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Characterization of Broiler Lines Divergently Selected for Breast Muscle Color

Sara Katherine Orlowski
University of Arkansas, Fayetteville

Characterization of Broiler Lines Divergently Selected for Breast Muscle Color

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Sara Katherine Orlowski
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_____
Dr. Nicholas B. Anthony, Ph.D.
Thesis Director

_____
Dr. Susan Watkins, Ph.D.
Committee Member

_____
Dr. Fred W. Pohlman, Ph.D.
Committee Member

ABSTRACT

An increase in the consumption of poultry has generated an increase in demand for higher yielding broilers. This has led to an increase in atypical meat and issues with appearance. Color is a direct result of a pH decline as meat goes through rigor mortis with meat generally becoming lighter. If the pH declines too rapidly or too slowly, meat quality can suffer. Physical properties of meat can be altered by pH. A fast pH decline results in pale meat with decreased tenderness. A slow pH decline can result in darker meat with a reduced shelf-life.

With a known relationship between pH and color, lines were divergently selected for breast muscle color (L*). After 10 generations of selection, the HMC and LMC lines (selected for high and low L* respectively) as well as a random bred control base population (RBC) were characterized through several studies.

Experiment 1 characterized the pH decline of the lines under two different chill methods. The chill method did not have an effect on the rate of decline or final pH. The HMC line exhibited a quicker pH decline and a lower pH when compared to both the RBC and LMC line. Although there were no statistical differences between the LMC and RBC line, the general trend showed the LMC line having a slower pH decline and higher pH.

Experiment 2 characterized all lines for pH and color, drip loss, and moisture uptake at the processing ages of 5, 6, 8 and 10wks of age. For the HMC and RBC lines, 24h color did not differ between the processing ages. For the LMC line, the 5wk group was significantly lighter than the remaining age groups, possibly due to the thickness of the muscle at 5 wks.

Experiment 3 looked at the effect of feed-withdrawal times. Birds were separated randomly be line into groups and feed was removed at 0, 6, 12, 18 and 24h prior to processing.

Line differences were detected for color and pH but not for treatment groups. In muscle color lines, feed-withdrawal times did not appear to have a significant impact on meat-quality.

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INTRODUCTION

Between the 1950s and today, broiler production in the United States has changed dramatically. According to the USDA and the National Chicken Council, in 1950, broiler production in the United States weighed in at a mere 1,381 million pounds of ready to cook chicken. It is currently projected that in 2016, broiler production in the United States will be at 40,586 million pounds (National Chicken Council, 2016). This increase in demand for the production of poultry products has led to dramatic changes in approach to formulation of poultry diets, genetic selection and house designs.

In fact, one of the main reasons for the increase in production of poultry meat is a direct result of improvements in genetics and intense genetic selection. Havenstein and coworkers (1994a, b, 2003a, b) compared the broiler of 1957 to the broiler of both 1991 and 2001, 85 to 90% of the observed changes between the broiler lines was a result of intense genetic selection. Unfortunately, as a result of the intense selection practices utilized, many unwanted consequences have arisen. One thing that is of major concern to the industry is atypical poultry meat which has a direct impact on consumer acceptability of a product (Barbut, 1997, 1998; Anthony, 1998). Atypical poultry meat can be caused by many things but is often directly related to post-mortem metabolism and the decline in pH that is observed after slaughter.

pH and Meat Quality

When an animal is slaughtered, whether it be pigs, cattle or broilers, muscle pH declines as the muscle is converted to meat. This pH decline and the ultimate pH that the muscle reaches after the process of rigor mortis is critical when considering meat quality attributes. Several studies have shown pH to be highly correlated with meat quality traits such as color, water holding capacity, tenderness and fresh product shelf life (Allen et.al., 1998, Qiao, et.al., 2001).

These traits are critical when evaluating consumer acceptability, palatability and quality of a fresh or further processed product. When discussing both pH and color, the two can almost be used interchangeable of one another. A breast fillet exhibiting a pale fillet will typically have a low pH and a darker fillet will have a higher pH. This has been shown not only in broilers and turkeys but also in muscles of both cattle and swine. Typically, a "normal" broiler fillet will have a pH around 5.8 to 5.9 (Qiao et al., 2001; Duclos et al., 2007) and a typical L* or color value of a "normal" fillet may vary but in general are between 50 and 54. Deviations above or below these values can result in detrimental poultry muscle myopathies and poor meat quality that has limited consumer acceptability

Muscle Myopathies Associated with pH

The rate of muscle pH decline and the ultimate pH that muscle reaches can have detrimental effects on the quality of meat produced. In red meat species, two well documented conditions exist related to pH and its decline that negatively impact meat quality and consumer acceptability. In cattle, a condition exists called DFD or dark firm and dry muscle. It can also be known as a dark-cutting condition and occurs when the pH of the muscle going through rigor mortis has a very minimal decline resulting in a high ultimate pH (Miller, 2007). DFD-like meat has also been documented in broilers (Harford, et al., 2014). Another condition exists that was first documented in swine called PSE or pale soft and exudative meat. A PSE-like condition has been documented to exist in both turkeys and broilers (Barbut, 1997; Woelfel et al., 2002; Petracci et al., 2009). The PSE-like condition is a result of a very rapid pH decline early in the rigor mortis process while carcass temperatures are still high followed by a low ultimate pH after rigor mortis is complete. Variations in meat quality associated with pH can occur and have been considered in broilers to be both PSE- and DFD-like muscle myopathies (Fletcher, 2002). Both

conditions have a negative impact on consumer acceptability of a product, further processing techniques and the wholesomeness of a fresh product.

Meat Color

Meat color is a highly valuable trait that can serve as a strong indicator towards the wholesomeness and quality of both fresh and further processed meat products. Meat color is easy to measure on a commercial basis while also having a strong negative correlation with muscle pH, an indicator of meat quality. Typically, a portion of meat with a high ultimate pH will have a very dark color as it is able to hold onto myoglobin (heme containing protein) that is the main pigment found in skeletal muscle. If meat has a low ultimate pH, it is possible that protein denaturation has occurred during the rigor mortis process and the muscle has lost its ability to hold on to water. Since myoglobin is a water soluble protein, it is lost from the muscle resulting in a fillet that is lighter in color.

Differences in color instruments, illuminant, angle and aperture can all affect the outcome of the color measurement (Tapp et al., 2011) as well as differences in breeds, age, and sex (Ngoka et al., 1982; Smith et al, 2002; Dadgar et al., 2011; Janisch et al., 2011). These factors as well as biological factors can influence the "cutoff" values used for determining if a broiler breast fillet is exhibiting PSE- or DFD-like characteristics.

DFD meat, as indicated by its name, will have a dark color and a high pH whether it be a breast fillet or a cut of steak. In broilers, DFD-like meat has been characterized in the quantitative genetics lab at the University of Arkansas as anything with an L* value <48 although the cutoff value varies between research groups. For example, Sheard et. al., (2012) used an L* cutoff value of <52 to classify a DFD-like fillet, while Mallia et. al. (2000) used an L* cutoff value of <41. PSE meat, will have a lighter opaquer color when compared to a normal

fillet (Van Laack et al., 2000; Le Bihan-Duval et al., 2001; Woelfel et al., 2002; Fraqueza et al., 2006; Berri et al., 2007). PSE-like meat in broilers has been classified at the U. of Arkansas as anything with an L* value >54 while Sheard et al, (2012) classified a PSE-like fillet >58.

When considering color, it is important to keep in mind not only its relationship to pH and the meat quality myopathies that are related to it but also consumer acceptability of a fresh product. If a breast fillet has a darker or lighter than normal color, it is possible that a consumer will be less likely to purchase that product (Viljoen, et al., 2002; Droval et. al., 2012). Consumer acceptability may also decline with a package containing multiple fillets that vary in color that are not considered "normal" to the average consumer (Barbut, 2009). Uniform packaging of breast fillets exhibiting a normal color may help ensure that a consumer purchases a fresh product.

Water Holding Capacity

Water holding capacity (WHC) is a complex meat quality trait that can be affected by the pH decline associated with the conversion of muscle to meat. Unfortunately, the mechanisms that control it are not very well understood. Much of what is known about its mechanisms has been established through research on PSE and DFD meat, particularly in swine (Bendall and Swatland, 1988). It can be measured many different ways whether it be through cook loss, drip loss or expressible moisture. According to Bowker and Zhuang (2015), High-WHC fillets exhibited lower L* reading and high pH at both 2 and 24 h post-mortem. On the contrary, low WHC fillets exhibited high L* and low pH at the same time points measured. It is known and documented that pH is correlated with WHC in broiler fillets (Froning et al., 1978; Barbut, 1993; Northcutt et al., 1994; Bianchi et al., 2005).

In PSE-like meat, WHC is a major concern that can lead to issues with tenderness and cook loss. The idea behind pale, soft, and exudative meat is that protein denaturation is occurring due to rapid pH decline while carcass temperatures are still high. With the occurrence of protein denaturation, muscle fibers lose the ability to hold on or retain water resulting in soft fillets that exude water (Barbut, 1993; Allen, et al., 1998; Sams, 1999). Harford et al. (2014) reported in lines that have been selected to exhibit a PSE-like myopathy have a drip loss percentage of over 2%. Qiao et. al. (2001) reported a strong positive correlation between WHC and pH when investigating pale, normal and dark fillets. In DFD fillets that have a high pH, it is likely that protein denaturation has not occurred during the rigor process resulting in a fillet with a high WHC (Cornforth, 1994). The improved WHC of a DFD-like fillet may help improve tenderness and decrease cook loss of a breast fillet but may lead to issues with shelf life and microbial spoilage of a fresh product.

Shelf-Life

Several studies have shown pH to be correlated to the shelf life of a fresh product (Allen et al., 1997). In general, a broiler breast fillet exhibiting a higher pH will have a higher WHC as it is able to hold on to water easier. This ultimately leads to a greater amount of moisture in the breast fillet. This high moisture content fillet is a more desirable environment for microbial growth and spoilage. It is thought that a DFD-like fillet will have a shorter shelf life and will spoil much quicker than a PSE-like fillet that does not retain as much water. In a study by Allen, et.al. (1997), broiler fillets categorized as both “dark” and “light” were evaluated for several shelf-life indicators. The results of the study suggest that the differences in pH observed between light and dark fillets may be responsible for the differences in the shelf life and spoilage of a fresh product.

Emulsification Capacity

The emulsification capacity of proteins found in fresh meat products is imperative when considering the production of further processed products such as sausages, nuggets and patties. Emulsification capacity can be defined as the ability of proteins found in meat to mix with fat particles. Emulsions in meat are typically an oil in water emulsion and consist of a two-phase system involving a dispersed phase and a continuous phase. The emulsification capacity of broiler meat is thought to be affected by pH and color interchangeably.

Studies in broilers have shown varying degrees of emulsification capacity. Qiao, et al. (2001) showed that emulsification capacity had a significant positive correlation with pH (0.9572) and a significant negative correlation with color (-0.9237). PSE-like fillets will have a lower emulsification capacity than normal broiler fillets, making it hard to use them in further processed products. Sorting techniques may be beneficial to remove the product from the line and be used in products or with products that help improve emulsification capacity. Broiler breast fillets that are considered DFD-like should exhibit a higher emulsification capacity than both normal and PSE-like fillets although it has not been documented in broilers.

Cook Loss and Tenderness

Tenderness of a product is also important when considering the consumer preference and acceptability of a product. It can be measured and evaluated through the use of subjective measurements such as trained panelists or product testing with consumers as well as objective measurements such as shear force. In meat species, the Warner Bratzler Shear Force (WBSF) is widely used and accepted as the method of choice. In poultry, three separate shear force methods have been evaluated including the razor blade shear force, Allo-Kramer, and Warner-Bratzler methods. According to Cavitt, et al., (2005b) all shear tests performed similarly when compared

to consumer sensory analysis. An advantage of using the razor blade shear force is that no sample cutting or weighing is necessary and is not as destructive to the muscle sample as the other methods. Fillets with a higher razor blade shear force are typically less tender (Cavitt et al., 2005a).

Cook loss, and drip loss or purge are also useful measurements of tenderness of a product. Cook loss is simply the difference between the initial weight and the cooked weight of a product on a percentage basis of the initial weight. Fillets that exhibit a higher percentage cook loss are in general a less tender product than fillets that exhibit a lower percentage cook loss.

Differences exist between cook loss and shear values in both PSE- and DFD-like fillets. Broiler breast fillets considered to be PSE-like have a higher cook loss and higher shear force measurements as well as decreased marinade uptake (Allen et al., 1998; Owens et al., 2000a; Woelfel et al., 2002, Galobart and Moran, Jr., 2004). In comparison, DFD-like fillets exhibit opposite characteristics including a lower shear force and a lower cook loss possibly leading to improved tenderness of a DFD-like product.

Factors Affecting Ultimate pH and Color of Meat

When considering the ultimate pH and color of meat as it goes through rigor mortis, there are numerous ante mortem and post-mortem factors that can ultimately affect the outcome (Maga, 1994; Froning, 1995). Physiological pH of a broiler is around 7.0 (de Fremery and Pool, 1960) but can drop to pH values below 5.0 during the rigor mortis process resulting in PSE-like product and acidic meat. Typical pH of broiler breast meat is in the range of 5.8 to 5.9 (Qiao, et.al. 2001, Duclos et.al. 2007). During the rigor mortis process, many biochemical reactions occur to initiate the conversion of muscle to meat. Variations in broiler breast meat color are

typically a result of the amount of glycogen or glycolytic potential in the muscle prior to slaughter (Warriss et al., 1999).

At the time of death, which in the case of a broiler is when the bird is cut for exsanguination, blood flow to and from the muscle ceases to occur. With a lack of blood flow, no new energy substrates or oxygen can arrive at the muscle and there is no possibility for removal of waste products. At this time the muscle is still contracting and post-mortem metabolism or glycolysis is occurring for a brief time through aerobic respiration. A muscle needs ATP for contraction and relaxation to occur. To attain this ATP, the muscle uses its glycogen stores, the main carbohydrate present in muscle cells to generate ATP. Glycolysis can generate 2 ATP molecules as well as two pyruvate molecules which through the TCA cycle and oxidative phosphorylation in the electron transport chain of the mitochondria can yield 36 more ATP, resulting in a net yield of 38 ATP.

Once oxygen is depleted, which occurs fairly shortly after exsanguination, anaerobic muscle metabolism begins and is a much less efficient process than aerobic metabolism. With anaerobic metabolism, the pyruvate molecules cannot enter into the mitochondria where oxidative phosphorylation occurs. Instead, the pyruvate is broken down by fermentation to yield only 2 ATP molecules, carbon dioxide and most importantly lactic acid. This buildup of lactic acid that cannot be removed from the muscle due to lack of blood flow, is what is responsible for the decline in pH observed in muscles during the conversion of muscle to meat. Eventually, ATP is depleted in the contracting muscle and permanent cross bridges form between actin and myosin, resulting in rigor mortis or stiffening of a muscle. The ante mortem and post-mortem factors that will be discussed have a substantial impact on the glycogen content of muscles prior to slaughter but also how rapidly the break-down of glycogen into lactic acid can occur.

Glycolytic potential can affect the rate and extent of the biochemical reactions that occur during rigor mortis. It is the estimate of the amount of glycogen in a muscle and can be obtained by measuring the amount of glycogen, glucose and other major metabolites such as glucose-6-phosphate (Monin and Sellier, 1985). A broiler that has a high glycolytic potential will have a large amount of glycogen at the time of slaughter resulting in the possibility of a lower pH. It is hypothesized the PSE-like meat is the result of a high glycolytic potential. On the contrary, broilers with low glycolytic potential will have a minimal pH decline and a higher ultimate pH. DFD-like broilers most likely have a low glycolytic potential prior to slaughter.

Ante-mortem Factors

Fiber type plays a major role in how a muscle will respond to changing environments during the rigor mortis process. There are three different types of muscle fibers; red, white and intermediate (which is a combination of both red and white fibers), and all three function very differently in both aerobic and anaerobic environments. All muscles contain both red and white fibers, only differing in proportion. If a muscle is considered a white muscle, it will have a higher proportion of white to red fibers and vice versa.

Red fibers, also known as slow-twitch oxidative or type I fibers, have a slower contraction time and high concentration of myoglobin leading to its dark appearance (Klont, Brocks, and Eikelenboom, 1998). Myoglobin is a heme containing protein that carries and stores oxygen and is only found in muscle cells. When red fibers go through rigor mortis, they already have a low glycogen content which is the main substrate used in post-mortem metabolism. Because of this low glycogen content and low glycolytic potential ante mortem (Monin et al., 1987; Fernandez et al., 1994), the result is a minimal pH decline, a high ultimate pH and subsequently, a dark color. It has been shown in cattle that animals that exhibit a dark cutting or

DFD condition have a higher proportion of oxidative or red fibers (Zerouala and Strickland, 1991) and similarities are likely to exist in broilers.

White fibers, also known as fast twitch, glycolytic or type IIb fibers are larger fibers with a very fast contraction time and a low myoglobin content. These muscles appear much lighter than muscles containing a high proportion of red fibers in vivo. Muscles containing a high proportion of white fibers such as the pectoralis major and pectoralis minor have a high glycogen content antemortem. With a high glycogen content, when a bird is slaughtered, there is more substrate available for post-mortem metabolism resulting in an increase in the production of lactic acid. Since lactic acid is responsible for the pH decline, a much more drastic pH decline followed by a lower ultimate pH is observed in muscles containing white fibers. Studies in swine have shown that there is a negative correlation between percentage of Type IIb fibers and pH at 45 min post-mortem and this correlation is also associated with an increase in post-mortem metabolic rate (Ryu and Kim, 2005). Broilers already exhibit lighter muscles due to a higher proportion of anaerobic fibers (Aberle et.al., 1979).

It has not been determined if the age of a broiler effects the outcome of the ultimate pH and color. The amount of myoglobin, the main pigment found in skeletal muscles, typically increases with age (Nishida and Nishida, 1985). With an increase in the amount of myoglobin, one would expect a darkening (decreased L*) and reddening (increased a*) of a muscle as birds age. This was shown in a study by Froning and Hartung (1967) in turkeys where increased age resulted in darker, redder meat. Studies in broilers have been inconclusive as to whether or not age has an effect on color like those effects exhibited by turkeys. Several studies show that age does not have an effect on the ultimate color of broiler breast fillets (Smith et al., 2002; Dadgar et al., 2011). Moreover, there are studies that do show that the older a broiler gets, the lighter the

fillet becomes (Ngoka et al., 1982; Janisch et al., 2011). With all the factors that affect the outcome of the color and pH of a breast fillet, age may not have a significant enough impact in relation to all other factors combined. Sex differences have been reported for both pH and color. Females in general have a lower pH, higher L* and subsequently a higher drip loss (Dadgar et al., 2011; Schneider et al., 2012).

A majority of the broiler production in the United States occurs in the southern part of the country with the top three broiler producing states being Georgia, Arkansas and Alabama (USDA-Poultry Federation). These states being in the south are generally of a warmer climate during a majority of the calendar year leading to the possibility for heat stress of broilers both in the house and during transportation to the plant prior to processing. Heat stress is a major concern for producers not only for the increased mortality and stress levels of the broilers, but also for its impact on meat quality and carcass characteristics. It has been documented that high environmental temperatures prior to processing has increased the incidence of PSE-like breast fillets (Bianchi et al., 2007; Wang et al., 2009). Babji et al. (1982) reported that heat stress had an effect on the color of broiler meat resulting in an increased L* and decreased a*. High environmental temperatures prior to slaughter may result in an increase in PSE-like meat.

In turkeys, cold stress has been reported to increase lightness and decrease redness of the breast muscle (Froning et al., 1978) but other researchers have reported no effect on meat color as a result of exposure to cold stress (Babji et al., 1982). In broilers, cold stress has been shown to decrease fillet lightness and increase the incidence of DFD-like fillets (Dadgar et al., 2011). With a period of cold stress, maintenance of body temperature is going to use up the metabolic substrates needed for post-mortem muscle metabolism, resulting in a minimal pH decline and the possibility of an increase in DFD-like meat.

Genetics has been reported as a principal factor in the determination of breast fillet color and meat quality in broilers (Berri et al, 2001; Debut et al., 2003). Much of the variation is attributed to difference in genetic strains of broilers as a result of intense genetic selection from different companies. While genetic strain has an impact on the outcome of color and meat quality, it is possible that certain genes or a combination of genes may be responsible for the variation in meat color observed in both turkeys and broilers.

Several genes have been identified to be directly responsible for the incidence of PSE meat in pork. A gene named the Rendement Naple (RN) is directly related to the glycolytic potential of a porcine animal. While there is no genetic test available to assess the genotype, individuals with a high glyocolytic potential are either homozygous dominant (RN-RN-) or heterozygous (RN-rn+) although the two are indistinguishable and those with low glyocolytic potentials are considered to be homozygous normal (rn+rn+) (Miller, 1998). If an individual is classified as RN-RN- or RN-rn+, the likelihood of the animal developing PSE is much more likely due to the large amount of glycogen already present in the animal. Swine that are considered rn+rn+ are less likely to develop PSE meat.

With swine, a condition exists called malignant hyperthermia or porcine stress syndrome (PSS). This condition is one of the main causes for the PSE condition exhibited in porcine animals. A single autosomal recessive gene called the stress, hal or halothane gene is responsible for this condition (Hall et al., 1980). If a pig is considered to be halothane-positive, they are homozygous (nn) for a mutation that occurs in the ryanodine receptor (RyR1) gene (Fujii et al., 1991; MacLennan and Phillips, 1992). This gene is responsible for the calcium release channel of all skeletal muscles which controls the flow of calcium from its storage locations to the area surrounding the proteins responsible for contraction, actin and myosin. This calcium release

channel is critical during post-mortem metabolism. In halothane-positive swine, upon slaughter, this channel can become locked open resulting in rapid contraction and post-mortem metabolism causing a rapid pH decline while carcass temperatures are still high. If environmental stress does not kill the animal prior to slaughter there is a high chance (>80%) that the individual considered to be halothane positive will exhibit PSE meat as a result of the malfunctioning calcium channel (Lee and Choi, 1999).

A similar gene to the RyR gene in swine has been documented in turkeys but has not been identified as a major factor contributing to the PSE-like condition (Owens et al., 2009). The reason this may be is that in poultry, two forms of the RyR genes exist in skeletal muscles (Percival et al., 1994). Mutations have been reported to exist (Chiang and Strasburg, 2003) but not of the same mutation that has been known to cause PSS and PSE meat in swine. Not much is understood on the functioning of the RyR gene in poultry but variation in breast meat color may be a result of this gene as well as others that have not yet been identified. Due to the fact that the RyR gene does not play a major role in meat color in poultry, poultry meat can only be considered as PSE-like compared to the PSE syndrome in porcine. With little knowledge of the genetics behind this condition, little can be done for genetic screening of the condition like is implemented in swine although several researchers have studied tests without full development or implementation of the methods (Wheeler et al., 1999; Owens et al., 2000b, Cavitt et al., 2004).

DFD meat in cattle has been shown to be the result of a minimal genetic effect. Cattle that have parentage involving a Chianina cross produced the highest number of carcasses considered “unacceptably” dark or DFD. *Bos indicus* crosses are more likely to be scored as “normal” carcasses compared to all other parentage crosses (Shackelford et al., 1994). Although differences do exist in genetic strain of cattle for the incidence of DFD meat, most of the

variation and issues with carcass color are the result of environmental influences. In poultry, while a genetic effect does exist for the variation in meat color, no single gene has been identified to help in selection programs to help control both variation and incidence in extreme L* values. Management of environmental factors may be more critical in managing the incidence and severity of both PSE- and DFD-like broiler meat.

In broilers, two divergent selection programs at two separate locations have undergone selection procedures to product lines representing both a PSE- and DFD-like condition in the industry. The first selection program was implemented at the University of Arkansas in which lines were divergently selected for breast muscle color. The HMC line which was selected for a high L* at 24 h is characteristic of a PSE-like product with a low pH and a low WHC. On the contrary, the LMC line was selected for a high L* at 24 h and is representative of a DFD-like product (Harford et al., 2014). The second selection program was carried out at the National Institute for Agronomic Research (INRA) in France. The selection criteria chosen was ultimate pH of the breast muscle at 24 h post-mortem. The pHu- line, selected for a low ultimate pH exhibited a higher L* and a higher drip loss representing a PSE-like condition while the pHu+ line, selected for a high ultimate pH exhibited a lower L* and lower drip loss (Alnaahhas et al., 2014). Both selection programs, although selecting on different traits, produced lines that behave like and represent a PSE- and DFD-like condition indicating that there is a significant genetic effect with the incidence of the two myopathies.

What is included in the diet of an animal can have a significant impact on the composition and quality of any meat product produced. Several studies have been conducted across all meat species that exhibit how changes in the diet of an animal can impact meat color and meat quality (Froning et al., 1969; Wu et al., 1994; Geay et al., 2001; Rosenvold et al., 2001;

Smith et al., 2002; Lyon et al., 2004). Broilers that are fed a wheat based diet will have significantly lighter breast fillets than those fed a corn or milo based diet (Smith et al., 2002; Lyon et al., 2004). The addition of nitrates to a broiler or turkey diet increased the redness (a*) of broiler fillets (Froning et al., 1969). Rosenvold et al., (2001) showed that the muscle color of pigs was affected by the composition of the diet. Feeding a mold culture to turkeys increased the redness of the breast fillets (Wu et al., 1994). A review was conducted that reported the impact of feed types and additives on ruminant meat color with multiple additives having an impact on color and meat quality (Geay et al., 2001). When considering the impact of nutrition on meat quality, only a small percentage of the variation viewed is a result of nutrition and environmental factors may have a much larger impact than other factors.

Prior to being processed, a broiler must travel from the farm to the processing plant. In some cases, the duration of the travel to the plant may take upwards of 3 hours creating a stressful environment for the broiler prior to arriving at the plant. This stressful environment can have a negative impact on meat color and pH (Owens and Sams, 2000; Perez, et al. 2002; Leheska, et al., 2003). Pre-slaughter stress can be divided into two different groups; acute and chronic pre-slaughter stress. Acute pre-slaughter stress is something that is going to increase the rate of glycolysis at the time of slaughter. As a result, metabolism in the bird will increase and lactic acid will accumulate. Since the broiler is killed and processed shortly after such a stress, the circulatory system is no longer functioning and the lactic acid cannot be removed from the muscle resulting in a change in pH and a lighter muscle. Acute pre-slaughter stress is a main cause of the development of PSE-like meat (Siegel, 1995).

Chronic pre-slaughter stress is much different and slightly more difficult to define. With chronic pre-slaughter stress, the amount of glucose and glycogen in the body is depleted well in

advance of euthanasia. This can be a stress that occurs on a daily basis such as temperature stress. It can also be something that occurs 1-3 days prior to slaughter such as feed withdrawal or transportation. Since the glucose and glycogen in the muscle is being depleted prior to slaughter, when slaughter actually occurs, there is little substrate to be broken down into lactic acid. The result is a minimal pH decline and the product becomes more DFD-like.

Transportation, depending on the duration, may be considered either an acute or chronic pre-slaughter stress in broilers. With transportation comes many other stressors on the broiler. It includes the noise and vibrations of the truck, the movement and shifting of the broilers in the crates along with crowding as well as heat stress, and issues with airflow and humidity (Mitchell and Kettlewell, 1998). All of these factors involved in transportation are going to impact the level of metabolic substrates prior to slaughter which will alter the pH decline and ultimate color that a broiler fillet reaches.

In meat species including broilers, transportation times as well as holding times at the plant have been shown to alter the pH and color of breast fillets. In general, it has been shown that longer transport and holding times have resulted in breast fillets that are darker in color than those that had shorter transport and holding times (Bianchi, et al., 2006; Dadgar, et al., 2011). Since studies have shown that transportation and holding times have darkened fillets, it is more likely to be considered a chronic pre-slaughter stress. Due to the complexity of the stress, glucose and glycogen substrates are rapidly being depleted but, since it occurs well in advance of slaughter, the lactic acid produced can be processed normally through aerobic respiration. When slaughter does occur and anaerobic respiration takes over, the result is a minimal pH decline due to a lack of substrate, and a darker fillet. Transportation and holding times may result in an

increase in DFD-like product in a plant and efforts should be made to manage time off feed as related to transportation times.

In 2009, a study was conducted by Zhang, et al. that looked at the impact of short term and long term transportation times in addition to a resting period prior to slaughter on glycogen content and glycolytic potential in broiler breast fillets. Glucose concentration in the long-term transported group exhibited a dramatic decrease in glucose concentration and a decreased concentration of breast glycogen. This supports the idea that a chronic stress, such as transportation can limit the substrates available in the bird to be broken down into lactic acid and may increase the incidence of DFD-like fillets.

Prior to slaughter, broilers typically undergo a period of feed withdrawal for various reasons. Withdrawal times that are too short can increase the amount of feed still present in the broilers' system and ultimately result in fecal contamination and condemnation of a carcass (Smidt et al., 1964; Bilgili, 1988). On the contrary, feed withdrawal times that are too long can ultimately decrease the yield of a carcass (Veerkamp, 1978). Not only can feed withdrawal times affect the yield and quality of a processed carcass, it may also have a significant impact on meat quality characteristics.

The amount of time off feed prior to slaughter, depending on the length of time, would most likely be considered an acute stress. In the industry, the typical feed withdrawal time is 10 h but varies depending on company, age, sex and weight of the broilers. By 6 h off feed, the glycogen reserves in the liver are nearly depleted, by 12 h reserves are reduced in the biceps femoris but little to no effect of fasting is observed in the breast muscle (Warriss et al., 1987). If the glycogen reserves are depleted prior to slaughter, there will be less substrate to be broken down to produce lactic acid which would result in a decreased rate of pH decline and a higher

ultimate pH. It is possible that long periods of stress prior to slaughter such as feed withdrawal could negatively affect meat quality of broilers (Kannan et al., 1997, 1998).

Lyon and coworkers (1991) evaluated four different feed withdrawal programs including 0, 8 16 and 24 h off feed. A quadratic relationship was determined for muscle pH in which the 0 h and 24 h had the lowest pH readings. The 0 h feed withdrawal time could be considered an acute stress which upon catching and processing the birds caused a quick increase in the rate of metabolic activity. With the bird being processed shortly after being taken off feed, the increased metabolic activity may have resulted in a lower pH. It is unclear why the 24 h withdrawal group had the lowest pH but is possibly due to the elevated stress level of the broiler undergoing a long feed withdrawal period.

Several studies have shown feed withdrawal to impact color, pH and other meat quality traits. Smith et al., (2002) showed an increase in fillet lightness (increased L*) between a group of broilers that were feed deprived versus full fed. Broilers subjected to feed withdrawal also exhibited a decrease in redness which is characteristic of a broiler fillet exhibiting a PSE-like myopathy. This was shown again by Lyon et al., (2004) in which regardless of dietary treatment, the breast muscle color of the broilers from a feed withdrawal group of 8 h was significantly lighter than those in the 0 h withdrawal group. Contreras-Castillo et al. (2007) showed a decrease in L* with increasing feed withdrawal times. Other studies have shown little to no impact of feed withdrawal times on meat quality parameters such as drip loss, ultimate pH and color (Ngoka et al., 1982; Kotula and Wang, 1994; Delezie et al., 2007). It remains unclear whether an ante mortem stress such as a period of feed withdrawal can have a negative impact on meat quality indicators such as pH, color and WHC as well as the incidence of PSE-like and DFD-like myopathies.

Post-mortem Factors

Stunning was introduced into poultry processing as a way to help render broilers and turkeys insensitive to pain and induce unconsciousness prior to being cut for bleed out (National Chicken Council, 2016). Several different stunning methods are used in the industry with the most common being electrical stunning typically through a head-only water bath treatment (Bilgili, 1999; Goksoy et al., 1999). Alternative methods to electrical stunning include gas and low atmosphere pressure stunning. All three types of stunning methods can impact meat quality. With electrical stunning, various levels of voltage can be implemented including low voltage (~15 V at 750 Hz) and high voltage (~100 V at 50 Hz). The result of any level of stunning is a supercontraction of muscle which may aid in the quickening of post-mortem metabolism and pH decline. Voltage levels and duration vary between companies, processing plants and flocks based on the age and size of the birds. In a study by Huang et al. (2014), various levels of voltages and duration were evaluated. Color and final pH were not affected by any of the voltage treatments. The control or no stunning group and medium stunning group had a significant increase in drip loss and a significant decrease in muscle pH indicating that the level of voltage has a significant impact on some but not all meat quality characteristics. Head-only stunning which is typically implemented in the industry resulted in darker (decreased L*) and redder (increased a*) breasts when compared to whole body stunning (Hillebrand et al., 1996). Higher pH have been reported in broilers that have been stunned compared to broilers in an un-stunned group (Papinaho and Fletcher, 1996; Craig et al., 1999) or in an air pressure stunning group (Goksoy et al., 1999; Lambooij et al., 1999).

Gas stunning is a common alternative to electrical stunning and is commonly used in the swine industry as a means of rendering an animal unconscious. With gas stunning, animal stress

can be reduced because the birds do not have to be shackled prior to death. Common gases used include carbon dioxide the most common as well as Argon. Mohan Raj et al. (1990) indicated that gas stunning through the use of Argon resulted in an increased amount of wing flapping and a decreased pH at 20 min post-mortem when compared to carbon dioxide stunning. It is apparent that gas stunning with Argon has the ability to increase the rate of early post-mortem metabolism in broilers. Gas stunning has also been shown to affect color and water holding capacity (Savenije et al., 2002).

The use of low atmosphere pressure stunning (LAPS) is a fairly modern concept to the poultry industry. With LAPS, broilers are placed in compression chambers and through the reduction of the atmospheric partial pressure of oxygen, the broilers lose the metabolic use of their brain and are "stunned" (Cheek and Cattarazzi, 2010). Changes in early post-mortem pH have been detected in LAPS (Battula et al., 2008). The changes in the partial pressure of oxygen most likely results in changes in post-mortem metabolism resulting in the differences in initial pH although severe meat quality differences have not been detected (Schilling et al., 2012).

PSE-like meat has been known to be caused by rapid pH decline while carcass temperatures are still high (Offer, 1991). These effects can be limited if proper chilling techniques are implemented at the plant. In turkeys, chilling at higher temperatures (40^0C) compared to rapid chilling (0^0C) can increase the rate of glycogen depletion, decrease pH, increase L* and increase drip and cook loss (McKee and Sams, 1998). Other studies in turkeys also found that chilling at a higher temperature resulted in an increased L* and had negative effects on meat quality measurements such as drip loss and gel strength (Alvarado and Sams, 2002, 2004). Faster chilling of a carcass can help alleviate issues from protein denaturation that occurs at high carcass temperatures in PSE-like meat.

After broilers are slaughtered and chilled, breasts are typically removed from the carcass unless it is being processed as a whole bird. This can occur anywhere from 2 h to 24 h post-mortem and can have a large impact on the quality of meat produced. If a broiler is deboned very early on in the rigor mortis process it has the ability to contract and become rigid due to remaining energy reserves (Castillo and Custodio, 2002). In general, the most tender meat is produced when a carcass is not deboned until after 6 h post-mortem, after which time rigor mortis has completed. Consumers prefer later deboned breast fillets to early deboned fillets (Schilling et al., 2012) although processors debone early as a cost saving technique. Much like with several of the ante mortem factors, it is unclear whether deboning time has an impact on pH and color. Several studies show that muscle pH is higher in carcasses that were deboned immediately after chilling (Lyon et al., 1985; Souza et al., 2005). Others studies indicate that deboning time has no effect on post-mortem pH (Sams et al., 1990)

After a broiler is slaughtered, there is typically a length of storage prior to use including time in the processing plant, in the truck, at the grocery store and prior to consumption by the consumer. The length of storage time varies for each product but both length of time and storage temperature which can vary between companies can impact the color and variation in color in poultry meat. Most color changes in broiler and turkey breasts have been reported between 2 and 48 h post-mortem with a lightening of the meat occurring as the fillet cools and ages (Le Bihan-Duval et al., 1999; Alvarado and Sams, 2000; Mallia et al., 2000; Owens et al., 2000a,b; Owens and Sams, 2000; Qiao et al., 2001). Most color changes occur very rapidly within a breast muscle during the first 4 h after slaughter. This is due to the fact that rapid anaerobic metabolism and rigor mortis are occurring resulting in a more acidic environment and a change in color. Small changes in color will occur up to 48 h post-mortem. Long term storage (more than 48 h) of

poultry meat has little to no impact on the color of fresh meat but further changes in color may be a result of processing or holding conditions (Petracci and Fletcher, 2002). Variation in color may be a result of long term storage and can affect consumer acceptability of a product.

CONCLUSION

No single factor is responsible for the outcome of the ultimate pH and color of a broiler fillet post-mortem. A combination of ante mortem stressors, genetic predisposition, physiological factors and post-mortem handling techniques all are capable of producing changes in both color and pH. If the incidence and severity of both PSE- and DFD-like muscle increase, it may be hard to determine what factor or set of factors is responsible for the increase in myopathies.

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Chapter 1: Post-mortem pH decline in broiler carcasses subjected to either air or ice chill environments

ABSTRACT

Previous research has shown that chill method and cooling rates may have an effect on the rate and extent of pH decline in meat. The current study characterizes the pH decline for broilers exposed to different post chill environments. The lines studied consisted of a random bred control broiler population (RBC) as well as populations that have been selected for either high (HMC) or low (LMC) L* at 24 h. The HMC and LMC lines have been selected to represent PSE/DFD-like conditions in poultry meat.

To accomplish this, three replications of twenty-four male broilers, eight from each line were reared on litter floor pens to 8 wk at which time they were processed. Carcasses were equally and randomly assigned by line to either open air or ice-bath chilling. Temperature and pH measurements were collected on the breast and thigh muscles immediately after exsanguination, 15 minutes,1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h post-mortem. Breast meat color was measured at 4, 12 and 24 h. Cook loss and tenderness was measured using the Meullenet-Owens Razor Shear blade method on the breast muscles 2 wks after collection. Within the HMC and LMC lines, no treatment differences were observed for pH decline or 24 h breast meat color. When averaged across treatments, the rate of pH decline was greatest with the HMC line and lowest with the LMC line with the RBC line being an intermediate. For all lines, pH decline plateaued between 6 and 8 h post-mortem indicating the completion of rigor mortis. Differences were observed between lines for 24 h L* indicating that the divergent lines behaved as expected. The HMC line had the highest cook loss and highest MORSF and MORSE readings, indicating a product with decreased tenderness. Characterization of pH decline and tenderness in broiler lines known to exhibit PSE- and DFD-like properties has demonstrated similarities between red meat and poultry species. Understanding of the interaction of pH and

temperature decline for lines known to vary in meat characteristics will allow for implementation of ante and post-mortem management techniques designed to improve meat quality.

INTRODUCTION

Meat quality is an ongoing concern to the broiler industry. Current commercial selection practices focus on an increase in yield, particularly of white meat, decreased feed conversion and rapid growth. Unfortunately, selection for such traits has has impacted negatively correlated traits such as fertility (Siegel and Dunnington, 1985, Qureshi and Havenstein, 1994), disease (Julian, 1998, 2000, Cook, 2000) and most importantly, meat quality defects (Anthony, 1998, Barbut, 1996, 1997, 1998). Several of the meat quality defects that are detected in the broiler industry are a direct result of the pH decline of a muscle and the ultimate pH that muscle achieves as it goes through rigor mortis. Regardless of the success of the breeding program built around performance traits, if the consumer does not like the appearance, taste and texture of a product, they will not purchase it.

Post-mortem pH decline has been heavily documented in red meat species such as beef and swine for both normal carcasses as well as those that exhibit meat color and quality abnormalities. In cattle, a condition known as dark, firm and dry (DFD) or a dark cutting condition can occur. This condition is a result of a lack of pH decline as muscle goes through rigor and a high ultimate pH (Park et.al., 2007). The resulting product has a greater ability to hold water, resulting in a tenderer product (Silva et.al., 1999). Unfortunately, DFD meat can lead to issues with atypical flavor, reduced shelf life and decreased consumer acceptability of the product produced (Allen et.al., 1997, 1998, Fletcher, 1999, 2002,).

Another condition that has been of major concern particularly in swine and turkeys regarding post-mortem pH decline is pale, soft and exudative or PSE meat. Meat exhibiting the PSE condition occurs when there is a rapid pH decline early on the rigor process while carcass temperatures are still high. This pH decline at high carcass temperatures, results in protein

denaturation, which leads to an inability of the muscle to retain water, a lower water holding capacity (WHC) and a tougher product (Barbut, 1997, Woelfel et al. 2002). Time course evaluations of pH decline have been documented for these conditions in red meat species as certain muscles go through rigor mortis (Jeacock, 1977) but little has been done to characterize the pH decline in broiler carcasses that may exhibit similar meat quality myopathies.

Both Harford, et.al. (2014) and Alnahaas, et.al. (2014), implemented divergent selection programs in broilers for ultimate color (L*) and ultimate pH respectively. Both selection programs have exhibited differences in ultimate pH, ultimate L*, cook loss (CL), and shear values. The results found in studies with the HMC/LMC and the pHu-/pHu+ lines behave in similar fashions as to what is to be expected from broiler meat that would be considered PSE- and DFD-like.

Chilling method has also been shown to impact the rate and extent of pH decline in poultry. Slow chilling of a carcass can elongate the rigor mortis process and the extent of the pH decline and has been shown to induce PSE-like symptoms in turkeys (Alvarado and Sams, 2002, 2004, McKee and Sams, 1998, Molette et. al. 2006). Increasing the chilling rate of a broiler carcass may help limit the detrimental effects that are a direct result of protein denaturation that can occur when carcass temperatures are still high. This increased chill rate can result in a breast fillet with a higher WHC as well as an improved color and texture.

Ultimate pH and the rate of pH decline have a significant impact on color, water holding capacity (WHC), tenderness, and many other processing characteristics. Post-mortem pH decline has been known to differ in red meat species that exhibit meat quality abnormalities such as pale soft and exudative (PSE) in swine and dark-cutters (DFD-dark, firm and dry) in cattle. Minimal research has been done to characterize the pH decline of both PSE- and DFD-like product in

poultry meat that may exhibit similar abnormalities to those found in swine and cattle. The rate of decline and ultimate pH has also been known to be affected by chilling regimes, whether it be fast chilling or slow chilling. After 10 generations of divergent selection for breast muscle color in broilers, 56 d males from the selected lines as well as a random bred control population were characterized for pH decline over a 24 h period in both air and ice chill environments.

MATERIALS AND METHODS

Broiler populations

Three lines were evaluated in this study for post-mortem pH decline under different chilling environments. The lines used included a random bred control (RBC) line as well as high (HMC) and low meat color (LMC) lines which were selected from the RBC for high and low 24 h L*, respectively (Harford, et. al. 2014). The study consisted of three replications of 24 male broilers, eight from each line. The birds were reared under normal industry conditions on litter floor pens to 8 wks of age. Water and a corn-soybean based diet that was formulated to meet or exceed the National Research Council (NRC) (1994) requirements were provided ad libitum. Access to water remained constant throughout the study with feed being removed 12 h prior to processing. The male broilers were preassigned equally and randomly by line to either an ice bath or air chill treatment

Slaughter, Early Carcass Measurements

On processing day, birds were cooped and transported to the processing plant. All males were processed at the same time in one batch. On the dock, birds were weighed and hung on a shackle-line. They were processed using inline equipment. Once placed on the line, birds were electrically stunned (11V, 11 mA, 10), manually cut through the carotid left artery and jugular

vein and allowed to bleed out for 2 min. Following bleed-out, carcasses were removed from the line, and a small incision made in the skin covering the left pectoralis major and left thigh muscle. Temperature and pH were recorded using a Testo 205 pH meter in both locations. Following initial measurements, carcasses were then returned to the line, scalded (55^0C) and then picked with an in-line commercial de-feather machine.

Chilling Treatment

Birds were hand eviscerated and pH and temperature were recorded for both the breast and thigh at 15 min post-mortem. Following these measurements, carcasses were placed into their preassigned chilling treatments. Carcasses assigned to the air chill were placed breast up on racks in a 3^0C cooler with continuous air movement from fans. Carcasses assigned to the ice bath chill were submerged completely in ice water and placed two per tub. Carcasses were not allowed to touch resulting in even cooling on all sides. Once in the chill treatments, pH and temperature were recorded for both the breast and thigh at 1, 2, 3 and 4 h post-mortem. Carcasses in the ice bath chill were towel dried prior to measurements being taken, then immediately placed back into the ice water bath.

Debone and Meat Quality Measurements

After the 4 h measurements, birds were removed from their chilling treatments and manually deboned. Breast fillets and thighs were labeled and bagged separately in zip lock bags and placed on trays in a 3^0C cooler for the remainder of the 24 h. Temperature and pH were recorded of the breast and thigh at 6, 8, 10, 12, 16, 20 and 24 h post-mortem. Color was recorded of the breast fillet at 4, 12 and 24 h post-mortem and of the thigh muscle at 24 h post-mortem using a Minolta CR-400 colorimeter. All pH and temperature measurements were done in a 3^0C cooler to ensure that fillets did not heat up prior to or in-between measurement times.

Statistical Analysis

A two-way ANOVA analysis was carried out using the generalized linear model procedure of SAS software (SAS Institute, 2010). Separation of means was done using Least Square means with the Tukey adjustment (HSD). The main effects analyzed in the model included line, treatment and their interaction.

RESULTS AND DISCUSSION

The results of this study characterize the pH decline of the breast and thigh muscle for HMC, LMC, and RBC lines under two different chill methods. As previously stated, the HMC line has been known to exhibit PSE-like characteristics and the LMC line exhibits DFD-like characteristics after 10 generations of selection. In red meat species such as cattle and swine, similar conditions exist in which the pH decline has been characterized. Cattle considered to be DFD or dark cutters have a significantly higher ultimate pH when compared to a normal carcass and a very minimal pH decline. Swine carcasses considered to be PSE had a very rapid pH decline early post-mortem and a low ultimate pH when compared to normal carcasses. While poultry is a white meat species, similarities are likely to exist between red meat species and poultry. With three processing dates across the span of a month and a half, hatch differences were infrequent and inconsistent and therefore hatch was excluded from the analysis.

Previous studies have shown that chilling rate can affect the rate of pH decline and ultimate pH in all types of meat (Offer, 1991). Chilling times to a final holding temperature of 3^0C differed between the treatment groups by 4 h (Figure 1). In the current study, no chilling treatment effects were observed for any of the pH measurements collected. It is possible that the low number of carcasses evaluated may not have been large enough sample size to show a

chilling treatment effect for pH decline and ultimate pH although the results from this study so no general trend for the pH decline of the chilling treatments (Figure 2). A large scale study evaluating the selected populations under different chilling methods may be beneficial to fully understand the effects that chilling method may have on color and quality of a product known to exhibit a meat quality abnormality.

With no chilling treatment effects observed, treatments within a line were combined to evaluate the effects of line on post-mortem pH decline. No differences were observed at 0 h post-mortem which was the measurement taken immediately after exsanguination. It is at this time is when metabolism switches from aerobic to anaerobic. It was expected that muscle pH be near physiological pH (~7.0) and line differences were not expected (Qiao, et.al., 2001, Duclos et.al., 2007). Differences begin to appear between the lines at 15 min post-mortem, the measurement taken immediately after scalding and evisceration. Previous work with the HMC and LMC lines have shown differences at this time measurement (Harford-Dissertation, 2014). At 15 min post-mortem, the HMC line had the lowest pH, the RBC behaved as an intermediate and the LMC line had the highest pH. A slight increase in pH was observed at 1 h post-mortem. This may be due to the fact that muscle has a high buffering capacity and attempts to maintain a normal physiological pH even after an animal is slaughtered. Following the 15 min measurement, the HMC line remained the lowest for pH with no difference observed between the LMC and RBC lines (Figure 3).

Although the LMC and RBC lines do not differ at any time post-mortem, the general trend of the decline of the lines indicate that the LMC line had a slower decline and a numerically higher ultimate pH than the RBC line at all time measurements (Figure 4). With a low number of birds per line evaluated in this study, increasing the sample size may have

resulted in statistical differences between the LMC and RBC lines instead of exhibiting only a visual trend.

Following debone at 4 h post-mortem, very little pH decline was detected in any of the lines after 6 h. The general trend again shows that the HMC line had the most rapid pH decline, with the lowest ultimate pH, the RBC line remained an intermediate while the LMC line had the slowest decline and highest ultimate pH. Since no further decline is seen after 6 h, it is likely that rigor mortis, or the conversion of muscle to meat is likely complete.

The time frame for the completion of rigor mortis is much more rapid than in red meat species which can take upwards of 24 h. With a decline in pH occurring so rapidly in a broiler, chilling temperature may play a crucial role in the rate and extent of pH decline. More rapid chilling of a broiler carcass may help reduce the severity of protein denaturation in carcasses exhibiting PSE-like characteristics by reducing carcass temperatures during a time when pH is rapidly dropping (first 4 h after slaughter). While rapid chilling has the possibility of helping slow post-mortem pH decline, extremely rapid chilling can cause issues with cold-shortening of muscle and may lead to a tenderness issue throughout the carcass.

Chilling treatment did not have an effect on the color measurements (L*) taken at 4, 12 and 24 h for the breast muscle (Figure 5). As to be expected based on the selection technique used, the HMC line had the highest L* at all times. At 24 h post-mortem, the RBC and LMC did not differ for breast muscle L*. Since pH and L* have a high negative correlation, it is possible that the lack of differences seen in L* for the RBC and LMC lines explains the lack of differences in pH decline. Chilling treatment had no effect on cook loss and shear values within a line. The HMC line had the highest cook loss, MORSF and MORSE while no difference was detected between the LMC and RBC lines (Table 1). The HMC line, which has been selected to

represent a PSE-like abnormality in broilers, has exhibited decreased tenderness and an inability to hold water similar to what is found in red meat species exhibiting a similar condition. Numerically, the LMC line had the lowest MORSF, MORSE, and cook loss and with an increase in sample size, differences are likely to be detected.

Similarities between red meat species and poultry in terms of post-mortem pH decline do exist for carcasses exhibiting meat quality abnormalities. In both swine and broilers, both PSE and PSE-like carcasses exhibit a rapid pH decline very early on in the process of rigor mortis followed by a low ultimate pH. With DFD in cattle and DFD-like conditions in broilers, a very minimal pH decline is seen followed by a higher ultimate pH when compared to a "normal" carcass or a control population. Differences between red meat species do exist in the time that it takes for rigor mortis to be completed. Completion times in cattle and swine are much longer than in poultry, specifically broilers. This is due to both the smaller carcass size in poultry but also may be due to the higher amount of anaerobic fibers in poultry. A higher amount of anaerobic or white fibers will result in a much more rapid pH decline. White fibers contain a large amount of glycogen prior to slaughter which is broken down to form lactic acid in post-mortem anaerobic metabolism (Berri et. al. 2005). The production of lactic acid is what is responsible for the pH decline so muscles containing large amounts of white fibers will exhibit a rapid, more drastic pH decline.

Also analyzed in this study was the characterization of pH and color of the thigh muscles of the broiler. The pectoralis major muscle is predominately white fibers but the thigh muscles, such as the satorius contain a higher proportion of red or aerobic fibers. Red fibers typically have a lower concentration of glycogen prior to slaughter, resulting in a smaller amount of lactic acid produced and a minimal pH decline. No line or treatment differences were detected for the thigh

muscle in the study. Averaged across all lines, the ultimate pH of the thigh muscle at 24 h was 6.58±0.06 which when compared to the breast muscle was pointedly higher. A line difference was detected for L* at 24 h. The HMC and RBC lines were the lightest and the LMC line the darkest (Figure 5). This was the first time that thigh pH had been evaluated within the research populations. With the selection trait implemented being breast muscle color, it appears that selection practices have begun to effect other muscles of the broiler.

Selection for breast muscle color in the HMC and LMC populations have led to differences in not only L*, but also in pH decline, ultimate pH, tenderness and WHC. These lines represent two economically important meat quality abnormalities: PSE- and DFD-like. Similarities between red meat species and broilers in terms of pH decline, ultimate pH and tenderness have been exhibited in the present study.

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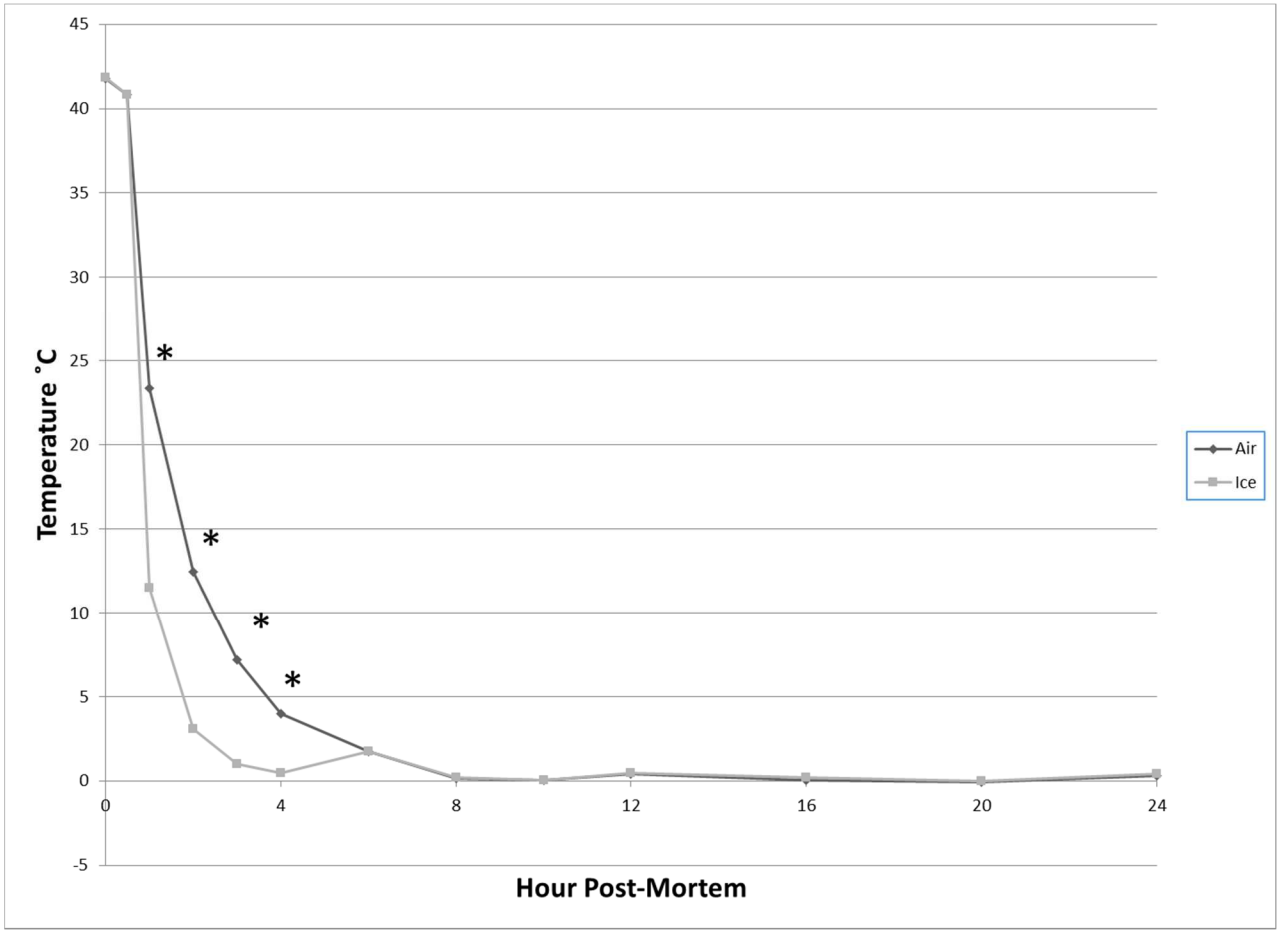
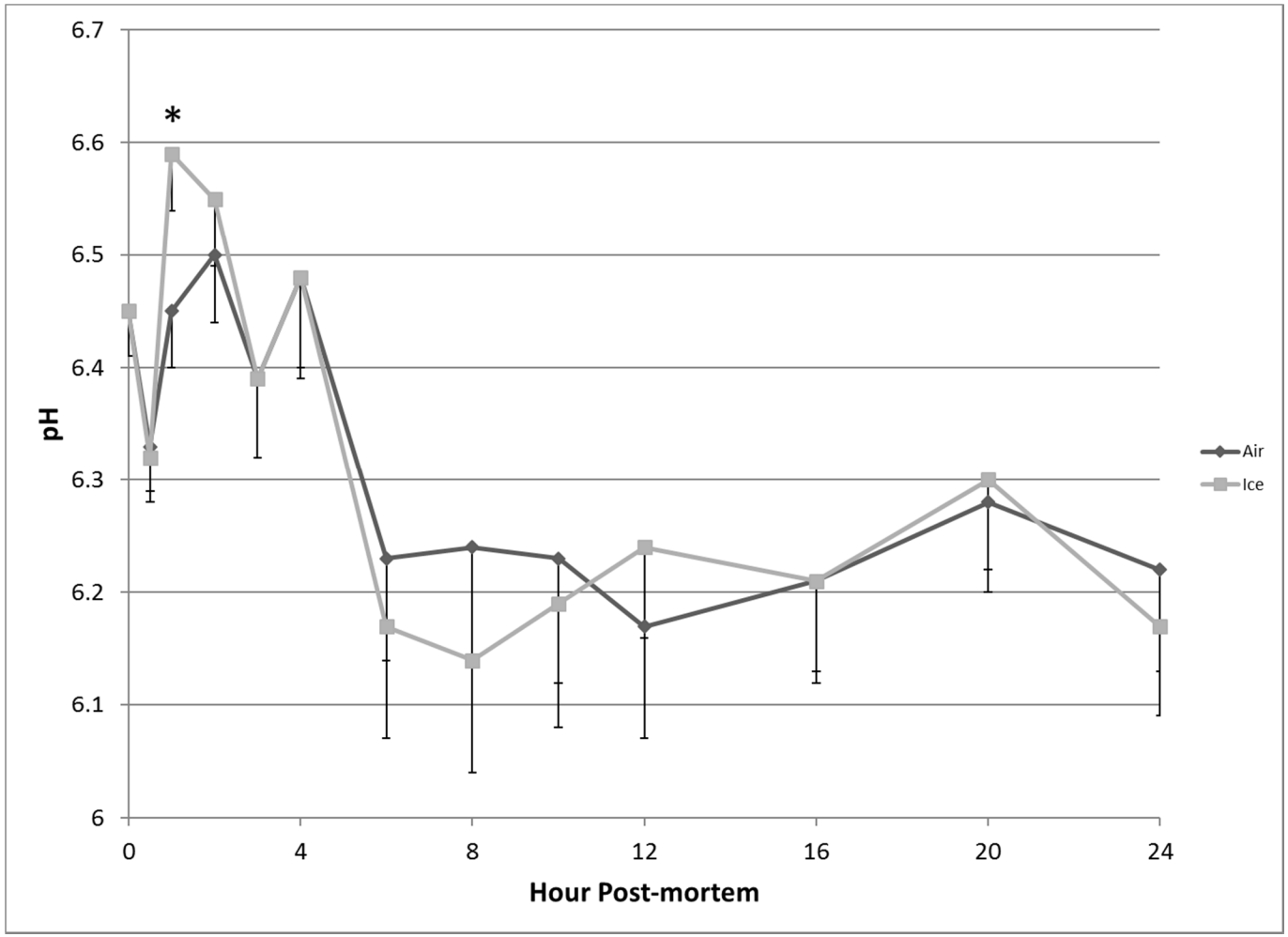
Figure 1. Temperature decline for all lines under both air and ice chill environments.



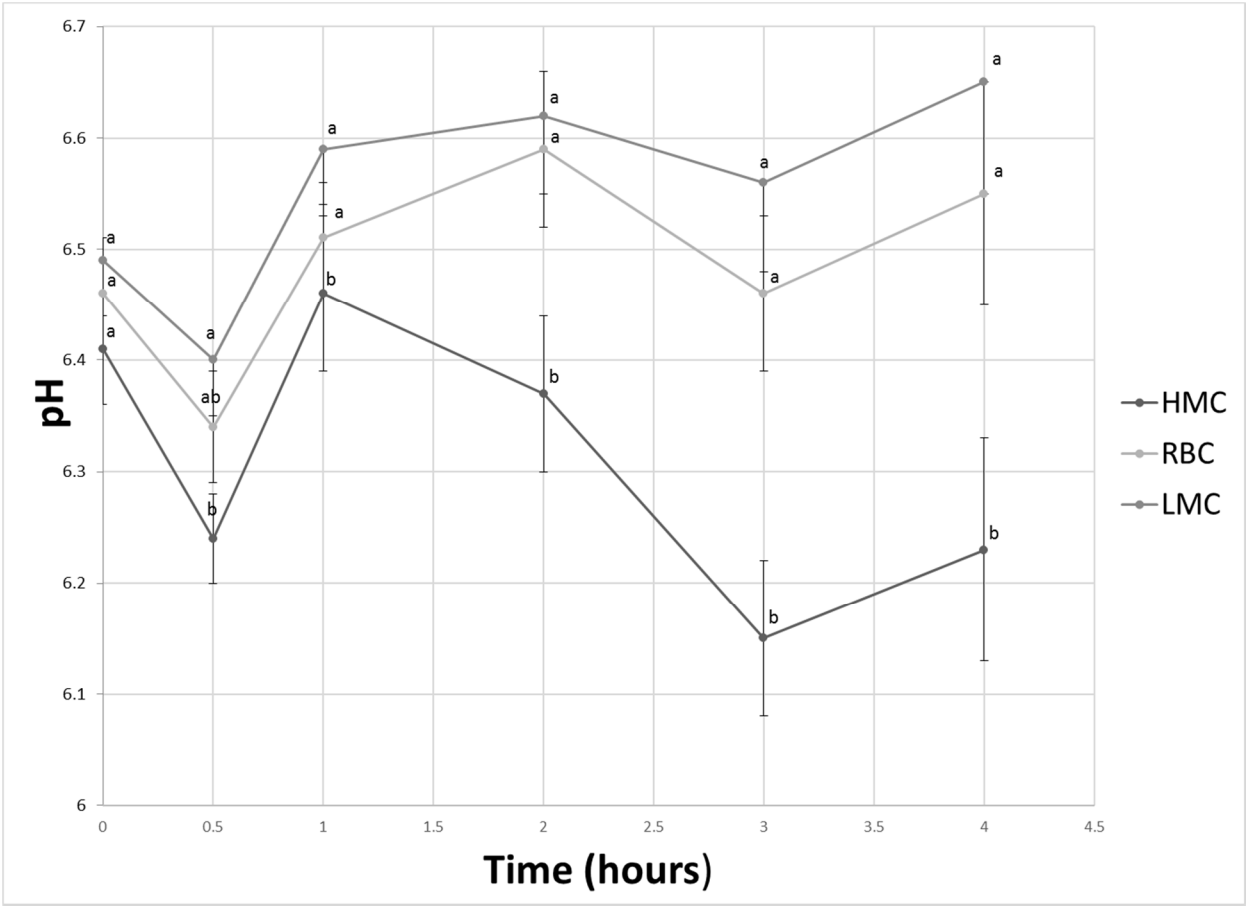
* Indicates difference in temperature between air and ice chill environments for breast muscle at ($P \leq 0.05$)

Figure 2. Post-mortem pH decline for air and ice chill environments.



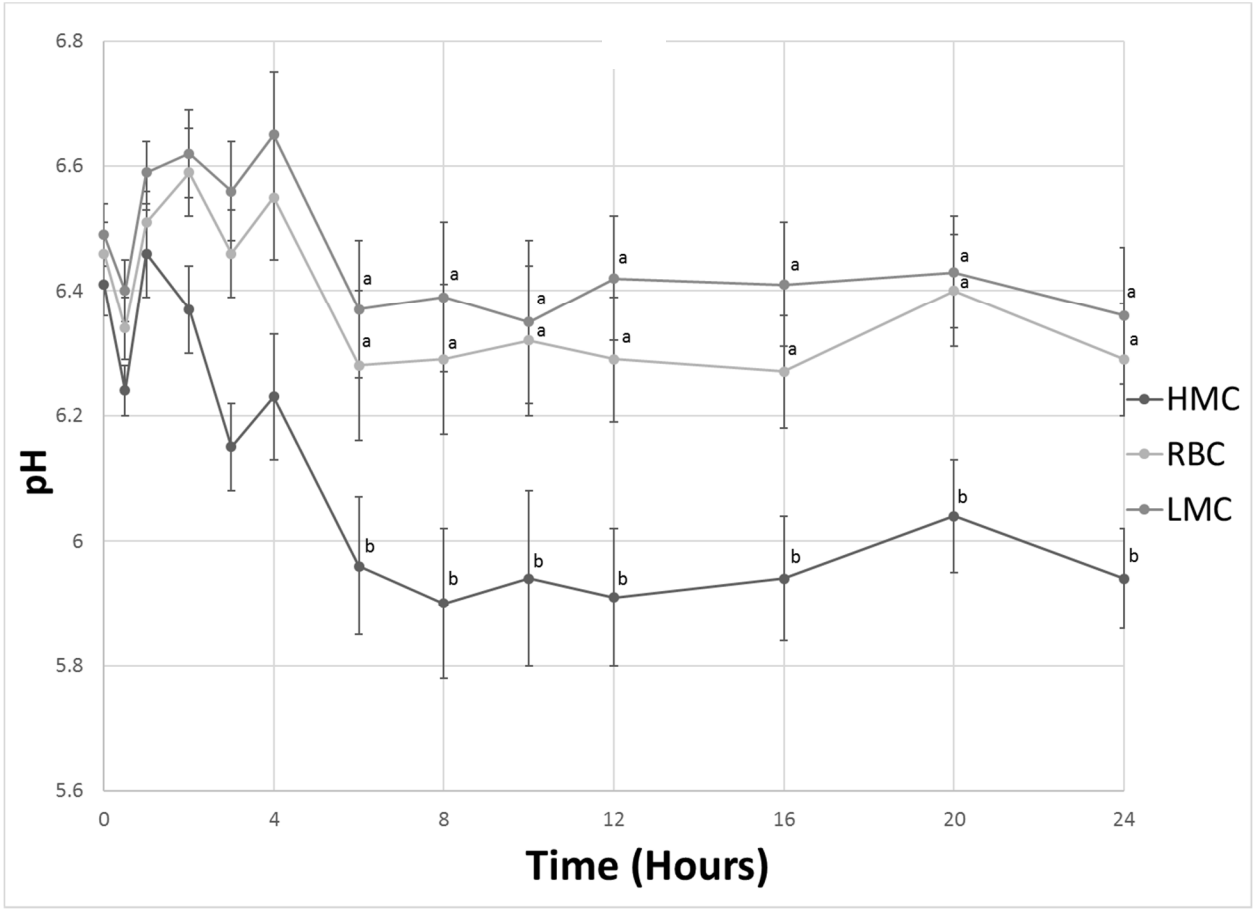
***** Indicates difference in temperature between air and ice chill environments for breast muscle at ($P \leq 0.05$)

Figure 3. Early post-mortem pH decline for muscle color lines[1].



[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control
[2] a-b At each time point, means with no common letters are different between lines at (P≤0.05)

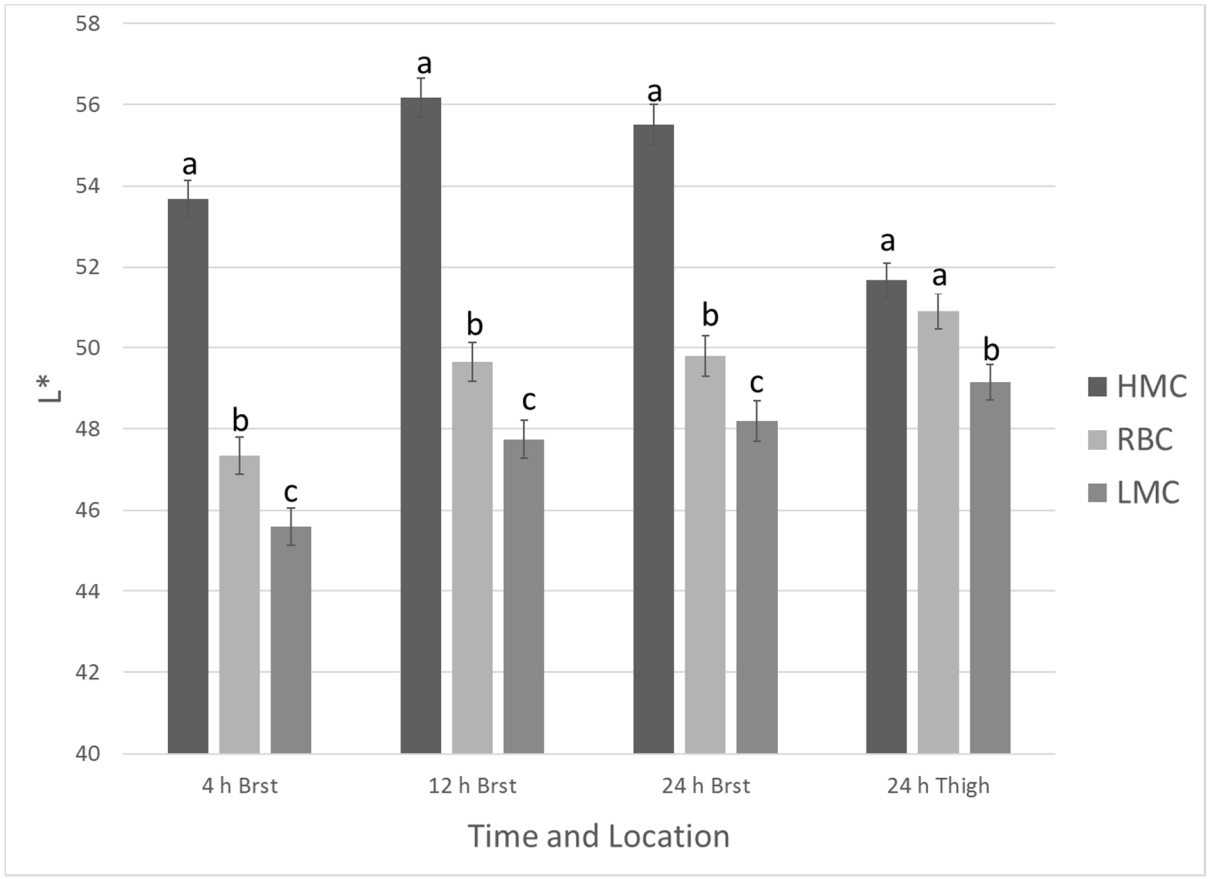
Figure 4. Post-mortem pH decline for muscle color lines[1] over 24 h.



[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control

[2] a-b At each time point, means with no common letters are different between lines at (P≤0.05)

Figure 5. Color (L*)[2] measurements for breast and thigh muscles at different times post-mortem for muscle color lines[1]



[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control
[2]L*=lightness
[3]a-c Within each time and location, lines with no common letter are different at (P≤0.05

Table 1. Tenderness measurements for the muscle color lines[1] after 10 generations of selection for L*

Tenderness[2]	HMC	RBC	LMC
Cook Loss (%)	29.87±0.84a	26.20±0.91b	25.17±0.88b
MORSF	13.50±0.20a	12.00±0.24b	11.27±0.21b
MORSE	171.64±2.80a	156.6±3.32b	149.87±2.86b

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control
[2]MORSF=Meullenet-Owens Razor Shear Force, MORSE=Meullenet-Owens Razor Shear Energy, Cook Loss= ((Cooked Brst Wt-Raw Brst Wt)/Raw Brst Wt)*100
[3]a-b Means with no common letters are different within a treatment at ($P \leq 0.05$).

Chapter 2: Effect of age and feed withdrawal times on broiler lines divergently selected for breast muscle color

ABSTRACT

Any factor that influences the appearance and consumer acceptance of a poultry product is of concern to the industry. One such condition for broilers is called pale soft and exudative (PSE) meat. PSE-like meat is characterized by having a high L*, low pH, poor cohesiveness of product and a high drip loss. Pale fillets are of concern for producers as it lessens the consumer acceptability of a fresh product but also creates issue with emulsification capacity and ability to form gels in further processed products. Previous research has been inconclusive on whether or not processing age or feed withdrawal times have an impact on breast muscle color. Two separate experiments were conducted out to characterize broiler research lines that have been selected for either PSE-like (HMC) or DFD-like (dark, firm and dry) characteristics (LMC) along with their random bred control (RBC) at four different processing ages (Experiment 1) and under four different feed withdrawal programs (Experiment 2).

For experiment 1, four separate hatches of straight run broilers from the three lines were raised under normal industry conditions and processed on the same day. The processing ages included 5, 6, 8 and 10 wk of age. Broilers were processed randomly across four batches. Color and pH were recorded for the pectoralis major muscle at both 4 and 24 h post-mortem. Line differences were observed for L* at 24 hr post-mortem. The HMC line exhibited the highest L*, the LMC the lowest average L* with the RBC line intermediate. Within the HMC and RBC lines, no color differences were seen between any of the ages investigated. For the LMC line, the 5 wk old broilers exhibited a significantly higher L* than the remaining ages. This is most likely a result of the smaller and thinner breasts collected from the LMC line at 5 wks of age. In lines that had been selected for 24 hr L*, bird age did not appear to have an impact on the color within each line.

Experiment 2 consisted of two replications of 20 broilers per line and feed withdrawal treatment group. The day before processing, birds from the three lines were equally and randomly sorted into five treatment pens. Feed was removed from treatment groups at 24, 18, 12, 6 and 0 h prior to processing with constant access to water. All birds were randomized by line and treatment and processed at the same time. Carcasses were evaluated for 15 min and 24 h pH and 24 h drip loss. The 0 h withdrawal group had the lowest 24 h pH but that difference did not correlate with color or drip loss as no differences were detected between the feed withdrawal treatments. In the muscle color lines, feed withdrawal times do not appear to have a substantial impact on the meat quality.

INTRODUCTION

It has been reported that the age at which a broiler is processed can have a huge impact on the quality of product produced (Petracci et al., 2014). As a broiler ages, muscle fiber size increases. This leads to tenderness and meat color issues that impact the ability of that muscle to retain water. Many meat quality abnormalities tend to increase in incidence and severity as the age of the bird increases. This has been shown with an increase in both woody breast and white striping at later processing ages (Petracci et al., 2014; Tijare et al., 2016). Muscle color may also be affected with increasing ages although the literature is inconclusive.

Two meat quality related abnormalities that are of recent concern to the poultry industry are PSE-like and DFD-like meat. Meat exhibition the PSE-like condition, defined as pale, soft, and exudative, is the result of a rapid pH decline early in the rigor mortis process while carcass temperatures are still high resulting in protein denaturation, low water holding capacity (WHC) and pale fillets (Barbut, 1997; Woelfel et al., 2002; Petracci et al., 2009). The second condition, defined as dark, firm and dry (DFD-like), is the result of a minimal pH decline and high ultimate pH with characteristics including dark fillets, with a high WHC but a reduced shelf life and atypical after flavor (Allen et al., 1997; Fletcher, 2002; Miller, 2007). There are many ante-mortem, post-mortem and physiological factors that can affect meat quality and lead to the development of both PSE- and DFD-like breast fillets. Factors documented to affect the incidence of these muscle myopathies include genetics (Berri et al, 2001; Debut et al., 2003; Alnahhas et al., 2014; Harford et al., 2014), nutrition (Smith et al., 2002; Lyon et al., 2004), pre-slaughter stress (Froning et al., 1978; Owens and Sams, 2000; Bianchi et al., 2007; Wang et al., 2009) and post-mortem handling (Hillebrand et al., 1996; Alvarado and Sams, 2002, 2004).

It is unclear as to whether the age of a broiler has an effect on the ultimate pH and color of meat and the incidence of PSE- and DFD-like myopathies. As a broiler ages, the amount of myoglobin in the skeletal muscles increases (Nishida and Nishida, 1985) and one would expect a decreased L* and reddening (increased a*) of the muscle. However, various experiments have shown a darkening of muscle as age increases (Froning and Hartung, 1967), a lightening of muscle as age increases (Ngoka et al 1982; Janisch et al., 2011) or no effect at all (Smith et al., 2002; Dadgar et al., 2011).

Similar to age, impact to feed withdrawal time prior to slaughter has also been inconsistent in the literature. Glycogen reserves, the main substrate responsible for post-mortem pH decline, would be expected to be depleted as fasting times increase. Therefore, the longer a broiler is fasted or remains off feed, a lower amount of glycogen reserves will be available when slaughter transpires which should result in a minimal pH decline and an increase in the incidence of DFD-like myopathies. Experiments have shown feed withdrawal programs to increase fillet lightness (Smith et al., 2002; Lyon et al., 2004) while others have shown a decrease in L* with increasing feed withdrawal times (Contreras-Castillo et al., 2007). Other experiments have shown no impact of feed withdrawal times on meat quality parameters related to meat myopathies such as drip loss, ultimate pH and color (Ngoka et al., 1982; Kotula and Wang, 1994; Delezie et al., 2007). These inconsistencies however, may simply be the result of bird source, rearing environment, or slaughter conditions. To address this, research lines were developed to assess consistency in product studied.

To determine if age and feed withdrawal times have an impact on meat quality indicators, and the incidence and severity of both PSE- and DFD-like myopathies, two experiments were conducted on lines divergently selected to represent PSE- and DFD-like myopathies. Meat

quality parameters including pH, color, drip loss and moisture uptake were evaluated in both experiments to determine if slaughter age and pre-slaughter feed withdrawal times have an impact on selected populations.

MATERIALS AND METHODS

Experiment 1

Three broiler lines were used in this experiment to evaluate the effect of age on breast muscle color. First is the random bred control line (RBC) which was established in 1995 from commercial stocks available at the time (Harford et al., 2014). The RBC line served as a base population for high meat color (HMC) line which has been selected for a PSE-like condition, the low meat color (LMC) line which has been selected for a DFD-like condition. Both the HMC and LMC lines have undergone selection for breast meat color (L*) at 24 h post-mortem and were evaluated after 10 generations of selection.

The experiment consisted of one processing date in which 30 straight run birds per line were evaluated at 5, 6, 8 and 10 wks resulting in a total of 360 birds evaluated across all line and age combinations. This was accomplished by arranging the hatch dates so that all age groups were processed on the same day. All birds were reared under normal industry conditions on litter floor pens to their respective processing ages. Water and a corn-soybean based diet that was formulated to meet or exceed the National Research Council (NRC, 1994) requirements were provided ad libitum. Access to water remained constant throughout the experiment even during the 12 h feed withdrawal period.

On processing day, birds were cooped and transported to the processing plant. The birds were processed randomly by line and age in three batches. On the dock, birds were weighed and hung on a shackle-line. They were processed using inline equipment. Once placed on the line, birds were electrically stunned (11V, 11 mA, 10), manually cut through the carotid left artery and jugular vein and allowed to bleed out for 2 min, scalded (55^0C) and then picked with an in-line commercial de-feather machine. Birds were eviscerated by hand and weighed (WOG) prior to being placed in an ice bath chill tank. Carcasses remained in the chill tank for four hours. After the chill, carcasses were weighed (Chilled WOG) and manually deboned. Breasts of the deboned carcasses were stored in zip lock bags at 3^0C.

Color was recorded of the interior right lobe of the breast fillets at 4 and 24 h post-mortem using a Minolta CR-400 colorimeter. Breast muscle pH was also recorded of the cranial portion of the left lobe at 4 and 24 h using a Testo 205 pH probe. Fillets were blotted dried prior to the 24 h weight being recorded. Meat quality parameters were measured for the HMC, LMC and RBC lines including moisture uptake (calculated as ((Chilled WOG-WOG)/WOG)*100) and drip loss ((calculated as ((24 h Brst wt-4 h brst wt)/4 h brst wt)*100).

Experiment 2

The effect of feed withdrawal times on breast muscle color was analyzed in broiler lines that have been divergently selected for breast muscle color after 10 generations. Three broiler lines were used in this experiment to evaluate the effect of age on breast muscle color. The same lines evaluated in experiment 1 (HMC, LMC and RBC) were evaluated in experiment 2. The experiment consisted of two replications of 8 wk old broilers, processed two weeks apart. Broilers from all lines and treatment group were grown in the same little floor pen for a majority of the experiment similar to the conditions described in experiment 1. Two days prior to each

processing date, straight run birds from all 3 lines were equally and randomly divided by line into one of five treatment pens (n=10 birds/line/trt/replication). The treatment pens included feed withdrawal periods of 0, 6, 12, 18 and 24 h off feed prior to processing. Twenty-four hours prior to processing, all birds in all treatments were weighed and weight contemporary cohorts established to eliminate bias in starting weights. Each treatment group was then weighed prior to being taken off feed and then again on the dock to calculate shrink percentage. All broilers were cooped and transported to the plant and processed at the same time, comparable to the process described in experiment 1.

Carcass measurements were taken at various times throughout the rigor mortis process. The first pH reading occurred at 15 min post-mortem (Testo 205 pH probe) which was immediately after scalding and evisceration and a carcass weight prior to chilling was recorded. Carcasses were allowed to chill for 4 h and then were manually deboned. At 4 h post-mortem, carcass weights, breast weights, and breast color (Minolta CR400 colorimeter) were recorded on all carcasses. Breast fillets were then placed in zip lock bags and stored in a cooler at 3 ^{0}C. At 24 h post-mortem, breast fillets were removed from the bags, blotted dry, weighed and again evaluated for pH and color.

Statistical Analysis

For both experiments, data were analyzed using a two-way ANOVA in the Fit Model platform in JMP Pro 12 (SAS Institutes, 2014). Means were separated using the least square means with the Tukey adjustment (HSD). The main effects included in the model for experiment 1 were line and age as well as their interaction. For experiment 2, the main effects included line and feed withdrawal treatment as well as their interaction. For experiment 2, hatch differences were detected but was excluded from the analysis. Main effects for both experiments are

presented and the interactions will be discussed if relevant. Data are presented as the LS Mean ± standard error.

RESULTS AND DISCUSSION

Experiment 1

For all body and carcass weight measurements, a line effect was present. The LMC line was lower than the HMC and RBC lines for dock wt, with-out-giblets (WOG) wt, and chilled-WOG wt. This is potentially due to the founder effect as these line differences in body and carcass weight have been detected since generation 1. The line effect does not appear for percentage breast in which all lines had similar breast percentages. As expected, an age effect was seen for the same traits measured in which the 5 wk age group was the lightest for all weight measurements and the 10 wk age group was the heaviest (Table 1).

An age by line interaction was present for all meat quality traits measured except for 24 h pH and moisture uptake. Breast pH measurements were recorded at 4 and 24 h post-mortem (Table 2). For the HMC line, the 10 wk age group had the highest pH with no difference among the other age groups. At 24 h, the age difference for the LMC is no longer present and all age groups had similar pH measurements. The 10 wk group for the LMC line had the lowest pH at 4 h while the 5 wk group acted as an intermediate and the 6 and 8 wk groups having the highest 4 h pH. At 24 h, the 10 wk age group again had the lowest pH with the 6 wk age group having the highest. For the RBC line, no difference was present for the age groups at the 4 h pH measurement while at the 24 h measurement, the 10 wk age had the lowest pH. Differences in size seen between the age groups for each line may be responsible for the differences in pH post-mortem due to varying amounts of glycogen reserves in the broilers.

In this study, understanding the effect of increasing age on color was vastly important. At 4 h, color measurements (L*) between age groups differed within each line. At 4 h, the process of rigor mortis is still occurring resulting in changing pH and color from post-mortem anaerobic metabolism. The rigor mortis process is not fully complete in broilers until around 6 h. It is possible that different age groups have a higher rate of post-mortem metabolism which would result in differences in pH and L* at an early time point such as 4 h. For the HMC and RBC lines, color differences between the age groups were not present at the 24 h measurement (Table 2). In a random bred population as well as a line selected to represent a PSE-like myopathy, age does not appear to have a significant effect on color, which is known to be a strong indicator of issues related to meat quality. For the LMC line, the 5 wk age group was significantly lighter at both 4 and 24 h. At 5 wk, the breast fillets taken from the LMC line were smaller and thinner than those from the HMC and RBC lines. It is possible that when color measurements were taken on the thinner fillets, light reflectance from the tray could have been a possibility, resulting in the 5 wk age group having a lighter fillet than the remaining age groups within the LMC line.

The redness of the fillets, recorded as a*, was different for both line and age with an interaction being present. For the HMC line, a linear effect was observed between age and a* at both 4 and 24 h. For the RBC and LMC lines, the 5 and 6 wk age groups had the highest a*, followed by the 8 wk and 10 wk age groups. A negative correlation between age and a* was present in all lines. This is contrary to previous research that shows that a fillet redness will increase with age due to an increase in the concentration of myoglobin in the muscle (Nishida and Nishida, 1985).

Moisture uptake, which occurs when a carcass is being chilled in the ice bath was different between the age groups. For both the HMC and LMC lines, moisture uptake was

highest in the 5 wk age group with no difference among the remaining age groups. These carcasses do tend to be smaller than the RBC line, leading to more surface area for moisture uptake to occur. For the RBC line, moisture uptake was highest in the 5 wk age group, and lowest in the 10 wk age group. With the 5 wk age group being a significantly smaller bird, an increase in weight between the WOG and chilled-WOG measurements is much more likely to be detected in the analysis as a result of amount of surface area being higher.

Line differences within each age group are presented in Tables 3 and 4. For each age group, the HMC line had the highest 4 and 24 h L*, the lowest pH, lowest A* and highest b*. Drip Loss was also highest in the HMC line for each age group. The LMC line had the lowest 4 and 24 h L*, highest a* and lowest b*. The RBC line remained an intermediate for most traits. Selection techniques for the HMC and LMC lines are implemented at 6 wk of age each generation. Regardless of what age the lines are processed, the HMC, RBC and LMC line still behave as they have been selected (Harford et al., 2014).

Age does not appear to have a very significant impact on several of the meat quality traits measured in this study. It is possible that due to the slow growing nature of the populations compared to modern industry broilers, that meat quality abnormalities (including those characteristic of the HMC and LMC lines) are not heavily impacted by increasing age. Perhaps if the research lines were processed at ages closer to sexual maturity, the color changes would have been observed.

Experiment 2

Prior to slaughter, broilers typically undergo a period of feed withdrawal. Feed withdrawal is an important part of broiler processing but if withdrawal times are either too long

or too short, there can be negative consequences on both carcass and meat quality (Smidt et al., 1964; Bilgili, 1988) as well as yield (Veerkamp, 1978). Typical withdrawal times in the industry are around 10 h but transportation and holding times at the processing plant can extend those times. Not only can feed withdrawal times affect the yield and quality of a processed carcass, it may also have a significant impact on meat quality characteristics. The results of this experiment look at several meat quality parameters of broilers from three different lines, processed under different feed withdrawal periods.

Several meat quality parameters were evaluated in this experiment including 15 min and 24 h pH, 24 h color (CIE-L*, a*, b*), moisture uptake, drip loss and carcass shrink. Data were analyzed by line and treatment (feed withdrawal group). For weight measurements, no interactions were detected so only line and treatment differences will be presented.

No differences were detected for initial weights (24 h prior to processing) for any of the treatment groups. A line difference was present for initial weights in which the LMC was lighter than the RBC and HMC lines (Table 5). This is a result of the founder effect created at the beginning of the selection program and the line difference continues for all live weight and carcass weight measurements (Harford et al., 2014). For the withdrawal treatments, the 24 h feed withdrawal group had the lowest withdrawal wt, dock wt, WOG (without giblets) and chilled WOG which was to be expected with the duration of feed withdrawal (Table 6). No differences were detected in breast weights at 4 and 24 h between the treatment groups indicating that the feed withdrawal had no effect on absolute breast weight of the broilers regardless of line.

Fifteen-minute pH differed between the lines with the HMC line having the lowest pH and the RBC line having the highest pH. At 24 h, the HMC line had the lowest pH and the LMC line had the highest pH with the RBC line being an intermediate. A similar breakout was

recorded for L* in which the HMC line was the lightest and the LMC line was the lowest. Drip Loss was highest in the HMC line (Table 7). These traits indicate that the lines are behaving as they have been selected according to Harford and coworkers (2014). Moisture uptake did not differ within the lines and shrink percentage was highest in the LMC line.

For feed withdrawal periods, it was predicted that pH differences would be detected early post-mortem. With longer feed withdrawal times, glycogen and glucose reserves have the potential to be used up because of the state of fasting. Most of the glycogen reserves used will be hepatic glycogen but it is possible that breast muscle glycogen may also be used. If these reserves are used up, it is likely that early post-mortem pH will remain higher than a broiler who remained on feed the entire time prior to processing. No treatment differences were observed for 15-minute pH (Table 8). It is possible that this time point was too early to detect differences. A treatment difference does appear for the 24 h pH measurement as shown in Table 8. The 0 h withdrawal group had the lowest 24 h pH when compared to all other treatment groups. Without a withdrawal period, there is no time for glycogen reserves to be used up so that when the broiler is slaughtered, there is a higher amount of substrate available to be broken down resulting in a lower pH. While it is known that pH and color (L*) are highly correlated, the differences seen in pH at 24 h does not correlate with the differences in L*. No treatment differences were observed for L*.

A difference in moisture uptake was observed between the feed withdrawal treatment groups (Table 8). The 0 h withdrawal group had the lowest moisture uptake while the 24 h withdrawal group had the highest. Moisture loss is likely to occur with extended fasting periods which would allow for a higher moisture uptake during the carcass chilling (ice bath chilling) as indicated by the treatment differences. No treatment differences were observed for drip loss but

the effect was present for shrink percentage. The 24 h feed withdrawal group had the highest shrink percentage while the 0 h feed withdrawal group was the lowest. This was as expected as the longer a broiler is off feed, the more body mass it is a capable of losing during a period of fasting.

Longer feed withdrawal periods did not appear to have an effect on meat quality in broiler lines that have been divergently selected to represent both PSE- and DFD-like abnormalities. Longer feed withdrawal times had a negative impact on shrink of a carcass which ultimately affects while visual characteristics such as color (L*) and indicators of tenderness (drip loss) did not differ among the treatment groups. Feed withdrawal periods longer than 24 h may have an impact on the glucose and glycogen reserves of a broiler but a feed withdrawal period of that duration is a rare occurrence in an industry setting.

CONCLUSION

The literature has been inconclusive as to whether or not both age and feed withdrawal times have an effect on meat quality and if there is an effect, what that effect might be. In lines divergently selected to represent to meat quality myopathies, both age and feed withdrawal times had little to no effect on meat quality parameters measured, indicated by little to no difference seen within a line for L*. Contrary to previous research, the redness of a fillet decreases with increasing age for lines selected for both PSE- and DFD-like myopathies as well as their random bred control from which they were developed. In addition, extension of a feed withdrawal times past 24 h or grow out of longer than 10 wk may still have the potential to affect meat quality but are not likely to be relevant to normal industry practices.

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Table 1. Weight measurements for the muscle color lines[1] in each age group (Experiment 1)

	0	Line		
Age	Parameter	HMC	RBC	LMC
5	Dock Wt	1144.2±22.5a	1166.2±26.9a	1000.6±23.9b
	WOG	736.4±15.5a	757.1±18.5a	643.8±16.3b
	Chilled-WOG	766.4±15.9a	784.2±19.3a	673.0±16.7b
	% Breast	19.9±0.4a	19.6±0.5a	20.7±0.4a
6	Dock Wt	1659.6±27.8a	1704.0±30.9a	1484.0±25.7b
	WOG	1105.2±18.7a	1134.2±20.8a	980.9±17.3b
	Chilled-WOG	1143.4±19.6a	1175.8±17.9a	1016.9±17.9b
	% Breast	21.3±0.37a	20.8±0.4a	21.2±0.3a
8	Dock Wt	2516.8±48.0a	2564.0±45.1a	2290.1±44.6b
	WOG	1716.8±35.4a	1761.8±33.2a	1555.1±32.9b
	Chilled-WOG	11771.9±36.2a	1815.4±33.6a	1609.3±33.6b
	% Breast	22.0±0.3a	22.4±0.3a	22.0±0.3a
10	Dock Wt	3721.5±86.6a	3769.2±82.0a	3332.0±73.5b
	WOG	2602.6±66.2a	2618.5±62.7a	2322.5±56.2b
	Chilled-WOG	2683.8±66.6a	2690.8±63.1a	2380.6±57.1b
	% Breast	25.6±0.3a	24.8±0.3a	24.8±0.3a

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L*
RBC=Non-selected random bred control
[2]a-b Means within a row without a common letter are different at ($P \leq 0.05$)

Table 2. Meat quality traits broken out by age group within each line[1] (Experiment 1)

			Age (wk)			
Line	Time	Parameter	5	6	8	10
HMC	4 h	pH	5.64±0.02b	5.58±0.02b	5.59±0.02b	5.76±0.02a
		L*	54.4±0.4a	54.3±0.4a	53.3±0.4ab	52.8±0.4b
		a*	5.37±0.13a	4.62±0.13b	4.08±0.13c	3.35±0.13d
		b*	4.03±0.16a	3.23±0.16b	3.91±0.16a	3.68±0.16ab
	24 h	pH	5.67±0.03a	5.65±0.03a	5.64±0.03a	5.56±0.03a
		L*	55.6±0.4a	55.8±0.4a	54.8±0.4a	55.7±0.4a
		a*	4.83±0.12a	4.28±0.13b	3.78±0.12c	2.74±0.12d
		b*	4.77±0.20a	4.03±0.21a	4.55±0.20a	4.27±0.20a
		MU (%)	4.12±0.15a	3.55±0.15b	3.24±0.15b	3.14±0.15b
		DL (%)	1.52±0.26a	1.80±0.27a	0.96±0.24a	1.64±0.25a
RBC	4 h	pH	5.90±0.03a	5.96±0.03a	5.87±0.03a	5.94±0.03a
		L*	49.2±0.5a	46.9±0.4b	48.1±0.4ab	48.1±0.4ab
		a*	6.62±0.18a	5.84±0.18b	4.79±0.14c	4.10±0.15d
		b*	3.82±0.23a	1.73±0.22c	2.18±0.22bc	2.64±0.19b
	24 h	pH	5.97±0.04ab	6.01±0.04a	5.87±0.03bc	5.82±0.03c
		L*	51.0±0.4a	49.6±0.4a	50.1±0.3a	50.6±0.3a
		a*	6.01±0.18a	5.36±0.17a	4.43±0.14b	3.66±0.14c
		b*	3.82±0.25a	1.93±0.24b	2.43±0.19b	2.55±0.20b
		MU (%)	4.04±0.20a	3.70±0.19ab	3.13±0.16bc	2.83±0.16c
		DL (%)	1.25±0.25a	0.27±0.24b	0.80±0.19ab	0.34±0.20b
LMC	4 h	pH	6.17±0.03ab	6.26±0.03a	6.18±0.02a	6.08±0.02b
		L*	46.3±0.4a	43.5±0.3bc	42.5±0.3c	44.5±0.3b
		a*	8.00±0.16a	7.61±0.15a	6.35±0.14b	4.81±0.13c
		b*	5.15±0.24a	2.87±0.21b	2.33±0.21bc	1.83±0.19c
	24 h	pH	6.17±0.03b	6.28±0.03a	6.21±0.03ab	6.04±0.02c
		L*	49.1±0.3a	46.6±0.3bc	45.8±0.3c	46.9±0.3b
		a*	7.12±0.15a	6.85±0.14a	5.68±0.13b	4.37±0.12c
		b*	4.34±0.21a	2.32±0.19b	1.97±0.19b	1.67±0.17b
		MU (%)	4.54±0.19a	3.65±0.17b	3.46±0.17b	3.11±0.15b
		DL (%)	0.18±0.19ab	0.04±0.17b	0.21±0.16ab	0.68±0.14a

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control

[2]L*=lightness, a*=redness, b*=yellowness, MU=moisture uptake, DL=drip loss

[3]a-d Means within a row are different without a common letter at (P≤0.05).

Table 3. Meat quality traits broken out by line[1] within each age group at 4 h (Experiment 1)

		Line		
Age	Parameter	HMC	RBC	LMC
5	pH	5.64±0.02c	5.90±0.03b	6.17±0.03a
	L*	54.4±0.4a	49.2±0.5b	46.3±0.4c
	a*	5.37±0.13c	6.62±0.18b	8.00±0.16a
	b*	4.03±0.16b	3.82±0.23b	5.15±0.24a
6	pH	5.58±0.02c	5.96±0.03b	6.26±0.03a
	L*	54.3±0.4a	46.9±0.4b	43.5±0.3bc
	a*	4.62±0.13c	5.84±0.18b	7.61±0.15a
	b*	3.23±0.16a	1.73±0.22b	2.87±0.21a
8	pH	5.59±0.02c	5.87±0.03b	6.18±0.02a
	L*	53.3±0.4a	48.1±0.4b	42.5±0.3c
	a*	4.08±0.13c	4.79±0.14b	6.35±0.14a
	b*	3.91±0.16a	2.18±0.22b	2.33±0.21b
10	pH	5.76±0.02c	5.94±0.03b	6.08±0.02a
	L*	52.8±0.4a	48.1±0.4b	44.5±0.3c
	a*	3.35±0.13c	4.10±0.15b	4.81±0.13a
	b*	3.68±0.16a	2.64±0.19b	1.83±0.19c

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L*
RBC=Non-selected random bred control
[2]L*=lightness, a*=redness, b*=yellowness
[3]a-c Means within a row are different without a common letter at ($P \leq 0.05$)

Table 4. Meat quality traits broken out by line[1] within each age group at 24 h (Experiment 1)

		Line		
Age (wk)	Parameter	HMC	RBC	LMC
5	pH	5.67±0.03c	5.97±0.04b	6.17±0.03b
	L*	55.6±0.4a	51.0±0.4b	49.1±0.3c
	a*	4.83±0.12c	6.01±0.18b	7.12±0.15a
	b*	4.77±0.20a	3.82±0.25b	4.34±0.21ab
	MU (%)	4.12±0.15a	4.04±0.20a	4.54±0.19a
	DL (%)	1.52±0.26a	1.25±0.25ab	0.18±0.19b
6	pH	5.65±0.03c	6.01±0.04b	6.28±0.03a
	L*	55.8±0.4a	49.6±0.4b	46.6±0.3c
	a*	4.28±0.13c	5.36±0.17b	6.85±0.14a
	b*	4.03±0.21a	1.93±0.24b	2.32±0.19b
	MU (%)	3.55±0.15a	3.70±0.19a	3.65±0.17a
	DL (%)	1.80±0.27a	0.27±0.24b	0.04±0.17b
8	pH	5.64±0.03c	5.87±0.03b	6.21±0.03a
	L*	54.8±0.4a	50.1±0.3b	45.8±0.3c
	a*	3.78±0.12c	4.43±0.14b	5.68±0.13a
	b*	4.55±0.20a	2.43±0.19b	1.97±0.19b
	MU (%)	3.24±0.15a	3.13±0.16a	3.46±0.17a
	DL (%)	0.96±0.24a	0.80±0.19a	0.21±0.16b
10	pH	5.56±0.03c	5.82±0.03b	6.04±0.02a
	L*	55.7±0.4a	50.6±0.3b	46.9±0.3c
	a*	2.74±0.12c	3.66±0.14b	4.37±0.12a
	b*	4.27±0.20a	2.55±0.20b	1.67±0.17c
	MU (%)	3.14±0.15a	2.83±0.16a	3.11±0.15a
	DL (%)	1.64±0.25a	0.34±0.20b	0.68±0.14b

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control

[2]L*=lightness, a*=redness, b*=yellowness, MU=moisture uptake, DL=drip loss

[3]a-c Means within a row are different without a common letter at (P≤0.05)

Table 5. Body weights, carcass weights and breast weights of broilers, pooled from all feed withdrawal groups for the muscle color lines[1] (Experiment 2)

Withdrawal (h)	24 hr BW (g)	WD Wt (g)	Dock WT (g)	WOG (g)	Chilled WOG (g)
HMC	1894.6±19.5a	1951.7±20.2a	1924.1±20.2a	1270.8±14.1a	1321.7±14.4a
RBC	1883.0±19.2a	1941.4±19.9a	1912.5±19.9a	1265.5±13.8a	1312.9±14.1a
LMC	1646.5±19.2b	1704.2±19.9b	1676.3±19.9b	1107.7±13.8b	1151.1±14.1b

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control
[2]a-b Means within a column without a common letter are different at ($P \leq 0.05$)

Table 6. Body weights and carcass weights of broilers, pooled from the muscle color lines under different feed withdrawal programs of 0, 6, 12, 18 and 24 h off feed prior to slaughter (Experiment 2)

Withdrawal (h)	24 hr BW (g)	WD Wt (g)	Dock WT (g)	WOG (g)	Chilled WOG (g)
0	1886.2±25.0a	1864.0±25.9ab	1843.4±25.9a	1240.8±18.1a	1297.0±18.4a
6	1851.0±25.0ab	1903.5±25.9a	1823.4±25.9a	1246.3±18.1a	1284.3±18.4a
12	1817.7±24.7ab	1887.5±25.8ab	1857.4±25.6a	1229.6±17.8a	1275.9±18.2a
18	1783.2±24.9bc	1878.5±25.8ab	1868.7±25.8a	1211.3±17.9ab	1258.5±18.3ab
24	1702.0±24.9c	1795.1±25.8b	1795.1±25.8a	1145.2±18.0b	1193.6±18.3b

[1]a-c Means within a column without a common letter are different at ($P \leq 0.05$)

Table 7. Meat quality traits pooled from feed withdrawal groups for the muscle color lines[1] (Experiment 2)

	pH		24 h L*	Moisture Uptake	Drip Loss	Shrink
Line	15 min	24 h		(%)	(%)	(%)
HMC	6.10±0.02c	5.67±0.01c	55.6±0.2a	4.05±0.13a	1.39±0.07a	2.90±0.12b
RBC	6.24±0.02a	5.99±0.02b	49.7±0.2b	3.78±0.13a	0.76±0.07b	3.00±0.11b
LMC	6.17±0.02b	6.23±0.01a	45.6±0.2c	3.98±0.13a	0.58±0.07b	3.39±0.11a

[1] HMC=selected 10 generations for high L*, LMC=selected 10 generations for low L* RBC=Non-selected random bred control
[2]a-b Means within a column without a common letter are different at ($P \leq 0.05$)

Table 8. Meat quality traits pooled from the muscle color lines under different feed withdrawal programs of 0, 6, 12, 18 and 24 h off feed prior to slaughter (Experiment 2)

Withdrawal	pH		24 h L*	Moisture Uptake	Drip Loss	Shrink
(h)	15 min	24 h		(%)	(%)	(%)
0	6.18±0.02a	5.88±0.02b	50.55±0.25a	3.53±0.16b	0.90±0.09a	1.20±0.15d
6	6.16±0.02a	5.97±0.02a	50.37±0.25a	4.12±0.16ab	1.00±0.09a	2.74±0.15c
12	6.17±0.02a	6.02±0.02a	50.38±0.24a	3.84±0.16ab	0.89±0.09a	3.70±0.15b
18	6.18±0.02a	5.99±0.02a	49.85±0.24a	3.94±0.16ab	0.83±0.09a	5.07±0.15a
24	6.16±0.02a	5.96±0.02a	50.37±0.24a	4.27±0.16a	0.91±0.09a	5.17±0.15a

[a-d]Means within a column without a common letter are significantly different at ($P \leq 0.05$)

CONCLUSION

Poultry meat consumption in the United States has steadily increased over the past 20 years. This increase in consumption resulted in a need for faster growing and higher yielding birds accomplished through genetic selection and changes in nutrition and rearing conditions. These changes have resulted in several unwanted atypical meat quality characteristics including issues with color, water holding capacity, and tenderness. These characteristics are a direct result of the decline in pH as the muscle goes through rigor mortis. With a known relationship between pH and color, lines were divergently selected for breast muscle color (L*). After 10 generations of selection, the HMC and LMC lines (selected for high and low L* respectively) have been known to represent two meat quality abnormalities including both PSE- and DFD-like myopathies respectively. The purpose of this thesis was to characterize those lines, as well as their random bred control (RBC) through several studies.

The pH decline as a muscle goes through rigor mortis is directly responsible for the variation in meat quality that is observed. If the pH decline is too slow or too fast, meat quality can suffer. The HMC line, known to represent a PSE-like condition has been shown through the first study to have a very rapid pH decline early on in the rigor process and a low ultimate pH. This decline has negatively affected color, drip loss, cook loss and tenderness. Although the RBC and LMC lines did not differ in their pH decline, the general trend is that the LMC or DFD-like line has a higher ultimate pH at all time points measured. No effect of chilling regime was seen for any of the lines.

Several studies have shown that both the duration of feed withdrawal times and the age of the broiler may have an impact on meat quality and breast meat color. What is unclear is what that effect, if any, will be. Using lines that have been selected to represent specific meat quality

abnormalities (based on the breast muscle color), the effect of both feed withdrawal times and age could be easily understood. In lines divergently selected to represent to meat quality myopathies, both age and feed withdrawal times had little to no effect on meat quality parameters measured, indicated by little to no difference seen within a line for L*. Extension of a feed withdrawal time past 24 h or grow out of longer than 10 wk may still have the potential to affect meat quality but are not likely to be relevant to normal industry practices.

No single factor is responsible for the outcome of the ultimate pH and color of a broiler fillet post-mortem. A combination of ante mortem stressors, genetic predisposition, physiological factors and post-mortem handling techniques all are capable of producing changes in both color and pH. If the incidence and severity of both PSE- and DFD-like muscle increase, it may be hard to determine what factor or set of factors is responsible for the increase in myopathies.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony

FROM: Craig N. Coon, Chairman

DATE: Apr 3, 2015

SUBJECT: IACUC Approval

Expiration Date: Apr 5, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Protocol: 15039 "General Rearing of Selected Chicken and Quail Populations" to begin April 6, 2015

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 Apr 5, 2018 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 IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199