-test when only two factors and Tukey’s HSD for more than two comparisons at a significance *$P<0.05$* using JMP® Pro 14. The Pearson correlation was done using multivariate and the scores were considered continuous. The WB categories were polled in four categories for most the analyses, Normal-0: pool of 0 and 0.5, mild-1: contains 1 and 1.5, moderate-2: had scores 2 and 2.5, and severe-3 with all score 3. However, the association of different parameters was done with nominal logistic regression with a binomial score, severe and normal. The selection of the score were higher and equal to score 1.5 for severe and all under and equal to 1 were considered normal. The covariates or continuous variables include the carcass/meat quality parameters such as RTC weight, fillet length, fillet width, keel length, thickness, caudal length, pH, L*, a*, b*, CF, cook loss, peak counts, and MORSE. The data was analyzed using nominal logistic regression procedure with JMP® Pro 14. The model will test each one of the inputs (e.g., RTC, fillet width, fillet height, and so on) and proceed, adding the next most significant input until all the significant parameters are included in the model. The result from the analysis is reported mainly as the odds ratio (OR), 95% CI, and the respective P-values. Odds is the ratio of the probability of an event of interest (e.g., probability of NORM) to the probability that the event will not occur (e.g., probability of SEV) and OR are the ratios of 2 odds comparing 2 groups. The OR indicates the increased or decreased chance of a dependent category as a result of an increase in the continuous variable by one unit or with a categorical variable in comparison with a reference. An OR >1 indicates an increased chance whereas <1 denotes a decreased chance. When the OR is equal to 1, there is an equal chance for the category in question and the reference category (Kuttappan et al., 2013a). The estimated probability of occurrence of the 2 degrees of WB was determined for all the categorical variables.

Results

Out of the 206 fillets evaluated at 24 h postmortem, 45 (22 %) were normal; 51 (25 %) showed mild lesions of WB; 30 (62 %) showed moderate lesions of WB; and 23 (48 %) showed severe lesions of WB. The results of the compression force of WB categories comparing the breast fillets sides are summarized in Table 1. In the left breast side, a significant increase in CF ($P<0.05$) was observed as WB score increased. For the right breast side, score 3 (severe) had the highest CF, followed by scores 2 (moderate), 1 (mild), and Normal (0) fillets, showed the lowest CF. The right and the left sides were compared and interestingly, the right breast side showed highest CF on normal, mild, and severe fillets, when compared with the same score on the left breast side (Table 1).

Table 2 shows the results of the CF of the chilled and thawed breast fillets of the right side from the different WB categories. As the WB lesion score increased on chilled breast fillets, the CF also showed a significant increase ($P<0.05$). However, thawed breast fillets with severe WB score had the highest CF, but the same of the normal category of the chilled breast fillets. Normal thawed fillets showed the lowest CF. Remarkably, thawed right side breast fillets for each WB lesion score had a significantly lower CF when compared with chilled fillets respectively (Table 2).

The results of the measurements of breast fillets butterflies by the WB scores are summarized in Table 3. A significant increase in thickness of the fillets were observed as the severity of the WB score increased. However, the fillet length was significantly shorter ($P<0.05$) on fillets with a severe WB score. No significant differences were noted on the cranial width, caudal width fillet or keel length among the fillets, regardless of the WB lesion score (Table 3).

Table 4 shows the results of the evaluation of WB categories on meat quality and parameters in broiler chickens. A similar trend of severe score being higher was observed for cook loss (%). No significant differences were observed on MORS, however, fillets with severe WB score showed the highest MORS peak count, BMORS and BMORSE. Severe and moderate fillets also showed the higher measurement of BMORS peak count, and normal fillets showed the lowest BMORS peak count, as well as pH and L* value. Moderate and severe fillets showed the highest L* and b* values. At processing, chickens with no WB lesions, had the lowest bird and carcass weight, as well as breast yield, whereas chickens with that had severe WB lesions had opposite results (Table 4).

The results of the Pearson's correlation between the measurements, compression force, and breast weight with the WB scores are summarized in Table 5. Significant and positive correlations were observed between WB score and CF, thickness, fillet length, breast yield, L*, b*, pH, cook loss, MORS peak count, BMORS, BMORSE and BMORS peak counts (Table 5).

The results of the relationship of WB with various carcass and meat quality attributes are summarized in Table 6. The multinomial logistic model obtained in the present study showed that the main carcass and meat quality factors that are significantly ($P<0.05$) associated with severe WB are cranial thickness, color L* value, fillet length, breast weight, and caudal width. L* value (indicates lightness) had the higher OR value influence on the occurrence of severe fillets (OR 1.30; 95% CI 1.07 to 1.59), followed by cranial thickness (OR 1.29; 95% CI 1.14 to 1.49), indicating that as the cranial thickness of the fillets increases; there is a greater probability that it could have a severe degree of WB (Table 6).

Discussion

In recent years, myopathies have caused significant economic losses to the poultry industry due to consumer acceptance of raw cut up parts and the quality of additional processed poultry meat products (Kuttappan et al., 2016). White striping (WS) is a disorder characterized by the occurrence of white striations parallel to muscle fibers on breast, thigh, and tender muscles of broilers, whereas woody breast (WB) is produce harder consistency to raw breast fillets (Zhuang and Bowker, 2018; Dransfield and Sosnicki, 1999; Griffin et al., 2018). Even though histologically, both pathologies are characterized by myodegeneration, necrosis, fibrosis, lipidosis, and regenerative changes in modern broiler chickens, recent studies have indicated that WS and WB could have a different etiology (Soglia et al., 2015). In the present study, out of the two hundred and six breast fillets evaluated at 24 h postmortem, 45 (22 %) were normal; 51 (25 %) showed mild lesions of WB; 30 (62 %) showed moderate lesions of WB; and 23 (48 %) showed severe lesions of WB. Furthermore, the heavier the chicken more severe the WS lesion will be and is a concomitant myopathy with WB, already was reported by Kuttappan et al. (2012, 2013b) and Alnahhas et al. (2016). This effect was also correlated with the carcass weight and breast yield (Mudalal et al., 2015; Chatterjee et al., 2016; Zambonelli et al., 2016; Dalle Zotte et al., 2017; Kuttappan et al., 2017; Xing et al., 2017; Dalgaard et al., 2018). Interestingly, the pH was significantly different ($P<0.05$) only between 0 and 3 and the other categories were similar ($P>0.05$) to them, confirmed as well in previous studies (Dalle Zotte et al., 2017; Kuttappan et al., 2017; Xing et al., 2017; Cai et al., 2018), but not founded differences in many other studies (Mudalal et al., 2015; Trocino et al., 2015; Soglia et al., 2016a; Wold et al., 2017; Chen et al., 2018; Dalgaard et al., 2018). However, L* value increased with WB scores in this study, meaning with the severe of the WB the paleness increases agreeing with Wold et al.(2017), Dalle Zotte et al.(2017), and Cai et al.(2018). Although, other research did not observed differences in the L* value (Mudalal et al., 2015; Trocino et al., 2015; Chatterjee et al., 2016; Tasoniero et al., 2016; Xing et al., 2017; Chen et al., 2018) Also, the same increment over the WB severity happens with the b* that is correlated with the yellowness (Tasoniero et al., 2016; Kuttappan et al., 2017; Baldi et al., 2018). Similar effects were observed with cook loss (%), where fillets that had severe WB scores, lost more water during cooking process. Similar results were reported by Mudalal et al. (2015b), Trocino et al. (2015), Soglia et al. (2016), Tasoniero et al. (2016), Zambonelli et al. (2016), Dalle Zotte et al. (2017); Xing et al. (2017), and Dalgaard et al. (2018). Overall, the occurrence of different degrees of WS and WB were associated with changes in pH, color L* and b* values, cook loss, and MORS peak counts, BMORS, BMORSE, and BMORS peak counts (assessment of texture quality).

The multinomial logistic model from this study confirm that previous studies show that fillet thickness had a much greater impact on fillet weight when compared with the length and width of fillet (Lubritz, 1997; Griffin et al., 2018). Furthermore, the higher correlation between fillet weights and the cranial thickness of the fillets observed in the present study is in agreement with other researchers, confirming that higher degrees of WS and WB are associated with heavier or thicker fillets (Brewer et al., 2012a; Mudalal et al., 2015; Kuttappan et al., 2017). This was in accordance with the findings from the previous studies (Kuttappan et al., 2012a; 2013b). These data are in agreement with WS and WB previous studies between the breast weight (Petracci et al., 2013b; Alnahhas et al., 2016) .Among these studies, Griffin et al. (2018), reported the cumulative logit mixed, where the best model was using breast length, width, thickness, yield, and *P. minor* width, yield. However, these study presented an inverse relationship with the breast length and the width. Furthermore, the conclusions of increasing breast morphometric (thickness, width, and length) increase the changes for the breast fillet become to be a severe WB. Thus, the incompatibility of the results could be related by the number of samples, different strain birds, and different WB score methodology.

In addition, compression force of LS and RS sides of the breast fillet were significantly different, with the RS of the breast showing higher force. It was remarkable to observe that freezing significantly decreased the compression force of thawed fillets compared to pre-frozen fillets, the reduction on the CF is due to the ice crystal formation of the loss in membrane strength (Leygonie et al., 2012). Previous studies conducted in our laboratory reported that the higher degrees of WB are associated with increased occurrence of muscle damage characterized by myopathic changes in broiler breast fillets (Sun et al., 2018; Kuttappan et al., 2016). The results from the present study imply that enhanced growth, resulting in greater carcass weight and even cranial fillet thickness, put more stress on the broilers, resulting in muscle damage.

Conclusion

In conclusion, the thickness of the breast fillet may potentially be used for sorting purposes, possibly in combination with other sorting criteria. A model of prediction of WB could be used in industry to select the different WB categories. Freezing the breast meat improves the softness of the WB and differences exist between right and left fillets.

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Figures and Tables

Table 1. Compression force (N) of WB categories comparing the breast fillets sides.

WB lesion score	Left breast side	St. dev	Right breast side	St. dev	*P-value*
0 (n=45)	4.94^{dB}	1.65	5.62^{cA}	1.63	*0.001*
SE	0.541		0.509		
1 (n=51)	7.20^{cB}	2.57	8.57^{bA}	3.19	*0.001*
SE	0.508		0.478		
2 (n=62)	9.51^{b}	3.34	10.06^{b}	2.99	*0.007*
SE	0.460		0.434		
3 (n=48)	14.42^{aB}	5.69	15.37^{aA}	5.01	*0.001*
SE	0.523		0.493		
P-value	*0.001*		*0.001*		

SE = standard error of the columns. WB = Woody breast St. dev= standard deviation of the rows

$^{a-d}$ Means showing difference between the columns are significantly different.

$^{A-B}$ Means showing differences between the rows are significantly different.

Table 2. Compression force of the chilled and thawed of right side breast fillet from the different WB categories.

WB lesion score	**Chilled breast fillet**	**St. dev**	**Thawed breast fillet**	**St. dev**	***P-value***
0 (n=45)	5.62^{cA}	1.63	2.74^{cB}	0.68	*0.006*
SE	0.509		0.189		
1 (n=51)	8.57^{bA}	3.19	3.71^{bB}	1.45	*0.001*
SE	0.478		0.178		
2 (n=62)	10.02^{bA}	2.99	3.65^{bB}	0.99	*0.001*
SE	0.434		0.162		
3 (n=48)	15.37^{aA}	5.01	5.42^{aB}	1.75	*0.001*
SE	0.493		0.186		
P-value	*0.001*		*0.001*		

SE = standard error of the columns. WB = Woody breast St. dev= standard deviation of the rows

$^{a\text{-}d}$ Means showing difference between the columns are significantly different.

$^{A\text{-}B}$ Means showing differences between the rows are significantly different.

Table 3. Means (± standard error) of the measurements (mm) of chilled breast fillets butterflies by the WB scores.

WB lesion score	**Thickness (mm)**	**Cranial Width (mm)**	**Caudal Width (mm)**	**Keel Length (mm)**	**Fillet Length (mm)**
0 (n=45)	39.34±0.62^{d}	149.3±1.85	168.2±1.82	143.2±1.66	185.1±1.45^{a}
1 (n=51)	42.82±0.59^{c}	149.8±1.74	166.7±1.71	139.9±1.56	182.5±1.36^{ab}
2 (n=62)	47.09±0.55^{b}	147.6±1.63	168.0±1.60	143.7±1.46	184.1±1.27^{ab}
3 (n=48)	49.63±0.60^{a}	147.6±1.79	167.9±1.76	139.5±1.60	179.7±1.40^{b}
P-value	*0.001*	*0.742*	*0.9207*	*0.1303*	*0.0389*

$^{a\text{-}d}$ Means showing different letters in each score are significantly different. SEM = standard error of the means. WB = Woody breast

Table 4. Evaluation of WB categories on meat quality and processing parameters in broiler chickens.

WB lesion score	**0 (n=45)**	**1 (n=51)**	**2 (n=62)**	**3 (n=48)**	**MSE**	***P-value***
Meat quality						
Compression force (N)	5.28 ^{d}	7.89 ^{c}	9.79 ^{b}	15.01 ^{a}	0.73	*0.001*
Cook loss (%)	24.45 ^{c}	26.32 ^{bc}	28.58 ^{ab}	30.24 ^{a}	0.36	*0.001*
MORS (N)	15.10	14.86	14.90	15.56	1.49	*0.372*
MORSE (N.mm)	210.67 ^{ab}	205.68 ^{b}	209.41 ^{ab}	223.03^{a}	5.58	*0.039*
MORS Peak Count	9.52 ^{b}	9.68 ^{b}	10.66 ^{b}	12.89 ^{a}	1.66	*0.001*
BMORS (N)	20.00 ^{b}	19.24 ^{b}	20.92 ^{b}	25.46 ^{a}	2.14	*0.001*
BMORSE (N.mm)	267.17 ^{b}	252.63 ^{b}	277.42 ^{b}	331.29 ^{a}	7.81	*0.001*
BMORS Peak Count	5.08 ^{c}	6.37 ^{b}	7.14 ^{a}	7.72 ^{a}	1.23	*0.001*
pH	5.77 ^{b}	5.82 ^{ab}	5.84 ^{ab}	5.86 ^{a}	0.33	*0.004*
L* value	53.99 ^{c}	54.37 ^{bc}	55.42 ^{ab}	56.41 ^{a}	1.56	*0.001*
a* value	3.16	3.39	3.58	3.76	1.31	*0.311*
b* value	6.06 ^{b}	6.64 ^{ab}	7.73 ^{a}	7.74 ^{a}	1.75	*0.008*
Processing						
Bird weight (Kg)	3.782 ^{b}	3.847 ^{b}	4.025 ^{a}	4.084 ^{a}	0.59	*0.001*
Carcass (Kg)	2.997 ^{b}	3.061 ^{b}	3.225 ^{a}	3.287 ^{a}	1.71	*0.001*
Breast yield (%)	30.7 ^{b}	31.54 ^{b}	32.88 ^{a}	33.7 ^{a}	1.35	*0.001*

$^{a\text{-}c}$ Means showing different letters in each score are significantly different. MSE = mean square error WB = Woody breast

Table 5. Pearson's correlation between the measurements, compression force, and breast weight with the WB scores.

Variable	**WB categories**[1]	***P-value***
Compression force	0.77	*0.001*
Thickness	0.67	*0.001*
Cranial width	-0.06	*0.411*
Caudal width	-0.01	*0.970*
Keel length	-0.04	*0.548*
Fillet length	-0.15	*0.031*
Breast yield	0.53	*0.001*
L*	0.35	*0.001*
a*	0.09	*0.222*
b*	0.23	*0.001*
pH	0.24	*0.001*
Cook Loss	0.38	*0.001*
MORS	0.03	*0.671*
MORSE	0.09	*0.162*
MORS Peak Counts	0.37	*0.001*
BMORS	0.36	*0.001*
BMORSE	0.29	*0.001*
BMORS Peak Counts	0.53	*0.001*

[1] WB categories were polled in four categories for most the analyses, Normal-0: pool of 0 and 0.5, mild-1: contains 1 and 1.5, moderate-2: had scores 2 and 2.5, and severe-3 with all score 3

Table 6. Odds ratio (OR), 95% CI, and the probability (*P-value*) level for variables in the model of SEV or NORMAL WB.

Variables	**OR**	**95% CI**	***P-value***
Thickness	1.29	1.14 to 1.49	<*0.0001*
Caudal width	0.91	0.86 to 0.96	*0.0008*
Fillet length	0.84	0.77 to 0.91	<*0.0001*
Breast wt	1.02	1.01 to 1.03	<*0.0001*
L* color	1.30	1.07 to 1.59	*0.009*

Chapter III. **LIVE PALPATION AND BREAST MEASURES FEATURES FOR WOODY BREAST MYOPATHY**

Abstract

Woody breast (WB) is a meat quality defect characterized by the hardness of the meat, but it is normally detected after deboning. Due to this reason, three experiments were designed to relate WB severity scores with the manual palpation and breast size measurements in live birds during grow out. In each experiment, the breast of birds was measured weekly for various dimensions and palpated for WB incidence. The measurements were made with either a fabric tape or caliper on the cranial (wing to wing) region (TCRAN and CCRAN, respectively), central region over the keel bone (TCENT and CCENT, respectively), and caudal region (TCAUD and CCAUD, respectively) represented in the breast fillet area where the ribs end. Moreover, WB palpation scoring of live birds was based on established scoring methodology for deboned breast fillets, which was based on the degree and extent of hardness of the muscle. Processing was on d 57 for experiments1 and 2 but on d 52 in experiment 3. Live bird measurements and bird weight were correlated to postmortem fillet WB severity scores. Additionally, live bird measurements and the bird weight by bird group type were fitted to Gompertz growth curve and comparisons of the parameters were performed among the curves of WB categories. The correlations between WB palpation and fillet WB scores increased with increasing bird age; However, correlations between live weight and fillet WB scores were inconsistent and varied correlation over the ages. Correlations of the different live bird breast measurements to fillet WB severity score were affected by bird market type across bird ages ($P<0.05$). Central region measurements had the lowest correlations of all live breast measurements, especially after 2 wks. Growth curve parameters for the breast measurements differed according to bird market type. The fast-food and tray-pack birds had similar inflection points (~16d) and asymptotes (~29 cm), and although inflection points of high-breast yield lines varied approximated 3d (18.3 and 15.2), asymptotes were considerably greater at 31 and 33 d.

When data were pooled across the 4 birds market types, size of chicken breast (TCRAN) indicated a significant difference. The results presented that heavy birds had differences on the breast size likewise the age inflection point. Finally, bird measurements could help to differentiate the WB scores between the strains, but other factors should be considered in future research to determine models that are predictive of WB during growth.

Key words: broiler palpation, live woody breast, chicken breast growth

Introduction

Chicken production is reaching an all-time record of 97.8 million tons this year, as consumer demand increases due to the sensory and nutritional properties, the relatively lesser cultural and religious restrictions on chicken consumption, and more importantly, the low cost of the meat (USDA, 2018). In order to meet the growing demand for poultry products increases in bird growth are the responses of selection and improved nutrition. Enhancing chicken growth has led to the development of woody breast (WB), a muscular dysfunction that impacts consumer purchasing decisions of fresh breast meat. Woody breast fillets are characterized by paleness and hardness when palpated. Severe cases can also present clear exudate, with petechiae or hemorrhagic regions and different texture over the breast surface (Dalle Zotte et al., 2014; Sihvo et al., 2014; Petracci et al., 2015; Tijare et al., 2016; Kuttappan et al., 2016).

Evaluation of WB scores was described by Tijare et al. (2016), with scores ranging from 0 (normal breast fillets) to 3 (most severe WB); however, breast fillets are typically scored after deboning and preferably after chilling. Papah et al. (2017) observed WB in histology samples from 2 wks old chickens and palpated differences in breast firmness at 4 wks of age; however, these authors did not relate their ante mortem findings to WB severity scores at the processing day. There is evidence that breast fillet thickness is correlated with myopathies (Mudalal et al., 2015;

Zambonelli et al., 2016; Kuttappan et al., 2017; Mallmann et al., 2018), along with of the measurements and observation on the caudal shape of the bird at the processing day (Sun et al., 2017).

The ability to detect WB in live birds during grow out could provide a better understanding of development this myopathy and indispensable to breeding companies in the selection and breeding of broilers devoid of this meat quality defect. Thus, the aim of this research was to determine the association between growth measurements of the live bird and the *Pectoralis major* with the WB condition across the production life-cycle of broilers, and correlate results of WB between the live birds' measurements with the breast fillet WB.

Material and Methods

Birds and processing

The University of Arkansas Institutional Animal Care and Use Committee approved all procedures (protocol nos. 17044, 17080, and 18025). All birds were raised from day old until the processing day on diets formulated to meet NRC (1994) recommendations.

Live measurements were conducted by a single evaluator over the course of the study, using a caliper and a standard cloth measure tape on the cross section on wing insertion for the cranial region (CCRAN and TCRAN, respectively); over/along the keel bone considered the central area (CCENT and TCENT, respectively); and caudal region where the ribs end (CCAUD and TCAUD, respectively).

Also all birds were examined visually, and the breast muscle was bilaterally palpated in all the regions mostly concentrated on the cranial and caudal areas as described by Papah et al. (2017). Woody breast scores on live birds and processed were graded depending on the hardness of the breast on the 0 to 3 scoring system of Tijare et al.(2016) at 0.5 increments. Briefly, fillets were categorized as 0 if the fillets that were flexible throughout the fillet; 1 if the fillets were hard in the cranial region but flexible throughout the remainder of the fillet; 2 if the fillets that were hard throughout the fillet but remained flexible in mid-region to caudal-region of the fillet; and 3 if the fillets were extremely hard and rigid throughout the fillet. For live bird palpation, the hardness of the breast region was the primary factor.

Feed was withheld from all birds 10 h before the processing day, but birds had *ad libitum* access to water until transportation to the University of Arkansas Poultry Processing Plant (Fayetteville). All birds were weighed individually, electrical stunned at 11V, 11mA for 11s, bled for 1.5 min, soft scalded in 53.8ºC for 2 min, de-feathered, manually eviscerated in-line and pre-chilled for 15 min at 12°C in immersion tanks, followed by a 3-h chill at 1° C in immersion chill tanks. Carcasses were then transferred to ice filled tanks and aged for 2-h at 4°C before deboning. Ready-to-cook (RTC) weight of each carcass was recorded before the breast fillet was deboned from the carcasses, weighed, and scored for WB (Tijare et al., 2016) by the same evaluator. For statistical purposes, WB scores were merged into WB severity categories of normal (NORM; scores of 0 and 0.5), mild (MILD; scores of 1 and 1.5), and severe (SEV; scores ≤ 2). Breast fillets dimensions were also measured with calipers, including fillet length (at the longest point), cranial fillet width at the widest point, fillet thickness (thickest portion), and caudal length (1/3 of the fillet length) were measured using calipers (Mehaffey et al., 2006a).

Description of Each Experiment (Figure 1).

Experiment 1: A total of 60 day-of-hatch male heavy debone broiler chicks (A) were obtained from Cobb-Vantress (Siloam Springs, AR, USA). Chicks were individually neck-tagged and randomly located to 1 of 3 environmentally controlled chambers (3.7 m long × 2.5 m wide × 2.5 m high). Individual body weight, breast hardness palpation scores (WB palpation), and measurements were collected as previously described on cranial (CCRAN and TCRAN), central (TCENT), and caudal (CCAUD and TCAUD) regions were measured with a caliper (C) and a cloth measure tape (T) on d 19, 26, 33, 40, 47, and 54 of age. Birds were processed at 57 d of age at the University of Arkansas Processing Pilot plant as described previously.

Experiment 2: After hatch, a total of 140 day-of-hatch male heavy debone broiler chicks (A), from Cobb-Vantress (Siloam Springs, AR, USA), were placed in 7 floor pens (20 birds/pen). Bird had *ad libitum* access to water and commercial feed formulated appropriately the production phase. Individual body weight, breast hardness palpation scores, and measurements were collected as previously described on cranial (CCRAN and TCRAN), central (CCENT and TCENT), and caudal (CCAUD and TCAUD) regions were measured with a caliper (C) and a cloth measure tape (T) on d 1, 6, 13, 20, 27, 34, 48, and 55 by a single person. At 57 days, all birds were processed as described previously. After mortalities and processing, breast fillets from134 birds were scored for WB condition (Tijare et al. 2016) and fillet dimensional measures were recorded by the same scorer.

Experiment 3: After hatch, 320 male broilers from 4 different bird market types (heavy debone A, heavy debone B, fast-food, and tray-pack) were place in four litter pens (80 birds/pen). Individual body weight, breast hardness palpation, and measurements on TCRAN and CCAUD regions were measured at d 5, 12, 19, 26, 33, 40, 47 by a single person. At 52 d of age, birds were processed, but were not chilled in this experiment. Breast fillet was deboned from the carcass, weighed, and dimensions measured before being scored for WB (Tijare et al., 2016) by the same scorer, but compression force was not measured in this experiment.

Statistical analysis

Woody breast severity categories (NORM, MILD, and SEV) were considered as a binary response variable of 0 (NORM) or 1 (MILD and SEV). Spearman's correlations were calculated for all breast measurements, including live bird and breast fillet weights with the WB scores. All the experiments were combined according with the bird type. One-way ANOVA was used to detect differences in live bird weights and WB palpation measures throughout the growth phase. When a significant ($P \leq 0.05$) F-test was detected, means were separated using Tukey's HSD.

Three parameter Gompertz nonlinear growth curves were fit to model various breast measurements over the growing period comparing two WB categories and by the bird types. Tests of parallelism parameter estimate comparisons and equivalence tests were performed to compare the WB categories and further for the bird line differences. All data were performed with JMP® Pro 14 (SAS Institute Inc.,2018).

Results and Discussion

The present study evaluated live palpation hardness and physical dimensions of breast region in broiler chickens during their grow out period to approximately 8 wks. The aim was not to find the etiology of the abnormality, but to determine if there was an association with WB at market age.

Palpation and WB scores

Chickens were palpated as early 1 d of age (Exp. 2) to as late as 19 d of age (Exp. 1) for initial palpation scores and continued up to market age (52 to 57d depending on the study). When data form all the trials were combined, Spearman correlations were low ($r_s = 0.12$) initially but increased as birds got older (Figure 2). Even though, for all the data combined not counting on the experiments, correlations increased from 6 up to 19%, each week from 5 through 8 wks, and correlations reached r_s of 0.7 and 0.74 by weeks 7 and 8, illustrated in Figure 2. At the 2nd week it is possible to differentiate the affected ones with the normal ones, but the correlation is not significant (Figure 3). There was a significant correlation on week 3 of r_s =0.29, and then the correlation increases by 10% to the next week.

Since there is a correlation by the weeks and the WB palpation, it is possible to have a relationship with the different type of birds. At approximately 4 wks, all correlations were significant ($P<0.001$) and the correlation was r_s =0.38 for heavy deboned birds A (represented in all three experiments). These results agree with the study realized by Papah et al. (2017) that reported differences on the palpation with 4 wks of age. At 5 wks of age, birds had a correlation of r_s =0.53, an increase of 30% and the correlation increased linearly with the age of the birds. At 6 wks, there was a high correlation of r_s =0.56, and at 8 wks the correlation increased to r_s =0.82. Similarly, Griffin et al. (2018) reported in them results a detected tactile WB in live birds with 42 days, validating that is one of the most precise age to palpate the live birds on them results.

When data were analyzed by the types of birds the palpation correlation decreased. Tray pack birds showed a significant correlation only with 5 wks. WB palpation had a better correlation for the high breast yield birds (r_s =0.31) at 3 wks while the standard bird types were r_s =0.2 and not significant at the same age. Standard bird lines (fast food and tray pack birds) had a correlation above r_s =0.5 after the wk 6 while the high breast yield birds showed this correlation at wk 5. The type of bird will bias the WB palpation, because high breast yield broilers have more incidence of WB than standard yield ones (Petracci et al., 2013b; Lorenzi et al., 2014; Bailey et al., 2015; Trocino et al., 2015; Alnahhas et al., 2016; Livingston et al., 2019a). It is possible to differentiate the WB by manual palpation on live birds, also there is an impact with the bird strains, being the high breast yield ones easier to be detected in early ages.

While the correlation was increasing by the age as well as the severity of WB. It was able to find significant differences ($P<0.05$) on the WB palpation by the WB fillet scores on the 3rd week for the severe category and following a consistency over the other weeks for all the three categories of WB (Table 3, Figure 3). This interaction of WB palpation and weeks represents a possible selection during growout.

Bird weight and WB fillet scores

There are many studies that support that bird and breast weight are related with WB (Siller and Wight, 1978; Sihvo et al., 2014; Mudalal et al., 2015; Chatterjee et al., 2016; Zambonelli et al., 2016; Alnahhas et al., 2016; Dalle Zotte et al., 2017; Xing et al., 2017; Dalgaard et al., 2018). Bird weight correlations were not constant along the ages in this study. High breast yield bird B showed the most consistent linear increase over the ages, but the correlations were the max of r_s=0.4 at 6 and 7 wks and r_s =0.5 for the fast food birds at 7 wks (Table 2). Tray pack birds had a negative correlation with WB scores from week 1 to 3. The results showed the highest correlations at week 1 (r_s =0.28) and then at week 8 (r_s =0.24) for overall results (Table 4).

While the bird weight correlations were not significant, heavier birds seemed to present more severe WB than lighter birds (Papah et al., 2017). Further, body weight means in each WB palpation score for experiment 1 and 2 for each week was significantly different until week 6 (Figure 4). In this case, on week 4 and 6 had WB categories mild and severe, exhibited no significant difference in body weight from one another but for the unaffected birds (normal) exhibited the lowest means for body weight, and it was significantly different from the affected birds (mild and severe). On weeks 2, 3, and 5 there was a difference ($P<0.05$) between the three categories, normal birds were lighter than all the other categories. However, there were no differences (P>0.05) after the 6 weeks, meaning that on week 7 and 8, the birds had similar weights among the WB categories. The similar weights can explain the overall low correlation of body weight and WB scores.

Measurements and WB fillet scores

In this study, breast measurements, made with two dimensional (using caliper) or three dimensional to include depth (using tape), were made to assess relationship to palpation and WB fillet scores. Breast measurements could potentially improve the detection of WB during the growth period along with palpation. Overall, data presented the central areas as the most significant ones at the first week, though the correlation were weak (r_s =0.22 to caliper and 0.12 for the tape). However, correlations were not significant as the bird aged (Table 4). The measurements that were most correlated with WB scores were CCAUD and TCRAN. The difference in using tape from caliper was because of diameter dimensions around the breast with the tape and while caliper measures straight line of the breast not considering the shape, more related to 2D dimension. Following research results with breast footprint related to WB on the cranial, caudal height, and breast width (Mudalal et al., 2015; Zambonelli et al., 2016; Kuttappan et al., 2017). The correlations of the CCAUD was above 0.6 at 7 wks, agreeing with images studies done in carcasses (Owens et al., 2018).

However, when the bird type is considered, standard and high breast yield, mostly the high breast yield types were more correlated with the measurements TCRAN (r_s =0.5) and CCAUD (r_s =0.5) at 7wks. The strains had different peculiarities, such as tray pack birds showed a significant correlation only at CCAUD at 7 wks (r_s =0.39), while fast food birds had the highest correlations for TCRAN (linear growth from 0.34 to 0.55), and CCAUD (0.44 and 0.41 at 3rd and 4th week and then on week 7 r_s =0.48).

Since the correlations were mild for most of the measurements, the interaction by the weeks was highly significant ($P<0.001$). On Table 6, it is possible to observe the means separated by the WB categories and ages, supporting and maybe selecting the animals with more precision on the 4th week by the TCRAN, and on the 3rd by the TCAUD. However, the caliper measurements were not that classificatory between the ages and WB scores. It could separate the severe from the other two categories on the week 4 until the 6, but only at the week 8 was possible differentiate between all the three categories for CCRAN. The CCAUD did not show differences until the week 5 on all the WB scores representing a better selection, but at 8 weeks there is no difference between the means. Comparing all the results one of the best measurements is the TCRAN where even on week 1 it was able to differentiate the severe with the other categories. The differences on the WB scores are at least 0.7 cm from the normal to severe on week 4 then increasing to almost getting almost 2 cm of difference at 8 weeks.

Gompertz growth curves of breast fillet related with WB

A comparison of the growth curves using Gompertz model indicates the inflection point where the growth stops to increase, reaching them maximum rate. The asymptote is when the curve plateaus, and the growth rate is the velocity that the animal is growing.

WB live palpation start to differentiate around the second week (Figure 5) with a R^2 of 0.38. Supposedly, severe birds reach the maximum of WB with 24 days (Table 7), and it matches with the findings of the correlation. Nevertheless, WB severity does not stop at that age; otherwise, it could have a better correlation as well as the birds with early ages will present the abnormality more frequently in the processing plants. This growth curve is only representative.

Breast measurements change over time and by the bird type (Table 8). High breast yield A had a decrease in the growth (inflection point) for the cranial breast region with the measuring tape with 18 days of age while the other birds had them two days earlier. The two standard birds, fast food and tray pack birds had the same cranial size of the breast (28.9 cm) at the highest point (asymptote). Because of these similarities, they were pooled together in order to be able to differentiate the normal breast from the woody ones.

The measurements realized on the central region of the breast did not have differences between the normal and severe birds for the all the strains ($P>0.05$). Most of the differences were on the asymptote, but all the values were estimated outside with the model ahead of the ones collected.

The Gompertz curve ($R^2=0.98$) differences on standard birds (fast food and tray pack) at TCRAN had an inflection point earlier, meaning that these broilers change the growth with 16 day while the heavy birds change them growth two day later for the normal and three for the WB bird (Table 9). The size of the breast matters at the end, measurements were realized in other studies over the breast fillet and was able to create a model with the footprint (Griffin et al., 2018; Mallmann et al., 2018). Heavy birds had bigger breast but severe ones were 34.7 cm (CI of 33.8 – 35.7) while the normal was 31.7 cm (CI of 30.5 – 32.9). Standard birds also had different size of the breast by the WB, normal breast were 28.9 cm (CI of 28.1 – 29.8) and WB were 30.2 cm (CI of 28.8 – 31.5). Finally, bird measurements on live birds could help to differentiate the WB scores between the strains increasing the possibilities of intervention on the farm.

Conclusion

Overall, the findings of the present study reveal that is possible to palpate WB by a trained person in live broilers with 3 weeks old and increases with the age but can be susceptible to differences depending on the genotype. Therefore, these birds could be selected for better understanding of the preventive ways to decrease the incidence of this abnormality. In addition, breast live measurements reported to vary much depending on the type of birds. One of the most relevant measurement realized was the TCRAN showing the highest differences between the measurements by the WB birds and the non-affected.

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Figures and Tables

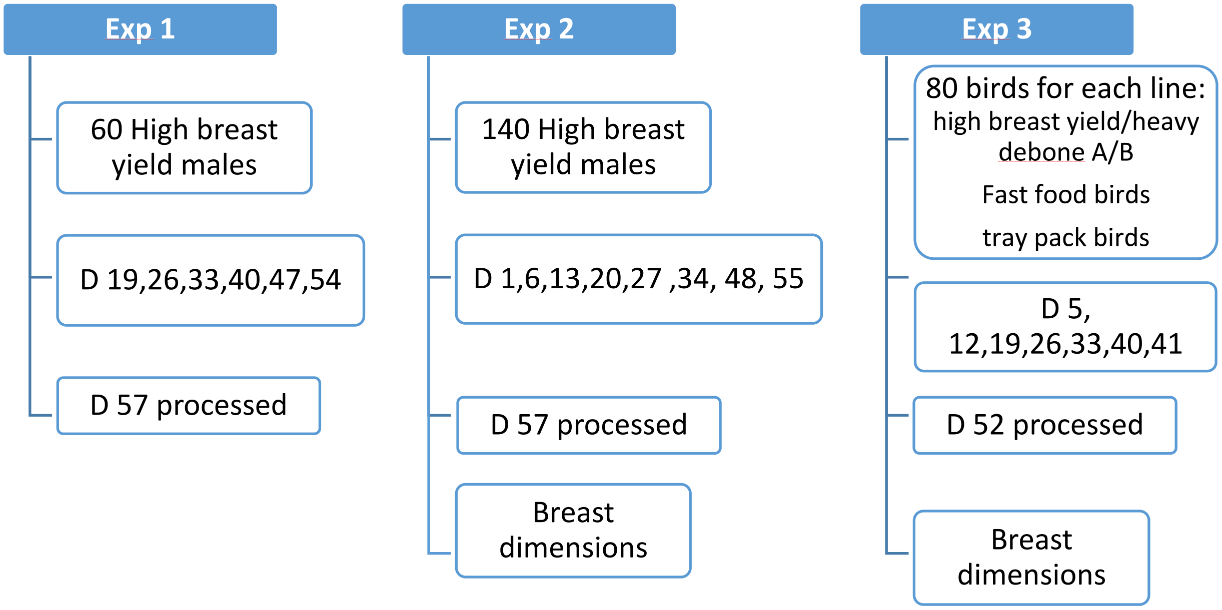

Figure 1. Summary of experiments data collection.

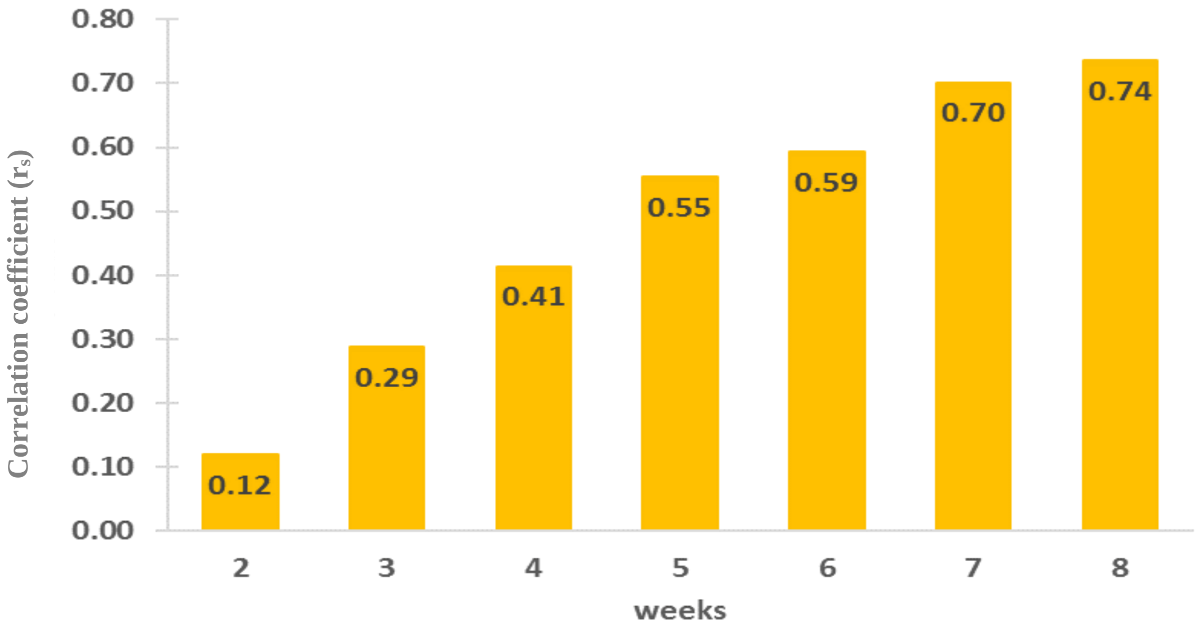

Figure 2. Correlation of WB palpation and WB score in the processing day. Data from all experiments were pooled for analysis. WB palpation was conducted at a given age and correlated withWB scored assessed at day of processing.

Table 1. Spearman's correlation (r_s) by week of WB palpation[1] and WB fillet scores[2] by the bird types[3].

Wks	**High yield birds A**	**High yield birds B**	**Fast food birds**	**Tray pack birds**
2	0.13	0.08	0.04	-0.13
3	0.23**	0.31**	0.19	0.19
4	0.38***	0.45***	0.37*	0.12
5	0.53***	0.41**	0.37*	0.24
6	0.56***	0.64***	0.55***	0.56***
7	0.69***	0.73***	0.60***	0.64***
8	0.82***			

[1] WB palpation realized in live birds weekly.[1]WB fillet scores evaluated on the processing day.

[2] WB fillet scores evaluated on the processing day.

[3] Birds divided into types based on their typical market segment.

Table 2. Spearman's correlation (r_s) by week of bird live weight and WB fillet scores[1] by the bird types[2].

Wks	Heavy debone birds A	Heavy debone birds B	Fast food birds	Tray pack birds
1	0.36***	0.09	0.13	-0.21
2	0.30***	0.20	0.19	-0.25
3	0.15*	0.33**	0.43***	-0.09
4	0.19**	0.33**	0.40**	0.02
5	0.26***	0.37**	0.30*	0.06
6	0.21**	0.40**	0.40**	0.08
7	0.19**	0.39**	0.51***	0.18
8	0.24**			

[1] WB fillet scores evaluated on the processing day.

[2] Birds divided into types based on their typical market segment.

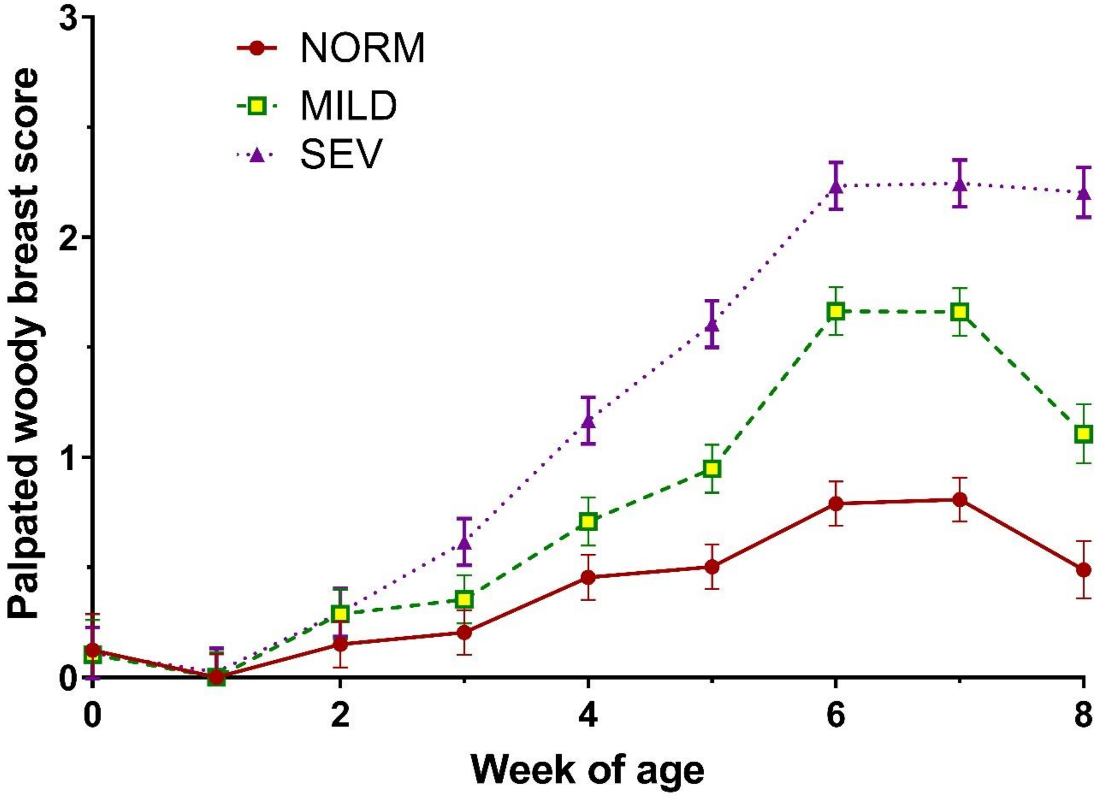

Figure 3. Differences over the weeks on WB palpation scores during growout. NORM (score 0), MILD (scores 1+1.5), and SEV (scores >2).

Table 3. Palpation WB score by the weeks and the different categories of WB.

	Palpated WB (WBS × Age, $P < 0.0001$)		
Wks	NORM	MILD	SEV
0	$0.1^{abc} \pm 0.17$	$0.1^{abc} \pm 0.16$	$0.1^{abc} \pm 0.12$
1	$0.0^{a} \pm 0.10$	$0.0^{a} \pm 0.11$	$0.0^{ab} \pm 0.11$
2	$0.2^{bc} \pm 0.10$	$0.3^{c} \pm 0.11$	$0.3^{c} \pm 0.11$
3	$0.2^{bc} \pm 0.10$	$0.3^{cd} \pm 0.11$	$0.6^{e} \pm 0.11$
4	$0.5^{d} \pm 0.10$	$0.71^{ef} \pm 0.11$	$1.2^{i} \pm 0.11$
5	$0.5^{de} \pm 0.10$	$0.9^{gh} \pm 0.11$	$1.6^{j} \pm 0.11$
6	$0.8^{f} \pm 0.10$	$0.8^{f} \pm 0.11$	$2.2^{kl} \pm 0.11$
7	$0.8^{fg} \pm 0.10$	$1.7^{j} \pm 0.11$	$2.2^{l} \pm 0.11$
8	$0.5^{de} \pm 0.13$	$1.1^{hi} \pm 0.13$	$2.2^{k} \pm 0.11$

$^{a-k}$Within a dependent variable, interactive least squares leans lacking a common superscripted letter differ ($P \leq 0.05$).

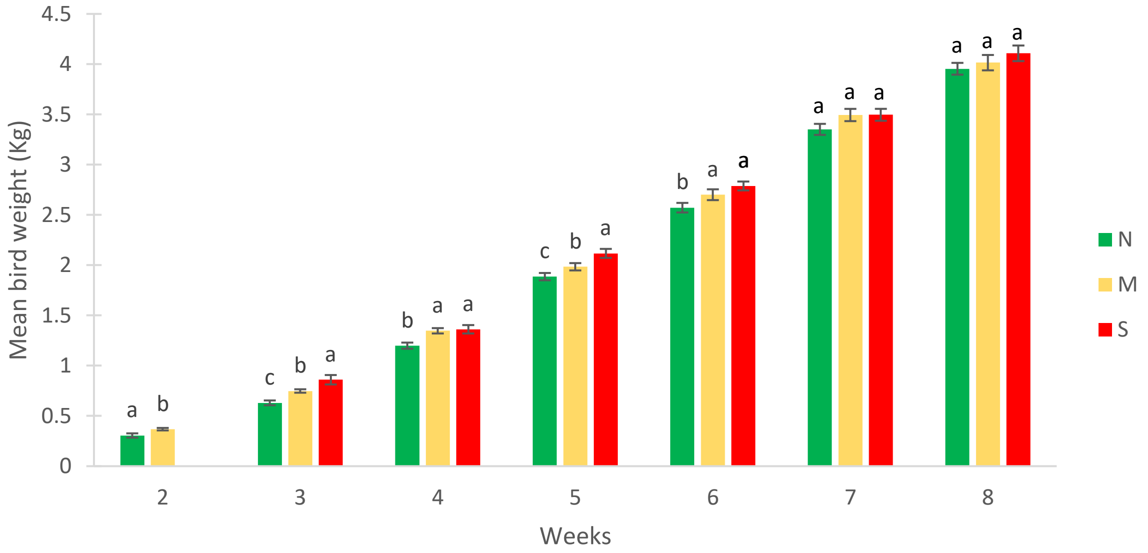

Figure 4. Weekly growth weights of live birds by WB palpation. Means showing different letters in each week are significantly different (*$P<0.05$*). WB palpation normal-N (0+0.5), mild-M (1+1.5), and severe-S (above 2). At 2 weeks it was only absence or presence, green represents the absence and yellow the presence.

Table 4. Overall Spearman's correlation of WB fillets score[1] with the bird weight and measurements by week.

Parameters	**Weeks**								
	0	1	2	3	4	5	6	7	8
Bird wt	-0.01	0.28***	0.13*	0.13*	0.12*	0.13**	0.1*	0.09*	0.24***
CCRAN[2]	0.07	0.21*	0.1	0.26**	0.41***	0.44***	0.39***	0.51***	0.11
TCRAN[3]	-0.07	0.39***	0.12*	0.23***	0.36***	0.41***	0.5***	0.5***	0.3***
CCENT[2]	0.22*	0.02	0.03	0.12	0.06	0.12	0.03	0.08	-0.09
TCENT[3]	0.14	0.12	0.04	-0.05	0.12	0.04	0.18*	0.13	0.18*
CCAUD[2]	-0.15	0.37***	0.32***	0.25***	0.33***	0.51***	0.54***	0.61***	0.05
TCAUD[3]	-0.08	0.07	0.13	0.41***	0.34***	0.54***	0.51***	0.48***	0.51***

[1] WB fillet scores evaluated on the processing day.

[2] Broiler breast measurements made with a caliper (2 dimensional).

[3] Broiler breast measurements made with a cloth measure tape (3 dimensional).

Table 5. Spearman's correlation of WB fillets scores[1] with the measurements by the bird type.

	Parameters	Weeks 1	2	3	4	5	6	7
TCRAN[3]	High yield birds A	0.26	0.25	0.16	0.24	0.31*	0.48***	0.53***
	High yield birds B	0.07	0.20	0.21	0.35**	0.43***	0.47***	0.56***
	Fast food birds	0.05	0.10	0.34**	0.36**	0.39***	0.56***	0.55***
	Tray pack birds	-0.13	-0.19	0.00	0.21	0.03	0.08	0.16
CCAUD[2]	High yield birds A	-0.14	0.13	0.09	0.17	0.38**	0.37**	0.53***
	High yield birds B	0.17	0.15	0.36**	0.31**	0.34**	0.35**	0.51***
	Fast food birds	0.24	0.22	0.44***	0.41***	0.23	0.24*	0.48***
	Tray pack birds	-0.21	-0.26	-0.02	0.16	0.09	0.18	0.39***

[1] WB fillet scores evaluated on the processing day.

[2] Broiler breast measurements realized with a caliper on the caudal region (end of the ribs).

[3] Broiler breast measurements realized with a cloth measure tape along the cranial region (wing to wing insertion).

Table 6. Differences of breast fillets WB score with the measurements by the weeks.

	TCRAN (WBS × Age, *P* < 0.0001)			**TCAUD** (WBS × Age, *P* < 0.0001)		
Wks	NORM	MILD	SEV	NORM	MILD	SEV
0	$4.8^{a} \pm 0.49$	$4.6^{a} \pm 0.49$	$4.7^{a} \pm 0.45$	$3.3^{ab} \pm 1.08$	$3.3^{ab} \pm 1.08$	$3.2^{a} \pm 1.07$
1	$5.4^{b} \pm 0.45$	$5.5^{b} \pm 0.45$	$5.8^{c} \pm 0.45$	$3.7^{b} \pm 1.08$	$3.4^{ab} \pm 1.08$	$3.7^{b} \pm 1.07$
2	$9.7^{e} \pm 0.45$	$9.6^{de} \pm 0.45$	$9.4^{d} \pm 0.45$	$5.3^{c} \pm 1.08$	$5.1^{c} \pm 1.08$	$5.5^{c} \pm 1.07$
3	$13.0^{f} \pm 0.44$	$12.9^{f} \pm 0.45$	$13.1^{f} \pm 0.45$	$8.3^{d} \pm 1.07$	$8.2^{d} \pm 1.07$	$8.6^{e} \pm 1.07$
4	$16.5^{g} \pm 0.44$	$16.6^{g} \pm 0.45$	$17.2^{h} \pm 0.45$	$10.9^{fg} \pm 1.07$	$10.6^{f} \pm 1.07$	$11.0^{g} \pm 1.07$
5	$19.6^{i} \pm 0.44$	$20.0^{j} \pm 0.45$	$20.7^{k} \pm 0.45$	$13.1^{h} \pm 1.07$	$13.0^{h} \pm 1.07$	$14.0^{i} \pm 1.07$
6	$22.4^{l} \pm 0.44$	$23.0^{m} \pm 0.45$	$23.9^{n} \pm 0.45$	$14.8^{j} \pm 1.07$	$15.0^{j} \pm 1.07$	$15.9^{k} \pm 1.07$
7	$24.5^{o} \pm 0.44$	$25.3^{p} \pm 0.45$	$25.8^{q} \pm 0.45$	$16.3^{l} \pm 1.07$	$16.6^{l} \pm 1.07$	$17.1^{m} \pm 1.07$
8	$25.8^{q} \pm 0.46$	$26.5^{r} \pm 0.47$	$27.1^{s} \pm 0.45$	$16.8^{m} \pm 1.07$	$17.5^{n} \pm 1.07$	$17.9^{o} \pm 1.07$

	CCRAN (WBS × Age, *P* = 0.0021)			**CCAUD** (WBS × Age, *P* < 0.0001)		
Wks	NORM	MILD	SEV	NORM	MILD	SEV
0	$1.6^{a} \pm 0.23$	$1.6^{a} \pm 0.22$	$1.7^{a} \pm 0.18$	$1.7^{a} \pm 0.43$	$1.6^{a} \pm 0.43$	$1.6^{a} \pm 0.41$
1	$2.4^{b} \pm 0.23$	$2.4^{b} \pm 0.22$	$2.6^{b} \pm 0.18$	$2.3^{b} \pm 0.41$	$2.3^{b} \pm 0.41$	$2.2^{b} \pm 0.41$
2	$4.3^{c} \pm 0.23$	$4.2^{c} \pm 0.22$	$4.4^{c} \pm 0.18$	$3.1^{c} \pm 0.41$	$3.0^{c} \pm 0.41$	$3.0^{c} \pm 0.41$
3	$5.7^{de} \pm 0.23$	$5.4^{d} \pm 0.22$	$6.0^{e} \pm 0.18$	$4.3^{d} \pm 0.41$	$4.3^{d} \pm 0.41$	$4.2^{d} \pm 0.41$
4	$7.7^{f} \pm 0.19$	$7.6^{f} \pm 0.19$	$8.1^{g} \pm 0.17$	$5.9^{e} \pm 0.41$	$5.9^{e} \pm 0.41$	$6.0^{e} \pm 0.41$
5	$9.8^{h} \pm 0.19$	$9.9^{h} \pm 0.19$	$10.5^{i} \pm 0.17$	$7.1^{f} \pm 0.41$	$7.4^{g} \pm 0.41$	$7.9^{h} \pm 0.41$
6	$11.9^{j} \pm 0.19$	$12.0^{j} \pm 0.19$	$12.5^{k} \pm 0.17$	$8.1^{i} \pm 0.41$	$8.5^{j} \pm 0.41$	$9.2^{l} \pm 0.41$
7	$13.1^{l} \pm 0.19$	$13.3^{l} \pm 0.19$	$13.7^{m} \pm 0.17$	$8.9^{k} \pm 0.41$	$9.4^{l} \pm 0.41$	$10.4^{m} \pm 0.41$
8	$14.6^{n} \pm 0.19$	$15.1^{o} \pm 0.19$	$15.5^{p} \pm 0.17$	$12.9^{n} \pm 0.43$	$12.9^{n} \pm 0.43$	$12.8^{n} \pm 0.41$

$^{a-s}$Within a dependent variable, interactive least squares leans lacking a common superscripted letter differ ($P \leq 0.05$).

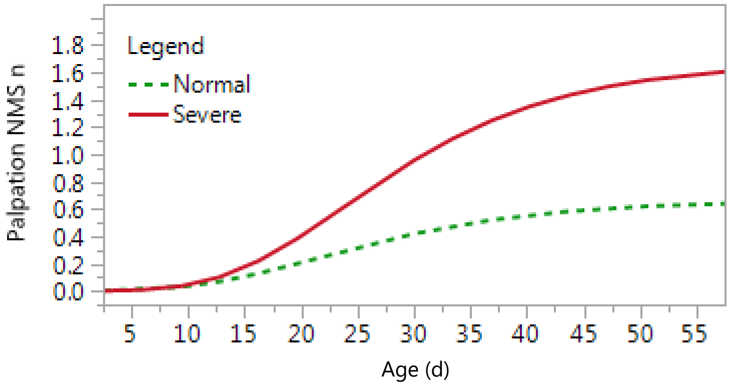

Figure 5. Gompertz curves differences of WB palpation by WB fillet scores over the weeks.

Table 7. Gompertz curve parameters for WB palpation by WB fillet scores.

Parameter	**WB**^{1}	**Estimate**	**Std Error**	***Prob > Chi-square***	**Lower 95%**	**Upper 95%**
Asymptote	Normal	0.66	0.08	*0.0001*	0.52	0.81
Growth	Normal	0.09	0.03	*0.0006*	0.04	0.15
Inflection Point	Normal	21.6	1.99	*0.0001*	17.7	25.5
Asymptote	Severe	1.68	0.07	*0.0001*	1.55	1.8
Growth	Severe	0.09	0.01	*0.0001*	0.07	0.11
Inflection Point	Severe	23.8	0.68	*0.0001*	22.5	25.1

[1] WB scores: Normal (0+0.5), Severe (1+1.5+2+2.5+3)

Table 8. Bird type differences on the breast chicken measurement (cm) at the cranial region with a cloth tape by the days.

Parameters	**Bird type**	**Estimate**	**Std Error**	**Prob > Chi-square**	**Lower 95%**	**Upper 95%**
Asymptote (cm)	High breast yield B	30.61	0.56	<.0001	29.5	31.7
	Fast food birds	28.91	0.5	<.0001	27.9	29.9
	Tray pack birds	28.97	0.32	<.0001	28.3	29.6
	High breast yield A	33.27	0.29	<.0001	32.7	33.8
Inflection Point (d)	High breast yield A	18.27	0.24	<.0001	17.8	18.7
	High breast yield B	16.43	0.44	<.0001	15.6	17.3
	Fast food birds	15.97	0.41	<.0001	15.2	16.8
	Tray pack birds	15.2	0.25	<.0001	14.7	15.7

Table 9. WB differences of polled bird type on the breast chicken measurement (cm) at the cranial region with a cloth tape by the days.

Parameter	**Bird type**	**WB[1]**	**Estimate**	**Std Error**	**Prob > Chi-square**	**Lower 95%**	**Upper 95%**
Asymptote (cm)	Heavy debone[2]	N	31.73	0.601	<.0001	30.5	32.9
		S	34.74	0.486	<.0001	33.8	35.7
	Standard[3]	N	28.91	0.429	<.0001	28.1	29.8
		S	30.15	0.689	<.0001	28.8	31.5
Inflection Point (d)	Heavy debone[2]	N	18	0.478	<.0001	16.6	18.5
		S	19	0.384	<.0001	18.4	19.9
	Standard[3]	N	16	0.343	<.0001	15.0	16.3
		S	16	0.527	<.0001	15.3	17.3

[1] WB scores: Normal (0+0.5), Severe (1+1.5+2+2.5+3).

[2] Pool of high breast yield A and B.

[3] Pool of fast food birds and tray pack birds

Chapter IV. **INCIDENCE OF WOODEN BREAST MYOPATHY BY BIRD AGE, GENDER, AND STRAIN**

Abstract

The objective of this study was assessing the impact of strain, sex, season, and age on breast myopathies. Fillet attributes were also assessed to evaluate the characteristics of wooden breast (WB) by the breast fillet weight and thickness by different ages. A total of 10,737 for three broilers types were processed on 5, 6, 7, 9, and 10 weeks of age in multiple trials over a three-year period. Following processing, carcasses were deboned and breast fillets were weighed, and measured for length, width of the caudal region, width of the cranial region and thickness of the cranial region. Fillets were then scored for WB using a scale from 0 to 3 with 0.5 (Tijare et al., 2016). Fillets were hot-boned and scored at approximately 1 h postmortem. For analysis, categories were combined and referred to as Normal (0+0.5), Mild (1+1.5), and Severe (2+2.5+3) and binary N (0 and 0.5) and Sev (above score 2). Data were analyzed using generalized regression and chi-square in JMP Pro 14 with strains, age, sex, breast fillet weight, and measurements fit as the effects. As age increased from 5 to 10 weeks, broiler size (body weight, fillet weight) and incidence of mild and severe WB increased (p<0.01). At 5 weeks of age, the incidence of mild and severe was 15.9% and this incidence increased to 52.8% at 10 weeks for overall data. Males had greater severity than females in all the age periods (p<0.01). Most of the data concentrates on 7 weeks of age (n=4630); males had 42.4% of Mild and Severe while Females show 21.2% of incidence of the same levels of WB. In addition, WS had a significant interaction by gender and age with females showing as age increases the differences are more prominent between the sexes. The thickness of the breast fillet has an impact on the WB (r_s=0.59), whenever the thickness increases the WB severity also increases. Spaghetti meat (SM) also was evaluated showing a highly difference (*$p<0.001$)* on 7 weeks of age, where females presented 10.2% of the breast analyzed on that age, while males

presented 3.2%. The presence of WB, WS and SM is still an issue in current commercial broiler flocks.

Key words: white striping, wooden breast, spaghetti breast, marked strains, frequency.

Introduction

Chicken meat is one of the most consumed meat around the world ("OECD-FAO agricultural outlook, 2008-2017," 2013). The consumption has increased over the years because how versatile, easy, healthy, cheaper than the other meats, and non-cultural and non-religious concerns. However, the inflation on consumption and production of chicken was able because the progress work of the genetic selection and the good nutrition requirements for the growth of the bird in seven weeks. Studies done by Havenstein et al. (2003) and Zuidhof et al. (2014) related old lined chicken breeds from 1950 with 2000's, they compared he differences on the bird growth and the performance of them as well the parts yield. Geneticists select the best performance broilers to be selected, looking for the lower feed conversion, and high processing yield. On the other hand, this selection affected birds' health, like lameness, ascites, and the meat quality of these animals by increasing the amount of myopathies.

Breast myopathies are a hot topic subject for the poultry meat quality. These myopathies harms meat quality by the appearance, texture, water holding capacity, and nutritional components (Petracci et al., 2019). Since the meat quality is compromise, industry downgraded increasing the economic losses (Huang et al., 2018). The most mentioned myopathies discussed now are white striping, wooden breast, and spaghetti meat. These three conditions affect the chicken breast muscle (*Pectoralis major)* and a classification depend on the degree of severity by visualization and/or manual palpation of this muscle. White striping (WS) consist in white striations following the muscle fibers on the surface of the raw chicken breast, normally appear on the cranial region close to the wing insertion and can extend through all the breast muscle (Kuttappan et al., 2013c; b; Ferreira et al., 2014) Wooden breast (WB) is represented by the hardness of the whole breast fillet and could be pale, exudative, and hemorrhagic on the surface (Sihvo et al., 2014; Kuttappan et al., 2016; Griffin et al., 2018).The toughness of the breast varies in intensity and region or broadcast (Tijare et al., 2016; Sihvo et al., 2018b). Spaghetti meat (SM) is characterized by the loosing, friability, and separation of bundles of muscle fibers, mostly focus on the cranial region, also it is possible to finger pinch the muscle(Sirri et al., 2016; Baldi et al., 2018; Zampiga et al., 2019).

The aim of this study is to evaluate the incidence of breast myopathies, body weight, and breast yield over three years of data collection in broilers with several factors: ages, sex, and strains along with carcass and breast yield.

Material and Methods

A total of 10,737 birds were processed at the University of Arkansas from 2016 to 2018. In each processing, there were equal number of males and females within the strains. Each trail consists of 280 broilers from the same age. Strain varied by trial and the birds used was from four different lines, the standard birds were separated in fast food birds and tray pack birds. The other two lines were pooled together in heavy debone birds. A commercial-style processing in-line system was used where the birds were electrically stunned, manually slaughtered by severing the left carotid artery and jugular vein, bled out, soft scalded, and defeathered. The carcasses were then manually eviscerated and deboned. Live birds weight, carcass weight and deboned parts were weighed and recorded. Fillets were hot-boned and scored at approximately 1 h postmortem. Breast fillets were measured for length, width of the caudal region, and width of the cranial region and thickness of the cranial region. Fillets were scored for WS, WB, and presence or absence of SM. White striping was scored by the thickness of the stripes according to Kuttappan et al. (2012) demonstrated on Figure 2. Woody breast used methods developed by Tijare et al. (2016), scores from 0 to 3 with 0.5 scale depending on the level of hardness of the fillets (Figure 1). For analysis, categories were combined and referred to as Normal (0+0.5), Mild (1+1.5), and Severe (2+2.5+3). Spaghetti meat was classified if present (1) when the meat was stringy and the surface was easily punctured through the muscle fascia or normal (0). Two people, experienced in scoring, scored fillets throughout the study period.

Statistical analysis

Data was analyzed with chi-square test ($P<0.05$) for the incidence of myopathies over the years, wks, sex, strain, and processing weights with JMP® Pro 14 (SAS Institute, 2018). Least squared means, interaction, and generalized regression were analyzed using ANOVA with the myopathies fixed source of variation. Means were compared using Tukey's HSD test considered significant at $P<0.05$ (SAS Institute, 2018). Bird weights were divided into equal-sized bins, to be able to visualize the relation between them with the myopathies. It was obtained by binning procedure in JMP), in 454g increments and mean score was plotted.

Results and Discussion

The presence and severity of muscle abnormalities in broilers of various ages, strains, and gender were assessed in a three year study. The incidence of WB and WS for overall dataset are presented in Table 1 and 2. The results showed that the severity of WB incidence (scores ≥ 2) increased (chi-square<0.05*)* in over the three years of analyses, the first year had a lower incidence of severe WB (18.2%) compared with the other two years (22.6 and 27.4%). Nevertheless, WS decreased from 19.6% to 13.9% in the severe cases and mild WS tended to decrease whereas the normal fillets tended to increase over that time period.

Since over the years the incidence was increasing for WB and decreased for WS, it could be related with the different type of birds or with the improvement of the meat quality. The fast food birds had the WB lower incidence of 81.3% while 72.3% for tray pack birds, and the heavy debone showing higher (19.3%) incidence of severe scores for WB (Figure 3). This data supports the trends that heavy debone broilers have higher WS incidence of 23.8% compared to standard birds (*i.e.* similar to tray pack in this study) (Table 2) (Petracci et al., 2013; Lorenzi et al., 2014; Bailey et al., 2015; Alnahhas et al., 2016; Livingston et al., 2019). Differently of this study, Trocino et al. (2015), did not find significant differences for WB between the two different strains of standard and high yield broilers at 46 d.

Age of broilers at processing age was highly significant (chi-square<0.05) showing an increase of severities of WS and WB with the increase of the age and the weight. In addition, the age is only based on a specific range of the bird weight for a product type. For example, , the severe WB incidence increases almost doubles (5.8% to 10.6% respectively from 6 to 7 wks as well as from 7 to 9 wks to20.2% (Figure 5). The results agree with the incidence presented by Cruz et al.(2017), Papah et al. (2017), and Griffin et al. (2018). White striping increases with the slaughter age as well, starting with 1.7% at 5 weeks increasing to 10.2% at 6 weeks and then almost 4 times more at 9 weeks with 37.7% of incidence for severe cases of WS.

The relationship by the bird weight and the myopathies were increasing by the weights (Figure 9). Bird weight had moderate correlation with WB (r_s =0.36) and WS (r_s =0.46). As well as the age, the same pattern happened with the bird weight (Figure 10). There is a 50% chance of birds that have 4.54 kg to present WB. There is an increase of 12% on the severity of WB for the increase of 1 kg. It is already known there is a higher probability of a high breast yield bird to have more WB than the other strains. Even though if the birds had the same range of weight like on Figure 11 at the range of 3.18 kg, the high breast yield had almost 50% of the birds with some level of WB while the fast food had 15.2% and the tray pack had the double of the fast food bird. However, it could be simple to change for a tray pack bird or a fast food bird to try to avoid WB, but also need to think about the cost of the growth and the yield produced at the end.

Breast yield is one of the most important features of broilers and correlated (r_s =0.5 and r_s =0.45) with the WS and WB, reported at Figure 6. White striping and WB increase the incidence with the breast yield growth, differently from the breast weight when the WB and WS does not have differences after 1.2 kg of breast fillet. The interaction of processing day by bird type and the WB categories showed that birds that have more than 30% of average of the breast yield have 50% of chances to present WB (Figure 7). This was the max for overall the animals, but when analyzing by the types it is possible to grown them until 30.3% at 9 wks for the heavy debone birds. The other two types were max of 28.6% at 10 wks for fast food birds and 28.7% at 9 wks for tray pack birds being heavier at that age than with 10 wks (Figure 8). Fast food birds are lighter than the other strains, for this reason there is less animals on the higher end of the breast yield.

Gender differences were significantly different (chi-square<0.05) for both myopathies, males had more mild (25% for WB and 45.8% for WS) and severe (18.4% for WB and 17.8%for WS) compared with females with mild WB of 17.4% and 42.4% for WS. The same significant difference also were found in other research from Kuttappan et al. (2013), Lorenzi et al. (2014), and Trocino et al. (2015). Some countries still sexing broilers for better fulfill them market with the specific products. As well as the bird strain; tray pack birds have the same amount of severe WB and for males and females of a mild score of 14.2%, showed in Figure 4. High breast yield male broilers had two times more WB than females for severe WB (26% and 13%, respectively).

Fast food birds represent the lowest incidence on WB where most of the fillets were normal, 88% for females and 75% for males.

Spaghetti meat has a different behavior where it manifests more in the lightweight birds and females. For the spaghetti meat myopathy, bird type differences do not follow the same pattern of WB incidence (Table 4). Heavy debone birds and the tray pack birds are not different between each other, 5.5% and 5.4% of incidence, respectively (Table 4). Differently from the WB, the females (7.8%) present three times more this abnormality than the males (2.3%).

Tray pack birds presents' higher incidences for females on SM with 6 and 7 weeks. There was a significant difference at 5 and 7 weeks of age, at 35 days tray pack birds presented 4.5% compared with 2% of high breast fillets and 0.8% for fast food birds (Figure 12). With 49 days of age, birds were significantly different (chi-square<0.005), high breast had 7.4%, followed by tray pack birds 7.1%, and 3.1% for fast food. At 49 d, females present three times more SM than males. Baldi et al. (2018) reported that breast fillets of SM were heavier than normal with 47d of age, and after 9 wks, the differences are opposite showing normal breast fillets heavier than the SM ones. As well as WS, SM have more incidences in females (7.5%) than in males (2.2%).

Conclusion

The results presented in this study showed the demographics on the most used bird strains in the U.S. broiler market. Among the type of birds there are differences in the incidence of breast myopathies, showing that high breast yield birds have more incidence on WB. As well as effect of gender where males had more WB and females had more SM. Age is another factor that is important for the breast myopathies. the WS and WB myopathies increase as the birds get older, but the majority of SM was observed at a younger market age (e.g., 7 wk). Breast yield and bird weight play a role on the problem as well. Overall, the larger birds with higher breast yield are more affected by myopathies (WB and WS), especially in the severe form.

The data presented could analyze the actual frequency and the differences on the effect of gender, breast and live weights, age and strains. The importance of this is to be able to help the industry to improve and guide for decision making to hopefully prevent the loss that myopathies are causing.

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Figures and Tables

Figure 1. Woody breast (WB) score methodology used to classify the different rigidity along the breast fillet.

Figure 2. White stripping score methodology used to classify the different white striations along the breast fillet (Kuttappan et al., 2016).

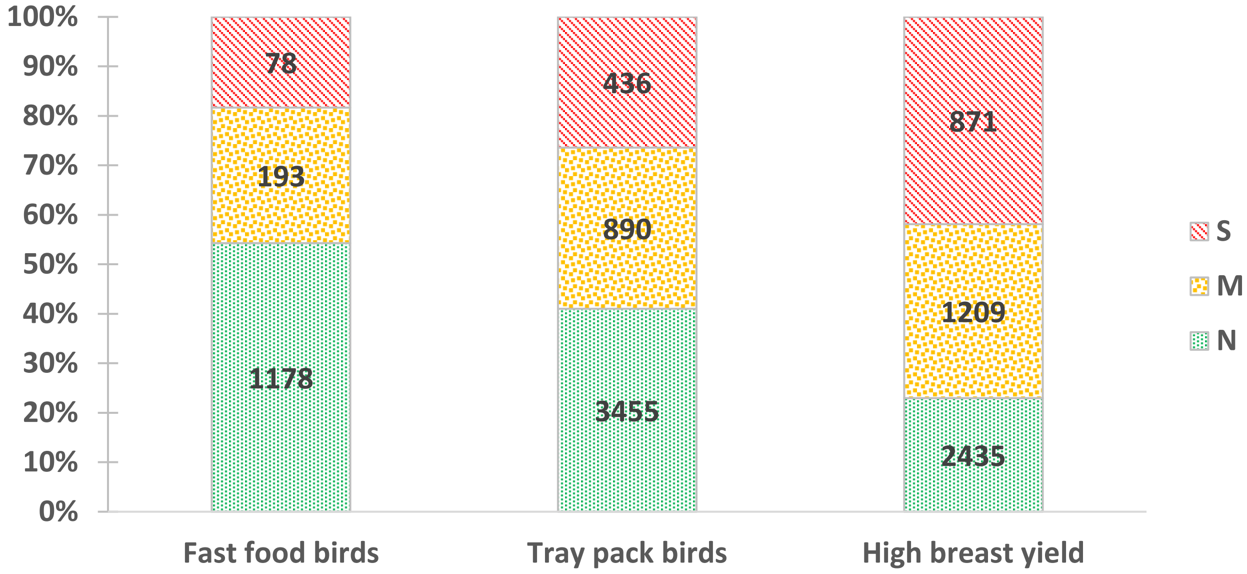


Figure 3. Incidence of WB over the different type of birds. Values in the bars represent the percentage of the total.

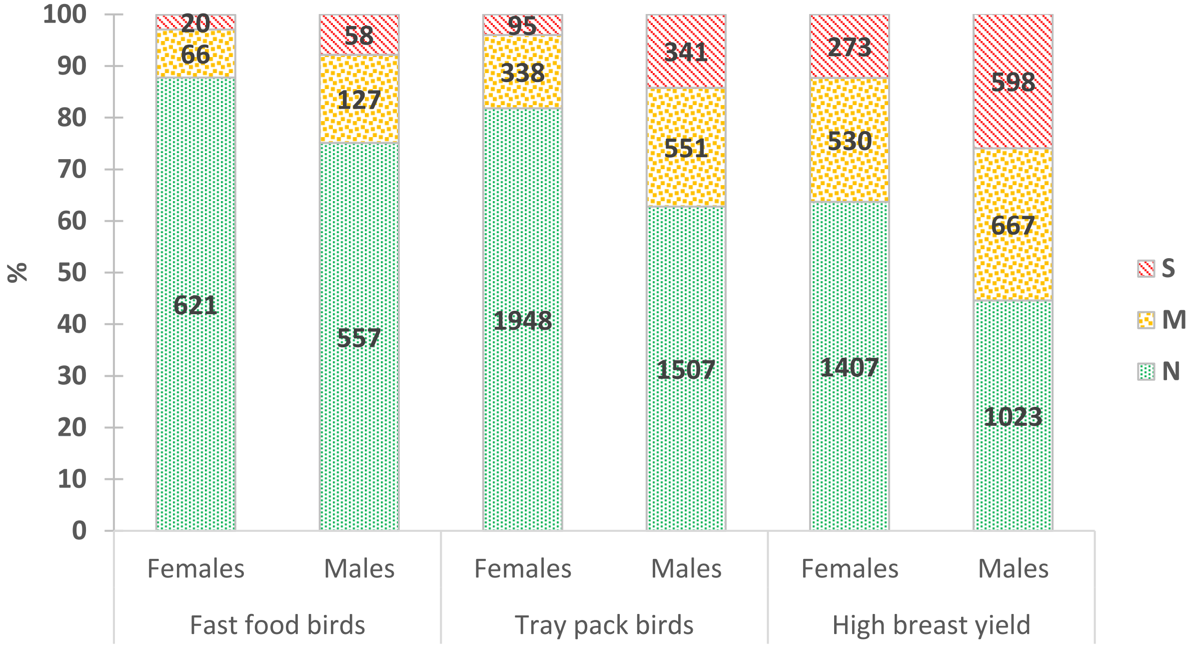


Figure 4. WB incidence by the bird type and the sex. Values in the bars represent the number of birds in each category.

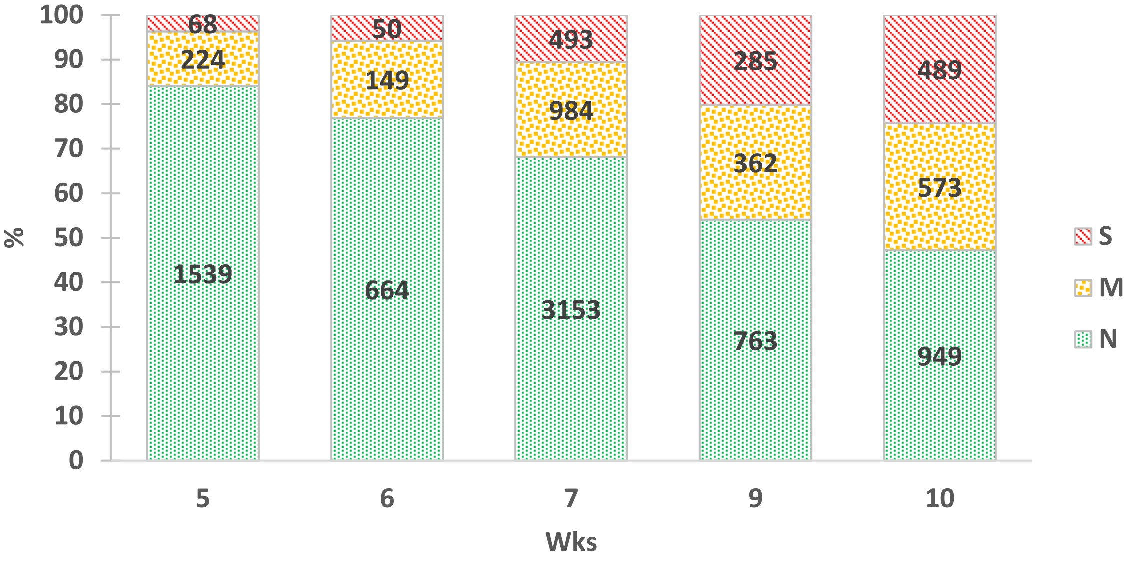


Figure 5. Incidence of WB by the age. Values in the bars represent the number of birds in each category.

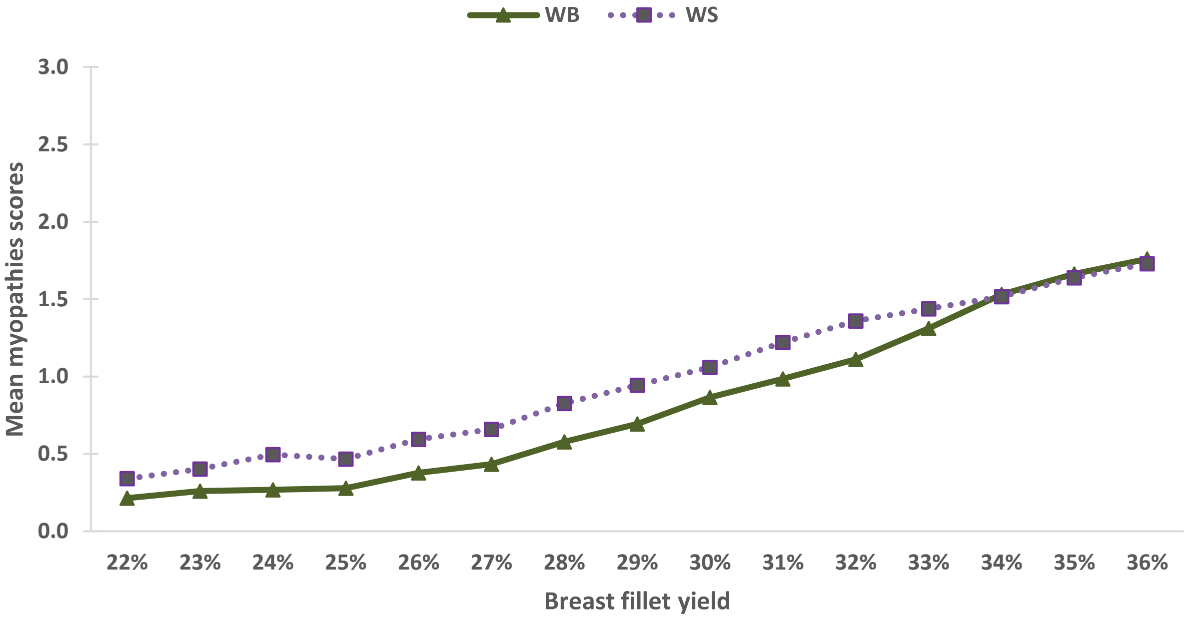


Figure 6. Relationship between breast fillet weight (g) and myopathies scores (WS – white striping (r_s=0.50) ; WB – wooden breast (r_s=0.45)) in broilers.

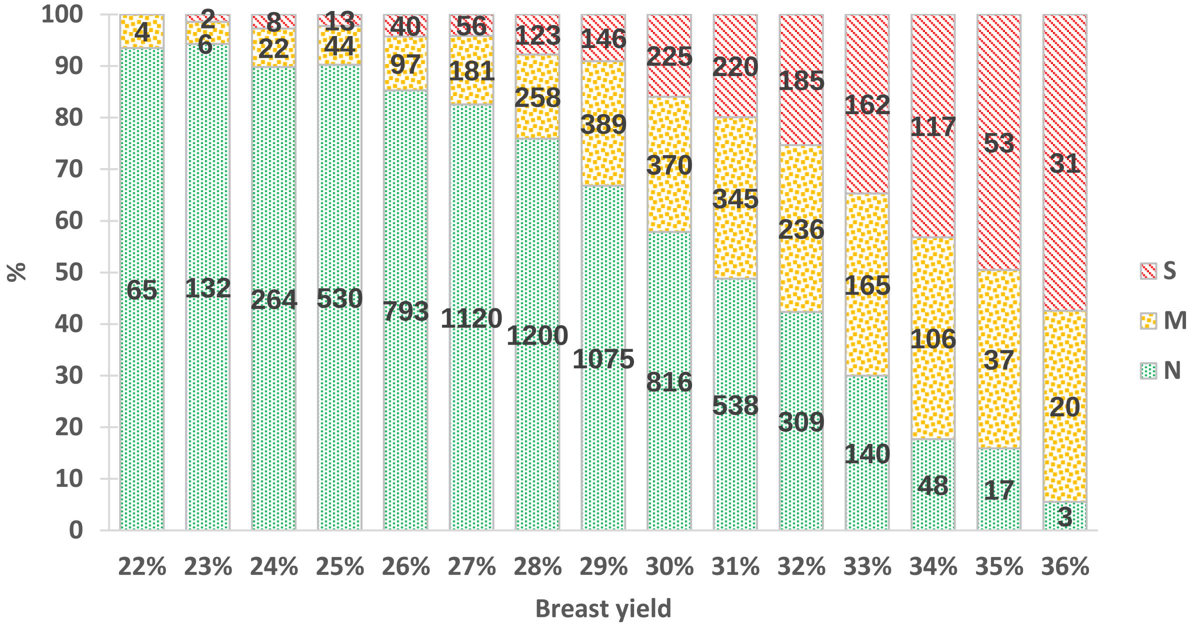


Figure 7. Distribution of WB scores by breast yield (%). Values in the bars represent the number of birds in each category.

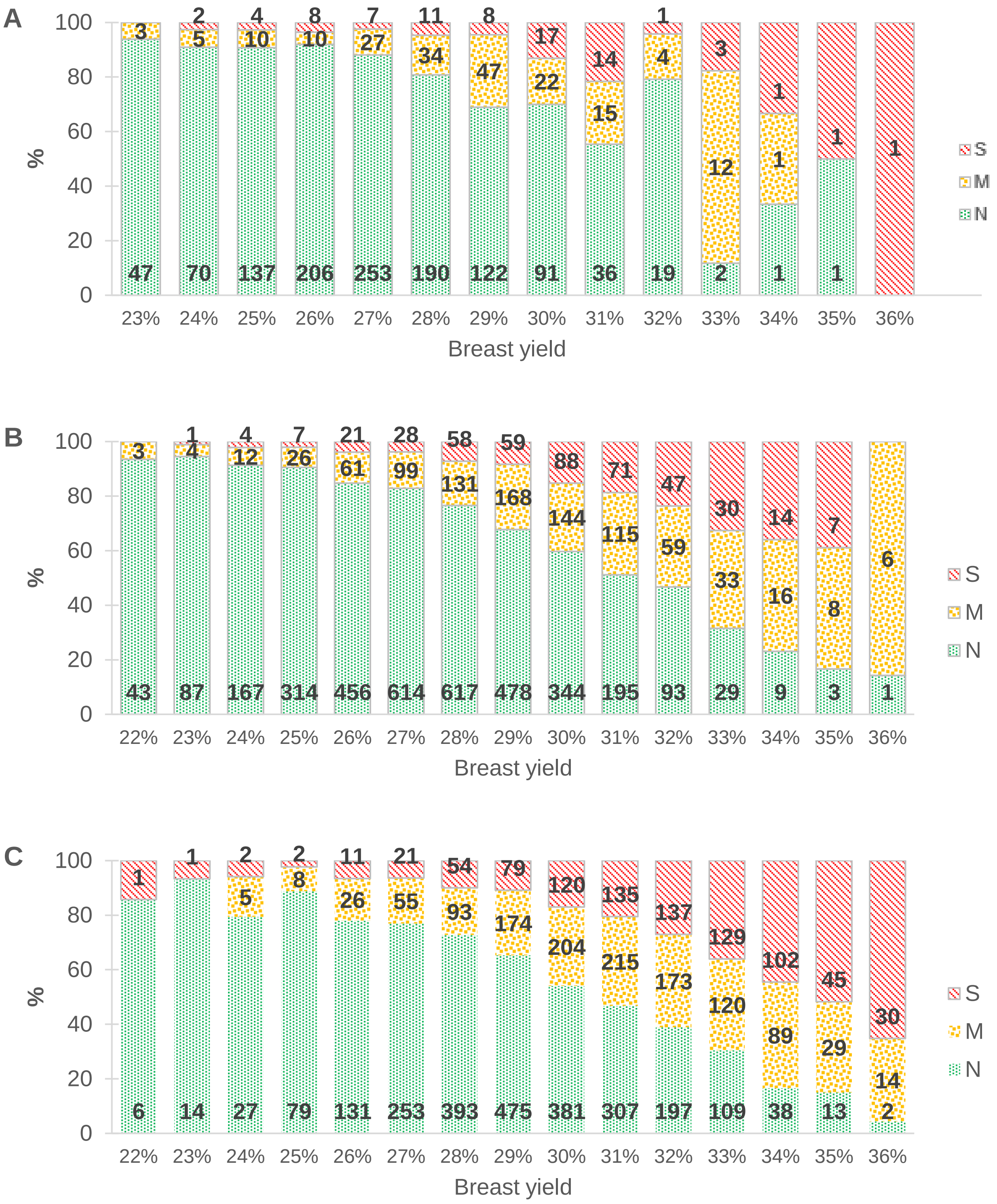


Figure 8. Incidence of WB scores by the breast weight (Kg) for fast food bird (A), tray pack bird (B), and heavy debone bird (C). Values in the bars represent the number of birds in each category.

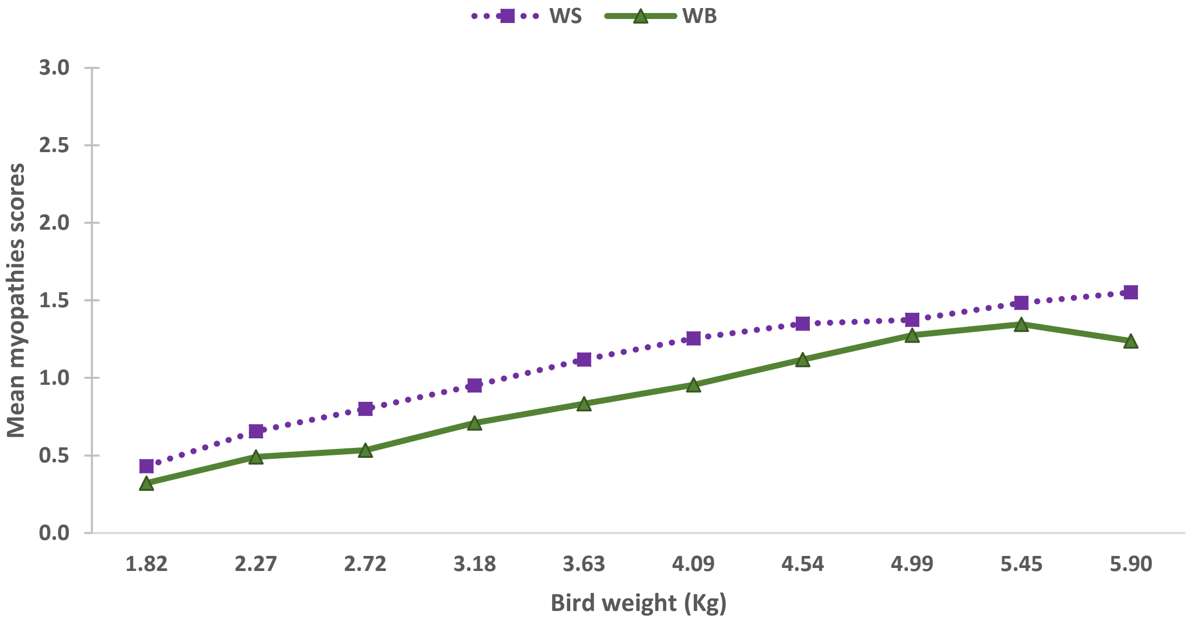


Figure 9. Relationship between bird weight (Kg) and myopathies scores (WS – white striping (r_s=0.46) ; WB – wooden breast (r_s=0.36)) in broilers.

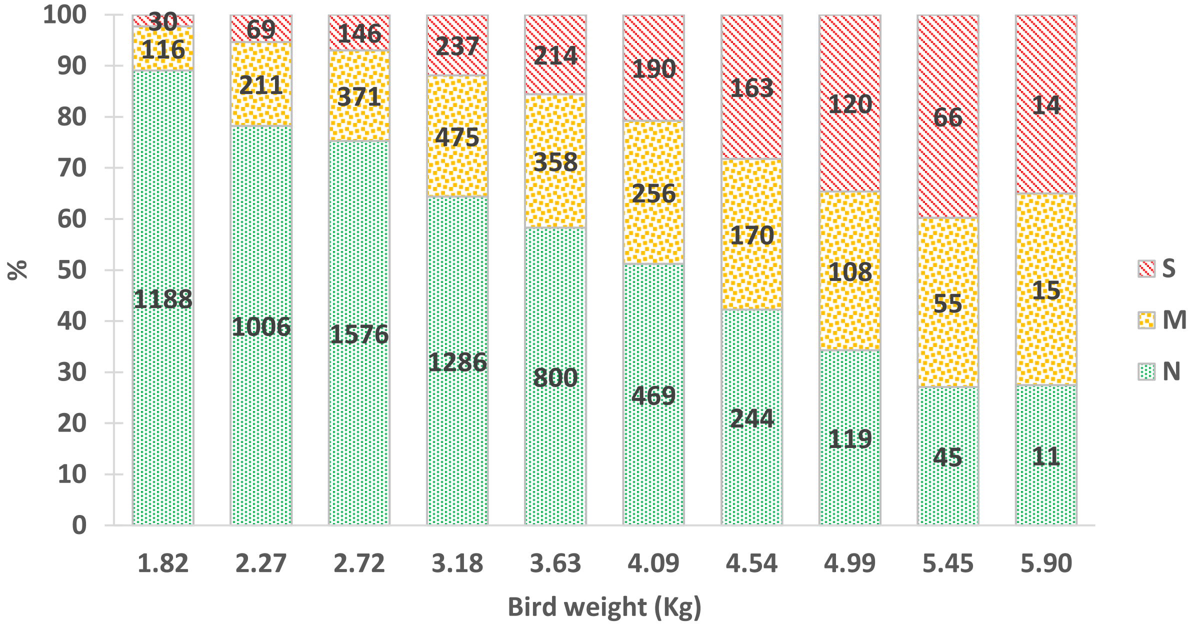


Figure 10. Distribution of WB scores by bird weight (Kg). Values in the bars represent the number of birds in each category.

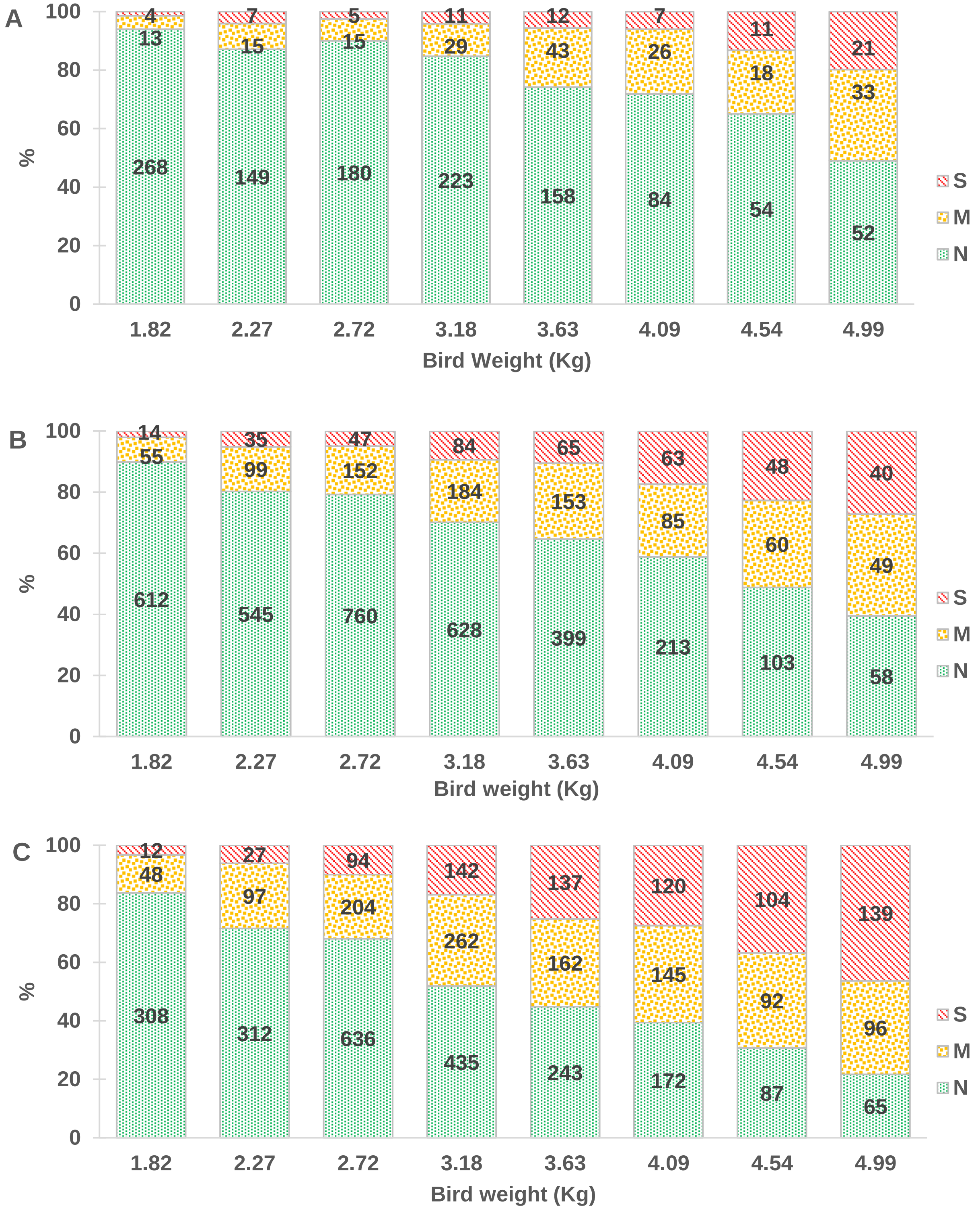


Figure 11. Incidence of WB scores by the bird weight (Kg) for fast food bird (A), tray pack bird (B), and heavy debone bird (C). Values in the bars represent the number of birds in each category.

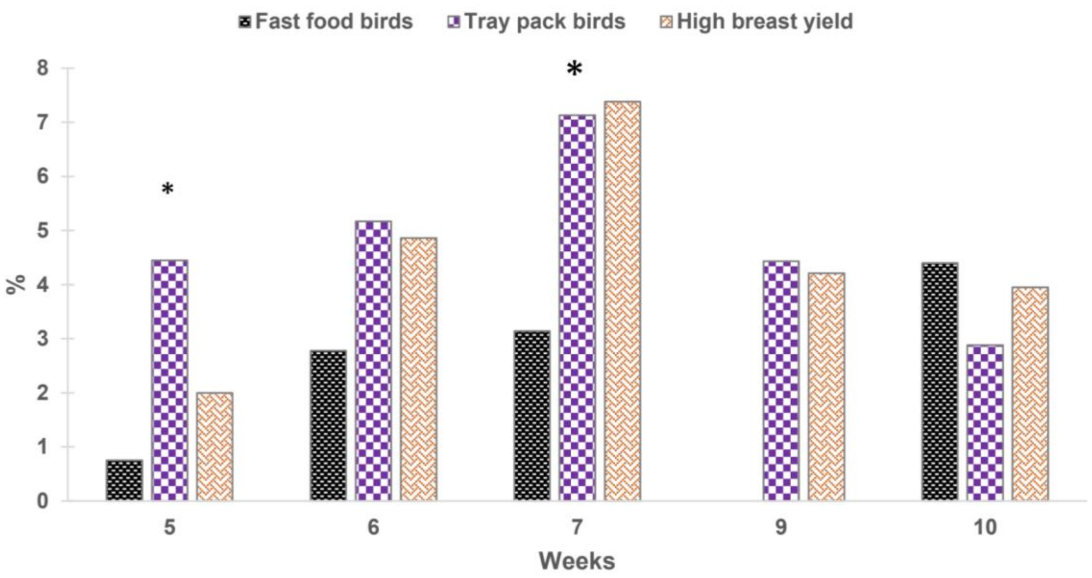


Figure 12. Spaghetti meat incidence by the bird type and weeks.

Table 1. Incidence of woody breast (WB) scores with respect to the factors or categorical independent variable.

Factor	Frequency (%) N[1]	M[1]	S[1]	$\chi 2$	Total
Year				0.0001	
2016	49.3	32.5	18.2		5255
2017	47.8	29.6	22.6		3306
2018	48.7	23.9	27.4		2184
Bird type				0.0001	
Fast food birds	81.3	13.3	5.4		1449
Tray pack birds	72.3	18.6	9.1		4781
Heavy debone	53.9	26.8	19.3		4515
Sex				0.0001	
Females	75.1	17.4	7.5		5302
Males	56.6	25	18.4		5443
Wks				0.0001	
5	84.1	12.2	3.7		1831
6	76.9	17.3	5.8		863
7	68.1	21.3	10.6		1477
9	54.1	25.7	20.2		1410
10	47.2	28.5	24.3		2011

[1] WB scores: N (0+0.5), M (1+1.5), and S (2+2.5+3).

Table 2. Incidence of white striping (WS) scores with respect to the factors or categorical independent variable.

	Frequency (%)				
Factor	**N[1]**	**M[1]**	**S[1]**	$\chi 2$	**Total**
Year				0.0001	
2016	33.5	46.9	19.6		5255
2017	47.8	38	14.2		2301
2018	42.6	43.5	13.9		1072
Bird type				0.0001	
Fast food birds	52.2	38.2	9.6		1166
Tray pack birds	43.9	42	14.1		3925
Heavy debone	27.8	48.4	23.8	0.0005	3537
Sex					
Females	40.5	42.4	17.1		4255
Males	36.4	45.8	17.8		4373
Wks				0.0001	
5	71.9	26.3	1.7		1496
6	56.2	33.5	10.2		576
7	38.6	47.8	13.5		3982
9	16.2	46	37.7		843
10	13.9	53.5	32.6		1731

[1] WS scores: N (0+0.5), M (1); S (1.5+2+2.5+3).

Table 3. Incidence of Woody breast (WB) between the bird type and weeks.

		N[1]	**M[1]**	**S[1]**
Fast food	n	%	%	%
5	398	30.6	13	15.3
6	72	5.4	3.6	1.3
7	637	44.8	43.5	32.1
9	69	4.1	7.2	9
10	273	15	32.6	42.3
Tray pack birds				
5	1033	25.1	14.3	8.5
6	503	11.2	8.8	8.5
7	1921	40.4	42.1	34.2
9	699	12	19	26.6
10	625	11.2	15.9	22.3
Heavy debone				
5	399	12.7	6	2.2
6	288	8.7	5.3	1.4
7	2072	50.4	43.4	36.6
9	642	12.4	14.8	18.6
10	1113	15.8	30.5	41.2

[1] WB scores: N (0+0.5), M (1+1.5), and S (2+2.5+3)

Table 4. Incidence of spaghetti meat (SM) with respect to the factors.

Factor	**SM**	χ^2	**Total**
Year		0.0001	
2016	70		5251
2017	27.2		3304
2018	2.78		2182
Bird type		0.0001	
Fast food birds	6.8		1449
Tray pack birds	47.8		4781
Heavy debone	45.4		4515
Sex		0.0001	
Females	77		5298
Males	23		5439
Wks		0.0001	
5	10.6		1831
6	7.8		863
7	57.2		1477
9	10.7		1410
10	13.7		2011

CONCLUSIONS

General Conclusion

In this dissertation research the model of inducing, predicting, growth curve, and demographics of breast myopathies in broilers was investigated. There is not a clear etiology or a model to increase or induce breast myopathies, specifically WB. In this study, inducing activity in birds through practical means such as human interaction and light intensity did not affect the woody breast myopathy.

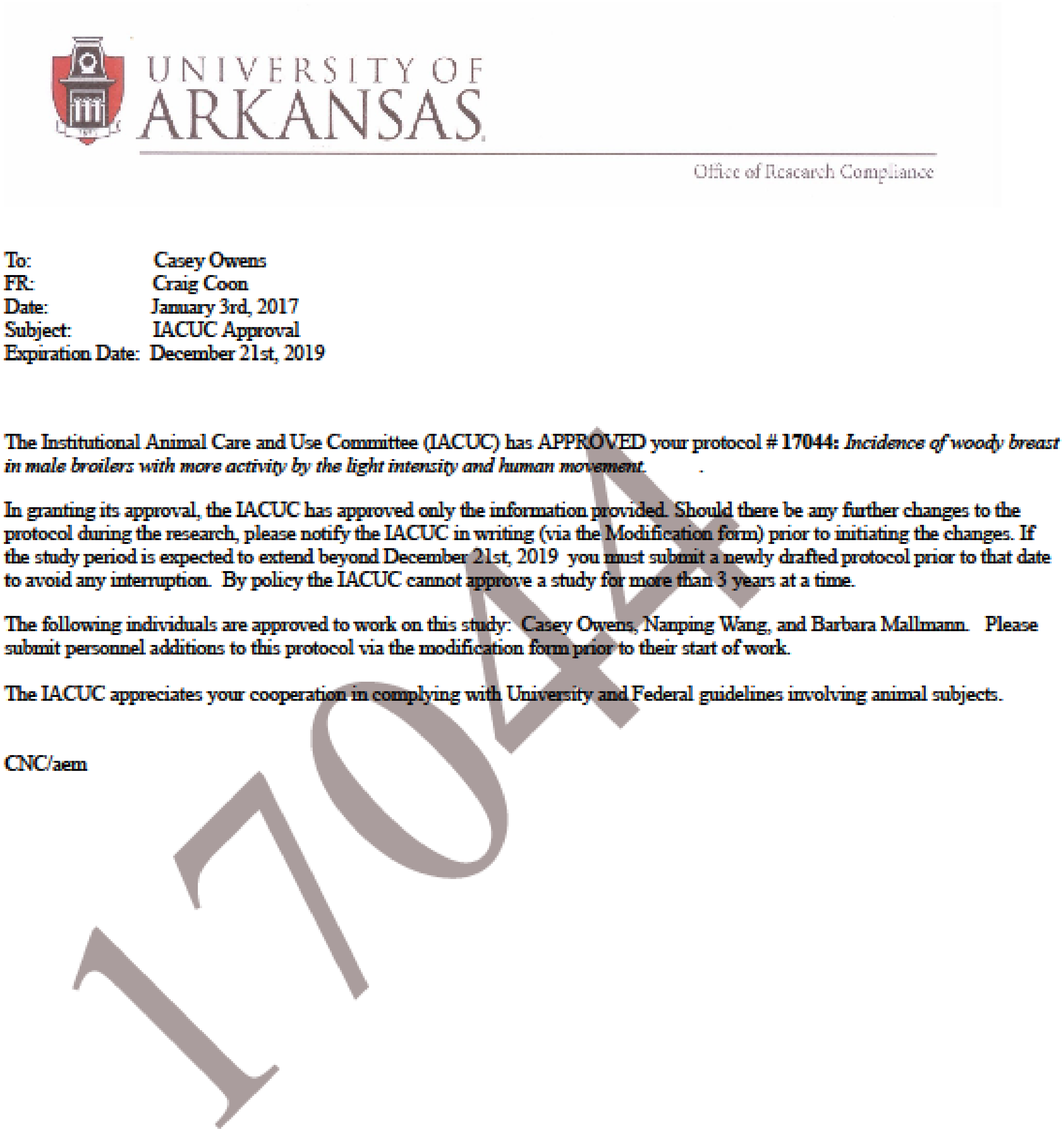
The need for a better methodology on WB scoring leaded a prediction model on the breast fillets measurements that was able to differentiate the non-affected by the affected ones. This model of prediction of WB could be used in industry to select the different WB categories. Freezing the breast meat improves the softness of the WB and differences exist between right and left fillets.

Since the WB prediction model for the breast fillet were successful it could be used in live birds to be able to select birds at the farm avoiding WB on the processing day. Then other way to evaluate the WB could be done by palpation on live birds, showing with 3 weeks old was correlated and had significant differences between the 3 categories and increases with the age but can be susceptible to differences depending on the genotype. Therefore, birds could be selected for better understanding of the preventive ways to decrease the incidence of WB. In addition, measurements on the breast of live birds was reported to vary much depending on the type of bird and age. However, the growth curves presented differences on the breast cranial and caudal region while the bird is growing. There is a high difference between the bird lines and a difference by the severe WB by the non-affected.

To conclude the research, demographics of breast myopathies realized on the market birds over the differences on the strains, sex, weights, and measurements. Mostly the males are heavier showing more WB than females, but females present more SM and WS. Heavy debone birds present more incidence of WS and WB but not of SM.

The importance of this work on the industry is to improve and guide for decision making in order to prevent the economic loss that myopathies are causing

APPENDIX



UNIVERSITY OF
ARKANSAS
Office of Research Compliance

To: Casey Owens
FR: Craig Coon
Date: January 3rd, 2017
Subject: IACUC Approval
Expiration Date: December 21st, 2019

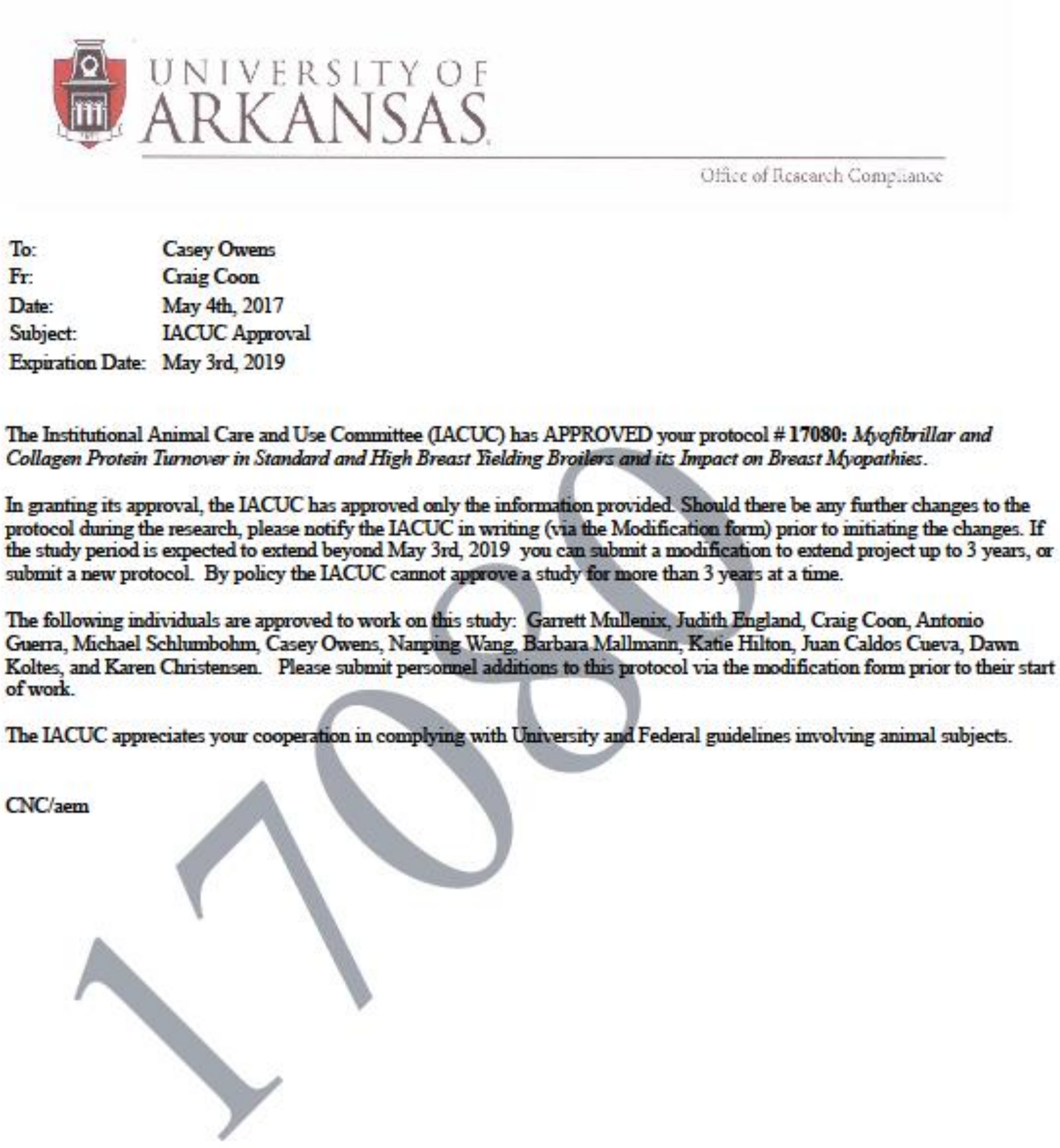
The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17044:** *Incidence of woody breast in male broilers with more activity by the light intensity and human movement.* .

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond December 21st, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Casey Owens, Nanping Wang, and Barbara Mallmann. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem



UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Casey Owens
Fr:	Craig Coon
Date:	May 4th, 2017
Subject:	IACUC Approval
Expiration Date:	May 3rd, 2019

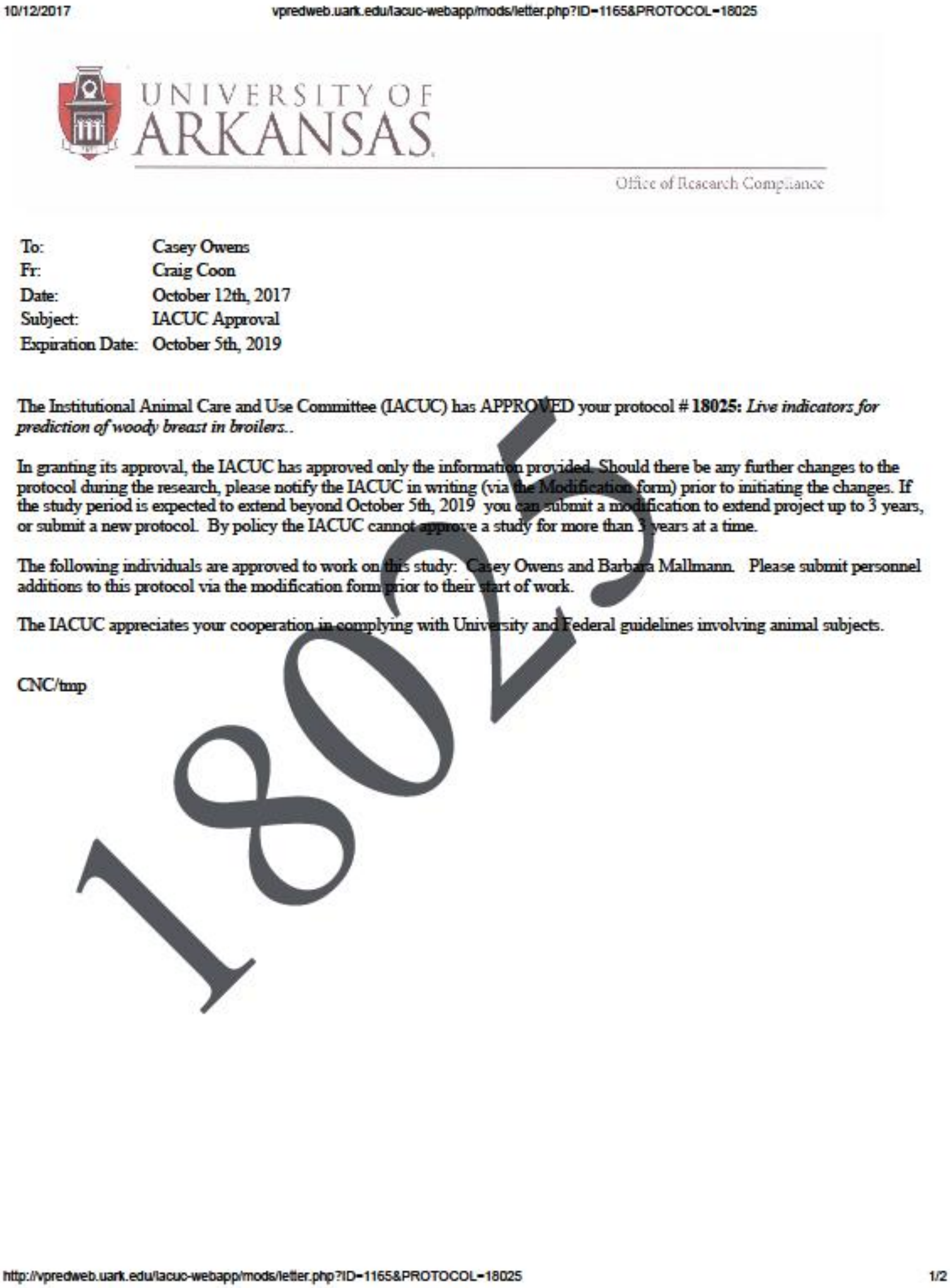
The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17080:** *Myofibrillar and Collagen Protein Turnover in Standard and High Breast Yielding Broilers and its Impact on Breast Myopathies*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 3rd, 2019 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Garrett Mullenix, Judith England, Craig Coon, Antonio Guerra, Michael Schlumbohm, Casey Owens, Nanping Wang, Barbara Mallmann, Katie Hilton, Juan Caldos Cueva, Dawn Koltes, and Karen Christensen. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem



10/12/2017 vpredweb.uark.edu/iacuc-webapp/mods/letter.php?ID=1165&PROTOCOL=18025

UNIVERSITY OF
ARKANSAS
Office of Research Compliance

To:	Casey Owens
Fr:	Craig Coon
Date:	October 12th, 2017
Subject:	IACUC Approval
Expiration Date:	October 5th, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18025:** *Live indicators for prediction of woody breast in broilers..*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond October 5th, 2019 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Casey Owens and Barbara Mallmann. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

http://vpredweb.uark.edu/iacuc-webapp/mods/letter.php?ID=1165&PROTOCOL=18025 1/2