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Evaluation of Genetic and Environmental Influences on Broiler Meat Quality

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Evaluation of Genetic and Environmental Influences on Broiler Meat Quality

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by

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ABSTRACT

The effect of both genetic and environmental influences on broiler meat quality were evaluated over three experiments. Selection response was assessed for broiler lines selected for high (HBY4) and low (LBY4) four day percentage breast yield which were formed from a random bred control (RAN). A modern random bred population (MRB) created in 2015 was evaluated for differences in 4 day percentage breast yield and the resulting differences in the incidence and severity of the woody breast and white striping myopathies. Additionally, the use of embryonic thermal manipulation to impact cell division and early caloric feed restriction to slow growth was analyzed in a yield type broiler population.

In the first experiment, histological analysis of the LBY4 and HBY4 lines was conducted after five generations of selection at various stages of development. At embryonic day 18 and 4 days post hatch, the HBY4 showed advanced muscle fiber development and formation when compared to the LBY4 line and random bred control. At the processing age of day 56, the only difference between the lines was for muscle fiber diameter in which the HBY4 line had larger muscle fibers with no difference in fiber number.

In the second experiment, the MRB line exhibited sire family differences in 4 day percentage breast yield. At processing age, the sire families with the highest average 4 day breast yield exhibited a higher dressing percentage and a higher percentage breast than the lower families. The sire families with the lowest average breast yield had a higher leg quarter percentage. No differences were reported for woody breast but the upper families had a higher score for white striping.

In the third experiment, a yield type broiler strain was subjected to a period of increased incubator temperature for 3 hours a day on embryonic days 15 through 17. The thermal manipulation treatment showed a slightly delayed hatch when compared to the control (C) with

no difference in the hatch of fertile. No differences were observed for body weight however, at processing, the 3 group had a higher percent breast yield and lower severity of woody breast.

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DEDICATION

I would like to dedicate my dissertation to my parents, Mark and Valerie. You never stopped believing in me, even when I changed my mind about being a physical therapist, or when I went off to Cornell, then Wisconsin or when I went and moved all the way to Arkansas. I am sure I gave you some headaches along the way but I am forever grateful for all you both have done for me throughout my life. For all those times you bailed me out, helped dry my tears, or welcome me back for some time away from graduate school, thank you. You gave me wings to fly. I love you both, forever and always.

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INTRODUCTION

Poultry consumption in the United States has sky rocketed over the last 50 years and with an increase in consumption has come changes in production of commercial broilers. It is projected that in 2019, broiler production in the United States will reach an estimated 42,955 million pounds of ready to cook chicken compared to the 1,381 million pounds in 1950 (National Chicken Council, 2018). An increase in the consumption and demand for poultry products has resulted in a need for dramatic changes in the formulation of poultry diets, house environments and genetic selection techniques

One of the reasons for the ability of producers to meet the increase in demand for poultry meat is a result of genetic selection for increases in body weight and growth rate, higher breast yield and a lower feed conversion. A study was done comparing a broiler from the 1950s to a broiler commercially available in both 1991 and 2001. From these studies, it was understood that 85 to 90% of the improvements made in modern broilers was the result of genetic selection (Havenstein et al., 1994a, b, 2003a, b). Unfortunately, as a result of genetic selection for intense growth and changes in management and environment the commercial broiler now grows at tremendous rates. This has exposed issues associated with fertility (Siegel and Dunnington, 1985; Quereshi and Havenstein, 1994), disease (Julian, 1998, 2000; Cook, 2000) and lastly, meat quality (Anthony, 1998; Barbut, 1996, 1997, 1998; Kuttappan et al., 2016). Two myopathies, woody breast and white striping, have recently been characterized and become a concern to the industry as they directly impact both the nutritive value and consumer acceptability of a product (Kuttappan et al., 2012; Petracci et al., 2014; Mazzoni et al., 2015). In order to alleviate the issues associated with these two myopathies, novel selection methods and changes to the environment need to be evaluated for their efficacy in reducing or even eradicating them.

MUSCLE DEVELOPMENT AND STRUCTURE

To understand the etiology of some of the myopathies facing the industry today, it is important to understand the development and structure of skeletal muscles and how certain cells contribute to growth and repair of muscle. The formation of muscle fibers is accomplished through a process called myogenesis which occurs largely during the embryonic period of broilers. During myogenesis, there are three waves of myoblast or muscle cell proliferation (Stockdale and Miller, 1987). The first wave of myoblast proliferation is the embryonic myoblasts which accumulate from embryonic day 3 to 7. Embryonic myoblasts will go on to fuse into primary myotubes, creating the general structure of a broiler skeletal muscle. The second wave of proliferation are fetal myoblasts from embryonic day 5 to 14. Fetal myoblasts will fuse into secondary myotubes which surround the primary muscle fibers. Myotubes are immature skeletal muscle fibers with their nuclei located near the center of the fiber. Upon maturation of the fiber, the nuclei will migrate towards the periphery (Aberle et al., 2012). The third and final wave of myoblast proliferation are adult myoblasts, more commonly known as satellite cells. These accumulate from embryonic day 15 onward and become quiescent in the muscle around 7 days post hatch (Stockdale and Miller, 1987). They will sit between the sarcolemma and basal lamina until they are needed for post hatch growth or repair. This embryonic process is more commonly known as hyperplasia, or the increase in cell number. Cell number is typically set by hatch.

Late in the embryonic development and early post hatch, myotubes will develop into muscle fibers, creating the general muscle structure of a broiler. A muscle is surrounded by a thick, protective sheath, otherwise known as the epimysium. Within the epimysium are muscle fiber bundles. Muscle fiber bundles are surrounded by another layer called perimysium. Within

the bundle are individual muscle fibers that are surrounded by a layer of connective tissue called the endomysium (Aberle et al., 2012). A muscle fiber contains a structure known as a myofibril which are made up by the basic contractile unit of muscle, the sarcomere (Liever, 2002) (Muscle structure shown in Figure 1). Sarcomeres are composed of two myofilaments; actin, a thin filament and myosin, a thick filament. Actin and myosin are arranged overlapping and parallel to each other to allow for muscle contraction and creation of the striated appearance of skeletal muscle (Wick, 1999). The M line separates the sarcomere with myosin filaments on either side. The Z disk or line represents the outer band of the sarcomere, connecting with actin filaments. Actin contains troponin and tropomyosin, two contractile proteins (Moos et al., 1995; Warriss, 2000).

Skeletal muscles are voluntary muscle that require a signal from the brain to contract. When a signal is sent, an electrical impulse, otherwise known as an action potential begins the contractile process. The electrical impulse arrives at a synapse between a neuron and the muscle fiber causing the release of a neurotransmitter called acetylcholine. The release of acetylcholine causes sodium ions to enter, depolarizing the membrane. This triggers a release of calcium ions that were stored in the sarcoplasmic reticulum (SR). The Ca^{+2} ions then bind to troponin C on tropomyosin, moving tropomyosin out of the binding site (Hedrick et al., 1994). Through the use of ATP, which is necessary for actin-myosin binding, the myosin head will attach to actin, thus forming the cross-bridge of the actomyosin complex. The myosin heads then pull the actin strands, resulting in contraction of the sarcomere and the muscle fiber (Huxley and Hanson, 1954; Pearson and Young, 1989; Aberle et al., 2012). The pulling of the actin by myosin uses the ATP resulting in the production of ADP and inorganic phosphate as waste products. Relaxation of muscle occurs when the action potential ceases. This causes a repolarization of the

sarcolemma and closes the calcium channels. Ca^{+2} ions are then sequestered back in the SR through the use of a pump and ATP. This causes tropomyosin to re-cover the binding site on actin and the muscle relaxes.

Late embryonic and early post hatch, muscle fibers transition from hyperplastic growth to hypertrophic growth. Hypertrophy is the accumulation of tissue due to an increase in cell size (Aberle et al., 2012). Since muscle fiber number is typically set at hatch, muscle fiber mass can increase in two distinct ways. Muscle fibers have the ability to grow in both length and diameter. To grow in length, new sarcomeres are added to the end of existing myofibrils. To grow in diameter is a little more complex. For a muscle fiber to grow, new myofibrils need to be added to the fiber. This can be done by the splitting of larger myofibrils within a muscle fiber up to 10 to 15 times during the lifetime of an animal. The smaller daughter fibers can grow through the addition of new myofilaments and the process will repeat resulting in an increase in muscle in the animal (Aberle et al., 2012).

While muscle fibers grow in size through protein accretion, eventually the protein to nuclei ratio becomes too large. To grow further, additional nuclei are necessary for increased protein accretion (Halevy et al., 2006). Satellite cells, originally described by Mauro (1961), which have accumulated during the embryonic growth period can help provide additional nuclear support for the growing muscle fiber in addition to aiding in repair (Hartley et al., 1992). When the protein to nuclei ratio becomes too high, satellite cells can donate their nuclei to allow for additional protein accretion and muscle growth. Exercise induced growth also occurs through satellite cell activity (Darr and Schultz, 1987). Muscle fiber damage occurs regularly (Wernig et al., 1990), whether it be through contraction, stretching or active damage. To avoid permanent damage to the cells, muscle fibers need additional nuclei to repair themselves quickly. Satellite

cells are activated and recruited to the site of damage, fuse with the damaged cell and donate their nuclei to aid in repair. Without satellite cells, growth and repair of damaged muscle fibers would not occur at a high enough level to accumulate the yield necessary in broiler production.

MUSCLE CHARACTERISTICS

Postmortem, muscles go through a muscle stiffening process called rigor mortis which converts muscle to meat (Aberle et al., 2012). The stiffening of the muscle is a result of permanent cross bridges forming between actin and myosin filaments. When an animal is euthanized, muscle metabolism switches from aerobic metabolism (with oxygen) to anaerobic metabolism (lack of oxygen). With blood flow ceasing, the amount of ATP declines until permanent cross bridges are formed and the waste product of anaerobic metabolism (Aberle et al., 2012), lactic acid, builds up in the muscle. The build of lactic acid results in a subsequent pH decline in the muscle. The rate of decline of muscle pH and the ultimate pH that a muscle reaches after the process of rigor mortis is complete is critical when determining meat quality. Several studies have shown muscle pH to be highly correlated with other traits useful in evaluating meat quality such as color, water-holding capacity, tenderness and shelf life (Allen et al., 1998; Qiao et al., 2001). A normal pH for a broiler breast fillet is typically between 5.8 and 5.9 (Qiao et al., 2001; Duclos et al., 2007). Variations in pH can result in changes in muscle characteristics and meat quality.

A visual trait that can be used to assess meat quality is meat color, being measured either with the naked eye or through the use of a colorimeter (L*, a*, b*). Meat pH and color have a strong negative correlation but with meat color being easier to measure, it is more widely used in meat quality evaluation. In general, a fillet with a low ultimate pH following the process of rigor mortis will have a high L*, or a pale fillet. When the pH declines while carcass temperatures are

still hot, proteins lose their ability to hold on to water. With myoglobin, the main pigment found in meat, being water soluble, proteins are unable to hold on to water and ultimately the myoglobin resulting in pale meat. On the other hand, a breast fillet with a high ultimate pH will have a better ability to retain water and myoglobin and will have a lower L*, or darker fillet (Vilojen et al., 2002; Droval et al., 2012).

Water holding capacity (WHC) is a trait that is positively correlated with muscle pH and can be measured through several methods such as cook loss, thaw loss, drip loss or expressible moisture. Breast fillets with a high pH have a better ability to hold on to water, improving their water-holding capacity (Bowker and Zhuang, 2015). If a fillet has a low ultimate pH, proteins have been denatured during the rigor process resulting in an inability of muscle to hold on to water and therefore have a lower water holding capacity. Several researchers have documented the relationship between these two traits in broilers (Froning et al., 1978; Barbut, 1993; Northcutt et al., 1994; Bianchi et al., 2005).

Several other meat quality characteristics are affected by the pH of meat including shelf life, emulsification capacity, cook loss and tenderness. The ability of a fillet to hold on to water, ultimately affects the shelf life of a product (Allen et al., 1997). High pH fillets with high WHCs results in a greater amount of moisture in the product. The combination of the fillet pH and high moisture creates a suitable environment for microbial growth resulting in a shorter shelf life and increase in spoilage bacteria in fresh products. Emulsification capacity (EC) is also affected by pH. EC is the ability of proteins in meat to mix with fat particles and other products when making sausages, nuggets or patties. A study by Qiao et al., (2001) showed that EC had a significant positive correlation with pH. The higher the pH, the higher the EC and vice versa. Cook loss and tenderness of a product are critically important when considering consumer

acceptability as it impacts the sensory and flavor aspects of broiler meat. In poultry, tenderness can be evaluated either through shear force analysis using the Allo-Kramer, Warner-Bratzler or Meulennet-Owens methods or through trained panelists who can evaluate the flavor and texture profiles of meat. Fillets that exhibit a high shear force are in general tougher pieces of meat (Cavitt et al., 2005). Fillets with a high pH tend to hold on to more water and in general have a lower shear force and an improved tenderness. Fillets with a low pH generally have a higher shear force and are less tender (Allen et al., 1998; Owens et al., 2000, Woelfel; et al., 2002).

FACTORS AFFECTING MEAT QUALITY

While measuring meat quality may be easy for researchers, elucidating which factor or factors contribute to it is difficult as there are many factors that influence meat quality. Genetics may be the underlying component to determining meat quality. Primary breeders select for performance traits, such as growth, breast meat, yield, feed conversion, disease resistance among many other traits. Several traits associated with meat quality have been shown to be highly heritable such as postmortem pH decline (0.35-0.49), lightness or color (0.5-0.75) and drip loss (0.55-0.64) (Mir et al., 2017). An increase in issues associated with pH and color have come about in recent years suggesting selection programs may be negatively impacting meat quality traits as not a lot of weight is placed on those traits each generation. Several companies have now been exploring the idea of using marker assisted selection to improve meat quality (Anadon, 2002).

While genetics may play a role in meat quality, the phenotypic expression of meat quality traits is also dependent upon the environment, both pre- and postmortem (Maga, 1994; Froning, 1995). Growth rate has been targeted as a cause for many meat quality abnormalities that occur in the industry. It has been observed that myopathies have a tendency to occur in the heaviest

birds (Kuttappan et al., 2016). Several studies have been done looking at the relationship between growth rate and meat quality (Le Bihan-Duval et al., 2001; Doherty et al., 2004; Berri et al., 2005; Fanatico et al., 2007; Le Bihan-Duval et al., 2008), showing meat quality issues can occur in both slow growing and fast growing broilers.

Nutrition can play a major role in meat quality as it can directly impact growth, fat deposition and carcass composition. As the energy in the diet increases, body fat increases with lean protein mass decreasing (Mir et al., 2017). Feeding a high energy, high protein diet can decrease fat yield and improve breast meat yield (Hess and Bilgilli, 2004). Supplementation with antioxidants, specifically α-tocopherol or vitamin E can reduce Thiobarbituric acid reactive substances (TBARS) in meat tissue and improve protein functionality (Guo et al., 2003). Broilers fed wheat based diets instead of the normal corn based diets have lighter breast fillets (Smith et al., 2002). While the general base of broiler diets may remain consistent, for energy, protein, fat, and amino acid levels, variations in ingredients have the potential to impact meat quality.

Broiler production in the United States is primarily concentrated to the southern states where the climate is generally warm to hot a majority of the year. Heat stress is a major concern not only in the house but also during transportation as it can impact not only meat quality but bird welfare such as stress levels and mortality. It has been reported that high temperatures prior to processing can result in lighter fillets with a lower pH (Babji et al., 1982; Bianchi et al., 2007; Wang et al., 2009). Cold stress can occur in broilers as well. However its effect on meat quality remains unclear with researchers reporting a decrease in fillet lightness (Dadgar et al., 2011) or no effect at all (Babji et al., 1982).

Pre slaughter handling can play a major role in the determination of meat quality. Prior to processing, broilers are subjected to a period of feed withdrawal. This is done to empty the

intestinal tract to decrease condemnation from fecal contamination without impacting yield. In general, feed withdrawal times of 8 to 12 hours are common in the industry while broilers still have constant access to water. Glycogen reserves in the liver are depleted with a 6 h withdrawal time (Warriss et al., 1987). With glycogen being a major component involved in the rigor mortis pH decline, decreasing muscle glycogen prior to slaughter can affect the ultimate pH of meat. With a depletion of glycogen reserves, there will be less substrate available for postmortem metabolism resulting in a higher ultimate pH and issues with meat quality. Feed withdrawal also increases stress levels in the birds which could negatively impact meat quality (Kannan et al., 1997, 1998). Transportation to the plant can not only increase the feed withdrawal period but may also allow for heat or cold stress depending on the time of the year, affecting meat quality.

At the processing plant, several techniques used can impact meat quality of broilers such as stunning and chilling. Electrical stunning is the most common method utilized in the broiler industry with gas stunning and low atmospheric pressure stunning (LAPS) being less common. Electrical stunning has been shown to result in super contraction of some muscles which may lead to tougher meat. It also increases the rate of postmortem metabolism which can impact pH decline and ultimate pH (Fletcher, 2002). Head only electrical stunning has resulted in darker and redder fillets when compared to whole body stunning (Hillebrand et al., 1996). Gas stunning can be done through the use of several gases including carbon dioxide and argon. Gas stunning has been shown to affect color and water holding capacity of breast muscle (Savenje et al., 2002). Low-atmospheric pressure stunning (LAPS) is the newest stunning method in which broilers are placed in compression chambers where the partial pressure of oxygen is decreased to a point where broilers lose all metabolic activity in their brain from a lack of oxygen. While this

method is fairly new, no severe differences in meat quality have been reported (Schilling et al., 2012).

Meat quality can be altered if carcasses are chilled too rapidly or too slowly. Chilling at higher temperatures in turkeys has been found to increase the rate of glycogen depletion, ultimately lowering the pH affecting the drip loss and cook loss of the breast muscle (McKee and Sams, 1998). It has also been found that chilling at higher temperatures increased L* and had a negative impact on drip loss and gel strength (Alvarado and Sams, 2002, 2004). Increasing the rate of chilling can help avoid issues associated with protein denaturation that can occur during the pH decline of breast muscle postmortem.

With so many factors contributing to the meat quality of poultry meat, alterations in the genetics and environment of broilers can lead to issues associated with meat quality, or the development of myopathies.

MUSCLE MYOPATHIES

Genetic selection and improvements in nutrition and management have allowed for a bird that is high yielding, fast growing and extraordinarily efficient. Unfortunately, these advancements have not come without a price. Several myopathies have emerged over the years that directly impact growth, yield, condemnation of product, meat quality and consumer acceptability. These myopathies include pale soft and exudative (PSE)- and dark firm and dry (DFD)-like, deep pectoral myopathy, white striping and woody breast. Changes in genetics, nutrition and management are necessary to help alleviate some of the issues associated with these myopathies.

PSE-and DFD-like

Two conditions exist that are associated with the postmortem pH decline of broilers. Both conditions affect several traits associated with meat quality and can be a concern to producers. These conditions are known as PSE- and DFD- like muscle myopathies as the variations in pH affect meat quality (Fletcher, 2002)

A condition called PSE or pale soft and exudative meat was first documented in porcine species (Aberle et al., 2012). However, a similar condition was also characterized in both broilers and turkeys and was termed PSE-like as the genetic basis between pork and poultry was not the same (Barbut, 1997; Woelfel et al., 2002; Petracci et al., 2009). A broiler breast fillet exhibiting a PSE like condition will have a very rapid pH decline early in the rigor process. This rapid pH decline while carcass temperatures are still elevated results in protein denaturation and the fillet loses its ability to hold on to water. The end result is a fillet with a low pH, a high L* or pale color and a low water-holding capacity and a shiny or exudative appearance with the loss of water.

Another condition that was originally characterized as a dark cutter condition in cattle is known as DFD-like or dark firm and dry meat (Miller, 2007). DFD-like meat has also been characterized in broilers (Harford et al., 2014). Breast fillets exhibiting this condition will have a minimal pH decline and a very high pH following the completion of rigor mortis (Miller, 2007). As a result of a lack of pH decline, a high amount of water will be retained in the muscle, resulting in a dark fillet with a dry exterior as the water is retained inside.

Two separate divergent selection programs have been implemented to create lines exhibiting the PSE- and DFD-like conditions. The first divergent selection program was done at the University of Arkansas. From a random bred population, two lines were created that were divergently selected based solely on the single trait of breast muscle L* at 24 h Color was chosen

based on the ease of measurement as well as the strong correlations with several other meat quality traits such as pH and water holding capacity. The HMC or high muscle color line was selected for a high breast muscle L*. This line represents a PSE-like condition. The LMC or low muscle color line was selected for a low L* and is representative of a DFD-like condition (Harford et al., 2014). Throughout several research studies, the HMC line has exhibited a rapid pH decline, a low ultimate pH, a higher drip loss and a higher shear force, indicating a tougher meat. The LMC line has exhibited a minimal pH decline, a high ultimate pH and a lower shear force, indicating a more tender meat (Orlowski, 2016). An additional study done looking at the shelf-life differences between the two lines found higher spoilage bacteria in the LMC line than the HMC line (data not published). The second selection program was done at the National Institute for Agronomic Research (INRA) in France. Instead of L*, the selection trait chosen for this selection program was 24 h breast muscle pH. Over several generations a pHu- and a pHu+ line were created. The pHu- line was selected for a low ultimate pH and exhibits a PSE-like condition while the pHu+ line was selected for a high ultimate pH and exhibits a DFD-like condition. The pHu- line has exhibited a higher L* and a higher drip loss while the pHu+ line has exhibited a lower L* and lower drip loss (Alnaahhas et al., 2014). Although these programs have selected for different traits, both sets of lines exhibit similar characteristics to both a PSE- and DFD-like condition. With these two myopathies, both genetics and the environment play a significant role in their incidence and severity.

Deep Pectoral Myopathy

A myopathy impacting the supercoracoideous otherwise known as the *pectoralis minor*, but most commonly known as the tender is called Deep Pectoral Myopathy (DPM). It was originally identified as a degenerative myopathy in turkeys by Dickinson and colleagues in 1968

and has been thoroughly studied since its discovery. DPM has also been called the Oregon muscle disease, green muscle disease or green tenders. While originally found in turkeys and later on in broiler breeders, the myopathy is becoming much more common in commercial broilers (Richardson et al., 1980; Bilgilli and Hess, 2002). Genetic selection has resulted in a broiler with an incredible amount of breast meat yield, but as a consequence DPM can occur.

As a broiler grows, the amount of room beneath the *pectoralis major* for the tender to grow and stretch decreases. In certain cases, particularly in high yielding birds, if a bird becomes stressed and moves rapidly, flapping their wings, blood flow to the tender can become cut off completely. With no blood supply to the muscle, the muscle quickly becomes necrotic (Harper et al., 1983; Siller, 1985). Due to the fact that broilers are relatively inactive in commercial settings, the tender generally is not exercised so if excessive wing flapping does occur, the tender is impacted. In the early stages of necrosis, the tender will appear a reddish-brown in color, followed by a green and shrunken stage as necrosis progresses (Bilgilli and Hess, 2002), similar to the development of a bruise. This can occur in either one or both of the tenders but it does not affect the overall health of the bird.

Unfortunately with DPM, it cannot be detected until processing when the breast meat is removed, displaying the tenders. Broilers affected by DPM will result in a trimmed condemnation in the plant as the product is not to be used for human consumption and a subsequent economic loss to the industry (Jordan and Pattison, 1998). Bilgilli and colleagues (2000) reported that several triggers for the development of DPM. The triggers include, human activity, loud noises, lack of feed and water or anything that may increase bird activity. It is necessary to avoid stress with handling, load-out, transportation and shackling as those are all periods were excessive flapping may occur. DPM has been shown to occur at an incidence of 5%

in broilers that were subjected to handling stress (Richardson et al., 1980), incidence in male roasters around 1% (Bianchi et al., 2007) while incidence in broiler breeders can be upwards of 43% in males and 22% in females (Bilgilli et al., 2000). The incidence in modern broilers however, remains unknown.

White Striping

White striping is a fairly recent condition affecting both the consumer acceptability and nutritive value of broiler meat. It is characterized as superficial and gross white striations of fat deposits that run parallel to the muscle fibers at varying degrees in broiler breast fillets and thigh meat (Kuttappan et al., 2012). In the field, white striping is reported to affect upwards of 12% of broiler breast fillets in a moderate or severe degree (Petracci, 2013) and upwards of 50% of fillets under experimental conditions (Kuttappan, et al., 2013). Fillets with white striping are suitable for human consumption, however it does impact the consumer acceptability of the fresh product as it is visually unappealing to the consumers. Fillets with white striping have an increase in the percentage of fat within the fillets, influencing the nutritive profile and caloric content (Kuttappan et al., 2012, 2013). There is also a decrease in protein quality creating issues with meat quality (Mudalal et al., 2014). As a result of protein degradation, there is a decrease in water holding capacity and marinade uptake as well as an increase in cook loss as the muscle fibers cannot hold onto water (Petracci et al., 2013). This condition most commonly occurs in heavier birds (Kuttappan et al., 2013; Petracci et al., 2013). It remains unclear why white striping develops in broilers and very few studies have found a positive impact on decreasing white striping. The addition of high levels of α-tocopherol to the diet was evaluated for its efficacy in reducing white striping but no effect was observed (Kuttappan et al., 2012).

Woody Breast

One of the biggest concerns to the industry today regarding meat quality is the woody breast myopathy. The woody breast myopathy is characterized as a breast fillet that has become hardened or stiffened with distinct caudal ridges and can be scored as mild, moderate or severe (Sihvo et al., 2014; Tijare et al., 2016) . Breast fillets affected by woody breast show necrosis and degeneration of muscle fibers, infiltration by immune cells such as macrophages and lymphocytes and deposition of collagen in place of healthy breast muscle tissue (Sihvo et al., 2014; Trocino et al., 2015; Brot et al., 2016). The deposition of collagen is what causes the hardening of the muscle creating a product that is unappealing to consumers.

In severe cases of woody breast, a yellow inflammatory exudate will form on the outside of the breast muscle below the skin. Woody breast is becoming an economic concern to the industry as the USDA has ruled that areas affected by the yellow inflammatory fluid would result in a trimmed condemnation in the plant (USDA:FSIS, 2017). It is estimated that woody breast is costing the US poultry industry over $200 million dollars in economic loss (Kuttappan et al., 2016; Poultry World, 2017) and research needs to be done to help reduce the economic impact of the myopathy in broilers.

The heritability of the woody breast myopathy has been shown to be relatively low in broilers (Bailey et al., 2015). As a result, non-genetic factors are responsible for a majority of the variations observed in woody breast (Trocino et al., 2015). Several methods have been evaluated for their efficacy in reducing the incidence of woody breast, mostly through dietary manipulation. Changes in nutrition have been shown to have a positive impact on the incidence of woody breast. This has been done through alteration of amino acids in the diet, supplementation with a high vitamin dosage (Bodle et al., 2018) or reduction in dietary energy (Meloche et al., 2018). While these methods may decrease the incidence of the myopathy, it does

come at a cost in the form of decreases in body weight, carcass and breast yield. These options, while viable for reducing the myopathy will ultimately have a negative impact on the industry as a result of a decrease in yield.

Livingston and colleagues (2018) suggests that longer periods of egg storage prior to incubation has a negative impact on the presence of both woody breast and white striping. Another study by Velleman and colleagues (2014) evaluated the effect of posthatch feed restriction on fat deposition. This study found that the timing of the feed restriction had a major impact on the development of muscle and adipocytes at processing ages with second week posthatch feed restriction showing little negative impacts and a first week posthatch feed restriction showing an increase in adipocyte deposition and poor muscle development (Velleman et al., 2014). Early posthatch feed restriction may contribute to the incidence of woody breast and white striping myopathies. Research needs to be done to find methods of reducing the woody breast myopathy without having a negative impact on yield or production cost

THERMAL MANIPULATION

Finding novel methods to decrease the incidence and severity of muscle myopathies without impacting yield is critical in the future progress of the broiler industry. As the number of days to reach processing age decreases the relative amount of time a broiler spends in the embryonic stage increases making the embryonic environment critical in the growth and development of a broiler (Halevy et al., 2006). Temperature has been shown to be a major factor when considering embryonic growth and development. Embryonic thermal manipulation (TM) is the changing of temperature in the incubator to either higher or lower temperatures compared to the normal 37.6°C as a means to alter development or growth (Meijerhof, 2000). The potential value of altering the embryonic temperature profile was first hypothesized and found to impact

post hatch thermal tolerance and thermoregulation (Piestun et al., 2008; Uni and Yahav, 2010). Broilers subjected to higher temperatures in the hatchery had lower body temperatures and were better able to cope with high temperatures should they occur. Throughout these studies, researchers also found that in addition to the improved thermoregulation, growth traits were also impacted in a positive way. Thermal manipulation applied between embryonic day 7 and 16 experienced a reduced feed intake and improved feed conversion as a result of a decreased body temperature (Piestun et al., 2013). It was also found that myogenin expression, important in muscle growth and development, was increased in broilers subjected to TM. This was confirmed by an increase in the amount of muscle growth and higher breast yields (Piestun et al., 2015). A study done by Collins and colleagues (2007) found that late stage embryonic TM showed higher breast meat yield compared to a control group. It was also found during this study that there was no negative impact on meat quality traits including breast meat pH and drip loss.

An increase in incubation temperature will result in increased cell division. Thermal manipulation during the period of satellite cell division (embryonic day 14 onward) could have the potential to increase the number of satellite cells available for post hatch growth and repair. TM has been shown to improve growth and yield with no negative impact on meat quality however its effect on the incidence of white striping and woody breast is still unknown.

COMPENSATORY GAIN

Broilers gain a majority of their weight at the fastest rate during their first 4 weeks of life (Marks, 1979). Unfortunately, this high growth rate at an early age can lead to issues with skeletal and metabolic diseases, mortality, as well as additional fat deposition (Leeson and Summers, 1988). With issues such as woody breast and white striping being of concern to the

industry, evaluating potential methods to help alleviate or correct some of these issues needs to be done.

During periods of undernutrition, particularly in the early life of a broiler, body weights can be impacted. However, a phenomenon can occur called compensatory gain that allows a broiler who has been underfed or restricted in their early life to reach or sometimes even exceed the body weights of birds fed ad libitum or at the proper nutritive levels (Plavnik and Hurwitz, 1989, 1991; Jones and Farrell 1992, a b). Compensatory gain is characterized in animals that have an abnormal growth relative to age (Bohman, 1955; O'Donovan, 1984). There have been two hypotheses stated to explain this concept; the central control hypothesis and the peripheral control hypothesis. The central control hypothesis states that an animal has an appropriate body size at a set age which is controlled by the central nervous system (Wilson and Osbourne, 1960). The peripheral control hypothesis states that the DNA of a cell has an effect on the growth following a period of undernutrition (Pitts, 1986). It is still unclear which hypothesis may be correct.

Early nutrient restriction programs have the potential to correct some of the factors associated with rapid growth such as fat deposition and death due to heart failure (Osbourne and Wilson, 1960). If the restriction program is done correctly, yield may not be impacted as a result of compensatory growth. Many factors contribute to compensatory growth including the severity and duration of the restricted nutrition program, the age of the restriction and the reimplementation of normal calorie food. The longer the period of undernutrition, the less likely compensatory gain will occur in broilers (Yu and Robinson 1992). Research has shown that a restriction of 6 days allowed for the regaining of body weight while birds were never able to recover after a restriction period of 12 days or more (Plavnik et al., 1989; Rosebrough et al.,

1986; Ballay et al., 1992). A study implementing a restriction period at 3 weeks of age showed no body weight compensation as little time was left for the bird to regain body weight (Washburn and Bondari, 1978). The severity of the restriction is usually done to meet the maintenance energy (ME) of the birds with levels below the ME hindering a birds ability to recover (Plavnik and Hurwitz, 1989), however other researchers have found complete recovery of body weight with restrictions below the ME level (Jones and Farrell, 1992a).

A study was conducted evaluating the effect of a 20% feed restriction in either the 1st or 2nd week of life on the muscle morphology and gene expression of broilers. The results of this study revealed that a 20% feed restriction in the first week of life, degraded the epimysial and perimysial spacing, decreased muscle fiber size and decreased the expression of myogenin which is required for satellite cell differentiation. However, the changes observed with the week one feed restriction were not observed in the group subjected to a feed restriction in the second week. Since satellite cells are most active in the first week of life before becoming quiescent, the timing of post hatch feed restriction is critical (Velleman et al., 2014). For early calorie or feed restriction to be a viable option for the industry in decreasing muscle myopathies, it will be necessary to evaluate the proper timing and restriction needed to improve meat quality while not impacting yield or feed efficiency. The concept of compensatory gain and when and why it occurs at this time, remains unknown.

CONCLUSION

Muscle myopathies have been around in the industry for a long time with DPM first being identified in the 1960s. The two newest myopathies, white striping and woody breast are of major concern for the industry as they are affecting consumer acceptability and yield creating an economic loss for the industry. While not much is yet understood about the myopathies and

why they occur, finding novel methods to help decrease their incidence is critical to avoid future loss to the industry. Methods that impact satellite cells, which are responsible for post hatch muscle growth and repair, may be an answer for controlling the myopathies. Embryonic thermal manipulation during a period of satellite cell development may help increase the number of satellite cells available for post hatch repair while the use of early feed restriction could also potentially impact myopathy development. Both of these methods will need to be extensively studied to determine their efficacy in reducing the myopathy while also being a viable option for the broiler industry. The current dissertation presents the results of a series of experiments evaluating the genetic (selection for 4-day percentage breast yield) and environmental (embryonic thermal manipulation and early feed restriction) on broiler meat quality.

REFERENCES

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of meat science. Fifth Edition. Kendall Hunt Publishing Company.

Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. Poult. Sci. 76:1042-1046.

Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. The relationship of broiler breast color to meat quality and shelf life. Poult. Sci. 77:361-366.

Alnahhas, N., C. Berri, M. Boulay, E. Baeza, Y. Jego, Y. Baumard, M. Chabault, and E. Le Bihan-Duval. 2014. Selecting broiler chickens for ultimate pH of breast muscle: Analysis of divergent selection experiment and phenotypic consequences on meat quality, growth, and body composition traits. J. Anim. Sci. 92:3816-3824.

Alvarado, C. Z., and A. R. Sams. 2002. The role of carcass chilling rate in the development of pale, exudative turkey Pectoralis. Poult. Sci. 81:1365-1370.

Alvarado, C. Z., and A. R. Sams. 2004. Turkey carcass chilling and protein denaturation in the development of pale, soft and exudative meat. Poult. Sci. 83:1039-1046.

Anadon, H. L. S., 2002. Biological, nutritional and processing factors affecting meat quality of broilers. PhD, Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA. 24061.

Anthony, N. B. 1998. A review of genetic practices in poultry. Efforts to improve meat quality. J. Muscle Foods. 9:25-33.

Babji, A. S., G. W. Froning, and D. A. Ngoka. 1982. The effect of preslaughter environmental temperature in the presence of electrolyte treatment on turkey meat quality. Poult. Sci. 61:2385–2389.

Bailey, R. A., K. A. Watson, S. F. Bilgili and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870-2879.

Ballay, M., E. A. Dunnington, W. B. Gross and P. B. Siegel. 1992. Restricted feeding and broiler performance: age at initiation and length of restriction. Poult Sci. 71:441-447.

Barbut, S. 1993. Colour measurements for evaluating the pale soft exudative (PALE) occurrence in turkey meat. Foo Red. Int. 26:39-43.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast. Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. Br. Poult. Sci. 38:355-358Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1998. Estimating the magnitude of the PSE problem in poultry. J. Muscle Foods 9:35-49.

Berri, C., B.-D. Elisabeth Le, B. Elisabeth, C. Pascal, P. Laurent, J. Nathalie, Q. Maxime, P. Michel, and D. Michel Jacques. 2005. Further processing characteristics of breast and leg meat from fast-, medium- and slow-growing commercial chickens. Anim. Res. 54:123–134.

Bianchi, M., D. L. Fletcher, and D. P. Smith. 2005. Physical and functional properties of whole and ground pale broiler breast meat. Poult. Sci. 84:803–808.

Bianchi, M., M. Petracci, F. Sirry, E. Folegatti, A. Franchini, A. Meluzzi. 2007. The influence of the season and market class of broiler chickens on breast meat quality. Poult. Sci. 86:959-963.

Bianchi, M., M. Petracci, A. Franchini, and C. Cavani. 2014. The occurrence of deep pectoral myopathy in roaster chickens. Poult Sci. 85:1843-1846.

Bilgili, S. F., J. B. Hess, R. J. Lien, and K. M. Downs. 2000. Deep pectoral myopathy in broiler chickens. Proc. XXI World's Poult. Congr. Montreal, Canada.

Bilgilli, S. F., and J. B. Hess. 2002. Green muscle disease in broilers increasing. World's Poult Sci J. 18:42-43.

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult Sci. 97:3298-3310.

Bohman V. R. 1955. Compensatory growth of beef cattle. The effect of hay maturity. J. Anim Sci. 14:249-255.

Bowker, B. and H. Zhuang. 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult Sci. 94:1657-1664.

Cavitt, L. C., J. F. Meullenet, R. K. Gandhapuneni, G. W. Youm and C. M. Owens. 2005. Rigor development and meat quality of large and small broilers and the use of Allo-Kramer shear, needle puncture, and razor blade shear to measure texture. Poult Sci. 84:113-118.

Collin, A., C. Berri, S. Tesseraud, F. E. Requena Rodon, S. Skiba-Cassy, S. Crochet, M. J. Duclos, N. Rideua, K. Tona, J. Buyse, V. Bruggeman, E. Decuypere, M. Picard and S. Yahav. 2007. Effect of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult Sci. 86:795-800.

Cook, M. E. 2000. Skeletal deformities and their causes: Introduction. Poult. Sci. 79:982-984.

Dadgar, S. E. S. Lee, T. L. V. Leer, T. G. Crowe, H. L. Classen and P. J. Shand. 2011. Effect of acute cold exposure, age, sex, and lairage on broiler breast meat quality. Poult. Sci. 90:444-457.

Darr, K. C., and E. Schultz, 1987. Exercise-induced satellite cell activation in growing and mature skeletal muscle. J. Appl. Physiol. 63:1816–1821.

de Brot, S., S. Perez, H. L. Shivaprasad, K. Baiker, L. Polledo, M. Clark and L Grau-Roma. 2016. Wooden breast lesions in broiler chickens in the UK. Vet Rec. 11.

Dickinson, E. M., J. O. Stevens, and D. H. Helfer.1968. A degenerative myopathy in turkeys. Page 6 in Proc. 17th West. Poult. Dis. Conf. Univ. California, Davis.

Doherty, M. K., L. McLean, J. R. Hayter, J. M. Pratt, D. H. Robertson, A. El-Shafei, S. J. Gaskell, and R. J. Beynon. 2004. The proteome of chicken skeletal muscle: Changes in soluble protein expression during growth in a layer strain. Proteomics 4:2082– 2093.

Droval, A. A., V. T. Benassi, A. Rossa, S. H. Prudencio, F. G. Paiao, and M. Shimokomaki. 2012. Consumer attitudes and preferences regarding pale, soft and exudative broiler breast meat. J. Appl. Poult. Res. 21:502-507.

Duclos, M. J., C. Berri, and E. L. Bihan-Duval. 2007. Muscle growth and meat quality. J. Appl. Poult. Res. 16:107-112.

Fanatico, A. C., P. B. Pillai, J. L. Emmert, and C. M. Owens. 2007. Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 86:2245–2255.

Fletcher, D. L. 2002. Poultry meat quality. Worlds Poult. Sci. J. 58:131-145.

Froning, G. W., A. S. Babji, and F. B. Mather. 1978. The effect of preslaughter temperature, stress, struggle and anesthetization on color and textural characteristic of turkey muscle. Poult. Sci. 57:630-633.

Froning, G. W. 1995. Color of poultry meat. Poult. Avian Biol. Rev. 6:83–93.

Guo, Y. G. Zhang, and J. Yuan. 2003. Effects of source and level of magnesium and vitamin E on prevention of hepatic peroxidase and oxidative deteoriation of broiler meat. Anim Feed Sci Technol. 107:143-150.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World's Poult. Sci. J. 62:485-497.

Harford, I. D., H. O. Pavlidis, and N. B. Anthony. 2014. Divergent selection for muscle color in broilers. Poult. Sci. 93:1059-1066.

Harper, J. A., P. E. Bernier, and L. L. Thompson-Cowley. 1983. Early expression of hereditary deep pectoral myopathy in turkeys due to forced wing exercise. Poult. Sci. 62:2303–2308.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev. Biol. 153:206-216.

Havenstein, G. B., P. R. Ferket and M. A. Qureshi. 2003a. Carcass composition and yield of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509-1518.

Havenstein, G. B., P. R. Ferket and M. A. Qureshi. 2003b. Growth, livability and feed conversion of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500-1508.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994a. Growth, livability, and feed conversion of 1957 vs, 1991 broilers when fed “typical” 1957 and 1992 broiler diets. Poult. Sci. 73:1785-1794.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and D. V. Rives. 1994b. Carcass composition and yield of 1991 vs. 1957 broilers when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 73:1795-1804.

Hedrick, H. B., E. D. Aberle, J. C. Forrest, M. D. Judge, and R. A. Merkel. 1994. Principles of Meat Science. 3rd ed. Kendall/Hunt Publishing Co., Dubuque, IA.

Hess, J. B., and S. F. Bilgili. 2004. Carcass yield response of small broilers to feed nutrient density. In: XXII World Poultry Congress, Istanbul, Turkey.

Hillebrand, S. J. W., E Lambooy, and C. H. Veerkamp. 1996. The effects of alternative electrical and mechanical stunning methods on hemorrhaging and meat quality of broiler breast and thigh muscles. Poult. Sci. 75:664-671.

Huxley, H. E., and J. Hanson. 1954. Changes in the cross-striations of muscles during contraction and structural interpretation. Nature 173:973-976.

Jones, G. P. D. and D. J. Farrell. 1992a. Early-life food restriction of chicken. I. Method of application, amino acid supplementation and the age at which restriction should commence. Br. Poult Sci. 33:579-587.

Jones, G. P. D., and D. J. Farrell. 1992b. Early-life food restriction of chicken. II. Effect of food restriction on the development of fat tissue. Br. Poult Sci. 33:589-601.

Jordan, F. T. W., and M. Pattison. 1998. Deep pectoral myopathy of turkeys and chickens. Pages 398–399 in Poultry Diseases. F.T.W Jordan and M. Pattison, ed. Saunders, London, UK.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773-1780.

Julian, R. J. 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathol. 29:519-527.

Kannan, G., J. L. Heath, C. J. Wabeck, and J. A. Mench. 1997. Shackling of broilers: Effects on stress responses and breast meat quality. Br. Poult. Sci. 38:323–332.

Kannan, G., J. L. Heath, C. J. Wabeck, S. L. Owens, and J. A. Mench. 1998. Elevated plasma corticosterone concentrations influence the onset of rigor mortis and meat color in broilers. Poult. Sci. 77:322–328.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91: 2677–2685.

Kuttappan, V. A., S. D. Goodgame, C. D. Bradley, A. Mauromoustakos, B. M. Hargis, P. W. Waldroup, and C. M. Owens. 2012b. Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. Poult. Sci. 91:3230–3235.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.

Kuttappan, V. A., B. M. Hargis and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult Sci. 95:2724-2733.

Le Bihan-Duval, E., C. Berri, E. Baeza, N. Millet, and C. Beaumont. 2001. Estimation of the genetic parameters of meat characteristics and of their genetic correlations with growth and body composition in an experimental broiler line. Poult. Sci. 80:839–843.

Le Bihan-Duval, E., M. Debut, C. M. Berri, N. Sellier, V. SantéLhoutellier, Y. Jégo, and C. Beaumont. 2008. Chicken meat quality: Genetic variability and relationship with growth and muscle characteristics. BMC Genet. 9:53

Leeson, S. and J. D. Summers. 1988. Some nutritional implications of leg problems with poultry. Brit. Vet J. 144:81-92.

Liever, R. L. 2002. Skeletal muscle structure, function and plasticity. The physiological basis of rehabilitation. 2nd Edition. Lippincott Williams & Wilkins.

Livingston, M. L., C. Landon, H. J. Barnes and J. Brake. 2018. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult Sci. 0:1-10.

Maga, J. A. 1994. Pink discoloration in cooked white meat. Food Rev. Int. 10:273–286.

Marks, H. L. 1979. Growth rate and feed intake of selected and non-selected broilers. Growth. 43:83-90.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

Mazzoni, M., M. Petracci, A. Meluzzi, C. Cavani, P. Clavenzani, and F. Sirri. 2015. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult. Sci. 94:123-130.

McKee, S. R. and A. R. Sams. 1998. Rigor mortis development at elevated temperatures induces exudative turkey meat characteristics. Poult. Sci. 77:169-174.

Meijerhof, R. 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group [WPSA Working Group 6 (Reproduction)], St. Edmand's Hall, Oxford, UK.

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili and W. A. Dozier III. 2018. Effects of reduced dietary energy and amino acid density on *Pectoralis major* myopathies in broiler chickens at 36 and 49 days of age. Poult Sci. 97:1794-1807.

Miller, M. 2007. Dark, firm and dry beef. Centennial, CO. Beef Facts-Product Enhancement. p 1–4.

Mir, N. A., A. Rafiq, F. Kumar and V. Singh. 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol. 54:2997-3009.

Moos, R. L., G. M. Diffee, and M. L. Greaser, 1995. Contractile properties of skeletal muscle fibers in relation to myofibrillar protein isoforms. Rev. Physiol. Biochem. Pharmacol. 126:1–63.

Mudalal, S., E. Babini, C. Cavani, and M. Petracci. 2014. Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. Poult. Sci. 93:2108–2116.

National Chicken Council, 2018. U. S. Broiler Production. Economic Research Service/USDA. https://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-production/.

Northcutt, J. K., E. A. Foegeding, and F. W. Edens. 1994. Water-holding properties of thermally preconditioned chicken breast and leg meat. Poult. Sci. 73:308-316.

O'Donovan, P. B. 1984. Compensatory gain in cattle and sheep. Nutritional. Abs and Rev. 54:389-410.

Orlowski, S. 2016. Characterization of broiler lines divergently selected for breast muscle color. Masters Thesis. University of Arkansas. Fayetteville, AR.

Osbourne, D. F. and P. N. Wilson. 1960 Effects of different patterns of allocation of a restricted quantity of food upon the growth and development of cockerels. J. Agri. Sci. 54:278-289.

Owens, C. M., E. M. Hirschler, S. R. McKee, R. Martinez-Dawson, and A. R. Sams. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. Poult. Sci. 79:553–558.

Pearson, A. M., and R. B. Young. 1989. Muscle and Meat Biochemistry. Academic Press, San Diego, CA.

Petracci, M., and D. L. Fletcher. 2009. Broiler skin meat and color changes during storage. Poult. Sci. 81:1589-1597.

Petracci, M., S. Mudalal, A. Bonfiglio, and C. Cavani. 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670–1675.

Petracci, M., S. Mudalal, E. Babini, and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:179–183.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake and S. Yahav. 2008. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermal tolerance. Poult Sci. 87:1516-1525.

Piestun, Y., S. Druyan, J. Brake and S. Yahav. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult Sci. 92:1155-1163.

Piestun, Y., S. Yahav and O. Halev. 2015. Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat type chickens. Poult Sci. 94:2528-2536.

Pitts, G. C. 1986. Cellular aspects of growth and catch-up growth in rats: a reevaluation. Growth. 50:419-436.

Plavnik, I. and S. Hurwitz. 1989. Effect of dietary protein, energy and feed pelleting on the response of chicks to early feed restriction. Poult Sci. 68:1118-1125.

Plavnik, I. and S. Hurwitz. 1991. Response of broiler chickens: and turkey poults to feed restriction of varied severity during early life. Br. Poult Sci. 32:343-352.

Poultry World. 2017. Reduce woody breast and white striping with dietary approach. https://www.poultryworld.net/Health/Partner/2017/10/Reduce-woody-breast-and-white-striping-with-dietary-approach-196197E/.

Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, water-holding capacity, and emulsification capacity. Poult. Sci. 80:676-680.

Qureshi, M. A. and G. B. Havenstein. 1994. A comparison of the immune performance of the 1991 commercial broiler with a 1957 randombred strain when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 73:1805-1812.

Richardson, J. A., J. Burgener, R. W. Winterfield and S. H, Dhillon. 1980. Deep pectoral myopathy in seven-week old broiler chicks. Avian Dis. 24:1054-1059.

Rehfeldt, C., I. Fiedler, and N. C. Stickland. 2004. “Number and size of muscle fibers in relation to meat production” Muscle Development of Livestock Animals. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Rosebrough, R. W., N. C. Steele, J. P. McCurty and I Plavnik. 1986. Effect of early feed restriction in broilers. Lipid Metabolism. Growth. 50:217-227.

Savenje, B., F. J. G. Schreurs, H. A. Winkelman-Goedhart, M. A. Gerritzen, J. Korf, and E. Lambooij. 2002. Effects of feed deprivation and electrical, gas, and captive needle stunning on early postmortem muscle metabolism and subsequent meat quality. Poult. Sci. 81:561-571.

Schilling, M. W., V. Radhakrishnan, Y. Vizzier-Thaxton, K. Christensen, P. Joseph, J. B. Williams, T. B. Schmidt. 2012. The effects of low atmosphere stunning and deboning time on broiler breast meat quality.

Sihivo, H. K., K. Immonen and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet Pathol. 51:619-623.

Siegel, P. B. and E. A. Dunnington. 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in Poultry Genetics and Breeding. Longman Group, Harlow, UK.

Siller, W. G. 1985. Deep pectoral myopathy: A penalty of successful selection for muscle growth. Poult. Sci. 64:1591–1595.

Smith, D. P., C. E. Lyon, and B. G. Lyon. 2002. The effect of age, dietary carbohydrate source and feed withdrawal on broiler breast fillet color. Poult. Sci. 81:1584-1588.

Stockdale F. E., and J. B. Miller. 1987. The cellular basis of myosin heavy chain isoform expression during development of avian skeletal muscles. Dev. Biol. 123:1-9.

Tijare, V., F. Yang, V. Kuttappan, C. Alvarado, C. Coon, and C. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult Sci. 95:2167-2173.

Trocino, A., A. Piccirillo, M Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, G. Xiccato. 2015. Effect of the genotype, gender and feed restriction on growth, meat quality, and the occurrence of white striping and wooden breast in broiler chickens. Poult Sci. 94:2996-3004.

Uni, Z., and S. Yahav. 2010. Managing prenatal development of broiler chickens to improve productivity and thermotolerance. In: P. L. Greenwood, (Ed). Managing the Prenatal Environment to Enhance Livestock Productivity. pp. 71-90. Springer, Netherlands.

USDA:FSIS. 2017. Disposition instructions for "woody breast" and "white striping" poultry conditions. https://www.fsis.usda.gov/wps/wcm/connect

Velleman, S. G., C. S. Coy and D. A. Emmerson. 2014. Effect of the timing of posthatch feed restrictions on broiler breast muscle development and muscle transcriptional regulatory factor gene expression. Poult Sci. 93:1484-1494.

Viljoen, H. F., H. L. de Kock and E. C. Webb. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. 2002. Meat Sci. 61:181-185.

Wang, R. R., X. J. Pan, Z. Q. Peng. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of pectoralis major in broilers. Poult. Sci. 88:1078-1084.

Warriss, P. D., S. C. Kestin, S. N. Brown and E. A. Bevis. 1987. Depletion of glycogen reserves in fasting broiler chickens. Br. Poult. Sci. 29:149-154.

Warriss, P.D. 2000. Meat Science: An introductory text. CABI Publishing, Oxon, UK.

Washburn, K. W., and K. Bondari. 1978. Effects of timing and duration of restricted feeding on compensatory growth in broilers. Poult Sci. 57:1482-1487.

Wernig A, A. Irintchev, and P. Weisshaupt. 1990. Muscle injury, cross-sectional area and fibre type distribution in mouse soleus after intermittent wheel-running. J. Physiol. 428:639–652.

Wick, M. 1999. Filament assembly properties of the sarcomeric myosin heavy chain. Poult Sci. 78:735-742.

Wikimedia Commons. Muscle Structure. https://commons.wikimedia.org/wiki/File:Illu_muscle_structure.jpg

Wilson, P. N. and D. F. Osbourbe. 1960. Compensatory growth after undernutrition in mammals and birds. Biol Rev. 35:325-363.

Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poult. Sci. 81:579-584.

Yu, M.E. and F. E. Robinson. 1992. The application of short-term feed restriction to broiler chicken production: a review. J Applied Poult Res. 1:147–153.

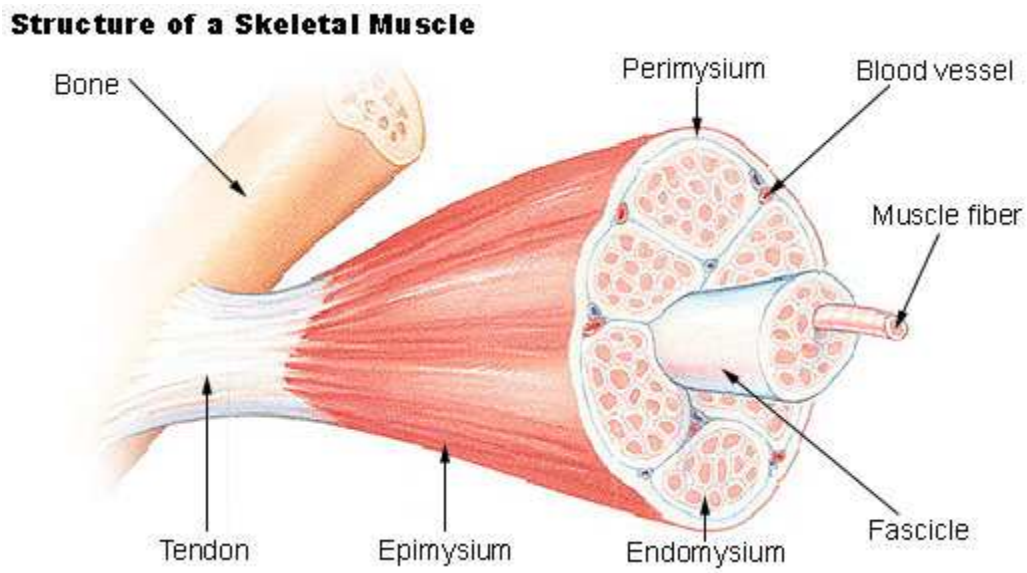


Figure 1. Structure of a skeletal muscle.

Source: Wikimedia Commons

Chapter 2: Histological analysis and gene expression of satellite cells in the pectoralis major muscle in broiler lines divergently selected for Percentage 4-day breast yield

ABSTRACT

Muscle development during embryonic and early post hatch growth is primarily through hyperplastic growth and accumulation of nuclei through satellite cell contribution. Post hatch, muscle development transitions from hyperplasia to hypertrophic growth of muscle fibers. Commercial selection for breast yield traditionally occurs at ages targeting hypertrophic rather than hyperplastic growth. This has resulted in the production of giant fibers and concomitant challenges with regard to muscle myopathies. The current study investigates the impact of selection during the period of hyperplastic growth. It is hypothesized that selection for percentage breast yield during hyperplasia will result in increased number of muscle cells at hatch and potentially impact muscle fiber characteristics at processing. This study characterizes the breast muscle histology of three broiler lines at various ages in the growth period. The lines include a random bred control (RAN) as well as lines which have been selected for high (HBY4) and low (LBY4) Percentage 4-day breast yield. Post-rigor pectoralis major samples from 6 males and 6 females of each age were collected and stored in formalin. The sample ages included embryonic d18, post hatch d4, and d56. The samples were processed using a Leica tissue processor, embedded in paraffin wax, sectioned, and placed on slides. Slides were stained using hematoxylin and eosin. Embryo D18 and D4 post-hatch analysis showed advanced muscle fiber formation for HBY4 and immature muscle development for LBY4 as compared to RAN. D56 samples were analyzed for fiber number, fiber diameter, epimysium and perimysium. Line HBY4 had the largest muscle fiber diameter (54.2±0.96) when compared to LBY4 (45.4±0.96). There were no line difference in epimysium spacing while perimysium spacing was higher for HBY4 males. Selection for Percentage 4-day breast yield has impacted the rate and extent of muscle fiber formation in both the LBY4 and HBY4 lines with no negative impact on fiber

spacing. The shift in processing age to later ages has exposed issues associated with muscle fiber viability. Selection during the period of muscle hyperplasia may result in additional nuclei per fiber and thus a decrease muscle myopathies.

INTRODUCTION

Broiler production in the United States has increased drastically over the past 50 years as demand for poultry meat by consumers has risen. Broiler genetic progress has resulted in a bird that is faster growing, high yielding and more efficient than the broiler from the 1950s. This has allowed for the poultry industry to meet the growing demand in a cost effective way (Barbut et al., 2008). Unfortunately, genetic progress as well as changes in management and environment have resulted in concomitant challenges in the areas of meat quality, with woody breast and white striping being two myopathies that have developed in recent years (Anthony, 1998; Barbut 1996, 1997, 1998). Woody breast is characterized as a hardening of the breast muscle at varying levels and the deposition of collagen in place of muscle fibers (Petracci et al., 2014; Tijare et al., 2016). White striping is superficial striated fat deposition that runs parallel to the muscle fibers and creates a visually unappealing fillet to consumers (Kuttappan et al., 2013). Changes in management and environment, specifically changes in nutrition have been hypothesized and shown to decrease the incidence and severity of these two myopathies but at the cost of a decrease in yield (Bodle et al., 2018; Livingston et al., 2018; Meloche et al., 2018). Heritabilities have been shown to be relatively low (Bailey et al., 2015) with a majority of the variation seen in woody breast associated with non-genetic factors (Trocino et al., 2015). Novel selection methods, however, are being evaluated for their effectiveness in controlling the development of both woody breast and white striping in the industry to avoid further economic losses from the muscle myopathies. Advancements in methods of characterization may also help increase the heritability of this trait by focusing on quantitative measures instead of subjective scoring systems.

One novel selection method being evaluated at the University of Arkansas focuses on an earlier form of breast muscle development. Muscle development in a broiler occurs primarily through two different forms. The first form of muscle development occurs during the embryonic growth period of a broiler and is called hyperplasia (Stockdale and Miller, 1987). Hyperplasia is the increase in cell number with cell number typically being set by hatch (Smith, 1963). Post hatch, muscle development transitions from hyperplasia to hypertrophy, or the accumulation of tissue due to an increase in cell size (Moss, 1968; Mozdziak et al., 1997). Current commercial selection practices in broilers typically focus on ages targeting hypertrophy typically between 6 and 8 weeks of age. Therefore, an emphasis is put on an increase in fiber size and not necessarily on fiber number. While great improvements have been made in the areas of growth rate, yield, feed conversion and disease resistance (Havenstein et al., 2003), genetic selection for hypertrophy or fiber size may be reaching a physiological limit (Mahon, 1999) as indicated by the recent development of muscle myopathies such as white striping and woody breast.

A majority of the breast yield at 4 days of age is going to be a result of fiber number as cells are just starting to enter into the hypertrophic growth period. Selection for 4-day percentage breast yield in broilers was hypothesized to focus not on fiber size but on fiber number. It has been shown in porcine breeds that additional fiber numbers has had a positive correlation with improved meat quality (Lengerken et al., 1994; Karlsson et al., 1999; Fiedler et al., 2003; Suzuki et al., 2003). Additionally, selection at a young age may have the ability to impact the number of satellite cells, also known as adult myoblasts. Satellite cells are important in post hatch growth and repair. According to Velleman et al., (2010), satellite cells have the ability to re-enter the cell cycle, fuse to damaged muscle fibers and contribute their nuclei to help air in post-hatch growth and repair of damaged muscle fibers. The additional nuclei allows for additional protein

accretion, or growth and repair of fibers (Fu et al., 2015). A study by Daughtry and colleagues (2017) found that satellite cells functionality decrease with age as they only have a certain number of divisions due to telomeric shortening. With a decrease in functionality at older ages where protein accretion is still occurring, it is possible that the satellite cells can no longer aid in repair of damaged muscle fibers resulting in the development of both woody breast and white striping. By selecting for additional satellite cells, it is possible to alleviate some of the concerns about aging satellite cells as there are more available to aid in repair.

The purpose of this study was to evaluate divergently selected broiler type lines after five generations of selection for 4-day percentage breast yield. Histological changes as well as any differences in gene expression markers associated with satellite cells will be documented. These factors will be used to determine if selection at 4 days post hatch has altered hyperplastic growth and cell number.

MATERIALS AND METHODS

Broiler Populations

Three broiler lines maintained and housed at the University of Arkansas were used in this study. The first line of birds has been maintained as a random bred population that originated by mating of 7 male and 6 female lines commercially available in the 1990s (RAN) (Harford et al., 2014). From this RAN line, divergent selection was utilized to create the high (HBY4) and low (LBY4) percentage breast yield lines. These lines have been selected through the use of sibling selection for 4 day percentage breast yield ((Breast Wt/(Body Wt-YolkSac)*100) for 5 generations. Since their creation, these lines have been maintained as closed populations and a randomly mated breeding structure is used with the avoidance of full and half siblings to decrease the rate of inbreeding accumulation.

Data Collection

For this study, one non-pedigreed hatch from the RAN, HBY4 and LBY4 lines were set and incubated at 99.5°F and 56% relative humidity from embryonic day 0 (E0) to embryonic day 18 (E18). At E18 they were transferred to a hatcher and hatched chicks were banded by line. Post hatch, chicks were randomly placed by line onto wood shavings litter floor pens with feed and water provided ad libitum throughout the study. Feed was formulated to meet or exceed NRC requirements with a commercial starter feed being fed from 0-21 days and a commercial finisher feed being feed from 22-56 days (NRC, 1994). At the processing age of D56, feed was removed from the birds 12 h prior to processing with access to water remaining constant.

Samples were collected at various ages throughout the embryonic and post hatch growth period including embryonic ages of E16, E18 and E20, and post hatch ages of d1, d4, d7, d14, d42 and d56. This document will only focus on E18, d4 (selection age) and d56. These ages were selected to evaluate three ages were muscle growth and development differ. At E18 and d4, samples were collected for all lines in a similar fashion. For histology, the left breast and keel was excised and stored in formalin solution until processing. At d56 post hatch, birds were euthanized using CO2 gas. The birds were placed in a cooler overnight to allow for the breast muscle to complete the process of rigor mortis to avoid contraction of the collected muscle sample. After 24 h in the cooler, a small rectangular segment running parallel to the muscle fibers was cut and stored in formalin solution. At E18 and d4, samples from 6 males and 6 females were collected from all three lines. At d56, samples from 6 males and 6 females were collected from the HBY4 and LBY4 lines. For gene expression at E18, D4 and D56, birds were euthanized and a small section of breast muscle was immediately flash frozen in liquid nitrogen

and stored at -80°C until further processing. Samples were collected from 6 males and 6 females per line at each sampling age.

Histology

Samples for histology at all ages were processed in a similar way. Small rectangular sections running parallel to the muscle fibers of each sample were cut and placed in plastic tissue processing cassettes. The samples were then processed using a Leica tissue processor to remove all moisture from the sample and replace it with paraffin wax. Once processed, samples were embedded in paraffin wax blocks and sectioned using a Leica microtome. Four sections of each sample were adhered to Starfrost polarized slides and stained using hematoxylin and Eosin Y according to Fischer et al., (2008). Four images per sample were taken using an Olympus BX50 microscope. At E18 and d4, 40X magnification was used and samples were compared for their stage of development. At d56, images were taken at 10X magnification and samples were evaluated for fiber number, fiber diameter, endomysium (spacing between muscle fibers) and perimysium (spacing between muscle fiber bundles) using the ImagePro ® software. For fiber number, a grid was overlaid over each image and the number of fibers within four randomly selected squares were counted and averaged. A fiber was counted in the square if over 50% of the fiber was located within the boundaries. For fiber diameter, 30 fibers were randomly selected, measured (in μm) and averaged per sample. Thirty random distances between fibers were measured and averaged for endomysium and 10 random distances between fiber bundles were measured and averaged for perimysium.

Gene Expression

Extraction of total RNA from the right breast muscle was done using Trizol reagent (Life Technologies, Grand Island, NY). Total RNA concentrations were determined for each sample

by Take 3 Micro-Volume Plate using Synergy HT multimode microplate reader (BioTek,Winooski, VT) after DNAse treatment and purification. RNA integrity and quality were assessed by both OD260/OD280 nm absorption ratio (>1.9). For cDNA synthesis, total RNA (1 μg) was reverse transcribed using qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD) in a 20-μL total reaction. Real-time quantitative (Applied Biosystems 7500 Real-Time PCR system) was performed using 5 μL of 10×-diluted cDNA, 0.5 μM of each forward and reverse specific primers for each gene, and SYBR Green Master Mix (ThermoFisher Scientific, Rockford, IL) in a total 20-μL reaction (Lassiter et al., 2015; Flees et al., 2017). Oligonucleotide primers specific for paired box protein 7 (Pax-7), paired box protein 3 (Pax-3), myogenic factor 5 (Myf5) and r18S as a housekeeping gene were utilized. Relative expressions of target genes were determined by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Samples extracted from the RAN line were used as a calibrator in this study.

Statistical Analysis

The d56 image analysis was analyzed using the PROC GLM procedure in SAS (2012). Images were analyzed for average fiber number, fiber diameter, endomysium spacing and perimysium spacing. The main effects analyzed were line and sex as well as the interaction between line and sex. Means were considered statistically different at a P value < 0.05 with means being separated using the Tukey's HSD. Gene expression results were analyzed using a one-way ANOVA in JMP with means separated by Tukey's HSD with the RAN line being used as a calibrator.

RESULTS

Image analysis in the Image Pro software was not utilized for age E18 and d4 as a result of lines being in different stages of development. No quantitative measures could be properly

evaluated. Images from E18 for each line are shown in Figure 1. At this age, lines appeared to be in different stages of development with the HBY4 line showing advanced muscle fiber development by 1-2 days and the LBY4 line lagging behind a day when compared to the RAN line. Of the 12 samples taken from the HBY4 line, 8 of the 12 showed advanced muscle fiber formation when compared to the random bred line while 4 of the 12 from the LBY4 line appeared to be lagging behind in development. At D4 (selection age), it is still apparent that the HBY4 line is slightly more advanced in muscle development and growth with all 12 of the images showing advanced muscle fiber formation. Images for the 3 lines are shown in Figure 2. For the LBY4 line, only 9 of the 12 samples appear similar to the RAN line in terms of fiber growth and development. Images from d14 are included in Figure 3 to show muscle fiber structure and development at a different time point.

At D56, no visual differences were observed between the HBY4 and LBY4 lines. A line*sex interaction was not present for fiber number, fiber diameter or endomysium and main effects are presented. Line effects were present for fiber diameter with the HBY4 line having a larger fiber than the LBY4 line. No differences were observed for the number of fibers of endomysium (fiber spacing) (Table 1). A sex effect was present for fiber number in which the males had a higher fiber number than the females. No differences were observed between males and females for fiber diameter or endomysium (Table 2). A line*sex interaction was present for perimysium (muscle fiber bundle spacing). For perimysium, the HBY4 males had the greatest perimysium spacing with no difference between the LBY4 males and females from either line (Table 3).

At E18, D4 and D56, gene expression was analyzed for Pax7, Pax3, and Myf5, all markers associated with satellite cell presence and functionality. At E18, no differences were

observed for Pax7, Pax3 or Myf5 (Figure 4). At day 4 (selection age), both Pax7 and Pax3 are down regulated in the HBY4 and LBY4 line compared to the RAN control while no differences were observed in Myf5 expression (Figure 5). At the processing age of D56, no differences were observed for Pax3 or Myf5 while Pax7 was down regulated in both the HBY4 and LBY4 lines when compared to the control (Figure 6).

DISCUSSION

The purpose of this study was to evaluate the HBY4 and LBY4 lines for differences in histological development and satellite cell associated gene expression after 5 generations of selection for four day percentage breast yield. Research done in previous generations have shown that the HBY4 line and LBY4 line do not differ in body weights throughout a 56 day grow-out period, however their percent breast yield differs at all ages measured (Mason, 2015). It has remained unclear from previous research what is driving the difference in breast meat yield, whether it be from an increase in fiber number, an increase in satellite cells or a combination of the two traits.

To better evaluate the effect of genetic selection, histology sections of the breast muscle from the LBY4, HBY4 and RAN lines were evaluated at 3 different ages. The first age evaluated was embryonic day 18 (E18). At E18, hyperplasia is nearly complete as the breast muscle development is in its final stage of proliferation, the satellite cell proliferation wave (Stockdale and Miller, 1987). Around this time, the number of fibers in a broiler will be at its highest point (Hartley et al., 1992). Evaluation of the lines at this age showed changes in the rate of development between the lines. The HBY4 line at this age appeared to be slightly more advanced than the RAN line in its muscle fiber development. Distinct spacing between the fibers, known as the epimysium (Aberle et al., 2012) is visible in the HBY4 line. However, in the LBY4 line,

muscle fiber spacing is lagging behind with a majority of the images taken at this age showing little fiber formation and only an organized mass of muscle cells. The RAN line appears to be in between the HBY4 line and LBY4 line in terms of fiber development and images from this line are consistent with the development of a modern commercial line at this age (Velleman, personal communication). As a result of selection, it appears that selection for a high percent breast yield at D4 has increased the rate of muscle fiber development embryonically while selection for a low percent breast yield has resulted in a decrease in the rate of muscle fiber development.

The next age evaluated was the selection age of post hatch day 4 (D4). At D4, muscle development has transitioned from hyperplastic growth that occurred embryonically to hypertrophy in which cells are growing through the accumulation of protein (Moss, 1968; Mozdziak et al., 1997). Satellite cells at this point are quiescent on the muscle siting between the sarcolemma and basement membrane on the muscle fibers (Mauro, 1961). At this age, through the use of H & E staining, it appears that the HBY4 line is again at a more advanced stage of development than the LBY4 and RAN lines. Images from the HBY4 line shown nuclei moving to the periphery of the muscle fiber where they will remain. The LBY4 line still shows nuclei spread throughout the fiber and may be lacking slightly in the rate of development. Because the lines appear to be at different stages of development, no numerical differences in fiber number or number of nuclei could be evaluated. It does appear though the HBY4 line has entered into rapid hypertrophic growth at an earlier age than the RAN and LBY4 lines.

The final age evaluated for histology was a typical processing age of commercial broilers, 56 days post hatch (D56). Histology samples of breast fillets affected by woody breast show severe fiber degeneration, fibrosis and lipidosis (Soglia et al., 2016). Only samples from the HBY4 and LBY4 lines were evaluated. At this age, muscle sections from both lines showed

healthy muscle fibers with only slight degradation of the fibers as a result of age. Because these lines were developed from a population that was commercial available in the 1990s, they do not exhibit current muscle myopathies such as woody breast or white striping and muscle sections tend to appear healthier than a modern commercial broiler breast fillet they may exhibit one or both of the myopathies described.

For the analysis, muscle fiber number, muscle fiber diameter, muscle fiber spacing (endomysium) and muscle fiber bundle spacing (perimysium) were evaluated. For muscle fiber number, no differences were present for fiber number between the HBY4 and LBY4 lines. As muscles grow muscle fibers can fuse together to form larger fibers (Aberle et al., 2012). It is still possible that at a younger age, muscle fiber number differs between the HBY and LBY4 lines. While fiber number did not differ, fiber size was larger in the HBY4 line than the LBY4 line supporting the theory that the HBY4 line may still have a higher fiber number but those fibers have fused together resulting in a larger fiber diameter. This larger fiber diameter is also responsible for the higher yields observed in the HBY4 line. No differences were present for the endomysium, meaning the spacing of muscle fibers was similar between the lines. Visual analysis showed healthy spacing between the fibers for both lines (Velleman, personal communication).

A sex effect was present for both fiber number and fiber diameter. Males had a higher fiber number and a smaller fiber diameter when compared to females. In broilers, females have been shown to have a higher breast meat yield, which could be a result of the larger fiber diameter and an increase in the amount of protein accretion. No difference was seen for endomysium with both males and females showing healthy fiber spacing regardless of line. An interaction was present for perimysium or the spacing between muscle fiber bundles. The HBY4

males had the largest perimysium spacing than the LBY4 males or either line of females. Results are inconsistent with a study done by An and colleagues (2010) in which a sex effect was present for the endomysial spacing with no effect on perimysium.

For gene expression, three genes associated with satellite cells were evaluated. Quiescent satellite cells exhibit the paired box protein 7 (Pax7) transcription factor (Seale et al., 2000). Upon activation, Pax7 is coexpressed with MyoD which is a member of the myogenic regulatory factor (MRF) family of proteins in addition to myogenic regulatory factor 5 (Myf5). The interplay between paired box proteins and myogenic regulatory factors are important in the indication of self-renewal of satellite cells. Pax7 expression decreases during activation and differentiation of satellite cells (Asakura et al., 2001). MRF family proteins are the main regulators in skeletal myogenesis with Myf5 being the earliest to be expressed. The expression of the paired box protein Pax3 and Pax7 along with the expression of Myf5 are key regulators in the myogenesis process and satellite cell proliferation and activation (Francetic and Li, 2011). All three genes in combination are good indicators not only of the presence of satellite cells but their activity as well.

No differences were seen in any of the genes measured at E18. At this time is when satellite cells are still proliferating and all 3 populations show high levels of Pax7, Pax3 and Myf5. However at D4, Pax7 and Pax3 are down regulated in both lines. It is possible though that for the HBY4 line, satellite cells are already active in contributing their nuclei to the fibers to aid in growth resulting in the differences in percent breast yield when compared to the Ran line at this age. Concurrently, the LBY4 line may not have as many satellite cells present as a result of selection resulting in a similarly low expression of both Pax7 and Pax3. Similarly at D56, the expression of Pax7 is lower in the HBY4 and LBY4 lines than the RAN line. This could again be

due to the fact that the satellite cells are more active in the HBY4 line and have already contributed their nuclei while in the LBY4 line they are at a lower number and have been used up. Evaluation of protein levels within these lines will result in a clearer understanding of the results of the selection program in relation to gene expression and will be evaluated in a future study.

CONCLUSION

A divergent selection program for 4-day percentage breast yield in broilers had been implemented for 5 generations. After 5 generations of selection, it appears that the embryonic and early post hatch development of the two broiler lines has been altered compared to their random bred control. The HBY4 line has exhibited an increased rate of muscle fiber formation while the LBY4 appears to be lagging behind. At processing ages, no difference exist between the lines for fiber number while the HBY4 line exhibited a larger fiber diameter. Differences between lines for satellite cell markers were not consistent at the three ages evaluated for satellite cell markers after five generations of selection for 4-day percentage breast yield. It appears as though selection for 4-day percentage breast yield may not be impacting satellite cell markers and selection in the upward direction has increased both the post hatch transition to and rate of hypertrophic growth.

REFERENCES

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of meat science. Fifth Edition. Kendall Hunt Publishing Company.

An, J. Y., J. X. Zheng, J. Y. Li, D. Zeng, J. L. Qu, G. Y. Xu and N. Yang. 2010. Effect of myofibrillar characteristics and thickness of perimysium on meat tenderness in chickens. Poult Sci. 89:1750-1754.

Anthony, N. B. 1998. A review of genetic practices in poultry. Efforts to improve meat quality. J. Muscle Foods. 9:25-33.

Asakura, A., H. Hirai, B. Kablar, S. Morita, J. Ishibashi, B. A. Piras, A. J. Chrst, M. Verma, K. A. Vineretsky and M. A. Rudnicki. 2007. Increased survival of muscle stem cells lacking the MyoD gene after transplantation into regenerating skeletal muscle. PNAS. 104:16552-16557.

Bailey, R. A., K. A. Watson, S. F. Bilgili and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870-2879.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast. Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. Br. Poult. Sci. 38:355-358Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1998. Estimating the magnitude of the PSE problem in poultry. J. Muscle Foods 9:35-49.

Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan, and E. W. Wilson. 2008. Progress in reducing the pale soft and exudative (PSE) problem in pork and poultry meat. Meat. Sci. 79:46-63.

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult Sci. 97:3298-3310.

Daughtry, M. R., E. Berio, Z. Shen, E. J. R. Suess, N. Shah, A. E. Geiger, E. R. Berguson, R. A. Dalloul, M. E. Persia, H. Shi, D. E. Gerrard. 2017. Satellite cell-mediated breast muscle regeneration decreases with broiler size. Poult Sci. 96:3457-3464.

Fiedler, I., K. Nürnberg, T. Harge, G. Nürnberg, and K. Ender. 2003. Phenotypic variations of muscle fiber and intramuscular traits in Longissimus muscle of F2 population Duroc x Berlin Miniature Pig and relationships to meat quality. Meat Sci. 63:131-139.

Fischer, A. H., K. A. Jacobson, J. Rose and R. Zeller. 2008. Hematoxylin and Eosin staining's of tissue and cell sections. Basic Methods of Microscopy.

Flees, J., H. Rajaei-Sharifabadi, E. Greene, L. Beer, B. M. Hargis, L. Ellestad, T. Porter, A. Donoghue, W. G. Bottje, and S. Dridi. 2017. Effect of morinda citrifolia (noni)-enriched diet on hepatic heat shock protein and lipid metabolism-related genes in heat stressed broiler chickens. Front. Physiol. 8:919.

Francetic, T., and Q. Li. 2011. Skeletal myogenesis and Myf5 activation. Transcription, 2:109-114.

Fu, X., H. Wang, P. Hu. 2015. Stem cell activation in skeletal muscle regeneration. Cell Mol Life Sci. 72:1663-1677.

Harford, I. D., H. O. Pavlidis, and N. B. Anthony. 2014. Divergent selection for muscle color in broilers. Poult. Sci. 93:1059-1066.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev. Biol. 153:206-216.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509-1518.

Karlsson, A. H., R. E. Klont, and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork quality. Livestock Prod. Sci. 60:255-270.

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, C. M. Owens. 2013. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult Sci. 92:811-819

Lassiter, K., E. Greene, A. Piekarski, O. B. Faulkner, B. M. Hargis, W. Bottje, and S. Dridi. 2015. Orexin system is expressed in avian muscle cells and regulates mitochondrial dynamics. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308:R173–R187.

Lengerken, G. V., S. Maak, M. Wicke, I. Fiedler, and K. Ender. 1994. Suitability of structural and functional traits of skeletal muscle for genetic improvement of meat quality in pigs. Arch. Tierz. 37:133-143.

Livingston, M. L., C. Landon, H. J. Barnes and J. Brake. 2018. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult Sci. 0:1-10.

Mahon, M. 1999. Muscle abnormalities-morphological aspects in: Richardson, R.I. and Mead, G.C. (Eds) Poult. Meat Sci., Poult. Sci. Symposium Series, pp 19-64. Wallingford, CAB INTL

Mason, J. G. 2015. Divergent selection for four-day relative breast yield and the effect of thermal manipulations on growth characteristic on lines selected for different processing dates. Dissertation.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili and W. A. Dozier III. 2018. Effects of reduced dietary energy and amino acid density on *Pectoralis major* myopathies in broiler chickens at 36 and 49 days of age. Poult Sci. 97:1794-1807.

Moss, F. P. 1968. The relationship between the dimensions of the fibres and the number of nuclei during normal growth of skeletal muscle in the domestic fowl. J Anim Annat. 122:555-563.

Mozdziak, P. E., E. Schultz, R. G. Cassens. 1997. Myonuclear accretion is a major determinant of avian skeletal muscle growth. Am J Physiol. 272:565-571.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Petracci, M., S. Mudalal, E. Babini, C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:179-183.

SAS Institute Inc. 2010. SAS/STAT® User's Guide. 2010 Edition of SAS Institute Inc., Cary, NC.

Seale, P., Sabourin, L. A., Girgis-Gabardo, A., Mansouri, A., Gruss, P. and Rudnicki, M. A. 2000. Pax7 is required for the specification of myogenic satellite cells. *Cell* 102, 777-78

Schmittgen, T. D., and K. J. Livak. 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3:1101–1108.

Smith, J. H. 1963. Relation of body size to muscle cell size in the chicken. Poult. Sci 42:283-290.

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

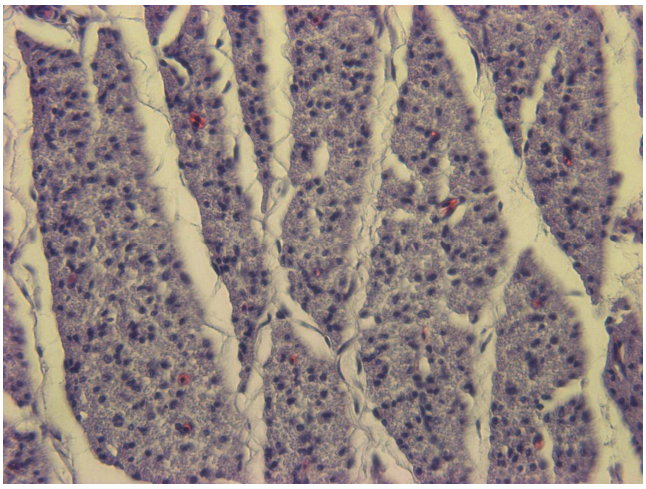
Stockdale, F. A. and J. B. Miller. 1987. The cellular basis of heavy chain isoform expression during development of avian skeletal muscles. Dev Biol. 123:1-9.

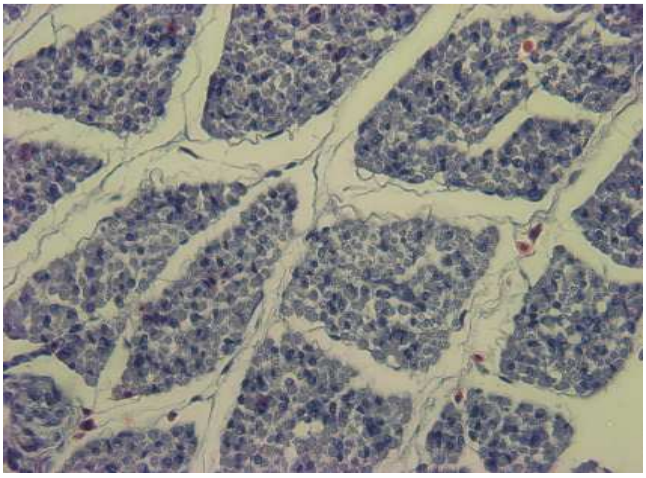
Suzuki, K., T. Shibata, H. Kadowaki, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64:35-42.

Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. 95:2167-2173.

Trocino, A., A. Piccirillo, M Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, G. Xiccato. 2015. Effect of the genotype, gender and feed restriction on growth, meat quality, and the occurrence of white striping and wooden breast in broiler chickens. Poult Sci. 94:2996-3004.

Velleman, S. G., X. Zhang, C. S. Coy, Y. Song, and D. C. McFarland. 2010. Changes in satellite cell proliferation and differentiation during turkey muscle development. Poult Sci. 89:709-715.

A. 

B. 

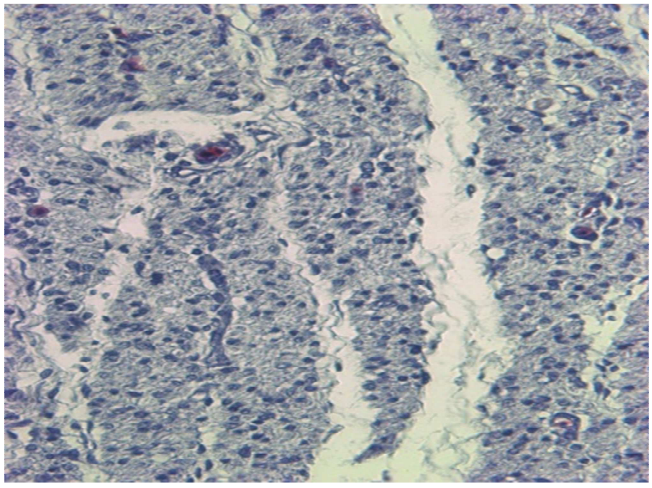
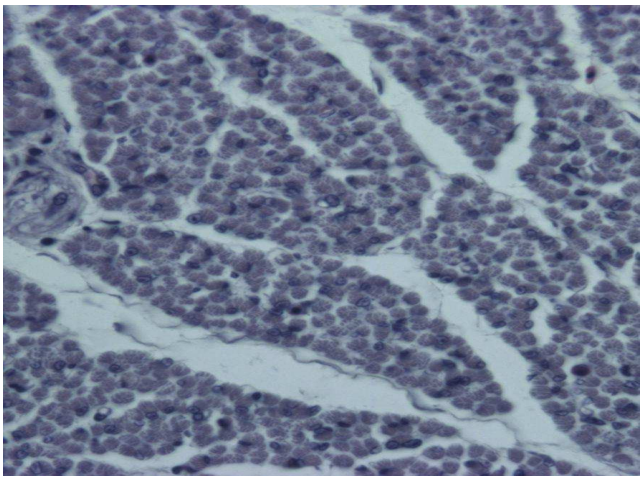
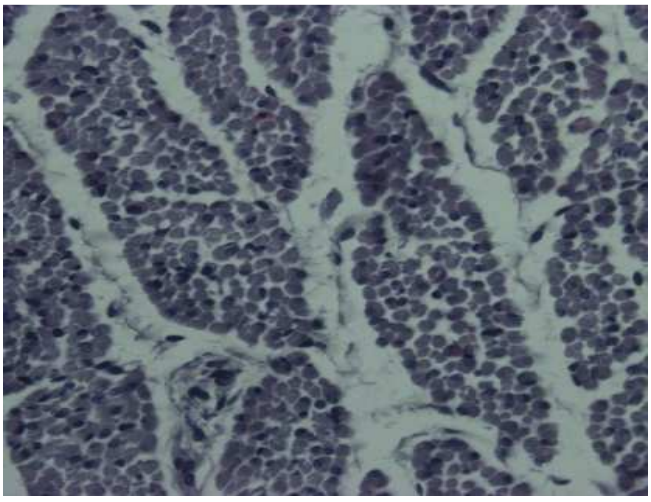
C. 

Figure 1. Histology images from the RAN (A), HBY4 (B) and LBY4 (C) lines at embryonic day 18 under 40X magnification

A. 

B. 

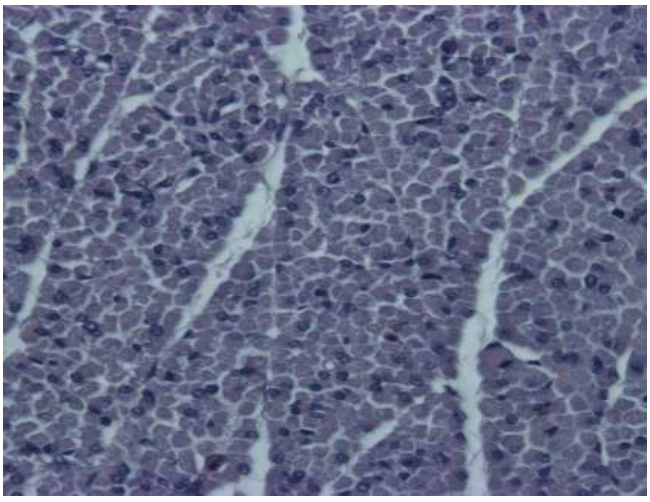
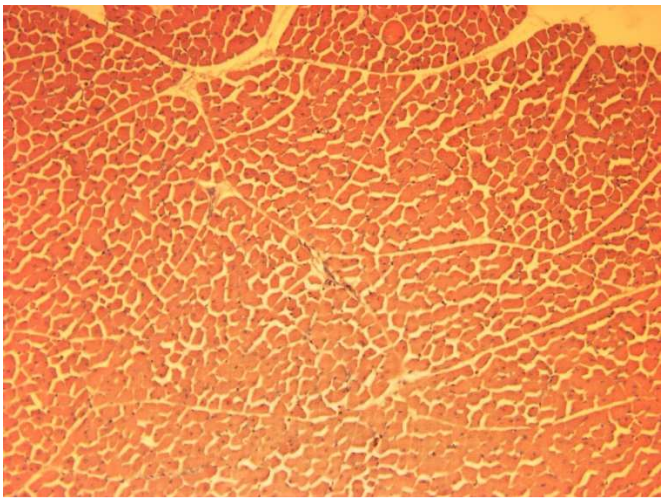
C. 

Figure 2. Histology images from the RAN (A), HBY4 (B) and LBY4 (C) lines at post hatch day 4 (selection age) under 40X magnification.

A.



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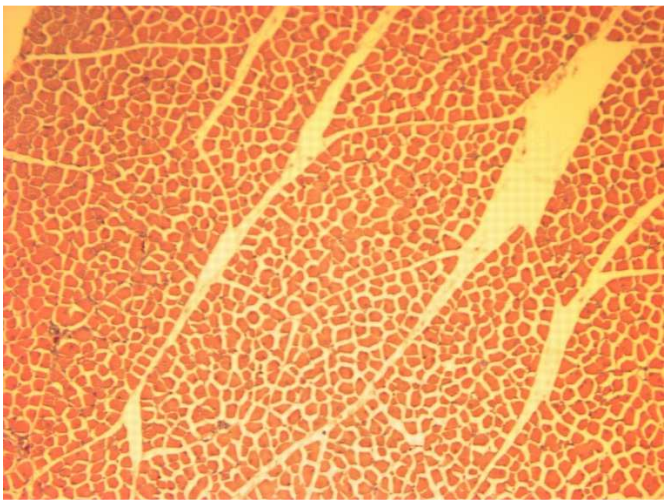

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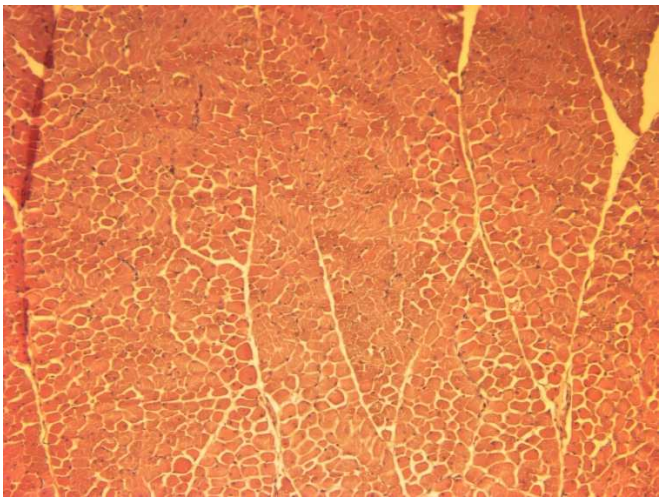


Figure 3. Histology images from the RAN (A), HBY4 (B) and LBY4 (C) lines at post hatch d14 under 40X magnification

Table 1. Line effects at D56 between the HBY4 and LBY4 lines[1] for histological analysis (Mean ± SE)

Trait	HBY4	Significance[2]	LBY4
Fiber Number	17.53 ± 0.39	NS	18.02 ± 0.40
Fiber Diameter	54.29 ± 1.13	***	45.39 ± 0.92
Endomysium	12.11 ± 0.42	NS	13.04 ± 0.33

[1]HBY4-High percent breast yield at 4 days post hatch, LBY4-Low percent breast yield at 4 days post hatch

[2] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 2. Sex effects at D56 between males and females for histological analysis (Mean ± SE)

Trait	Males	Significance[1]	Females
Fiber Number	18.38 ± 0.43	*	17.18 ± 0.34
Fiber Diameter	47.28 ± 1.31	***	52.40 ± 0.98
Endomysium	12.33 ± 0.39	NS	13.04 ± 0.39

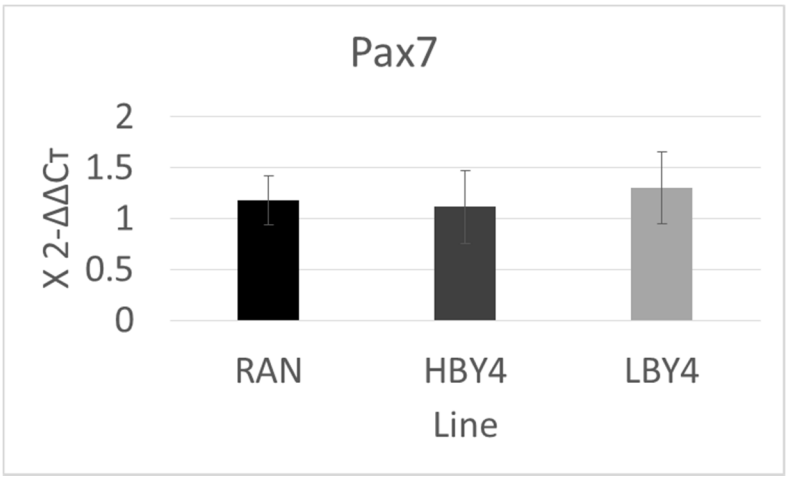
[1]Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 3. Line*Sex[1] interaction for perimysium measurement (μm) at D56 (Mean ± SE)[2]

	HBY4 - M	LBY4 - M	HBY4 - F	LBY4 - F
Perimysium	44.01 ± 2.84a	33.05 ± 1.60b	29.57 ± 1.30b	29.77 ± 1.35b

[1]HBY4-M-High 4-day breast yield males, LBY4-M-Low percent breast yield males, HBY4-F-High 4-day breast yield females, LBY4-F-Low percent breast yield females
[2]a-b For each trait, groups with no common letter within a row are different at P≤0.05

A.



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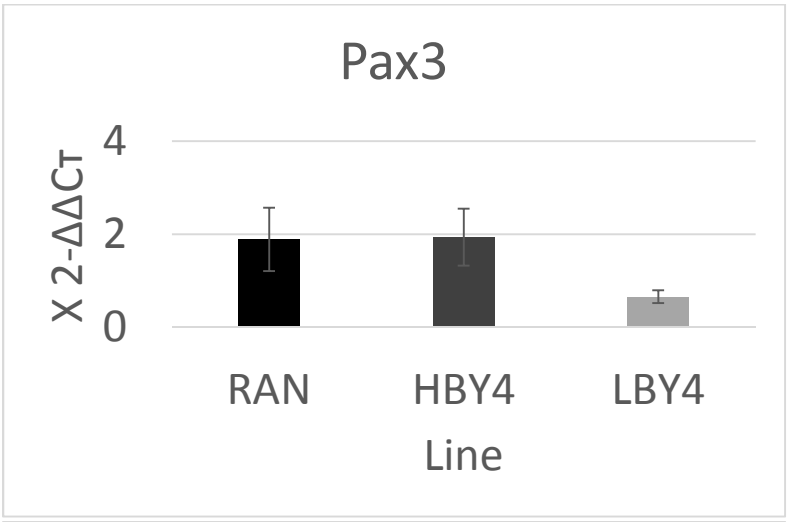


C.

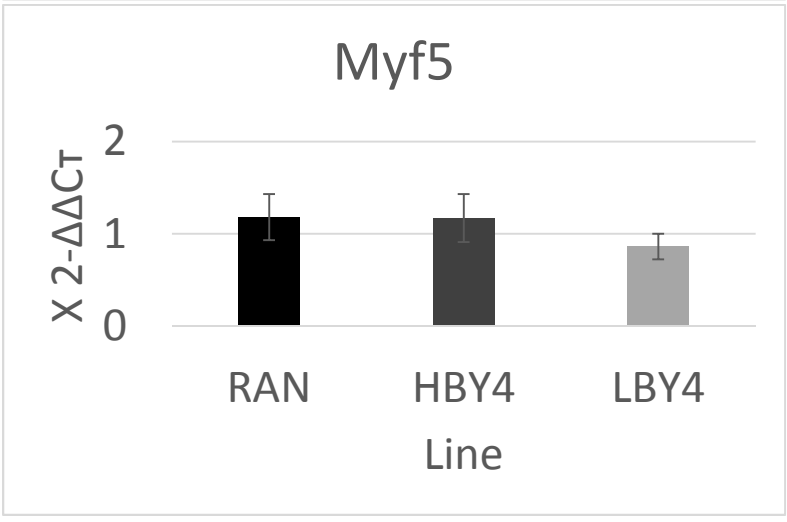
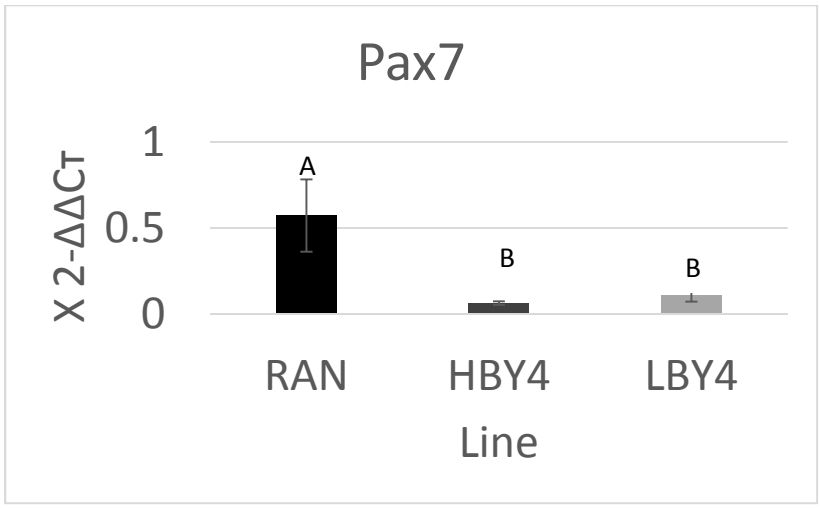


Figure 4. Gene Expression (Mean ± SE) of Pax7 (A), Pax3 (B) and Myf5 (C) at Embryonic Day 18 for the HBY4, RAN and LBY4 lines

[1]HBY4-High percent breast yield at 4 days post hatch, LBY4-Low percent breast yield at 4 days post hatch, RAN=random bred control



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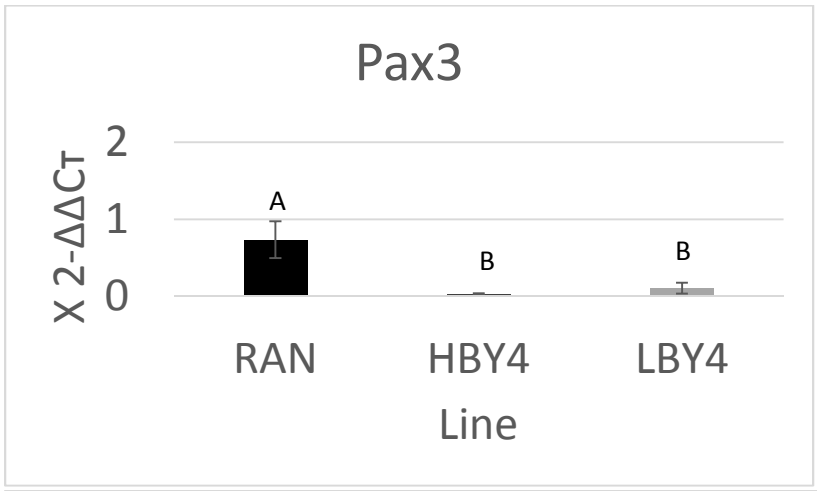

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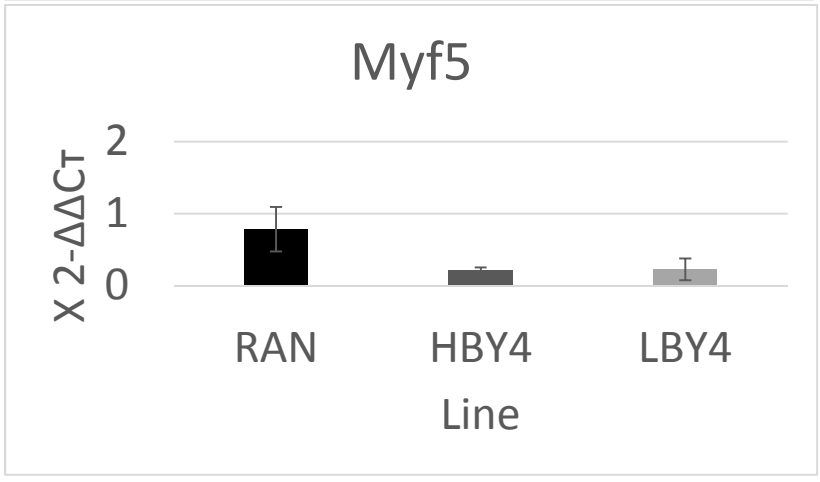
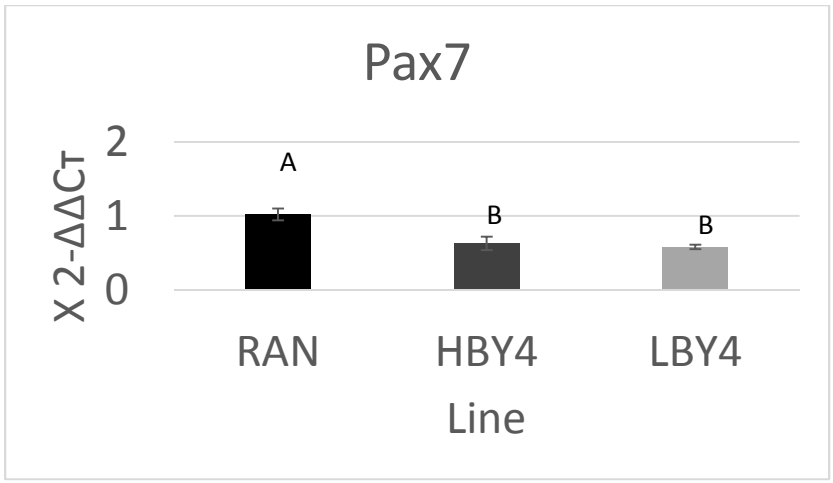


C.

Figure 5. Gene Expression (Mean ± SE)[2] of Pax7 (A), Pax3 (B) and Myf5 (C) at Post Hatch Day 4 (Selection Age) for the HBY4, RAN and LBY4 lines[1]

[1]A-B For each trait, groups with no common letter within a graph are different at P≤0.05
[2]HBY4-High percent breast yield at 4 days post hatch, LBY4-Low percent breast yield at 4 days post hatch, RAN=random bred control

A.



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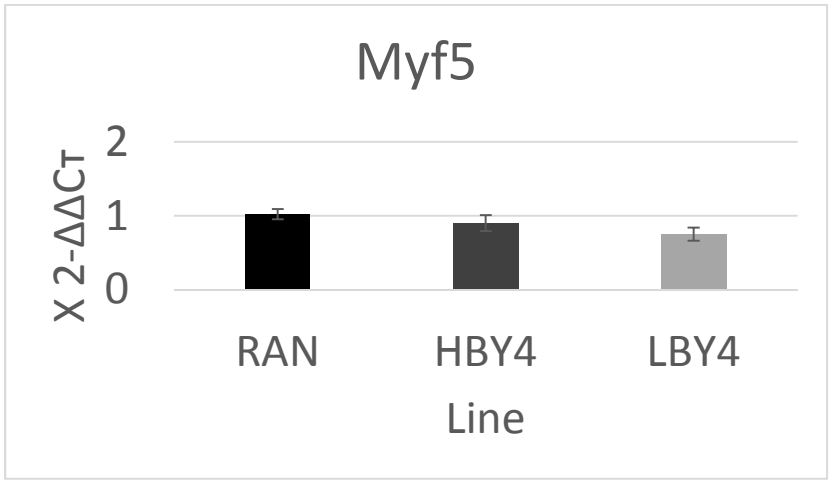


Figure 6. Gene Expression (Mean ± SE)[1] of Pax7 (A), Pax3 (B) and Myf5 (C) at Post Hatch Day 56 for the HBY4, RAN and LBY4 lines[2]

[1]A-B For each trait, groups with no common letter within a graph are different at P≤0.05
[2]HBY4-High percent breast yield at 4 days post hatch, LBY4-Low percent breast yield at 4 days post hatch, RAN=random bred control

Chapter 3: Relationship between 4 day percentage breast yield and incidence of woody breast and white striping muscle myopathies in broilers

ABSTRACT

Embryonic and early post hatch muscle development and growth is primarily through hyperplastic growth and accumulation of nuclei through satellite cell contribution. Post hatch, muscle development transitions from hyperplasia to hypertrophic growth of fibers. Commercial selection for breast yield traditionally occurs at ages targeting hypertrophic rather than hyperplastic growth. This has resulted in the production of giant fibers and concomitant challenges regarding muscle myopathies including woody breast and white striping. It has been hypothesized that selection for breast yield during the hyperplastic growth phase will result in an increase in the number of muscle cells and the number of nuclei at hatch and potentially impact muscle characteristics at processing ages. The current study investigates the relationship between 4 day percentage breast yield and incidence and severity of the woody breast and white striping myopathies based on sire family relationships in a randomly mated modern commercial broiler population. Average 4 day percent breast yield was calculated for 24 sire families (n=12 per sire family) over two separate hatches and separated into High (highest yielding 6 families ≥3.7%) and Low (lowest yielding 5 families ≤3.3%). A separate hatch of birds representing the High and Low sire families was grown to 8 weeks of age, processed and evaluated for muscle myopathies. Throughout the growth period, the High sire family birds were heavier from 4 days to 6 weeks but the weight differences between the groups disappeared by processing day. The High sire families had the highest percent breast yield at 8 weeks (28.3±0.2), when compared to the Low (26.8±0.2) sire families while the Low sire families had a higher percent leg. The High sire families had the most severe incidence of white striping but there was no difference between the High and Low sire families for the woody breast myopathy. There appears to be an association between early muscle growth and muscle characteristics at traditional processing ages.

INTRODUCTION

Over the past 30 years, major improvements in genetic selection have resulted in a modern broiler that is faster growing, higher yielding and has a decreased feed conversion (Barbut et al., 2008). However, with these improvements have come concomitant challenges in the areas of fertility (Siegel and Dunnington, 1985; Quereshi and Havenstein, 1994), disease (Julian, 1998, 2000; Cook, 2000) and lastly meat quality (Anthony, 1998; Barbut, 1996, 1997, 1998). Two meat quality abnormalities that have become a serious problem within the last decade include woody breast and white striping (Kuttappan et al., 2013; Petracci et al., 2014; Tijare et al., 2016). Woody breast is a hardening or stiffening of the breast fillet at varying degrees and deposition of collagen and in severe cases causes a yellow exudate to form on the outside of the breast muscle. In recent years, the USDA has ruled that areas affected by the yellow exudate or inflammatory fluid would have to be trimmed and/or condemned (USDA:FSIS, 2017) resulting in a loss in profit. It is estimated that woody breast is costing the US poultry industry over $200 million in economic loss (Kuttappan et al., 2016; Poultry World, 2017). White striping is a myopathy that is usually found to coincide with woody breast (Bowker and Zhuang, 2017). It is the deposition of fat that runs parallel to the muscle fibers resulting in superficial and visible white striations that results in a visually unappealing product for the consumers (Kuttappan et al., 2012). In addition to issues with consumer acceptability it has also been found to increase the cook loss of the breast fillet and have negative impacts on marinade uptake (Kuttappan et al., 2016).

Several methods have been evaluated in their effectiveness of decreasing the incidence and severity of both white striping and woody breast. Nutrition has been shown to decrease the incidence of woody breast slightly through alteration of amino acids or supplementation with a

high dosage of vitamins (Bodle et al., 2018) or reduction in dietary energy (Meloche et al., 2018). However, it has been shown that alteration of the diet, while it may alleviate some of the incidence and severity of the myopathies, also has a negative impact on body weight, carcass yield and breast yield and may not be economically sound. Livingston et al., (2018) suggests that longer periods of egg storage may have a negative impact on the presence of the two myopathies. Selection has also been looked at as a potential method to decrease the incidence of the myopathies although heritability's have been shown to be relatively low (Bailey et al., 2015) with non-genetic environmental factors such as nutrition and management resulting in most of the observed variance in the myopathies (Trocino et al., 2015).

One novel selection method has been implemented and evaluated at the University of Arkansas and focuses on selection for 4-day percentage breast yield in broilers. With muscle growth in broilers, muscle develops in two very different ways. The first and earliest process of development is through hyperplasia or the increase in cell number which occurs embryonically through several waves (Stockdale and Miller, 1987). Near or at hatch, hyperplasia is complete and cell number in a broiler is typically set. The second process is through hypertrophy or the accumulation of tissue due to an increase in cell size or additional protein accretion. This can be a result of satellite cell, also known as adult myoblast activation post hatch. Satellite cells are undifferentiated cells that accumulate embryonically but become quiescent on the muscle around 7 days post hatch until they are activated and recruited to aid in repair of damaged muscle fibers through nuclear contribution (Moss and LeBlond, 1971).

Current commercial selection practices focus on ages that target hypertrophic growth and not necessarily hyperplastic growth with the typical selection age between 6 and 8 weeks of age. The hypothesis behind the 4 day breast yield divergent selection program is that by selecting for

a high percent breast yield at a young age, most of the breast yield at this age will be a result of fiber number and not yet fiber size as it is very close to the period of hyperplastic growth. In addition, selection for a higher breast yield at this age should result in an increase in the number of satellite cells which can help aid in post hatch muscle repair and possibly hinder the development of muscle myopathies. While these lines are currently involved in a divergent selection program for both high and low 4-day percentage breast yield, they have been developed from a random bred line from the 1990's and do not exhibit currently relevant muscle myopathies including woody breast and white striping at a very high incidence or severity. To better evaluate the relationship between 4 day percentage breast yield and the incidence of woody breast and white striping, a study was done with a modern random bred broiler population developed in 2015 in an attempt to correlate the traits.

MATERIALS AND METHODS

Broiler population

The line used in this study was a modern random bred (MRB) broiler population that was established at the University of Arkansas in 2015. This line originally consisted of four commercial broiler packages (Ross544 x Ross308, Ross Yield+ x Ross 708, CobbMX x Cobb500, and Hubbard M99 x Hubbard HiY) from three different companies that in 2015 made up greater than 95% of the broiler sales in the United States (Cooper, personal communication). The MRB line, once established, has been considered a closed population and undergoes random mating each generation, avoiding full and half sibling matings. This line had exhibited both the woody breast and white striping myopathies at an incidence and severity consistent with industry prevalence.

Three pedigreed hatches from the MRB line were incubated at 99.5°F and 56 % relative humidity from embryonic day 0-18. At E18, they were transferred to a commercial walk in hatcher and bagged by pedigree family for hatch. At hatch chicks were pulled and banded by family. They were placed on litter shavings floor pens with feed and water being provided ad libitum throughout the study. All birds in the study received a commercial starter feed from day of hatch to 3 weeks and a commercial finisher feed for the remainder of the study. All diets were formulated to meet or exceed NRC requirements (NRC, 1994).

4-day Breast Yield-Hatch 1 and 2

For hatches 1 (n=142) and 2 (n=117), chicks were grown until 4 days of age. Twelve hours prior to processing, feed was removed. Chicks were euthanized using CO_2 gas, placed breast side up on trays and allowed to chill for 4 hours. After chilling, the body weights of the chicks were recorded as well of the weights of the breast (pectoralis major), tenders (pectoralis minor), keel, and yolk sac. If any feed remained in the crop after the withdrawal period, it was removed prior to the recording of body weight. Breast yield for each individual chick was calculated using the following equation: (Breast Wt/(Body Wt-YolkSac))*100. Sire families were then separated into two distinct groups based on natural numerical breaks observed in the dataset. Families with an average breast yield greater than 3.7% were labeled as the High sire families. This included 6 sire families. Families with an average breast yield less than 3.3% were labeled as the Low sire families. This included 5 sire families.

56 d Processing

Hatch 3 consisted only of pedigreed offspring from the High (n=78) and Low (n=82) sire families. Weights were recorded at hatch, d4, d7, d10, d14 and weekly until processing at d56. Feed was removed from the broilers 12 hour prior to processing while access to water remained

constant until load out. Dock weights were recorded, birds were hung on shackles, stunned and allowed to bleed out for 3 minutes. They were then scalded, feathers picked and neck and feet removed. Birds were manually eviscerated and weighed for a with-out-giblets (WOG) weight and placed in an ice bath for a 4 hour chilling period. Post chill, carcasses were reweighed for a chilled-WOG (CWOG) weight and manually deboned. The weight of the breast, tenders, wings, leg quarters and rack were recorded. Following deboning, breast fillets were subjectively hand scored for two muscle myopathies. A woody breast score was done on a scale from 0 to 3 with half scores being included (Tijare et al., 2016). This was a subjective score with 0 being a fillet that was flexible throughout with no signs of the woody breast myopathy and 3 being severe woody breast with firmness throughout the entire fillet and a hard caudal ridge. White striping was also scored subjectively for each breast fillet on a scale from 0 to 3 with 0 being no white striping and 3 being severe white striping throughout the entire breast fillet. Half scores were also used for white striping.

Statistical Analysis

Statistical analysis was done using the Fit Model platform in the JMP Pro 13 software (SAS Institute, 2010). For hatch one and two combined, sire families were separated by their LS Means calculated in the JMP software for 4 day percentage breast yield. From there, natural breaks in the 4-day breast yield averages were chosen to separate the sire families into two distinct groups (High and Low). For the 56 d growth and processing results, the main effect analyzed was the 4-day sire family that was calculated from the data recorded from hatch 1 and 2 through a one-way ANOVA. Means were separated using the Students t-test with a p value < 0.05. All data is presented as the LS Mean ± standard error.

RESULTS

Variations in 4-day percentage breast yield were present among the sire families of the MRB line ranging from average percentage breast yields of 2.89 to 4.04. Sire families were broken into two categories based on the average percentage breast yield of two pedigreed hatches. The High sire families had an average percent breast yield greater than 3.7% and the Low sire families had an average percent breast yield less than 3% at 4 days of age. The following results are from a single pedigreed hatch from the High and Low sire families.

Weight differences between the High and Low sire families were not present at hatch. However, at days 4, 7, 11 and 14, the High sire families were heavier than the Low sire families. No differences between the groups were observed after 14 days. Figure 1 shows the complete growth curve from hatch to processing of the two groups.

No differences were observed for both WOG, CWOG and average moisture uptake with the average WOG among the groups being 3035.1g and the average CWOG being 3099.0g. Table 1 shows the processing results for the full parts cut up between the High and Low sire families. There were no differences detected for absolute breast, tenders, wings, legs or rack. Percentage breast was highest in the High sire families and lowest in the Low sire families while the opposite was observed for percentage legs in which the Low sire families had the highest percent legs when compared to the high (Figure 2). Even though the same birds were not used for both the 4-day percentage breast yield and 56 d percent breast calculation, siblings from the families labeled as High at 4 days also had the highest percent breast at 56 days. Dressing percentage which was calculated as the (WOG/Dock Wt)*100 was highest in the Upper sire families at 76.8% ± 0.17 and lowest in the Low sire families at 75.3% ± 0.17

The severity scores for both woody breast (WB) and white striping (WS) are shown in Table 2. There were no differences observed between the High and Low sire families for the average WB severity score. For the average WS severity score, the High sire families had a more severe incidence than the Low sire families. Few differences were found in the frequency scores for both white striping (Figure 3) and woody breast (Figure 4).

DISCUSSION

Selection for 4-day percentage breast yield has the potential to impact not only the number of muscle fibers post hatch but also the number of satellite cells or adult myoblasts. Satellite cells, which are beneficial in post hatch muscle growth and repair (Moss and Leblond, 1971) may be useful in reducing the incidence of muscle myopathies such as woody breast and white striping. A modern random bred population (MRB) created in 2015 at the University of Arkansas exhibited variations for 4-day percentage breast yield at 4 days of age among the 24 sire families utilized. This variation ranged from 2.89 and 4.04. Even in a randomly mated population, it is possible to generate differences in breast yield at a young age and selection for 4 day percentage breast within this line remains a possibility.

Body weight differences between the High and Low sire families during the 8 week grow out were present at days 4, 7, 11 and 14 in which the High sire families were heavier at those ages. However, body weight differences are not present at any other time of the grow-out period. During the embryonic growth period, hyperplasia is occurring most prevalently as cells are dividing (Stockdale and Miller, 1987), but it is possible that some birds may be entering hypertrophy at an earlier age. With the High sire families, there is a possibility for more satellite cells and thus a possibility that these birds would enter a more rapid hypertrophic growth phase at an earlier age. This could have resulted in the heavier weights observed at those earlier ages.

However, the Low sire family birds had a 56 day growth period to catch up whether it be through a later increase in hypertrophic growth or through compensatory gain (Zubair and Leeson, 1996).

At processing a difference was observed for the High and Low sire families for percent breast. The High sire families, whose siblings had the highest percent breast at 4 days of age, also had the highest percent breast at processing age. Selection for 4-day breast yield (selecting those families with a high breast yield at a young age) should lead to increases in the breast yield at processing age resulting in more product and ultimately more profit for a company (Mason, 2015). Interestingly, a difference in percent legs was observed between the High and Low families with the Low families having a higher percent leg. The breast muscle and the leg muscles are two very different muscles. The breast muscle consists of predominantly white fibers which are fast twitch and glycolytic and anaerobic. The leg muscles are predominantly red fibers which are slow twitch, oxidative and aerobic (Klont et al., 1998). Families that had a lower percent breast yield, theoretically have less satellite cells for post hatch growth and repair. However, those families exhibited a higher percent leg. Selection pressure, particularly in yield type lines in the industry is put on white meat yield, specifically breast meat yield. By applying pressure to one type of muscle over the other, there may be a negative effect on the subsequent muscle group resulting in a lower yield. With this study, there was a "Selection" simulation in the MRB line for 4-day breast yield continuing to 56 day breast yield. By focusing solely on the breast muscle, those families with the Low breast yield while having similar carcass weights resulted in a higher percent leg.

Another theory behind selection for 4-day percentage breast yield is that the number of satellite cells available in the muscle for post hatch growth and repair will be altered. Selection for a high percentage breast yield at a young age should result in an increase in satellite cells as

they accumulate embryonically while selection for a low percent breast yield at a young age should decrease the number of available satellite cells. If more satellite cells are available in a muscle, the muscle should it become damaged, should have a better chance at repair through satellite cell activation, recruitment and nuclear contribution (Kornasio et al., 2011). In the case of muscle myopathies, specifically woody breast and to a lesser extent white striping, the muscle is damaged, becomes necrotic and cannot repair itself with the end result being the deposition of collagen and a hardening of the muscle (Tijare et al., 2016; Kuttappan et al., 2016). If selection for 4-day breast yield results in more satellite cells, the muscle should be better able to repair itself in the onset of woody breast development. In this study, the woody breast severity score did not differ between the High and Low sire families. As a result of a simulated selection, breast yield was increased in the High families with no negative impact on the woody breast myopathy which would equate on an industry to level to additional profit. However, the white striping severity score was higher in the High sire families. Satellite cells are undifferentiated multipotent cells and through different activation factors can go on to form muscle cells, bone cells or fat cells (Cooper et al., 1999). It is possible that some satellite cells were activated and recruited not to produce muscle cells but instead produced fat cells (Asakura et al., 2001; Harding et al., 2015) resulting in the higher severity of white striping in those High sire families. The ability to mark and identify what pathway satellite cells take in differentiation will be crucial in further understanding their role in muscle myopathy development and repair.

CONCLUSION

Variations exist between sire families for 4 day percentage breast yield in a modern random bred broiler population. Within these variations, those families with the highest average percent breast yield at 4 days of age also have the highest breast yield at the processing age of

d56 and the highest severity of white striping. No differences were observed for the average severity of the woody breast myopathy between the High and Low families. Selection for 4-day percentage breast yield may still be a useful method to improve yield in broilers while not altering the severity of the woody breast myopathy. It will be important in future studies to determine if satellite cells are being activated and recruited to produce fat cells instead of muscle cells in broilers exhibiting the white striping myopathy.

REFERENCES

Anthony, N. B. 1998. A review of genetic practices in poultry. Efforts to improve meat quality. J. Muscle Foods. 9:25-33.

Asakura, A., M. Komake, and M. A. Rudnicki. 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation. 68:245-253.

Bailey, R. A., K. A. Watson, S. F. Bilgili and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870-2879.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast. Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. Br. Poult. Sci. 38:355-358Can. J. Anim. Sci. 76:455–457.

Barbut, S. 1998. Estimating the magnitude of the PSE problem in poultry. J. Muscle Foods 9:35-49.

Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan, and E. W. Wilson. 2008. Progress in reducing the pale soft and exudative (PSE) problem in pork and poultry meat. Meat. Sci. 79:46-63.

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult Sci. 97:3298-3310.

Bowker, B., and H. Zhuang. 2017. Woody breast condition in broiler breast meat. Proc. 2017 Midwest Poultry Federation Convention (St. Paul, MN; March 14-16, 2017).

Cook, M. E. 2000. Skeletal deformities and their causes: Introduction. Poult. Sci. 79:982-984.

Cooper, R. N., S. Tajbakhsh, V. Mouly, G. Cossu, M. Buckingham and G. S. Butler-Browne. 1999. In vivo satellite cell activation via Myf5 and MyoD in regenerating mouse skeletal muscle. J. Cell Sci. 112:2895-2901.

Harding, R. L., D. L. Clark, O. Halevy, C. Coy, S. Yahav and S. G. Velleman. 2015. The effect of temperature on apoptosis and adipogenesis on skeletal muscle satellite cells derived from different muscle types. Phyisol Rep. Abstract/

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773-1780.

Julian, R. J. 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathol. 29:519-527.

Klont, R. E., Brocks, L., & Eikelenboom, G. 1998. Muscle fiber type and meat quality. Meat Sci. 49:219-229.

Kornasio, R., O. Kedar, O. Halevy, and Z. Uni. 2011. Effect of in ove feeding and its interaction with timing of first feed on glycogen reserves, muscle growth and body weight. Poult Sci. 90:1467-1477.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J. F. C. Meullenet, S. R. Mckee, C. M. Owens. 2012. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult Sci. 91:1240-1247.

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, C. M. Owens. 2013. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult Sci. 92:811-819

Kuttappan, V. A., B. M. Hargis and C. W. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult Sci 95:2724-2733.

Livingston, M. L., C. Landon, H. J. Barnes and J. Brake. 2018. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult Sci. 0:1-10.

Mason, J. G. 2015. Divergent selection for four-day relative breast yield and the effect of thermal manipulations on growth characteristic on lines selected for different processing dates. Dissertation.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili and W. A. Dozier III. 2018. Effects of reduced dietary energy and amino acid density on *Pectoralis major* myopathies in broiler chickens at 36 and 49 days of age. Poult Sci. 97:1794-1807.

Moss, F. P., and C. P. LeBlond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421–435.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Petracci, M., S. Mudalal, E. Babini, C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:179-183.

Poultry World. 2017. Reduce woody breast and white striping with dietary approach. https://www.poultryworld.net/Health/Partner/2017/10/Reduce-woody-breast-and-white-striping-with-dietary-approach-196197E/.

Qureshi, M. A. and G. B. Havenstein. 1994. A comparison of the immune performance of the 1991 commercial broiler with a 1957 randombred strain when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 73:1805-1812.

SAS Institute Inc. 2010. SAS/STAT® User’s Guide. 2010 Edition of SAS Institute Inc., Cary, NC.

Siegel, P. B. and E. A. Dunnington. 1985. Reproductive complications associated with selection for broiler growth. Pages 59-72 in Poultry Genetics and Breeding. Longman Group, Harlow, UK.

Stockdale, F. A. and J. B. Miller. 1987. The cellular basis of heavy chain isoform expression during development of avian skeletal muscles. Dev Biol. 123:1-9.

Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. 95:2167-2173.

Trocino, A., A. Piccirillo, M Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, G. Xiccato. 2015. Effect of the genotype, gender and feed restriction on growth, meat quality, and the occurrence of white striping and wooden breast in broiler chickens. Poult Sci. 94:2996-3004.

USDA:FSIS. 2017. Disposition instructions for “woody breast” and “white striping” poultry conditions. https://www.fsis.usda.gov/wps/wcm/connect/07e454eb-1803-4333-b5fb-efb0b50eb756/35-17.pdf?MOD=AJPERES&CONVERT_TO=url&CACHEID=07e454eb-1803-4333-b5fb-efb0b50eb756

Zubair, A. K. and S. Leeson. 1996. Compensatory growth in the broiler chicken: a review/ World’s Poult Sci J. 52:189-200.

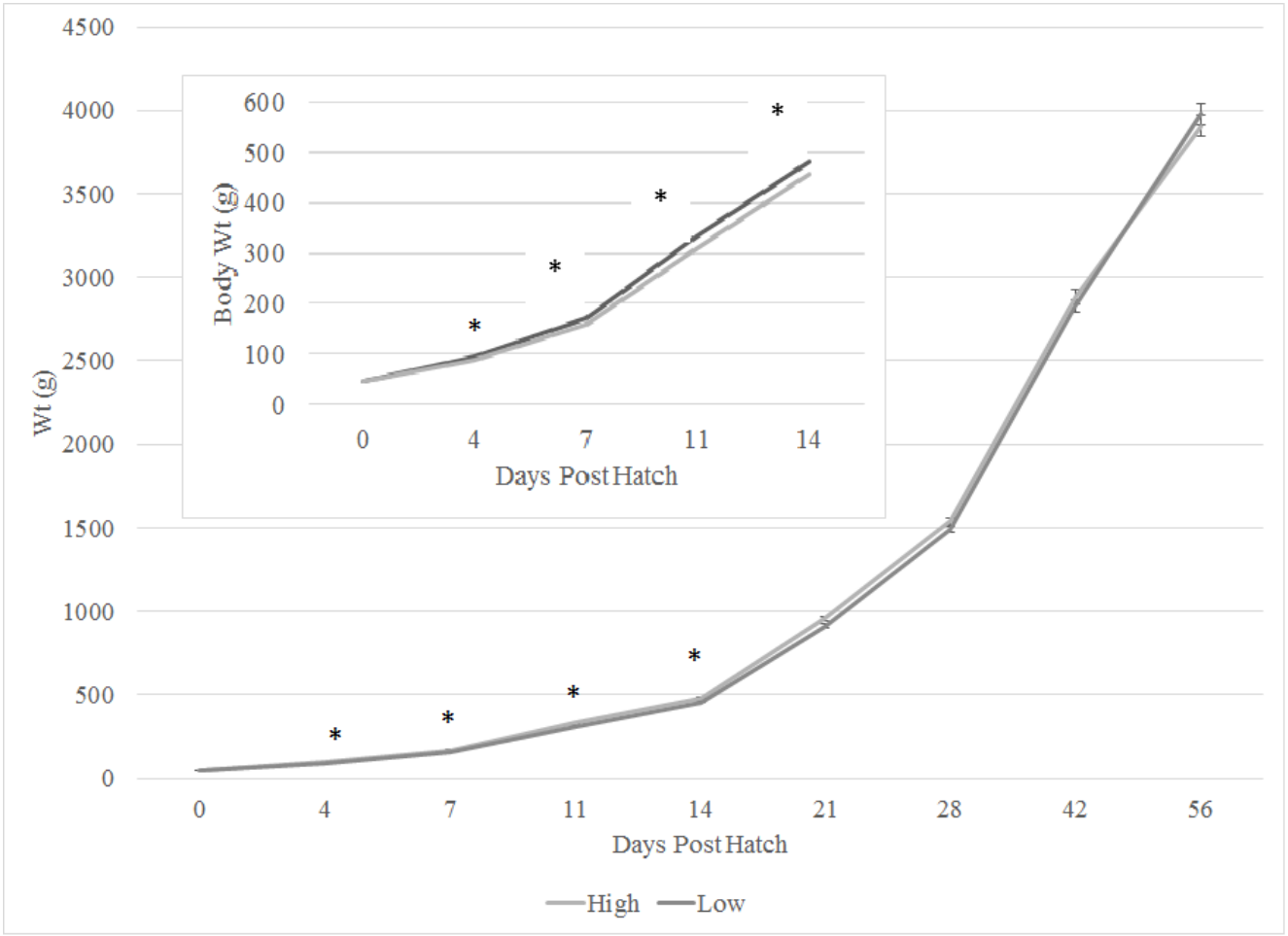


Figure 1. Growth curve for the High and Low sire families[1] through 8 weeks of age (Mean)[2]

[1]High-Highest 4-day percent breast yield sire families (6 families), Low=lowest 4-day percent breast yield families (5 families)
[2]*Indicates a difference in body weights between the High and Low sire families at ($P \leq 0.05$)

Table 1. Carcass trait measurements (mean ± SE) for the High and Low[1] sire family groups at 56 days of age

Trait	Low	High
Breast	815.4 ± 19.0	859.5 ± 19.2
Tenders	170.1 ± 3.25	171.9 ± 3.29
Wings	293.6 ± 5.4	292.3 ± 5.5
Legs	906.1 ± 19.8	876.1 ± 20.1
Rack	883 ± 17	862 ± 17.3

[1] High-Highest 4-day percent breast yield sire families (6 families), Low=lowest 4-day percent breast yield families (5 families)

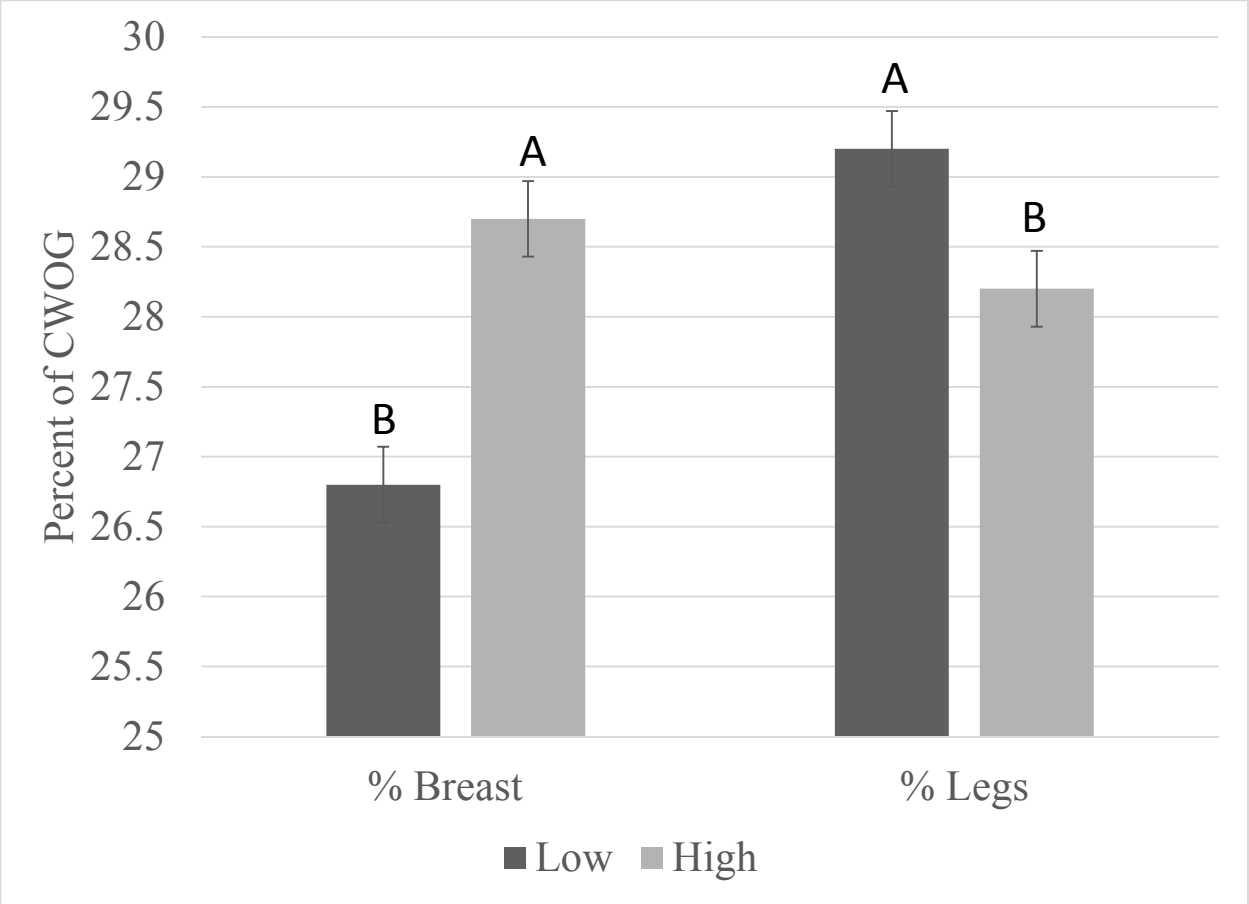


Figure 2. Percent breast and percent legs[1] for the High and Low[2] sire families (Mean ± SE)[3]

[1]%Breast = (Breast Wt/CWOG Wt)*100; %Leg = (Leg Wt/CWOG Wt)*100
[2] High-Highest 4-day percent breast yield sire families (6 families), Low=lowest 4-day percent breast yield families (5 families)
[3]A-B For each trait, groups with no common letter are different at P≤0.05

Table 2. Woody Breast and White Striping severity scores for the High and Low Sire Families[1] (Mean ± SE)[2]

Trait	Low	High	P-Value
WS	1.15 ± 0.07b	1.56 ± 0.08a	0.0086
WB	1.09 ± 0.1a	1.27 ± 0.1a	0.1986

[1] High-Highest 4-day percent breast yield sire families (6 families), Low=lowest 4-day percent breast yield families (5 families)
[2]a-b For each trait, groups with no common letter within a row are different at $P \leq 0.05$

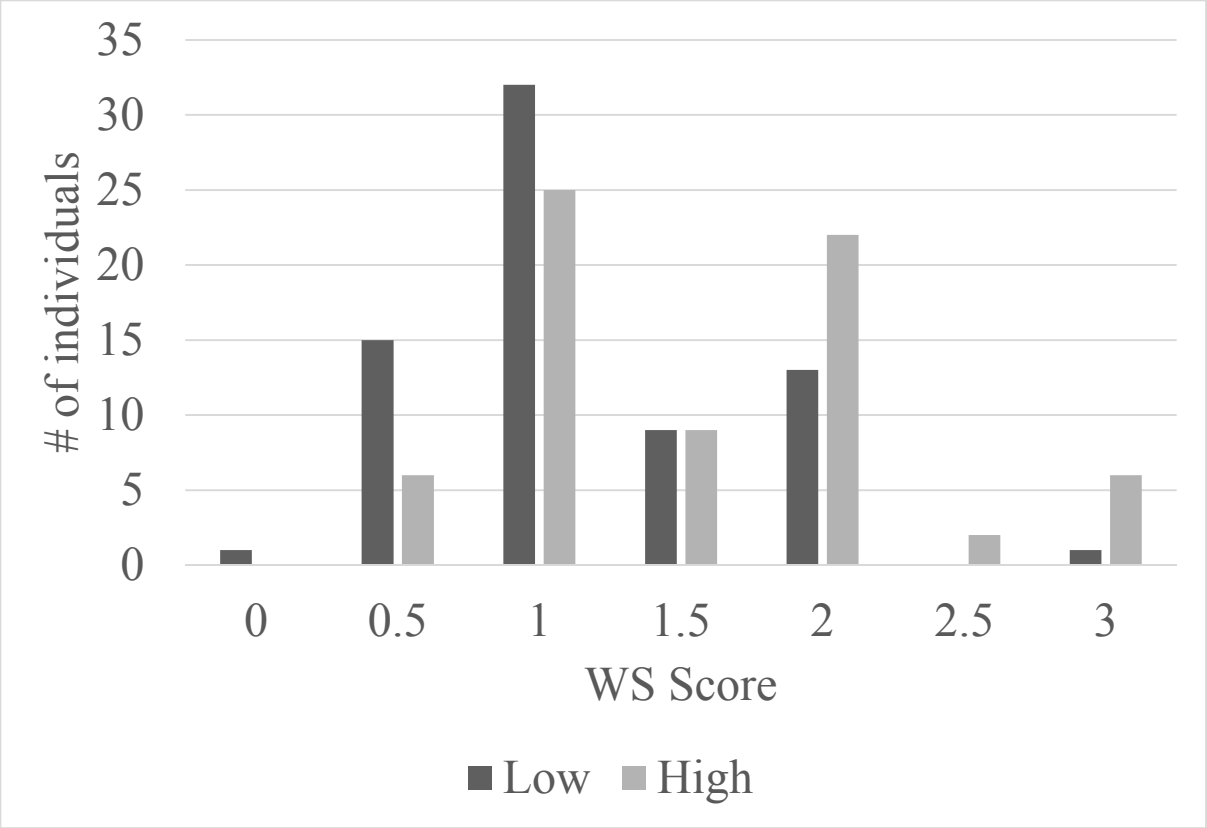


Figure 3. Frequency distribution for white striping scores in the high and low sire groups.

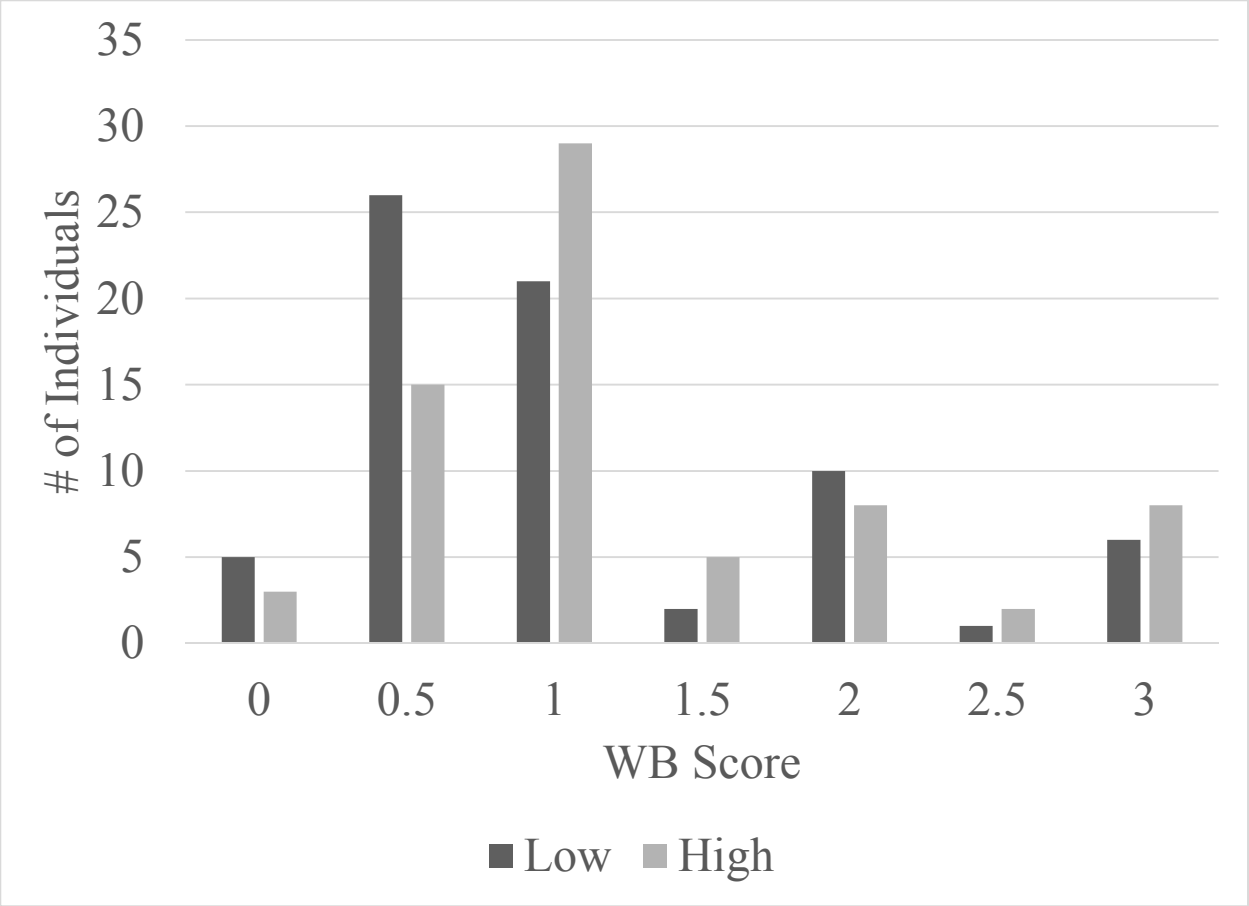


Figure 4. Frequency distribution for white striping scores in the high and low sire groups.

Chapter 4. Effect of late stage embryonic thermal manipulation on the growth and meat quality of a yield strain of broilers

ABSTRACT

Current commercial selection practices focus solely on selection for late stage hypertrophic (increase in muscle fiber size) growth to promote increased body weight and yield. Little research has been done to evaluate the effect of selection for growth at an earlier stage that focuses on hyperplastic growth or the increase in muscle fiber number and accumulation of nuclei through satellite cell proliferation. Thermal manipulation (TM) during embryogenesis has been shown to promote growth, possibly due to an increase in hyperplasia. Through two studies, the objective was to evaluate the effect of late stage embryonic thermal manipulation on the growth and meat quality parameters of a commercial yield line of broilers grown under normal conditions or a period of calorie restriction from 0-21 d. For both studies, fertile eggs from a yield strain of broilers were incubated to d14 and then separated into a control (C) (constant temperature of 37.8^{0}C) and either one or two TM treatments. For trial 1, the TM treatments consisted of either a 3 hour (3) or 6 hour (6) period of increased temperature (39.5^{0}C) on embryonic day 15, 16 and 17. For trial 2, a 3 h TM treatment was used similar to trial 1. For both trials, a hatch window was recorded and birds were grown out until processing age. For trial 1 and half the birds in trial 2, from placement to day 21, all treatment groups were subjected to a starter feed containing 500 kcals lower than the NRC requirement. After day 21 to processing, the birds were switched to a finisher diet that met the NRC requirement for calories. At d 47 (trial 1), or day 60 (Trial 2), birds were processed and evaluated for breast yield and the incidence of both the wooden breast and white striping myopathies. Data was analyzed using a 2-way full factorial ANOVA (line/sex) and means were separated using Tukey's HSD. Both the 3 and 6 h treatments began hatching earlier. For trial 1, the greatest hatch of fertile was in the 3 hour (96.39%) and the lowest in the control (89.22%) while the 3 and C did not differ in trial

(97.3%). Percentage breast was highest in the 3 hour treatment for both trials. The woody breast and white striping scores did not differ between treatment groups for trial 1, however in trial 2, the 3 hour treatment had a lower severity score for woody breast. Overall FCR did not differ between the treatment groups for either trial. Short periods of TM (3 hours) may help to improve breast yield with no negative impact on feed conversion and may have the ability to decrease the severity of the woody breast myopathy.

INTRODUCTION

Hyperplasia is the first stage of muscle development in a broiler and occurs during the 21 day embryonic growth period. During the myogenesis process, there are three waves of cell proliferation that go on to form the basic muscle fiber structure in a broiler. The first wave is embryonic myoblasts that are accumulating from embryonic day 3 to 7. Embryonic myoblasts will go on to form the primary muscle fibers. The second wave are fetal myoblasts from E5 to E14. Fetal myoblasts will go on to fuse into secondary muscle fibers. The third and final wave are adult myoblasts, more commonly known as satellite cells which are proliferating from E14 onward (Stockdale and Miller, 1987). Satellite cells are an undifferentiated population of cells that become quiescent in the muscle sitting between the sarcolemma and basement membrane around 7 days post hatch (Mauro, 1961).These cells, although quiescent in the muscle, have the ability to re-enter the cell cycle to aid in growth as well as to repair damaged muscle fibers (Velleman et al., 2010). When a muscle is damaged which could occur through normal everyday use of the fiber, the damaged fiber sends out a signal to the satellite cells near it. The satellite cell is then activated and recruited to the site of injury. Being a multinucleated cell, it fuses with the damaged fiber and donates its nuclei. The additional nuclei allows for additional protein accretion and repair of that fiber (Fu et al., 2015) making satellite cells important when considering both yield and meat quality at older ages.

In recent years, meat quality has become a major concern in the industry with several new muscle myopathies emerging. Two of those myopathies include both woody breast and white striping. Woody breast has been characterized by several researchers as a breast fillet that has become hardened or stiffened with varying degrees (Petracci er al., 2013; Tijare et al., 2016). Woody breast can be detected through palpation in both live birds and carcasses. Fillets

exhibiting woody breast have been a concern for the industry as they are visually unappealing to consumers (Kuttappan et al., 2012) and can have a decreased protein functionality when put into further processed products such as nuggets or patties (Mudalal et al., 2014, 2015). Additionally, fillets affected by woody breast will have a higher compression force (Sihvo et al., 2014), higher cook loss, lower uptake of marinade and a decreased tenderness (Petracci et al., 2013; Mudalal et al., 2015; Tijare et al., 2016). Histological analysis of fillets with woody breast show muscle degradation and an increase in collagen at the site of damage (Velleman et al,. 2015) and fillets with white striping having increased fat deposition (Kuttappan et al., 2009). White striping as another myopathy plaguing broilers that is characterized by having visible white striations of fat deposits that run parallel to the muscle fibers of the breast fillet (Kuttappan et al., 2013). Fillets affected by white striping have a higher fat content and ultimately lower protein content (Kuttappan et al., 2012, 2013) resulting in a change in nutritive value in addition to the negative impacts on consumer acceptability. The woody breast and white striping muscle myopathies, having separate etiologies, tend to occur in a breast fillet in conjunction with each other (Kuttappan et al., 2016).

The heritability of these myopathies, specifically woody breast has been shown to be relatively low (Bailey et al., 2015). As a result, non-genetic factors have been targeted as being responsible for the variation exhibited within the myopathy (Trocino et al., 2015). With woody breast being attributed to the rate of growth and yield of broilers, several researchers have found ways to reduce the incidence of the woody breast myopathy. Changes in environment and nutrition have been shown to decrease the incidence of the myopathies focusing on alteration of amino acids and high vitamin dosing (Bodle et a., 2018), reducing the energy availability in the diets (Meloche et al., 2018) or even reducing the time of egg storage (Livingston et al., 2018).

However, these alterations tend to come at a cost of decreasing yield in the broilers and new methods for reducing the incidence need to be evaluated.

One such method being evaluated is the use of embryonic thermal manipulation. With broilers being processed at an earlier age, the amount of time a broiler spends in the embryo has increased proportionally (Halevy et al., 2006) making changes to the embryonic environment critical in the development and post hatch growth of broilers. Temperature has been shown to be a major factor when considering the embryonic growth and development of a broiler (Meijerhof, 2000). Changes to the incubator profile otherwise known as thermal manipulation (TM) was originally hypothesized and found to impact post hatch thermal tolerance and thermal regulation (Piestun et al., 2008; Uni and Yahav, 2010). However, throughout these studies researchers found that TM had an impact on growth, feed conversion and yield. Piestun and colleagues (2013) found that broilers who were subjected to TM applied between embryonic day 7 and 16 had a lower feed intake, improved feed conversion and a lower body temperature. The same group also found that broilers subjected to TM had higher levels of myogenin expression, increased muscle growth and higher breast weights as a result of increased myoblast proliferation (Piestun et al., 2015). It has also been found that late stage embryonic thermal manipulation broilers had higher breast meat yield with no negative impact on breast meat pH or drip loss (Collin et al., 2007), two important factors in determining meat quality. It is possible that late stage embryonic thermal manipulation may have the ability to increase satellite cell proliferation. An increase in the number of satellite cells should allow for improved growth and an improved ability for repair should a muscle become damaged. Therefore, the purpose of the following studies was to determine the effect of late stage embryonic thermal manipulation on the growth,

yield, meat quality characteristics and incidence of woody breast and white striping muscle myopathies in a high yielding strain of broilers

MATERIALS AND METHODS

Broiler Population and Early Incubation

For both trial 1 and 2, a commercially available broiler strain that has been selected for breast meat yield was utilized. This line of broilers has exhibited both the woody breast and white striping myopathies throughout the industry. In trial 1, 720 hatching eggs were set in the winter months from a breeder flock that was 39 weeks old and for trial 2, 1080 hatching eggs were set in the summer months from a breeder flock that was 28 weeks old. Eggs were pre-warmed to 26.6°C for 12 hours prior to set. All eggs were incubated at 37.6°C and 56% relative humidity from embryonic (E) day 0 to E15. Eggs were candled at E14 and fertile hatching eggs were divided evenly among the treatment groups for each trial prior to thermal manipulation (TM) treatments.

Trial 1

Embryonic TM treatments for trial 1 consisted of a control (C) treatment as well as a 3 and 6 hour TM treatment. For the TM treatment, eggs were placed in an incubator that was set at 39.5°C and 65% relative humidity for either 3 or 6 hours a day on E15, E16 and E17. When the eggs were not in their treatment incubator, they were moved back to the control machine that was held at a constant temperature of 37.6°C. All eggs were transferred to a walk-in hatcher on E18. A hatch window was recorded which began at hour of incubation 468 and ended at hour of incubation 512. Chicks from each treatment were pulled, weighed and banded every four hours. A chick was considered hatched at a pull time if it was fully out of the egg, partially or fully dry

and could stand and hold up its head. All chicks remained in the hatcher until the hatch window was complete.

Chicks were then placed by treatment into litter shavings floor pens (10 pens/trt; 16 birds/pen). In this trial, all birds were fed a starter feed for the first three weeks that was averaging 2800 kcals/kg, roughly 500 kcals/kg less than the NRC requirements (NRC, 1994). A normal calorie starter feed was originally supposed to be fed but after a week of grow-out, it was apparent the chicks were not growing as they should and after testing the feed, it was found that the wrong feed was delivered. The study was continued to generate information on thermal manipulation and growth under a short term calorie restriction. Following the 3 week early calorie restriction, birds were switched to a normal calorie commercial finisher feed. Feed and water were provided ad libitum throughout the study. Feed was weighed into the pen and the feed consumption per week was recorded as well as the feed conversion (FCR) for each feeding period (starter and finisher).

Birds were processed at 47 days of age. Feed was removed from each pen 12 hours prior to processing while access to water remained constant until load out. Birds were weighed on the back dock of the processing plant, hung on a shackle line and stunned using an electric water bath stunner (11V, 11 mA, 10). They were manually cut through the left carotid artery and jugular vein and allowed to bleed out for 3 minutes. Following bleed out, carcasses were scalded (55^0C) and then picked with an in-line commercial de-feather machine and the head/neck and feet were removed. Carcasses were eviscerated by hand and placed in an ice-bath chill for four fours. Following the chilling period, carcasses were manually deboned and weights were recorded for the chilled carcass, breast, tenders, wings, leg quarters and rack. Breasts were then subjectively hand scored for woody breast and white striping myopathies. A score of 0 for both

myopathies indicated a normal fillet with either no visible striping or a soft, flexible fillet. A score of 3 for woody breast was a fillet that was hard throughout and had a distinct and visible caudal ridge. A score of 3 for white striping had thick white striations in the cranial region with thinner striations throughout the entire muscle.

Trial 2

Based on the results from trial 1, treatments were chosen for trial 2. The 3 h treatment in trial 1 resulted in an increase in breast yield, with no negative impacts on meat quality and was chosen for trial 2. Treatments for trial 2 included a control (C) that was held at a constant temperature of 37.6°F and 56% relative humidity throughout the treatment period and a 3 hour (3) thermal manipulation treatment which was placed in an incubator that was set at 39.5°F and 65% relative humidity for 3 hours a day of incubation 15, 16 and 17. Eggs were transferred by treatment to a walk in commercial hatcher on E18. A hatch window was recorded for each treatment beginning at hour of incubation 464 and ending at hour 496. At each pull time, hatched chickens (similar to the criteria described in trial 1) were pulled, weighed and banded.

Following the completion of the hatch, chicks were placed randomly by line into litter shavings floor pens (12 pens/TM treatment, 36 birds/pen). Additionally, a feed treatment was added for the first 3 weeks in which half of the pens (6 pens from each treatment group) received a low calorie starter feed. A calorie restriction similar to trial 1 was used, while the remaining 12 pens received a normal calorie commercial starter feed that was formulated to meet or exceed NRC requirements (NRC, 1994). This resulted in 4 treatment groups; C_N (control TM and normal calorie feed), 3_N (3 hour TM and normal calorie feed), C_R (control TM and restricted calorie feed) and 3_R (3 hour TM and restricted calorie feed). Following the initial 3 week feed treatment, all pens received a normal calorie finisher feed for the remainder of the trial. Pen

weights were recorded weekly and the feed conversion ratio (FCR) was recorded for each feeding period (starter-0-3wks, finisher-3-9 weeks) and adjusted for mortality.

On day 60, 6 males and 6 females per pen were randomly chosen for processing. They were processed in the same manner as those processed in Trial 1. In addition to breast scoring for woody breast and white striping post de-bone, the right half of the breast fillet was removed, weighed and stored in a plastic Ziploc bag in a 3°C cooler for 24 h. Following the 24 h holding period, breast fillets were removed from the bag, towel dried and reweighed to record drip loss ((Initial Breast Wt-Final Breast Wt)/Initial Breast Wt)*100). Breast meat color was also recorded at 24 h post debone on the backside of the right breast fillet using a Minolta CR-400 colorimeter. Three locations throughout the fillet were scanned and average measures for L*, a* and b* were used in the analysis.

Statistical Analysis

Statistical analysis was done using the JMP Pro 13 software (SAS Institute, 2010) using either a 2 or 3-Way ANOVA. For trial 1, the main effects of sex and TM treatment were analyzed in addition to the TM treatment by sex interaction with only the main effect of TM treatment being presented as no interactions were present. For trial 2, the body weight data was analyzed for the main effects of TM treatment and feed treatment as well as their interaction. The main effects of TM treatment, feed treatment and sex were analyzed for the processing data in addition to the interactions. No interactions were significant and only main effects are presented. Means were separated using the Student's t-test or Tukey's HSD were appropriate with pen being used as the experimental unit for both trials and a p value < 0.05 being used to measure significance. All data is presented as the LS Mean ± standard error.

RESULTS

Trial 1

The results from Trial 1 were used as initial data for trial 2. Following embryonic TM treatments, a hatch window was recorded. The 3 and 6 h TM treatments began hatching at hour of incubation 468 while the C did not start hatching until 8 hours later at hour 476. At the end of the hatch window the 3 and 6 h TM treatments had finished hatching by hour 500 while the control did not finish hatching until hour 508 (Figure 1). TM showed an early shift in the hatch window by 8 hours. The hatch of fertile was also impacted by TM with the highest hatch of fertile in the 3 h treatment (96.39%), and lowest in the C (89.22%) with the 6 h treatment acting as an intermediate (92.12%).

Post hatch, all birds from all treatments were subjected to a calorie restriction for 3 weeks. No body weight differences were observed between the treatments at any stage in the 47 day grow-out period (Figure 2). However when comparing the growth curve of all treatments to the expected bodyweights based off of the broiler guide for this yield line, it is apparent that the early calorie restriction hindered growth. At day 21, the birds in the trial were averaging 545g and were over 5 days behind in their projected growth while at day 47, they were averaging 2983g and were less than 2 days behind in their growth. The birds do appear to catch up to the curve near the end of the grow-out period as a result of compensatory gain. Feed conversion for each feeding period did not differ among the treatments (data not shown).

Processing results from trial 1 showed no differences between the treatments for carcass weight, chilled carcass weight, wings legs or rack. The tenders were heaviest in the 3 h treatment, lightest in the 6 hour treatment with the control as an intermediate. A similar result occurred for breast weight (Figure 3). However, when breast was put on a percent of chilled

carcass weight ((Breast Wt/Chilled Carcass WT)*100), the 3 h treatment had the highest percent breast, with no difference between the C and 6 h treatment. The percent of fillets within each treatment that fell within each score did not differ for woody breast (Figure 5) or for white striping (Figure 6). For both myopathies in this study, there were very few birds exhibiting a severe score with a majority of the fillets falling in a score of 0 (no woody breast/white striping) or 1 (mild woody breast/white striping).

Trial 2

A hatch window was recorded in trial beginning at hour of incubation 464 and ending at hour 496. Both the C and 3 h treatment began and ended hatching at the same time although it does appear that the C group hatched slightly faster than the 3 h treatment (Figure 6). The hatch of fertile for both the C and 3 h treatment were identical at 97.1%. Chick weights did not differ between treatment groups.

While birds were placed by their treatment group into pens, half of each treatment received a low calorie starter feed for the first three weeks while the remaining half received a normal calorie starter feed. No interactions were significant for body weight at any of the ages measured. At d 14, the C group was heavier than the 3 h treatment with no differences between the groups at any other ages (Figure 8). Feed conversions did not differ between the C and 3 treatment groups. Unsurprisingly, the restricted birds had lower body weights beginning at four days post hatch and remained lower throughout the 63 day grow out period (Figure 9). The FCR for the starter period was higher in the restricted birds with no difference between the groups for the FCR during the finisher phase (data not shown).

No interactions were significant for any of the processing characteristics. For the main effect of TM treatment, the only differences observed for the carcass characteristics was for

percentage breast yield (Table 1). Breast yield was highest in the 3 h treatment and lowest in the C group. For the feed treatments, the Normal fed birds had a higher dock, carcass, chilled carcass, and parts weights than the restricted fed birds. Percentage wings and percentage legs was higher in the restricted birds than the normal fed birds. Percentage fat was also higher in those fed a normal calorie starter feed (Table 2). A sex effect was present for all carcass characteristics except for absolute tender wt. Males had higher dock, carcass, chilled carcass, absolute breast, absolute and percent wings and leg quarters and absolute rack. Females had a higher percent fat, percent breast and percent tenders (Table 3).

Meat quality traits for TM treatments are shown in Table 4. The only difference seen was for the average severity score for woody breast in which the 3 h treatment had a less severe average score for woody breast. Drip Loss was approaching significance at a p-value of 0.07 with the 3 h treatment having a lower drip loss. Looking at the percent woody breast score within each treatment, the 3 h treatment had a higher percentage of fillets with a score of 0 or no woody breast. No differences were found for any of the other scores (Figure 10). There were no differences between the C and 3 h treatment for their percent of total for each white striping score (Figure 12). No difference was detected for dressing percentage between the Normal and Restricted fed birds. Moisture uptake and a* (redness) was higher in the restricted birds. Normal fed birds had a lower drip loss, a higher severity of both white striping and woody breast and a higher b* (yellowness) (Table 5). Restricted fed birds had a higher percentage of a score of 0 for woody breast and a lower percentage of the more severe scores of 2 and 3. Both normal and restricted fed groups had the same percentage of a score of 1 for woody breast (Figure 11). For white striping, the normal fed birds had a lower percentage of score 0 and higher percentage of scores 1, 2 and 3 when compared to the restricted fed birds (Figure 13). Females had a higher

moisture uptake and a lower L* when compared to males with no difference in any other meat quality trait measured (Table 6).

No statistical differences were found for the four groups analyzed for gene expression (Control Males, Control Females, TM Males and TM Females). However, it does appear that in males, genes associated with satellite cells appear to be upregulated in TM birds when compared to the control. Additionally, it appears that satellite cell markers appear at higher levels in females than in males (Figure 14).

DISCUSSION

With woody breast and white striping concern to the industry, finding a method to reduce the incidence without affecting yield is critical. Several nutritional methods may help decrease the incidence of the myopathies but it comes at a cost, whether it be through a decrease in yield or an increase in the cost of feed. Thermal manipulation has the potential to improve breast meat yield, decrease the incidence of severe woody breast while at the same time having no negative impact on body weight and FCR.

There was a concern that TM would significantly alter embryonic development and hatch window. For these trials. a hatch window was recorded for both studies in which chicks were pulled and banded every four hours during the hatching period. In trial 1, the TM treatments began hatching earlier than the C group and finished hatching earlier resulting in a hatch window shift of 8 hours. This trial was conducted in the winter months and it is possible that the TM birds were producing more heat allowing them to hatch earlier than the C group. In addition, research has shown that higher incubator temperatures accelerate hatch time of broiler chicks (Romanoff, 1960 Ricklefs, 1987; Leksrisonpong, et al., 2007) as eggs treated with high temperatures produce more heat (Yildirim and Yetisir, 2004).However, the hatch window for

trail 2 was not consistent with the results of the first trial. For trail 2, the C group began hatching sooner and appeared to have a slightly quicker hatch window than the 3 h TM treatment. Trial 2 was conducted during the summer months and it remains unclear why a difference in hatch window between the two trials was observed. Although purely observational, it is possible that a seasonal effect may be a result of the differences in hatch window between trial 1 and trial 2. The eggs incubated at a higher temperature in trial 1 may have exhibited the normal effect of high temperature on hatch time while in the summer, those embryos who were heat treated could not dissipate heat properly, slowing their hatch time slightly (Leksrisonpong, et al., 2007).

Hatch of fertile in trial 1 was positively impacted by the 3 h TM treatment with no difference between the C and 3 h treatment in trial 2. Decuypere and colleagues (2001) reported that the optimum temperature for hatchability was between 37-38°C with negative effects on hatch viewed at higher or lower temperatures but hatchability results have varied. Other researchers have found that TM during embryogenesis had no effect on hatchability (Yahav et al., 2004; Loyau et al., 2013). Hatchability has been shown to be affected by several other traits such as breeder age (Roque and Soares, 1994; Araujo et al., 2016), egg size and weight (Senapti et al., 1996; Abiola et al., 2006), making it hard to elucidate the differences in hatchability in trial 1 and the variations seen between trial 1 and trial 2.

In both trials, TM did impact the body weight or growth of the broilers. TM was applied during the period of satellite cell division. If the TM were to be applied, earlier, it would be targeting the myoblast proliferation waves that are responsible for primary and secondary muscle fibers and may have the ability to impact body weight. Previous research with embryonic TM have also noted no differences in body weight of TM birds compared to a control (Collin et al., 2007). No treatment effect was observed for feed conversion for both trials. When considering

the efficacy of a new method in the broiler industry, two traits that should not be negatively impacted are growth and feed conversion as both are related to the cost of production. With TM, in two separate studies, neither trait was negatively impacted making it a potentially beneficial technique for the industry.

One thing that is interesting to note is the compensatory gain observed in trial 1 and not in trial 2. In trial 1, all birds were subjected to 3 week early calorie restriction while half the birds in trial 2 were. In trial 1, comparing only to the broiler performance guide for the yield line as there was no control, it appeared that the broilers grew at a faster rate to recover some of the body weight lost in the early restriction period. In trial 2, the restricted fed birds never recovered from the early calorie restriction and their body weights remained consistently lower than both the normal fed birds and the broiler guide weights. Thermal manipulation did not have an impact on the compensatory gain of broilers used in these studies. Research on compensatory growth has been inconsistent in the literature (Zubair and Leeson, 1996), however the results do suggest a seasonal effect on the ability of bird to return to its potential weight following an early calorie restriction. At processing, feed restricted birds had lighter body parts than the control, less fat, and a lower severity of both woody breast and white striping. While early calorie feed restriction may alter body composition and decrease myopathies, it comes at a huge cost of a reduction in yield.

In both trials, TM did not have a substantial impact on carcass traits with no differences observed for several of the carcass characteristics measured. For trial 1, absolute breast weight was heaviest in the 3 h treatment, lightest in the 6 h and the C an intermediate. However, in both trials, percentage breast was highest in the 3 h treatment, regardless of feeding program. Satellite cells are important in post hatch muscle growth. Thermal manipulation during the period that

satellite cells are proliferating can increase the number of satellite cells available for post hatch growth. In both trials, the increase in breast yield is most likely correlated to an increase in the number of satellite cells that have accumulated embryonically.

While no differences were observed in trial 1 for the incidence and severity of white striping and woody breast, trial 2 produced promising results when considering meat quality. The severity score for woody breast was lowest in the TM treated birds and the percent of fillets with a score of 0, or no woody breast also being higher. Satellite cells, in addition to contributing to post hatch growth can also donate their nuclei to aid in repair, should a muscle fiber become damaged. With the woody breast myopathy, it is hypothesized that cells are growing too large, too quickly, creating a hypoxic condition and resulting in damaged fibers. An additional number of satellite cells could aid in repair of the fibers. No differences were detected in satellite cell markers between normal fed, C and 3 h TM males and females. It does appear that satellite cell marker expression is higher in females than in males (Figure 14). This could be indicative of the higher breast yield observed in females over males.

CONCLUSION

Manipulation of the embryonic environment to alter muscle cell proliferation, specifically during the late stage of muscle development during satellite cell proliferation has the ability to improve yield and may also lessen the impact of the woody breast muscle myopathy. Through two studies, a 3 hour TM treatment of 39.5°C and 65% relative humidity on embryonic day 15 through 17 increased absolute breast weight (Trial 1), increased percentage breast (Trial 1 and 2) and decreased the severity of WB while increasing the percentage of fillets with a score of 0 or no woody breast (Trial 2). TM did not have a negative impact on body weight, hatching time, hatch of fertile, growth or feed conversion. Additionally, satellite cell marker in males that

underwent a 3 hour TM treatment in Trial 2 showed an upregulation of genes associated with satellite cells although it was not statistically different. In conclusion, short periods of TM during the late stages of embryogenesis may be a beneficial way for the industry to improve yield, increase revenue and improve meat quality in lines known to be affected by current muscle myopathies. Large scale thermal manipulation in a commercial hatchery is the next step in determining the practicality of TM in a commercial broiler setting.

REFERENCES

Abiolo, S. S., O. O. Meshioye, B. O. Oyerinde, and M. A. Bamgbose. 2006. Effect of egg size on hatchability of broiler chicks. Arch Zootec. 57:83-86.

Araujo, I. C. S., N. S. M. Leandro, M. A. Mesquita, M. B. Café, H. H. C. Mello, E. Gonzales. 2016. Effect of incubator type and broiler breeder age on hatchability and chick quality. Braz. J Poult Sci. 18: 1516-1521.

Bailey, R. A., K. A. Watson, S. F. Bilgili and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870-2879.

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult Sci. 97:3298-3310.

Collin, A., C. Berri, S. Tesseraud, F. E. Requena Rodon, S. Skiba-Cassy, S. Crochet, M. J. Duclos, N. Rideua, K. Tona, J. Buyse, V. Bruggeman, E. Decuypere, M. Picard and S. Yahav. 2007. Effect of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult Sci. 86:795-800.

Decuypere, E., K. Tona, V. Bruggeman, and F. Bamelis. 2001. The day old chick: a crucial hinge between breeders and broilers. World's Poult Sci J. 57:127-138.

Fu, X., H. Wang, P. Hu. 2015. Stem cell activation in skeletal muscle regeneration. Cell Mol Life Sci. 72:1663-1677.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World's Poult. Sci. J. 62:485-497.

Kuttappan, V. A., V. B. Brewer, F. D. Clark, S. R. McKee, J. F. Meullenet, J. L. Emmert, and C. M. Owens. 2009. Effect of white striping on the histological and meat quality characteristics of broiler fillets. Poult Sci. 88:136-137 (Abstr).

Kuttappan, V. A. V. B. Brewer, A. Mauromoustakas, S. R. McKee, J. L. Emmert, J. F. Meullenet and C. M. Owens. 2013. Estimation of factors associated with the occurrence of WS in broiler breast fillets. Poult Sci. 92:811-819.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J-F. C. Meullenet, S. R. McKee, and C. M. Owens. 2012. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of WS. Poult Sci. 91:1240-1247.

Kuttappan, V. A., B. M. Hargis and C. W. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult Sci 95:2724-2733.

Lekrisompong, N., H. Romero-Sanchez, P. W. Plumstead, K. E. Brannan and J Brake. 2007. Broiler incubations. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. 86:2685-2691.

Livingston, M. L., C. Landon, H. J. Barnes and J. Brake. 2018. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult Sci. 0:1-10.

Loyau, T., C. Berri, L. Bedrani, S. Metayer-Coustard, C. Praud, M. J. Duclos, S. Tesseraud, N. Rideau, N. Everaert, S. Yahav, S, Mignon-Grasteua, A. Collin. 2013. Thermal manipulations of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. J Anim Sci. 91:3674-3685.

Mauro, A.1961. Satellite cell of skeletal muscle fibers. J. Biophys Biochem. Cytol. 9:493-495.

Meijerhof, R. 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group [WPSA Working Group 6 (Reproduction)], St. Edmand's Hall, Oxford, UK.

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili and W. A. Dozier III. 2018. Effects of reduced dietary energy and amino acid density on *Pectoralis major* myopathies in broiler chickens at 36 and 49 days of age. Poult Sci. 97:1794-1807.

Mudalal, S., E. Babini, C. Cavani and M. Petracci. 2014. Quantity and functionality of protein fractions in chicken breast fillets affected by WS. Poult Sci. 93:1-9.

Mudalal, S., M. Lorenci, F. Sogalia, C. Cavani, and M. Petracci. 2015. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal 9:728-734.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Petracci, M., S. Mudalal, A. Bonfiglio and C. Cavani. 2013. Occurrence of WS under commercial conditions and its impact on meat quality in broiler chickens. Poult Sci. 92:1670-1675.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake and S. Yahav. 2008. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermal tolerance. Poult Sci. 87:1516-1525.

Piestun, Y., S. Druyan, J. Brake and S. Yahav. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult Sci. 92:1155-1163.

Piestun, Y., S. Yahav and O. Halev. 2015. Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat type chickens. Poult Sci. 94:2528-2536.

Ricklefs, R. E.1987. A comparative analysis of avian embryonic growth. J. Exp. Zool. 51:309–324

Romanoff, A. L. 1960. The Avian Embryo. The Macmillan Co., New York, NY.

Roque, L., M. C. Soares. 1994. Effects of Eggshell quality and Broiler breeder age on hatchability. Poult Sci. 73:1838-1845.

SAS Institute Inc. 2010. SAS/STAT® User's Guide. 2010 Edition of SAS Institute Inc., Cary, NC.

Senapati, P.K, K.G. Dask Madal and A.K. Chatterjee. 1996. Relationship between egg weight, shape index, fertility and hatchability of Japanese quail eggs. Environ. Ecol. Stat, 14: 574- 577.

Sihivo, H. K., K. Immonen and E. Pualanne. 2014. Myodegeneration with fibrosis and regeneration of the pectoralis major muscle of broilers. Vet Pathol. 5:619-623.

Stockdale F. E., and J. B. Miller. 1987. The cellular basis of myosin heavy chain isoform expression during development of avian skeletal muscles. Dev. Biol. 123:1-9.

Tijare, V., F. Yang, V. Kuttappan, C. Alvarado, C. Coon, and C. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult Sci. 95:2167-2173.

Trocino, A., A. Piccirillo, M Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, G. Xiccato. 2015. Effect of the genotype, gender and feed restriction on growth, meat quality, and the occurrence of white striping and wooden breast in broiler chickens. Poult Sci. 94:2996-3004.

Uni, Z., and S. Yahav. 2010. Managing prenatal development of broiler chickens to improve productivity and thermotolerance. In: P. L. Greenwood, (Ed). Managing the Prenatal Environment to Enhance Livestock Productivity. pp. 71-90. Springer, Netherlands.

Velleman, S. G., X. Zhang, C. S. Coy, Y. Song, and D. C. McFarland. 2010. Changes in satellite cell proliferation and differentiation during turkey muscle development. Poult Sci. 89:709-715.

Velleman, S. G., and D. L. Clark. 2015. Histopathological and myogenic gene expression changes associated with wooden breast in broiler breast fillets. Avian Disease. 59:410-418.

Yahav, S., R. Sasson Rath and D. Shinder. 2004. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. J Thermal Bio. 29:245-250.

Yildirim, I., and R. Yetisir. 2004. Effects of different hatcher temperatures on hatching traits of broiler embryos during the last five days of incubation. S. African J Anim Sci. 34:211-216.

Zubair, K. and S. Leeson. 1996. Compensatory growth in the broiler chicken: a review. World's Poult Sci J. 52:189-200.

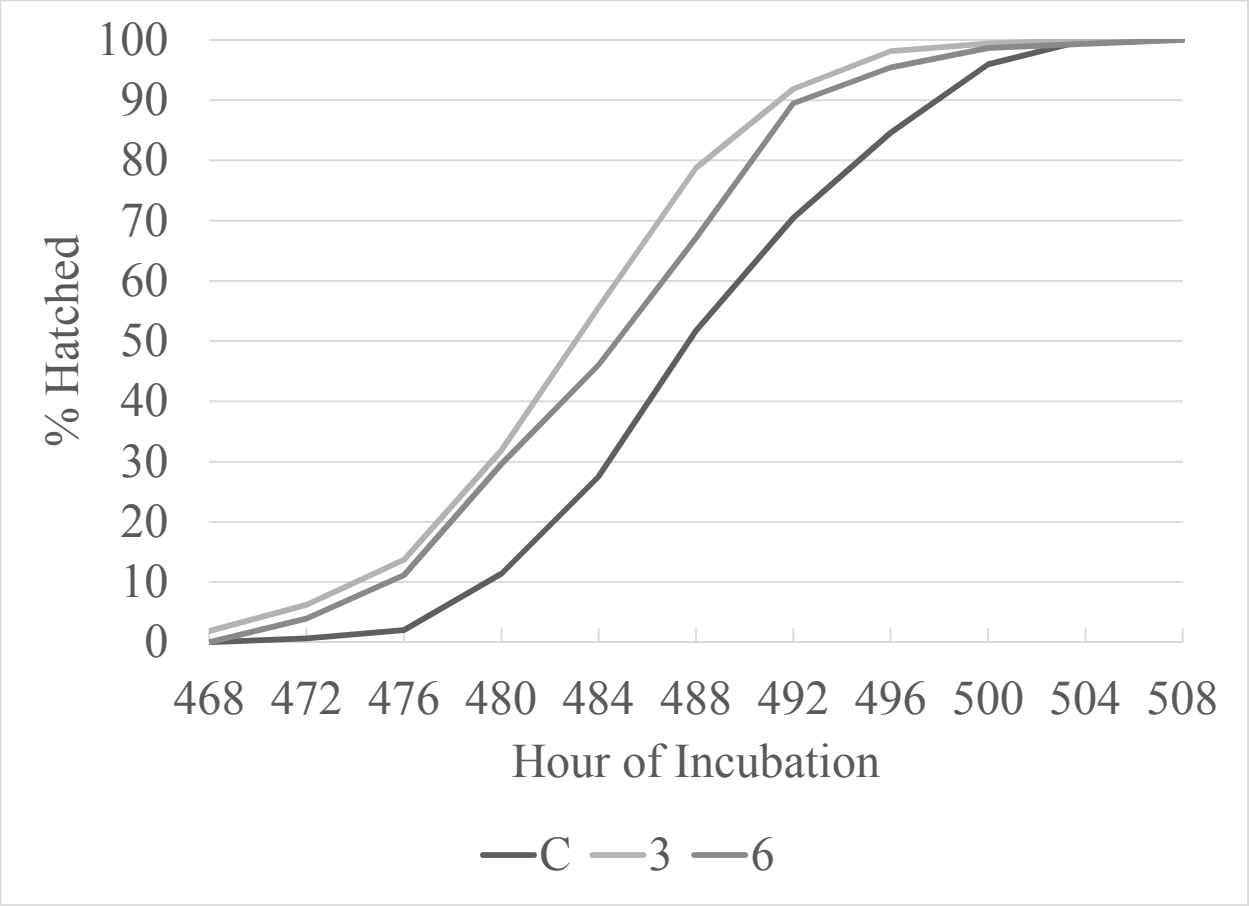


Figure 1. Trial 1 cumulative percentage of total hatch window of all 3 Treatments[1] from hour of incubation 468 to hour 508.

[1]C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17

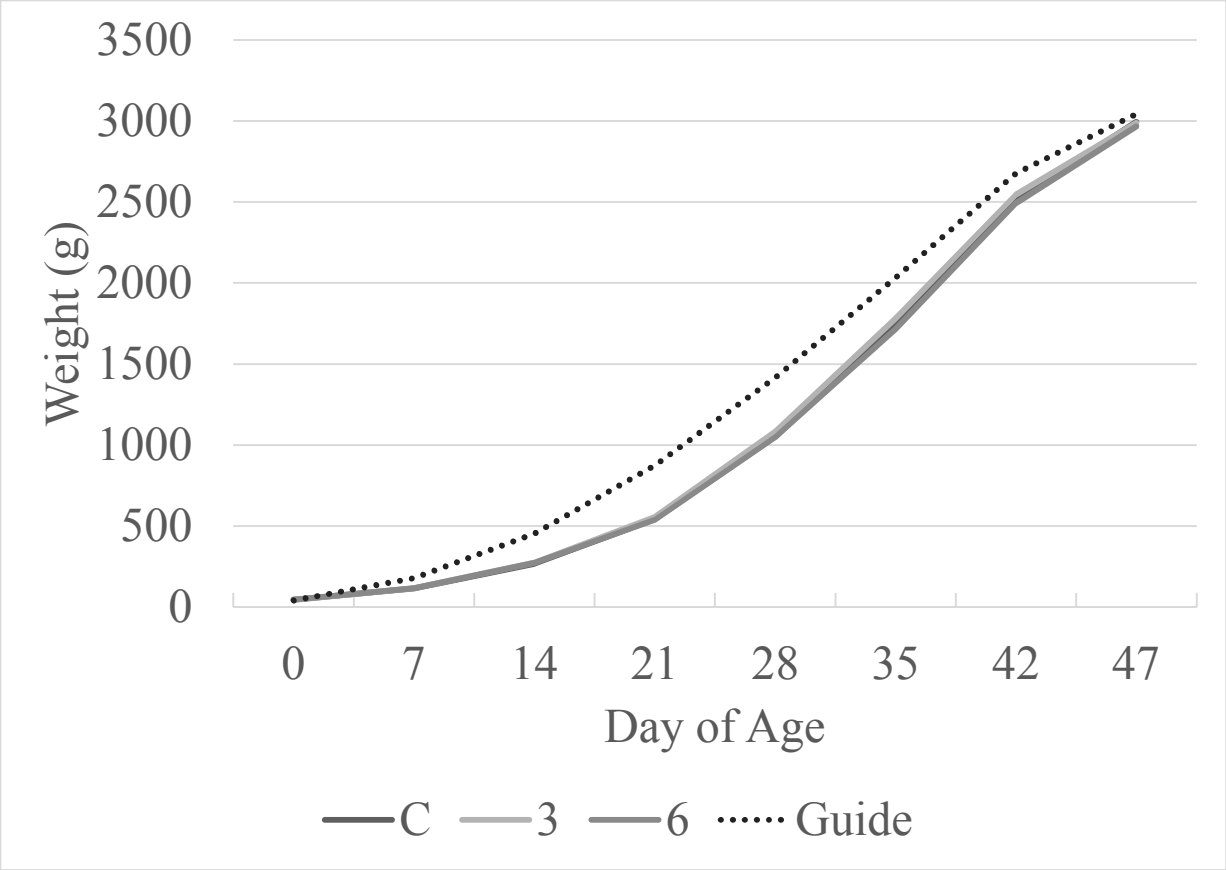


Figure 2. Trial 1 growth curve from 0 to 47 days for all treatments[1]. The growth curve from the broiler performance objective is included[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17
[2]Data obtained from 2014 Ross 708 Broiler Performance Objective Guide

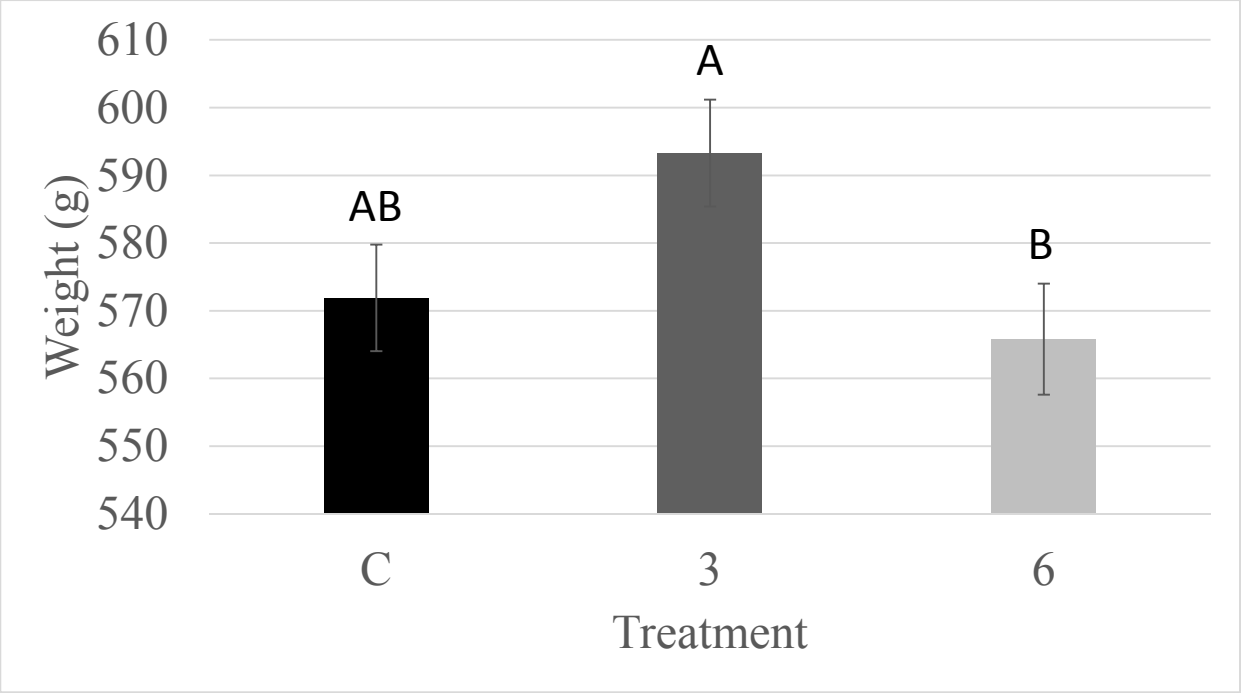


Figure 3. Trial 1 absolute breast weights for all three treatments[1] at 47 days of age (Mean ± SE)[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17

[2] A-B For each trait, groups with no common letter are different at $P \leq 0.05$

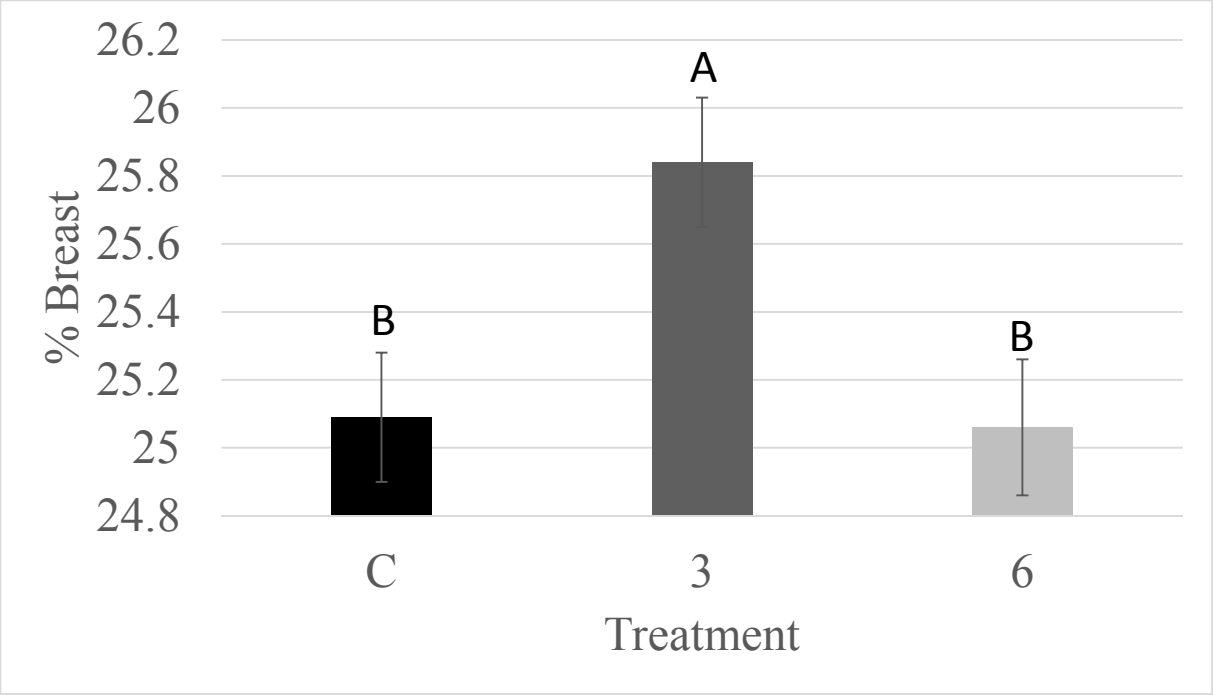


Figure 4. Trial 1 percent Breast ((Breast Wt/Chilled Carcass Wt)*100) for all three treatments[1] at 47 days of age (Mean ± SE)[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17

[2] A-B For each trait, groups with no common letter are different at $P \leq 0.05$

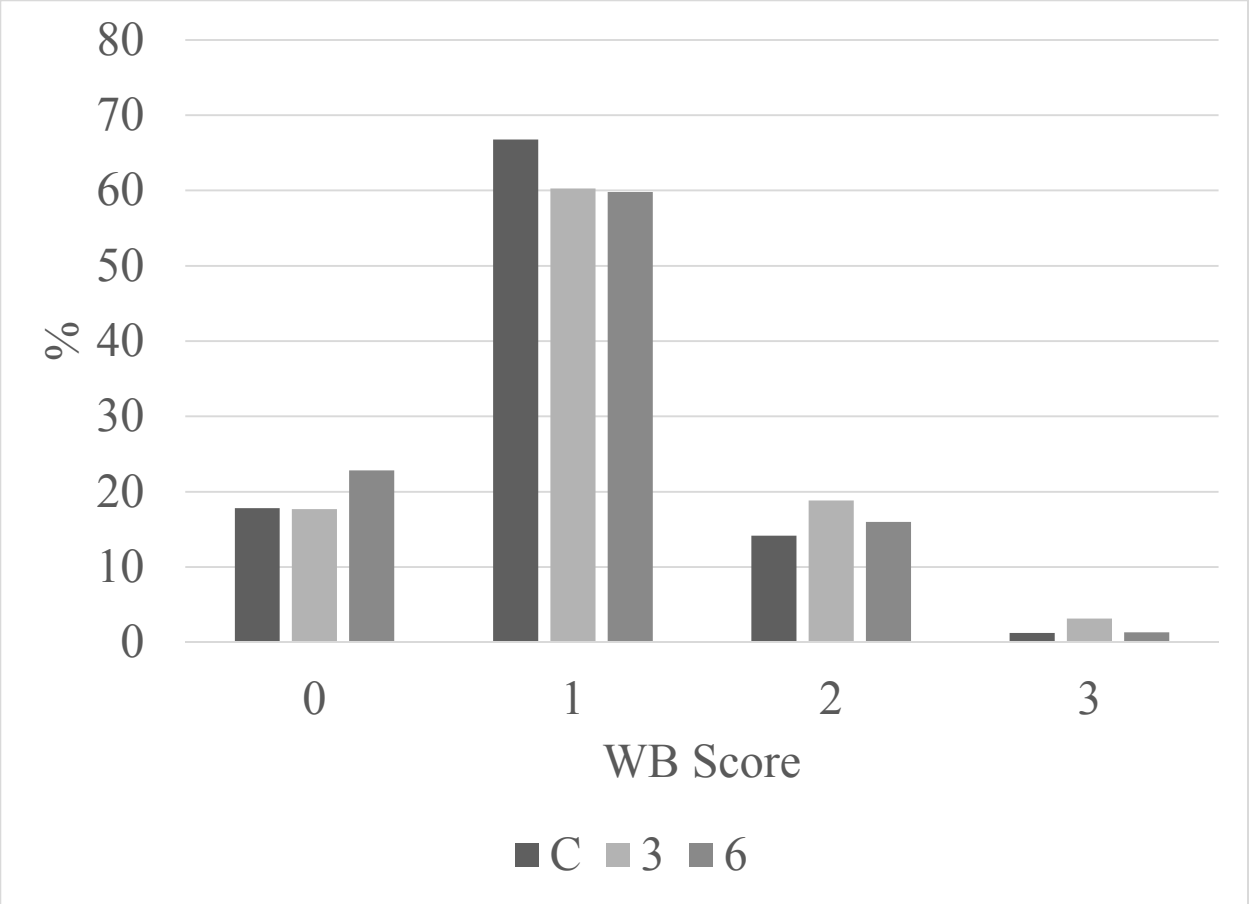


Figure 5. Trial 1 percent woody breast for each score within each treatment[1] at 47 d

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17

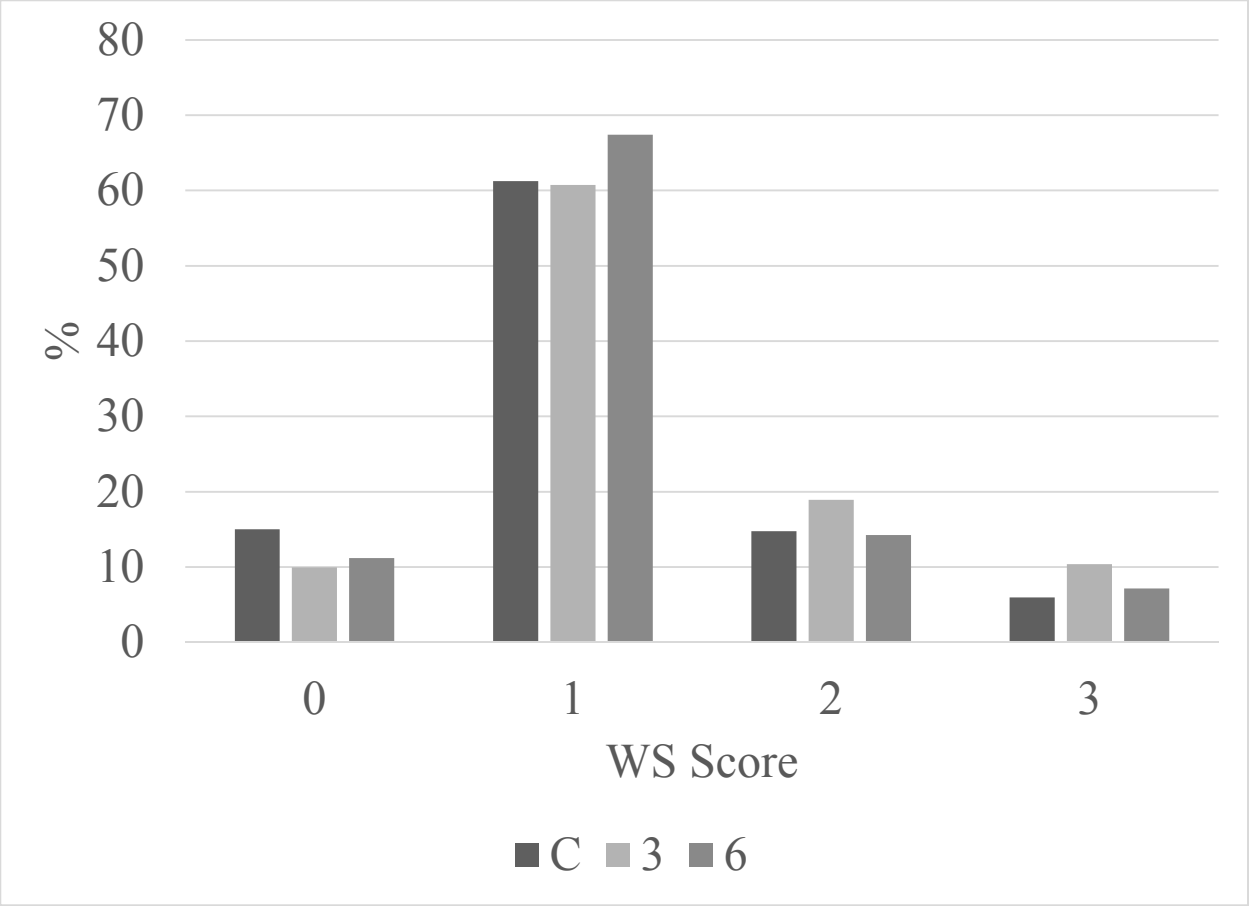


Figure 6. Trial 1 percent white striping for each score within each treatment[1] at 47 d

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17, 6=6 hour TM treatment from E15 to E17

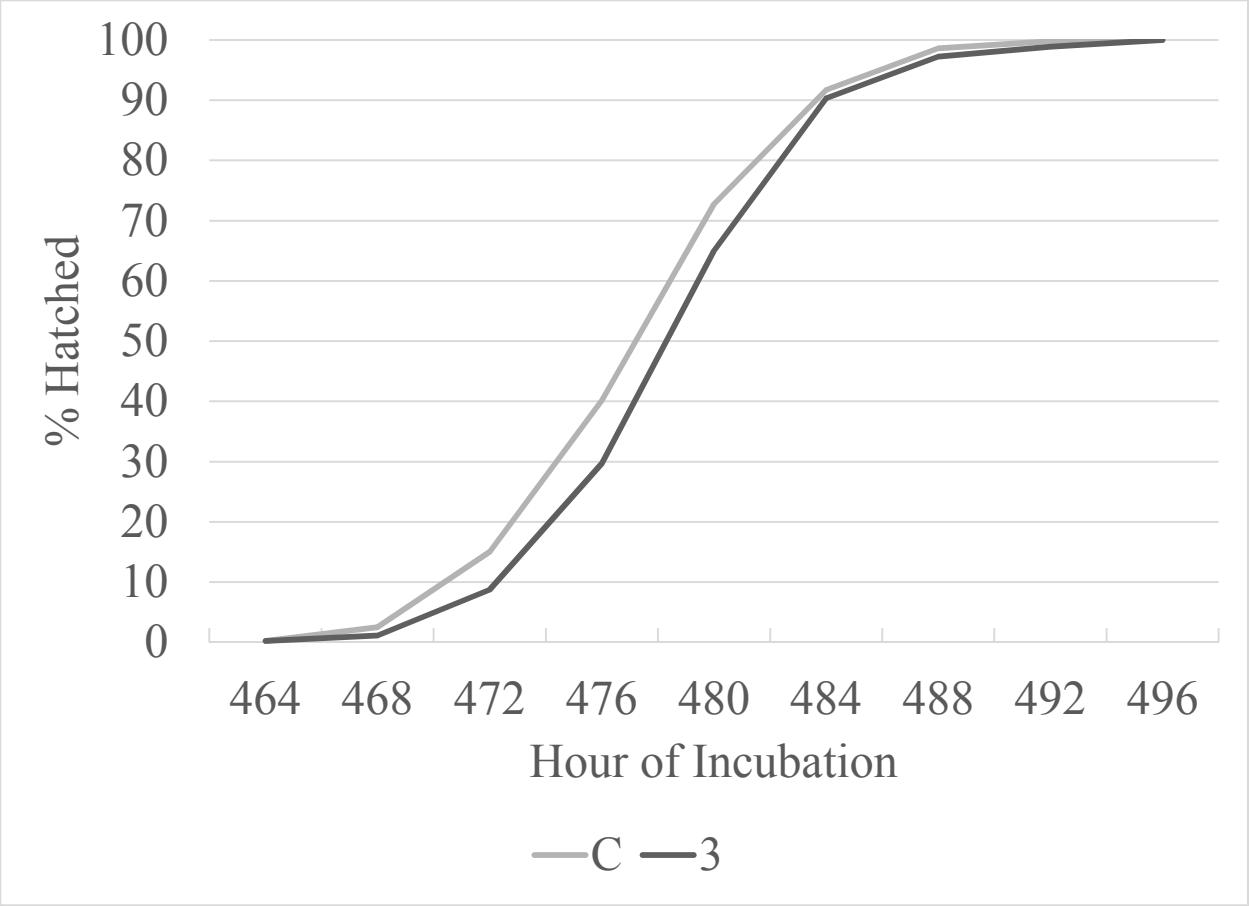


Figure 7. Trial 2 cumulative percentage of total hatch window of all treatments[1] from hour of incubation 464 to hour 496.

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17

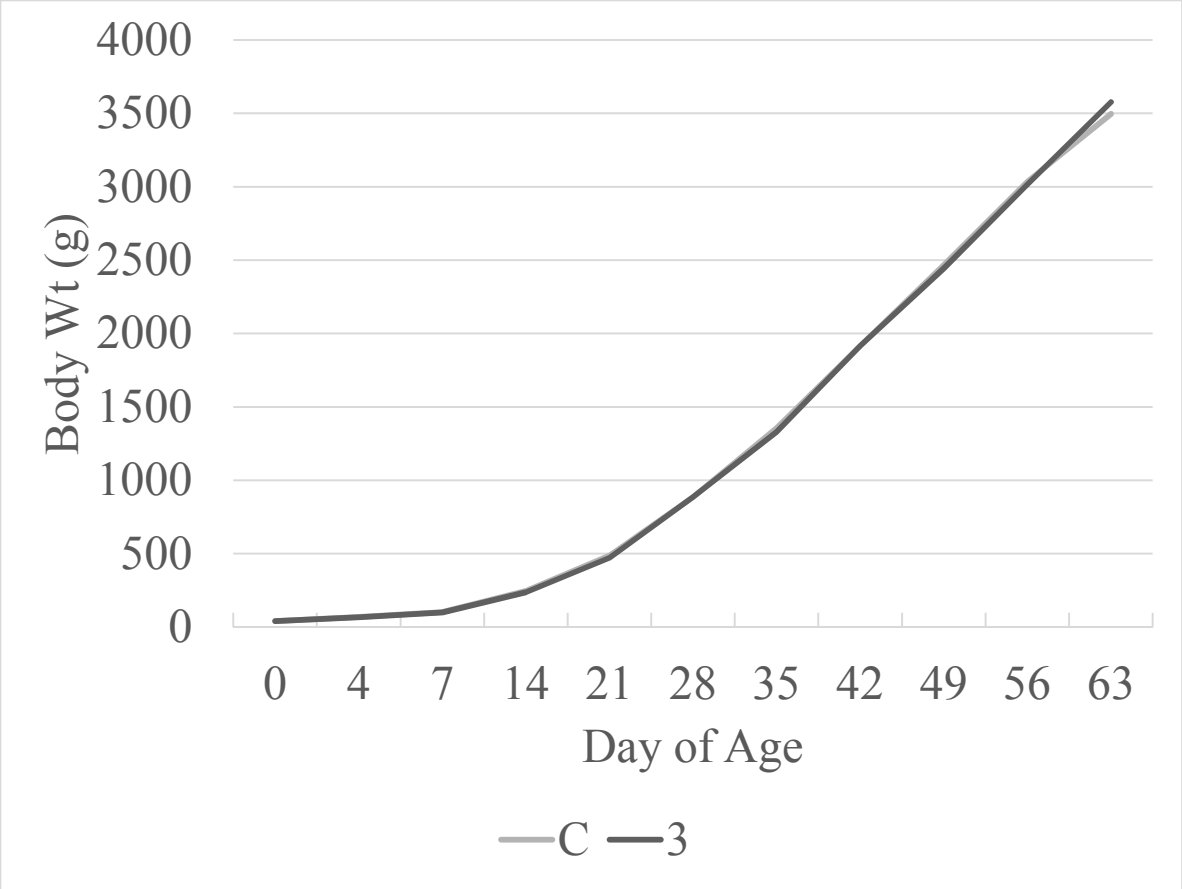


Figure 8. Trial 2 growth curve for Control and 3 hour TM[1] from 0 to 63 days[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17
[2] An * indicates a difference between the C and 3 group for body weight at P≤0.05

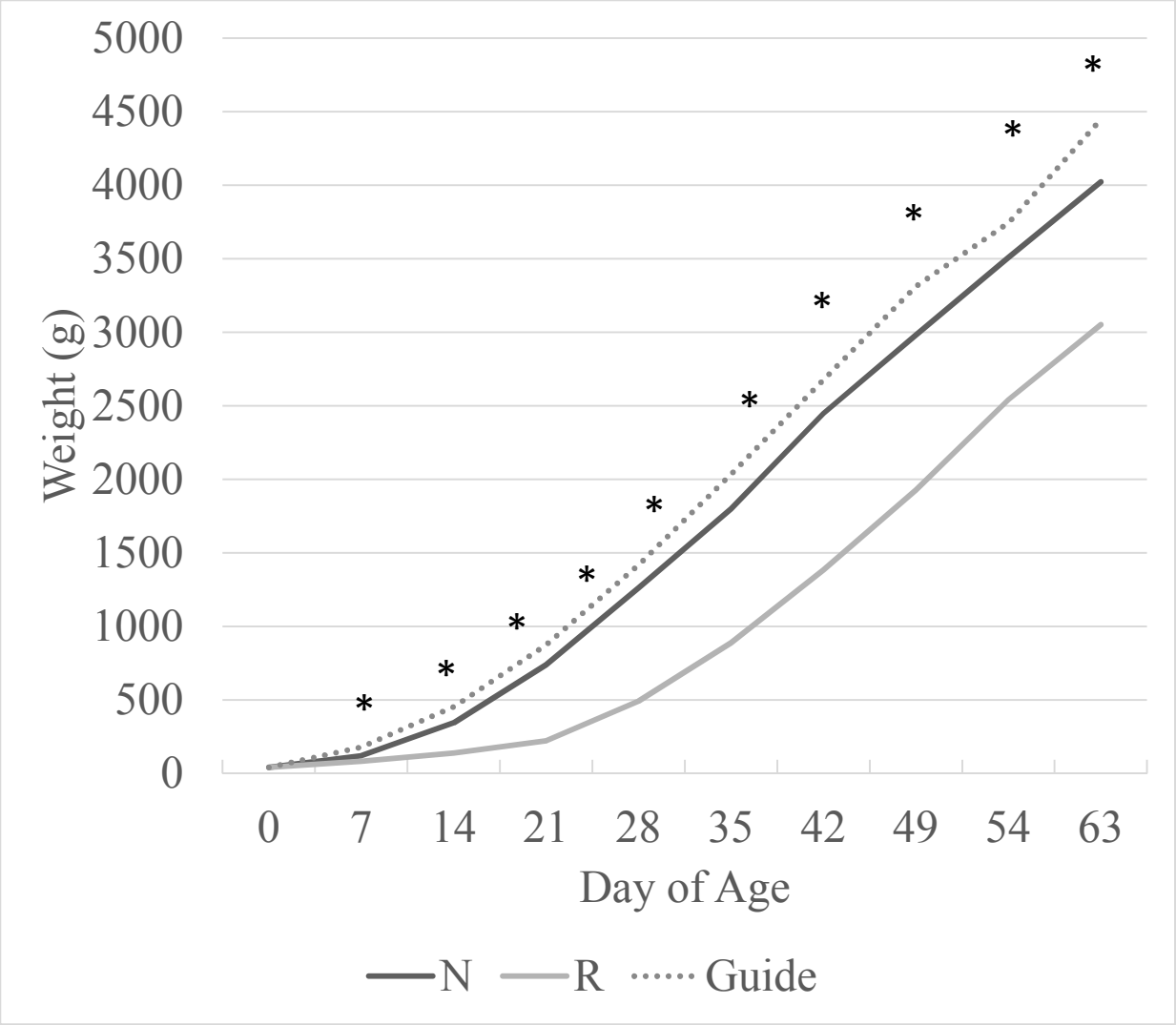


Figure 9. Trial 2 growth curve for Normal and Restricted[1] fed birds from 0 to 63 days[2]. The growth curve from the broiler performance objective is included[3]

[1]N=Normal calorie starter feed R=Low calorie starter feed
[2] An * indicates a difference between the Normal and Restricted groups for body weight at $P \leq 0.05$
[3]Data obtained from 2014 Ross 708 Broiler Performance Objective Guide

Table 1. Trial 2 carcass characteristics and weights broken out by main effect of TM treatment[1] (Mean ± SE)

Trait	3 Hour TM	Significance[2]	Control
Dock (g)	3429.8 ± 29.6	NS	3245.2 ± 29.6
Carcass (g)	2461.3 ± 23.9	NS	2453.1 ± 23.9
Fat (%)	2.17 ± 0.05	NS	2.16 ± 0.06
Chilled Carcass	2494.8 ± 24.1	NS	2486.9 ± 24
Breast (g)	637.4 ± 9.7	NS	627.8 ± 9.6
Breast (%)	25.34 ± 0.19	***	24.54 ± 0.2
Tenders (g)	143.8 ± 1.83	NS	145.2 ± 1.84
Tenders (%)	5.74 ± 0.04	NS	5.8 ± 0.04
Wings (g)	244.8 ± 1.9	NS	245.7 ± 2
Wings (%)	9.9 ± 0.04	NS	10 ± 0.04
Leg Quarters (g)	811.1 ± 7.26	NS	805.3 ± 7.3
Leg Quarters (%)	32.8 ± 0.7	NS	32.7 ± 0.16
Rack(g)	666.2 ± 6.7	NS	663.1 ± 6.7

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17

[2] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 2. Trial 2 carcass characteristics and weights broken out by main effect of Feed treatment[1] (Mean ± SE)

Trait	Normal			Significance[2]	Restricted		
Dock (g)	3713.6	±	29.6	***	2781.6	±	29.6
Carcass (g)	2870.1	±	23.9	***	2044.3	±	23.8
Fat (%)	2.26	±	0.06	**	2.08	±	0.05
Chilled Carcass	2905.8	±	24.6	***	2075.9	±	24.1
Breast (g)	794.6	±	9.5	***	470.6	±	9.6
Breast (%)	27.35	±	0.2	***	22.53	±	0.19
Tenders (g)	174.2	±	1.8	***	114.8	±	1.9
Tenders (%)	6.01	±	0.04	***	5.53	±	0.04
Wings (g)	280.3	±	1.9	***	210.3	±	1.9
Wings (%)	9.7	±	0.04	***	10.2	±	0.04
Leg Quarters (g)	906.2	±	7.26	***	710.3	±	9.3
Leg Quarters (%)	31.2	±	0.15	***	34.3	±	0.17
Rack(g)	759	±	6.8	***	570.3	±	6.7

[1]Normal=normal calorie starter feed, Restricted=low calorie starter feed

[2] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 3. Trial 2 carcass characteristics and weights broken out by main effect of Sex (Mean ± SE)

Trait	Males	Significance[1]	Females
Dock (g)	3464.6 ± 29.6	***	3030.5 ± 29.6
Carcass (g)	2618.9 ± 23.9	***	2295.6 ± 22.8
Fat (%)	1.88 ± 0.05	***	2.46 ± 0.05
Chilled Carcass	2650 ± 24.1	***	2331.6 ± 23.9
Breast (g)	648.5 ± 9.7	**	616.7 ± 9.4
Breast (%)	24.01 ± 0.19	***	25.85 ± 0.19
Tenders (g)	145.8 ± 1.8	NS	143.1 ± 1.8
Tenders (%)	5.43 ± 0.03	***	6.1 ± 0.04
Wings (g)	265.2 ± 1.8	***	225.4 ± 1.9
Wings (%)	10.1 ± 0.05	***	9.7 ± 0.04
Leg Quarters (g)	893 ± 7.1	***	723.5 ± 8.6
Leg Quarters (%)	34.1 ± 0.16	***	31.3 ± 0.17
Rack (g)	703 ± 6.7	***	626.3 ± 6.7

[1] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 4. Trial 2 meat quality traits broken out by the main effect of TM treatment[1] at 60 day processing (Mean ± SE)

Trait	3			Significance[2]	Control		
Dressing%	75.4	±	0.12	NS	75.2	±	0.1
Moisture Uptake	1.43	±	0.04	NS	1.43	±	0.04
Drip Loss	-1.14	±	0.06	NS	-1.28	±	0.05
Woody Breast	0.83	±	0.06	*	1.01	±	0.06
White Striping	0.76	±	0.05	NS	0.72	±	0.06
L*	0.4	±	0.21	NS	56.8	±	0.2
a*	3.9	±	0.07	NS	3.9	±	0.07
b*	0.88	±	0.09	NS	0.94	±	0.1

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17
[2] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 5. Trial 2 meat quality traits broken out by the main effect of Feed[1] treatment at 60 day processing (Mean ± SE)

Trait	Normal	Significance[2]	Restricted
Dressing (%)	75.4 ± 0.12	NS	75.2 ± 0.12
Moisture Uptake (%)	1.27 ± 0.04	***	1.57 ± 0.04
Drip Loss (%)	-1.02 ± 0.05	***	-1.41 ± 0.06
Woody Breast	1.23 ± 0.06	***	0.61 ± 0.06
White Striping	1.2 ± 0.05	***	0.28 ± 0.05
L*	56.5 ± 0.22	NS	56.7 ± 0.21
a*	3.7 ± 0.08	***	0.1 ± 0.07
b*	1.19 ± 0.09	***	0.63 ± 0.09

[1]Normal=normal calorie starter feed, Restricted=low calorie starter feed
[2] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

Table 6. Trial 2 meat quality traits broken out by main effect of Sex at 60 day processing (Mean ± SE)

Trait	Males			Significance[1]	Females		
Dressing %	75.5	±	0.12	NS	75.2	±	0.2
Moisture Uptake (%)	1.22	±	0.04	***	1.62	±	0.05
Drip Loss	-1.23	±	0.05	NS	-1.2	±	0.06
Woody Brest	0.98	±	0.05	NS	0.86	±	0.06
White Striping	0.69	±	0.05	NS	0.79	±	0.06
L*	57.2	±	0.21	***	56.1	±	0.21
a*	3.9	±	0.07	NS	3	±	0.06
b*	0.7	±	0.09	NS	0.95	±	0.09

[1] Significance: ***=Highly Significant (P value<0.001), **=Moderately Significant (P Value<0.01), *=Significant (P Value<0.05), NS=No Significance.

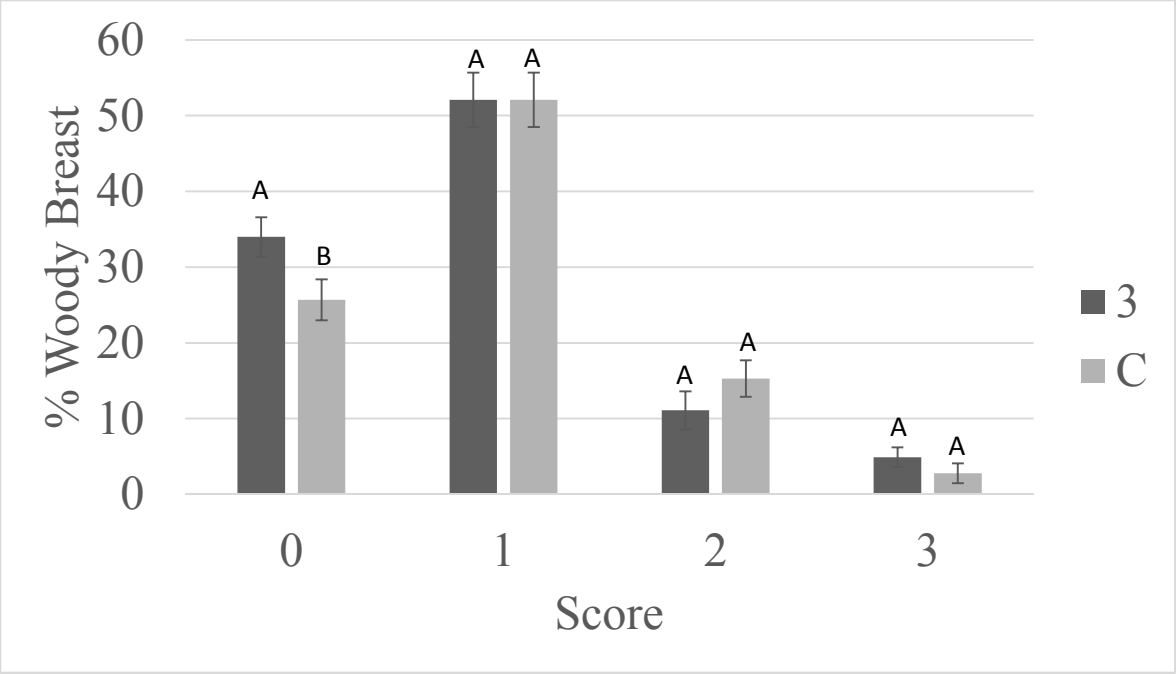


Figure 10. Trial 2 percent woody breast for each score within each treatment broken out by TM treatment[1] at 60 d[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17
[2] A-B For each score, groups with no common letter are different at P≤0.05

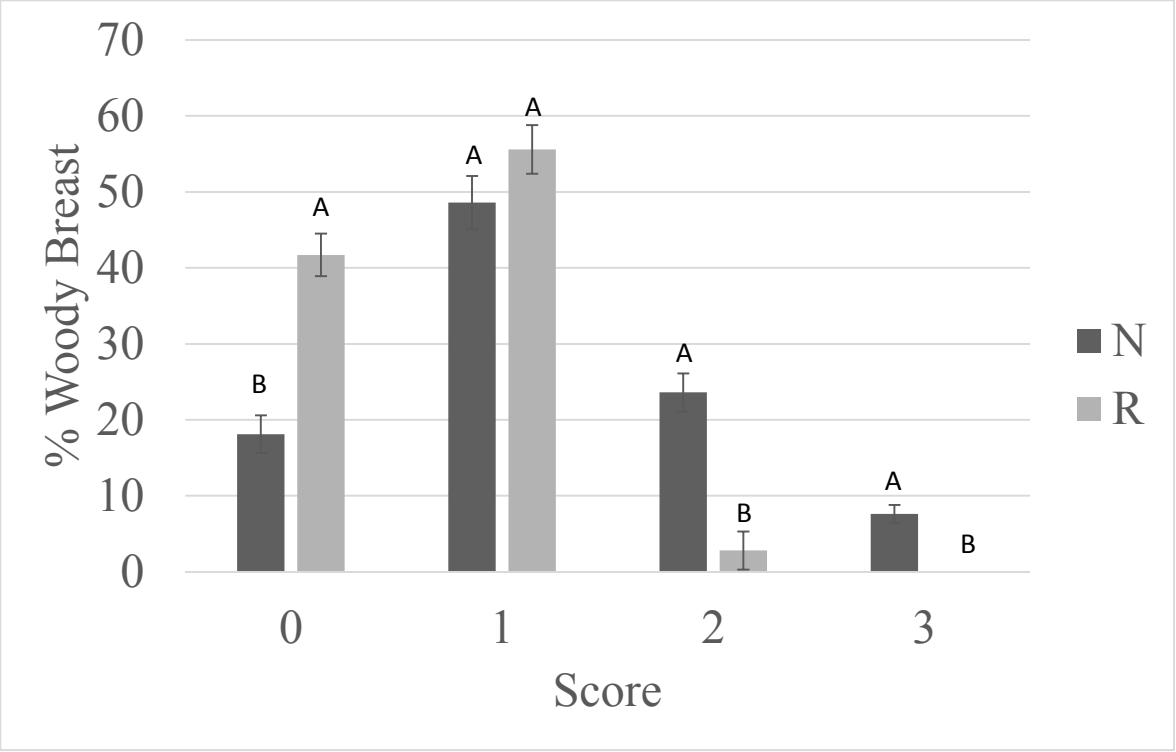


Figure 11. Trial 2 percent woody breast for each score within each treatment broken out by feed treatment[1] at 60 d[2]

[1]N=Normal starter feed, R=low calorie starter feed
[2] A-B For each score, groups with no common letter are different at P≤0.05

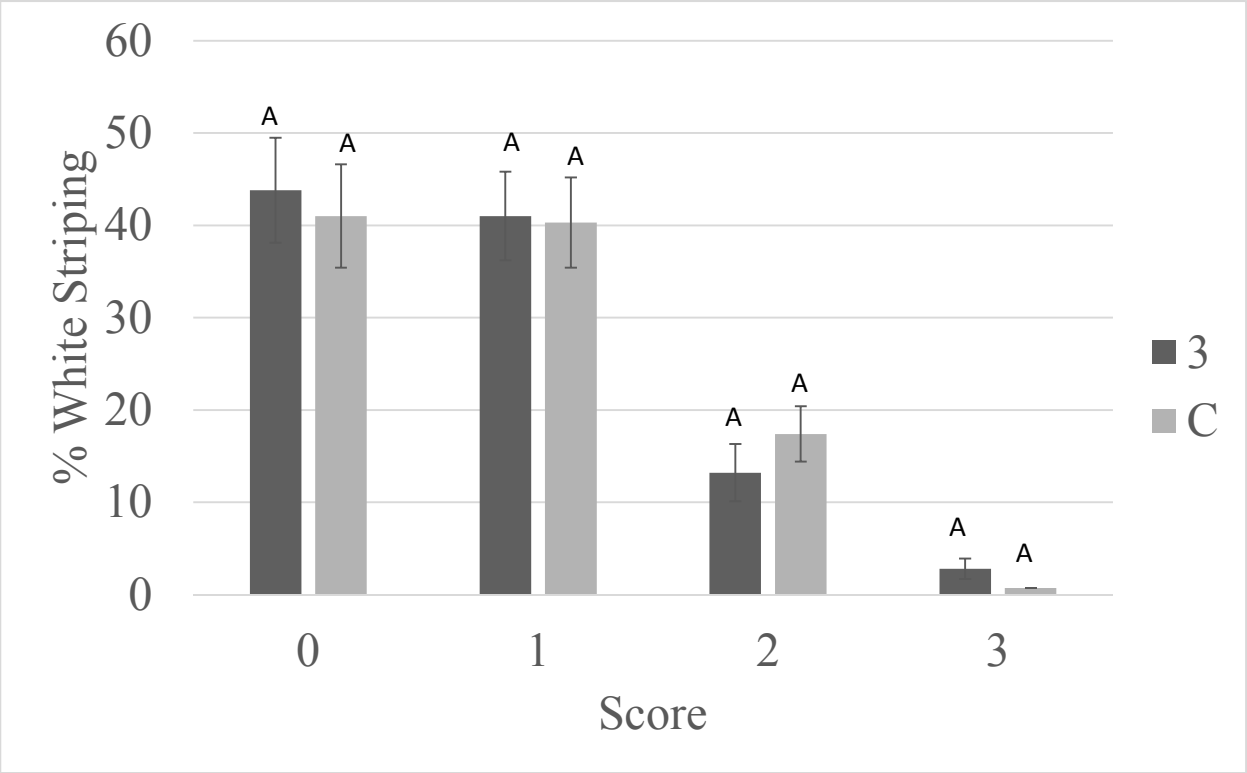


Figure 12. Trial 2 percent white striping for each score within each treatment broken out by TM treatment[1] at 60 d[2]

[1] C=control, normal incubation profile, 3=3 hour TM treatment from E15 to E17
[2] A-B For each score, groups with no common letter are different at P≤0.05

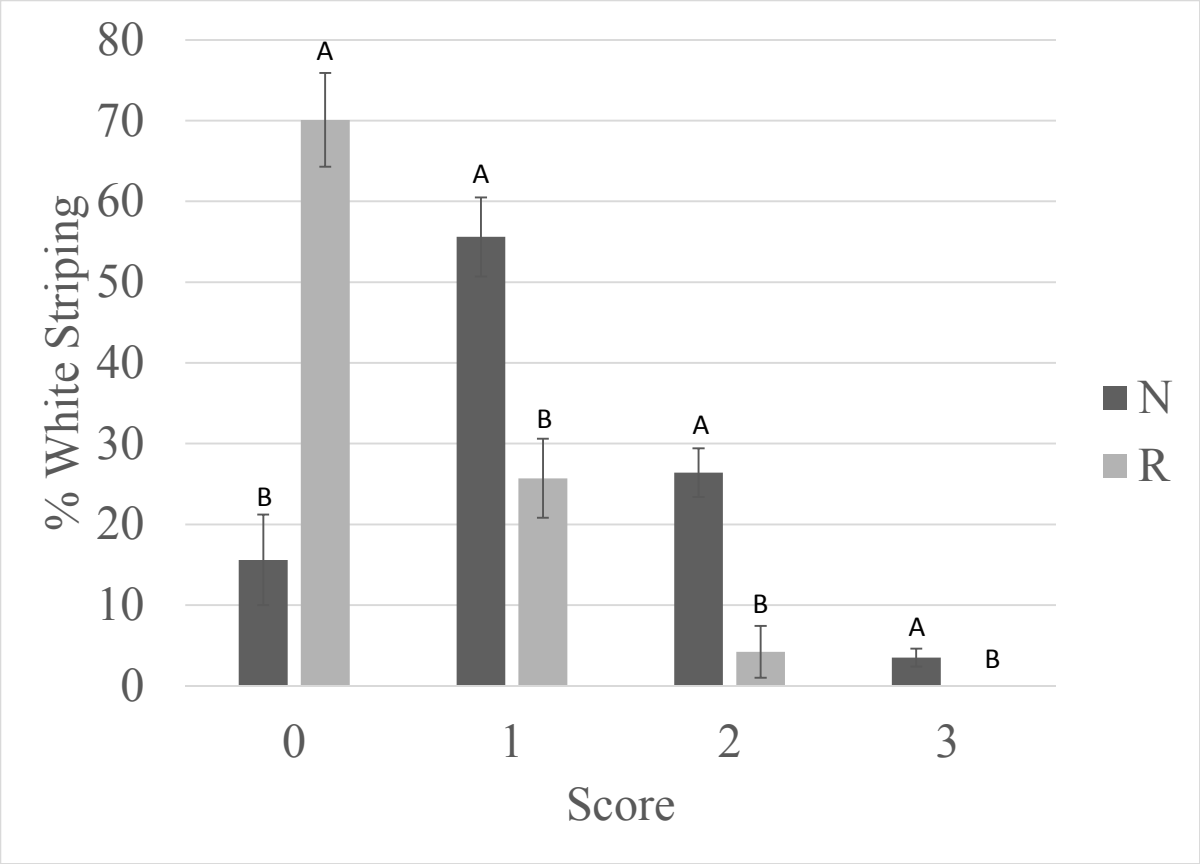
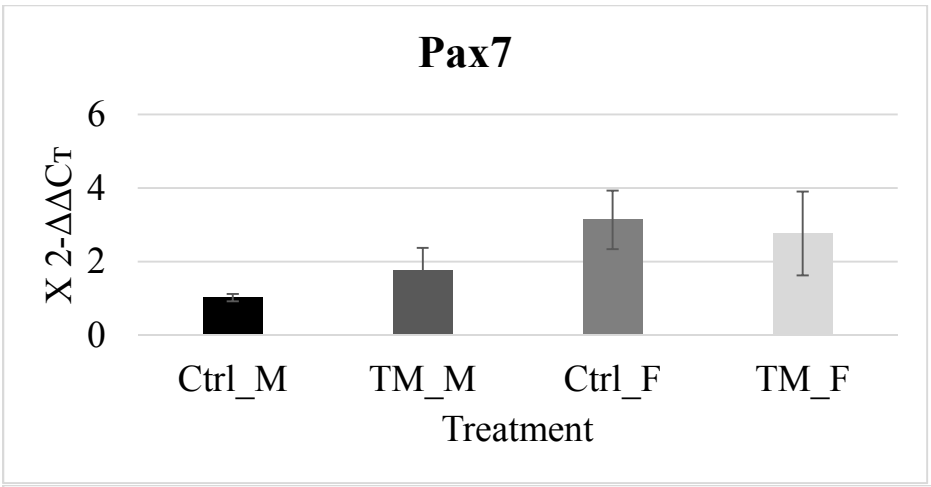


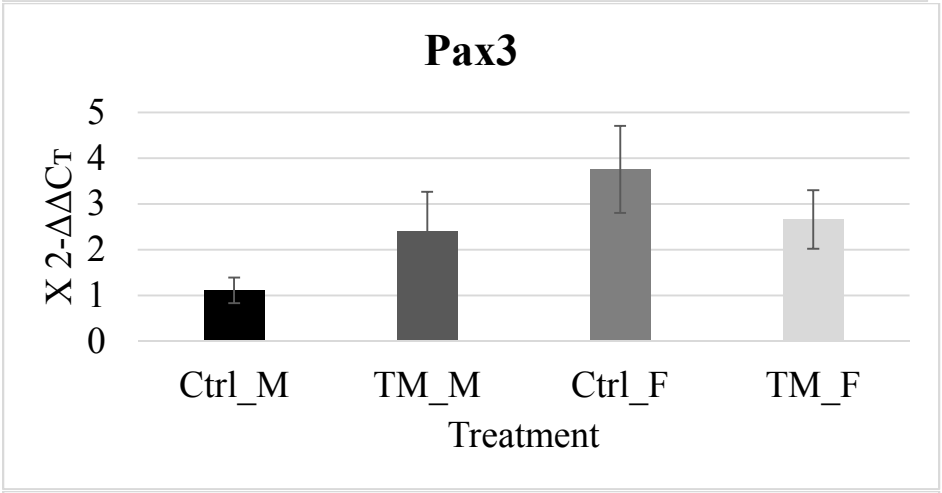
Figure 13. Trial 2 percent white striping for each score within each treatment broken out by feed treatment[1] at 60 d[2]

[1]N=Normal starter feed, R=low calorie starter feed
[2] A-B For each score, groups with no common letter are different at P≤0.05

A.



B.



C.

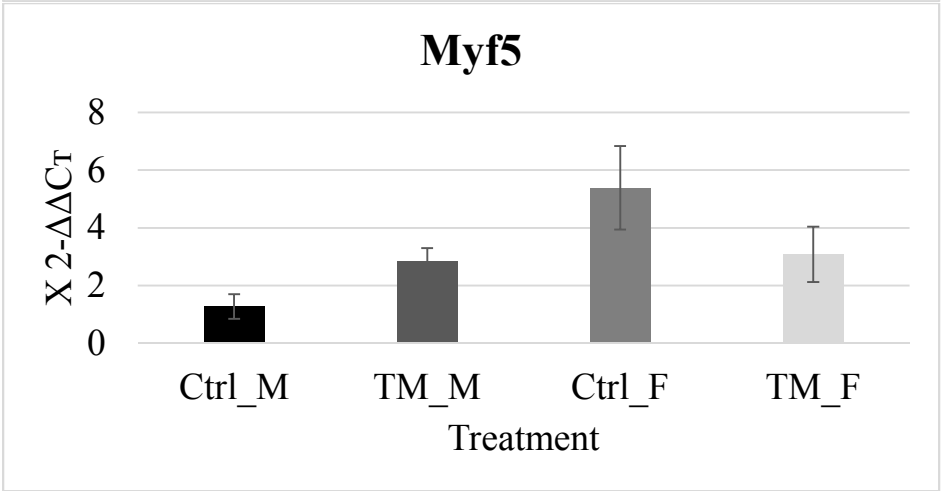


Figure 14. Trial 2 Gene Expression (Mean ± SE) of Pax7 (A), Pax3 (B) and Myf5 (C) at processing day 60 for males and females from the Control and 3 hour TM, normal fed pens.

GENERAL SYNTHESIS

Genetic progress has resulted in a bird that is higher yielding, faster growing and more efficient then the broiler produced 50 years ago. This genetic progress in addition to changes and advancements in the environmental management of the broiler has resulted in poultry being one of the cheapest and healthiest protein sources available to the growing population. While these advancements have altered things such as yield, feed efficiency and growth, it has also brought about issues associated with meat quality. The rapid growth and high processing weights of the modern broiler have led to the development of two muscle myopathies that are of concern to the industry; woody breast and white striping. Evaluating novel methods to help reduce or even eliminate these myopathies is critical to the advancement of the industry in feeding the growing population.

Selection for 4-day breast yield, which focuses not on selection for cell size (Hypertrophy) but cell number (hyperplasia) may still be a useful selection tool for decreasing the incidence of the two myopathies previously discussed. If selection for a high percent breast yield at 4 days of age results in an increase in the number of satellite cells available for post-hatch growth and repair, a broiler may be better able to hinder the onset of woody breast however future research needs to be done to evaluate the quantity of satellite cells in the selected populations.

Thermal manipulation (TM) and early post-hatch feed restriction may also be beneficial ways to decrease the woody breast and white striping myopathies. Embryonic TM during the period of satellite cell division can help increase the number of satellite cells available for post hatch growth and repair. Throughout two studies, embryonic TM of 3 hours from E15 to E17 resulted in a decrease in the average severity of white striping as well as improved breast meat

yield. Early post hatch feed restriction, if done properly may also help reduce the severity of woody breast. Both methods will need further evaluation for the efficacy in reducing both woody breast and white striping but the results of these studies are promising, going forward.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Nicholas Anthony
Fr:	Craig Coon
Date:	February 9th, 2018
Subject:	IACUC Approval
Expiration Date:	February 1st, 2021

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18083:** *General Rearing of Selected chicken and Quail Populations*.

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 February 1st, 2021 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: Nicholas Anthony, Sara Orlowski, and Joseph Hiltz. 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

18083